Synthesis and Cytotoxicity Evaluation of New 3-substituted 4-(4-methyloxy phenyl)-1*H*-Pyrrole Derivatives

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A new series of 3-substituted 4-(4-methyloxy phenyl)-1*H*-pyrrole derivatives were synthesized and biologically evaluated for potential anticancer activity. Fifteen targeted compounds showed high selectivity toward normal cells and cancer cells: that is, all targeted compounds had no obvious cytotoxicity toward normal human cells (HUVEC and NIH/3T3), but some compounds exhibited broad-spectrum proliferation inhibitory activity against the screened cancer cell lines. Among these pyrrole derivatives, compounds **3b** and **3o** showed potent anticancer activity against the MG-63 cell line, with IC_{50} values of 14.9 and 12.7 μM, respectively. Other pyrrole derivatives also showed promising proliferation inhibitory activity, including compound **3d** against A375 (IC_{50} = 18.6 μM), compound **3f** and **3j** against MGC80-3 (IC_{50} = 19.9 μM), and compound **3o** against MGC80-3 (IC_{50} = 11.9 μM). Because the developed pyrrole derivatives showed strong anticancer activity and high selectivity, this new series of pyrrole derivatives could be considered as promising lead compounds for further development of potent and safe anticancer agents.

Keywords: Pyrrole derivatives, Anticancer activity, Synthesis, MTT assay, Structure–activity relationship

Introduction

Pyrrole derivatives are an important class of heterocyclic compounds, and are found in many natural products,[1–3] pharmaceuticals,[4] and biological agents.[5–9] Usually, pyrrole derivatives are used as antifungal agents,[10–12] antimicrobial agents,[13–15] antiviral agents,[16,17] and cyclooxygenase inhibitors.[18–20] Pyrrole derivatives used as anticancer agents have only been rarely reported till now except for a few compounds separated from plant sources, such as alkaloids.[21,22] It is well known that anticancer agents in clinical use usually induce serious toxicity during the killing of cancer cells, and thus design and synthesis of novel anticancer agents with potent anticancer activity and low toxicity is a desirable approach to cancer treatment.

A series of novel 3,4-disubstituted pyrrole compounds have been reported by us, in which the moieties at the 3-position of the pyrrole ring are substituted by benzoyl, nitro, cyano, acetyl, and so on, and those at the 4-position of pyrrole ring by 4-methylthio phenyl, and 3-chloro-4-fluorophenyl. These reported compounds have shown the same level *in vitro* anticancer activity as paclitaxel. For example, some 3,4-disubstituted pyrrole derivatives have shown strong anticancer activity against the MGC80-3 cell line with IC_{50} values ranging from 4.5 to 9.6 μM, and some other compounds against A375 with IC_{50} values ranging from 6.1 to 8.5 μM. Moreover, these compounds have shown broad-spectrum anticancer activity against MGC80-3, MCF-7, CHO, CT-26, A375, and HCT-15 cell lines.[23,24]

More importantly, these compounds exhibit high selectivity to normal cell lines. They hardly induce cytotoxic effects in the screened normal cell lines when killing cancer cells. Therefore, our objective is to synthesize a novel series of 3,4-disubstituted pyrrole compounds, evaluate their biological activity, explore the structure–activity relationship, and try to find a lead compound with high selectivity and sensitivity.

In this paper, a novel series of 3-substituted 4-(4-methyloxy phenyl)-1*H*-pyrrole compounds were prepared by the method of Van Leusen pyrrole synthesis. The proliferation inhibitory activity of 15 pyrrole derivatives against two normal cell lines (HUVEC and NIH/3T3) and 16 cancer cell lines were determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay. Furthermore, the structure–activity relationship is discussed based on the activity data.

Experimental

Chemistry. All chemical reagents were purchased from commercial suppliers and were of analytical grade. Flash column chromatography was performed on a column packed with 200–300 mesh silica gel 60 or neutral aluminum oxide. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254}-plate with a fluorescent indicator. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Advanced III 400 spectrometer with DMSO-d_6 as solvent and TMS as the internal standard. High-resolution mass spectroscopy (HRMS) data were obtained on an AB SCIEX TripleTOF® 4600 mass spectrometer using the electrospray ionization (ESI) technique. Melting points were recorded on a microscopic melting point apparatus (SGW

X-4, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China) and were uncorrected.

General Procedure for the Synthesis of α,β-unsaturated Carbonyl Compounds (2a–2o). Na (0.01 g, 0.4 mmol) was cut to pieces and added into anhydrous MeOH (15 mL) under ice bath; the fresh MeONa/MeOH solution was prepared till no gas evolved. 4-Methoxybenzaldehyde (1.36 g, 10.0 mmol) was added to the fresh, cooled MeONa/MeOH solution (15 mL), and then 10.0 mmol acetyl aromatic compound was added dropwise. The mixture was stirred at room temperature for 2–8 h (36–54 h for **2n** and **2o** due to the steric hindrance of the naphthyl and biphenyl). The end of the reaction was monitored by TLC. Afterward, water (20 mL) and EtOAc (75 mL, 25 mL × 3) were added into the mixture. The inorganic precipitate was removed, and the organic solution was washed with brine three times and dried. The crude product was purified by silica gel column chromatography with EtOAc/hexane as eluent to get pure α,β-unsaturated carbonyl compounds (**2a–2o**).

General Procedure for the Synthesis of 3,4-disubstituted Pyrroles (3a–3o). One equivalent of α,β- unsaturated carbonyl compounds (**2a–2o**), 1.1 equiv TosMIC, and 1.2 equiv *t*-BuOK were added to an anhydrous THF (20 mL) solution, and the mixture was stirred in an ice bath for 1 h. Ice water (50 mL) was poured into the mixture, THF was removed under vacuum, and the solution was extracted by EtOAc (75 mL, 25 mL × 3). The combined organic layer solution was washed with saturated brine and dried over anhydrous Na_2SO_4. The crude pyrrole product was purified by aluminum oxide column chromatography with EtOAc/hexane as eluent to get pure 3,4-disubstituted pyrroles (**3a-3o**).

3-(4-Methoxyphenyl)-1-(2-fluorophenyl) propen-1-one (2a). Yellow solid, yield 98.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.81 (t, J = 6.0 Hz, 1H, ArH), 7.73 (d, J = 18.0 Hz, 1H, ═CH─), 7.59 (d, J = 9.0 Hz, 2H, ArH), 7.52 (m, 1H, ArH), 7.51 (m, 2H, ArH), 7.33–7.12 (m, 1H, ═CH─), 6.94 (d, J = 9.0 Hz, 2H, ArH), 3.86 (s, 3H, $-OCH_3$).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (2-fluorophenyl) methanone (3a). White solid, yield 77.7 %, mp 195–197 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.63 (s, 1H, pyrrole–NH), 7.55–7.42 (m, 2H, ArH), 7.38 (d, J = 9.0 Hz, 2H, ArH), 7.26 (d, J = 9.0 Hz, 1H, ArH), 7.22 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, ArH), 7.09 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, pyrrole H), 6.96 (t, J = 3.0 Hz, 1H, pyrrole H), 6.85 (d, J = 9.0 Hz, 2H, ArH), 3.74 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 186.45, 158.74 (1C, J_{CF} = 248.5 Hz), 157.70, 131.70 (1C, J_{CF} = 8.1 Hz), 129.79, 129.74 (2C), 129.70 (1C, J_{CF} = 3.6 Hz), 129.60, 127.19, 124.88, 124.10 (1C, J_{CF} = 3.3 Hz), 121.42, 119.70, 115.83(1C, J_{CF} = 21.7 Hz), 113.04 (2C), 54.97; ESI-HRMS *m/z*: calcd for $C_{18}H_{14}FNO_2$ ([M + H]): 296.1088; found: 296.0980.

3-(4-Methoxyphenyl)-1-(4-fluorophenyl) propen-1-one (2b). Pale yellow solid, yield 92.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.05 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 2H, ArH), 7.79 (d, J = 16.0 Hz, 1H, ═CH─), 7.60 (d, J = 8.0 Hz, 2H, ArH), 7.38 (d, J = 16.0 Hz, 1H, ═CH─), 7.17 (t, J = 8.0 Hz, 2H, ArH), 6.94 (d, J = 8.0 Hz, 2H, ArH), 3.86 (s, 3H, $-OCH_3$).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (4-fluorophenyl) methanone (3b). White solid, yield 90.8%, mp 221–223 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.55 (s, 1H, pyrrole–NH), 7.83 (t, J = 9.0 Hz, 2H, ArH), 7.34–7.28 (d, J = 9.0 Hz, 2H, ArH), 7.23 (s, 1H, pyrrole H), 7.04 (t, J = 9.0 Hz, 2H, ArH), 6.88 (s, 1H, pyrrole H), 6.84 (d, J = 9.0 Hz, 2H, ArH), 3.80 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 188.92, 163.95 (1C, J_{CF} = 249.0 Hz), 157.50, 136.57 (1C, J_{CF} = 2.9 Hz), 131.58 (2C, J_{CF} = 9.1 Hz), 129.41 (2C), 127.80, 127.53, 125.15, 120.24, 118.97, 115.01(2C, J_{CF} = 21.7 Hz), 113.17 (2C), 54.95; ESI-HRMS *m/z*: calcd for $C_{18}H_{14}FNO_2$ ([M + H]): 296.1088; found: 296.1003.

3-(4-Methoxyphenyl)-1-(2-chlorophenyl) propen-1-one (2c). White solid, yield 97.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.73 (d, J = 9.0 Hz, 2H, ArH), 7.62-7.42 (m, 4H, ArH), 7.34 (d, J = 18.0 Hz, 1H, ═CH─), 7.13 (d, J = 18.0 Hz, 1H, ═CH─), 7.68 (d, J = 9.0 Hz, 2H, ArH), 3.80 (s, 3H, $-OCH_3$).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (2-chlorophenyl) methanone (3c). White solid, yield 94.1 %, mp 211–213 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.63 (s, 1H, pyrrole–NH), 7.52–7.36 (m, 4H, ArH), 7.43 (d, J = 9.0 Hz, 2H, ArH), 6.97 (t, J = 3.0 Hz, 1H, pyrrole H), 6.93 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, pyrrole H), 6.87 (d, J = 9.0 Hz, 2H, ArH), 3.75 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 188.34, 157.74, 140.84, 130.41, 130.25, 129.72 (2C), 129.50 (2C), 128.47, 127.13, 126.77, 124.86, 120.89, 119.95, 113.05 (2C), 54.99; ESI-HRMS *m/z*: calcd for $C_{18}H_{14}ClNO_2$ ([M + H]): 312.0792, 314.0763; found: 312.0625, 314.0544.

3-(4-Methoxyphenyl)-1-(4-chlorophenyl) propen-1-one (2d). White solid, yield 96.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.07 (d, J = 9.0 Hz, 2H, ArH), 7.71 (d, J = 9.0 Hz, 2H, ═CH─), 7.60 (d, J = 6.0 Hz, 2H, ArH), 7.38 (s, 4H, ArH), 7.06 (d, J = 9.0 Hz, 2H, ArH), 3.98 (s, 3H, $-OCH_3$).

[4-(4-methoxyphenyl)-1*H*-pyrrol-3-yl] (4-chlorophenyl) methanone (3d). White solid, yield 88.1%, mp 230–232 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.62 (s, 1H, pyrrole-NH), 7.73 (d, J = 9.0 Hz, 2H, ArH), 7.52 (d, J = 9.0 Hz, 2H, ArH), 7.30 (d, J = 9.0 Hz, 2H, ArH), 7.23 (dd, J_1 = 3.0 Hz, J_2 = 1.2 Hz, 1H, pyrrole H), 7.00 (t, J = 3.0 Hz, 1H, pyrrole H), 6.84 (d, J = 9.0 Hz, 2H, ArH), 3.73 (s, 3H,$-OCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 189.04, 157.55, 138.76, 136.15, 130.71 (2C), 129.46 (2C), 128.30–128.10 (1C), 128.17 (2C), 127.46, 125.18, 120.11, 119.12, 113.17 (2C), 54.95; ESI-HRMS *m/z*: calcd for $C_{18}H_{14}ClNO_2$ ([M + H]): 312.0792, 314.0763; found: 312.0695, 314.0658.

3-(4-Methoxyphenyl)-1-(3-bromophenyl) propen-1-one (2e). Pale yellow solid, yield 93.5%. ^{1}H NMR (300 MHz,

$CDCl_3$) δ (ppm): 8.34–8.04 (m, 2H, ArH + ═CH═), 7.88 (d, J = 9.0 Hz, 2H, ArH), 7.87–7.68 (m, 3H, ArH + ═CH═), 7.64–7.47 (m, 1H, ArH), 7.01 (d, J = 9.0 Hz, 2H, ArH), 3.82 (s, 3H, ═OCH_3).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (3-bromophenyl) methanone (3e). White solid, yield 81.7 %, mp 215–217 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.64 (s, 1H, pyrrole–NH), 7.80–7.55 (m, 3H, ArH), 7.51–7.37 (m, 1H, ArH), 7.28 (d, J = 9.0 Hz, 2H, ArH), 7.25 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, pyrrole H), 7.00 (t, J = 3.0 Hz, 1H, pyrrole H), 6.83 (d, J = 9.0 Hz, 2H, ArH), 3.73 (s, 3H, ═OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 118.61, 157.57, 142.24, 133.93, 131.27, 130.34, 129.50 (2C), 128.43, 127.82, 127.39, 125.23, 121.44, 120.00, 119.23, 113.17 (2C), 54.96; ESI-HRMS *m/z*: calcd for $C_{18}H_{14}BrNO_2$ ([M + H]): 356.0287, 358.0266; found: 356.0163, 358.0192.

3-(4-Methoxyphenyl)-1-(4-bromophenyl) propen-1-one (2f). Pale yellow solid, yield 93.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.07 (d, J = 9.0 Hz, 2H, ArH), 7.85 (d, J = 9.0 Hz, 2H, ═CH═), 7.75 (d, J = 6.0 Hz, 4H, ArH), 6.99 (d, J = 9.0 Hz, 2H, ArH), 3.80 (s, 3H, ═OCH_3).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (4-bromophenyl) methanone (3f). White solid, yield 93.9 %, mp 250–252 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.62 (s, 1H, pyrrole–NH), 7.63 (s, 4H, ArH), 7.28 (d, J = 9.0 Hz, 2H, ArH), 7.21 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, pyrrole H), 6.99 (t, J = 3.0 Hz, 1H, pyrrole H), 6.82 (d, J = 9.0 Hz, 2H, ArH), 3.72 (s, 3H, ═OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 189.16, 157.55, 139.11, 131.11 (2C), 130.89 (2C), 129.46 (2C), 128.23, 127.45, 125.18, 125.13, 120.06, 119.13, 113.17 (2C), 54.96; ESI-HRMS *m/z*: calcd for $C_{18}H_{14}BrNO_2$ ([M + H]): 356.0287, 358.0266; found: 356.0203, 358.0199.

3-(4-Methoxyphenyl)-1-(4-methylphenyl) propen-1-one (2g). White solid, yield 94.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.93 (d, J = 8.0 Hz, 2H, ArH), 7.78 (d, J = 16.0 Hz, 1H, ═CH═), 7.50 9 (d, J = 9.0 Hz, 2H, ArH), 7.42 (d, J = 16.0 Hz, 1H, ═CH═), 7.30 (d, J = 8.0 Hz, 2H, ArH), 6.94 (d, J = 8.0 Hz, 2H, ArH), 3.86 (s, 3H, ═OCH_3), 2.44 (s, 3H, $PhCH_3$).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (4-methylphenyl) methanone (3g). White solid, yield 81.3 %, mp 215–217 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.52 (s, 1H, pyrrole-NH), 7.65 (d, J = 9.0 Hz, 2H, ArH), 7.27 (t, J = 6.0 Hz, 4H, ArH), 7.16 (dd, J_1 = 3.0 Hz, J_2 = 1.2 Hz, 1H, pyrrole H), 6.98 (t, J = 3.0 Hz, 1H, pyrrole H), 6.83 (d, J = 9.0 Hz, 2H, ArH), 3.73 (s, 3H, ═OCH_3), 2.36 (s, 3H, $PhCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 190.03, 157.46, 141.48, 129.35 (2C), 129.11 (2C), 128.65 (2C), 127,70, 127.51, 125.08, 120.46, 118.81, 113.17 (2C), 54.94, 21.01; ESI-HRMS *m/z*: calcd for $C_{19}H_{17}NO_2$ ([M + H]): 292.1338; found: 292.1012.

3-(4-Methoxyphenyl)-1-(2, 4-dimethyl phenyl) propen-1-one (2h). White solid, yield 91.8%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.52 (d, J = 9.0 Hz, 2H, ArH), 7.50–7.39 (m, 2H, ArH + ═CH═), 7.15–7.97 (m, 3H, ArH + ═CH═), 6.92 (d, J = 8.0 Hz, 2H, ArH), 3.85 (s, 3H,═OCH_3), 2.44 (s, 3H, $PhCH_3$), 2.38 (s, 3H, $PhCH_3$).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (2, 4-dimethylphenyl) methanone (3h). White solid, yield 93.9 %, mp 203–205 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.51 (s, 1H, pyrrole-NH), 7.40 (d, J = 9.0 Hz, 2H, ArH), 7.24 (d, J = 9.0 Hz, 1H, ArH), 7.06 (s, 1H, ArH), 7.02 (dd, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H, ArH), 6.95 (t, J = 3.0 Hz, 1H, pyrrole H), 6.89 (t, J = 3.0 Hz, 1H, pyrrole H), 6.85 (d, J = 9.0 Hz, 2H, ArH), 3.75 (s, 3H, ═OCH_3), 2.30 (s, 3H, ═$PhCH_3$), 2.22 (s, 3H,═$PhCH_3$); ^{13}C NMR (100 MHz, DMSO═d_6) δ (ppm): 192.14, 157.59, 138.59, 138.57, 135.14, 131.08, 129.63 (2C), 129.23, 127.88, 127.54, 125.44, 124.88, 121.87, 119.41, 113.02 (2C), 54.97, 20.79, 19.33; ESI-HRMS *m/z*: calcd for $C_{20}H_{19}NO_2$ ([M + H]): 306.1495; found: 306.1259.

3-(4-Methoxyphenyl)-1-(3-methoxo-phenyl) propen-1-one (2i). Pale yellow solid, yield 96.5%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.81 (d, J = 15.0 Hz, 1H, ═CH═), 7.62 (d, J = 9.0 Hz, 2H, ArH), 7.60–7.50 (m, 2H, ArH + ═CH═), 7.46–7.35 (m, 2H, ArH), 7.13 (dd, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H, ArH), 6.95 (d, J = 9.0 Hz, 2H, ArH), 3.90 (s, 3H,═OCH_3), 3.87 (s, 3H, ═OCH_3).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (3-methoxophenyl) methanone (3i). Pale yellow solid, yield 93.4 %, mp 193–195 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.56 (s, 1H, pyrrole–NH), 7.41–7.29 (m, 3H, ArH), 7.30 (d, J = 9.0 Hz, 2H, ArH), 7.32–7.26 (m, 2H, 1 pyrrole H +1 ArH), 7.12 (m, 1H, ArH), 6.99 (t, J = 3.0 Hz, 1H, pyrrole H), 6.82 (d, J = 9.0 Hz, 2H, ArH), 3.77 (s, 3H, ═OCH_3), 3.73 (s, 3H, ═OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 189.92, 158.86, 157.50, 141.51, 129.43 (2C), 129.20, 128.01, 127.62, 125.16, 121.36, 120.32, 118.99, 117.37, 113.57, 113.14 (2C), 55.12, 54.95; ESI-HRMS *m/z*: calcd for $C_{19}H_{17}NO_3$ ([M + H]): 308.1287; found: 308.1203.

3-(4-Methoxyphenyl)-1-(4-methoxo-phenyl) propen-1-one (2j). Pale yellow solid, yield 94.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.16 (d, J = 9.0 Hz, 2H, ArH), 7.83 (d, J = 18.0 Hz, 1H, ═CH═), 7.82 (d, J = 9.0 Hz, 2H, ArH), 7.68 (d, J = 18.0 Hz, 1H, ═CH═), 7.09 (d, J = 9.0 Hz, 2H, ArH), 7.02 (d, J = 9.0 Hz, 2H, ArH), 3.87 (s, 3H, ═OCH_3), 3.82 (s, 3H, ═OCH_3).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (4-methoxophenyl) methanone (3j). White solid, yield 93.3 %, mp 203–205 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.50 (s, 1H, pyrrole–NH), 7.75 (d, J = 9.0 Hz, 2H, ArH), 7.27 (d, J = 9.0 Hz, 2H, ArH), 7.17 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, pyrrole H), 6.99 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, pyrrole H), 7.00–6.98 (m, 1H, pyrrole H), 6.82 (d, J = 9.0 Hz, 2H, ArH), 3.82 (s, 3H, ═OCH_3), 3.73 (s, 3H, ═OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 189.26, 162.00, 157.40, 132.42, 131.24 (2C), 129.23 (2C), 127.75, 126.72, 124.98, 120.55, 118.55, 113.35 (2C), 113.20 (2C), 55.33, 54.93; ESI-HRMS *m/z*: calcd for $C_{19}H_{17}NO_3$ ([M + H]): 308.1287; found: 308.1212.

3-(4-Methoxyphenyl)-1-(2-pyridyl) propen-1-one (2k). White solid, yield 30.6%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.79 (d, J = 3.0 Hz, 1H, ArH), 8.20–7.98 (m, 3H, ArH + ─CH═), 7.88–7.75 (m, 3H, ArH + ─CH═), 7.69 (m, 1H, ArH), 7.03 (d, J = 9.0 Hz, 2H, ArH), 3.83 (s, 3H,─OCH_3).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (2-pyridyl) methanone (3k). White solid, yield 92.5 %, mp 139–141 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.58 (s, 1H, pyrrole–NH), 8.65 (d, J = 6.0 Hz, 1H, pyridine H), 7.96 (dt, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H, pyridine H), 7.72 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, pyrrole H), 7.81 (d, J = 9.0 Hz, 1H, ArH), 7.55 (m, 1H, pyridine H), 7.34 (d, J = 9.0 Hz, 2H, ArH), 6.93 (t, J = 3.0 Hz, 1H, pyrrole H), 6.86 (d, J = 9.0 Hz, 2H, ArH), 3.76 (s, 3H,─OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 187.36, 157.57, 156.87, 148.23, 137.09, 130.55, 129.76 (2C), 127.90, 125.75, 125.65, 122.99, 118.90, 118.84, 112.99 (2C), 54.96; ESI-HRMS *m/z*: calcd for $C_{17}H_{14}N_2O_2$ ([M + H]): 279.1134; found: 279.0886.

3-(4-Methoxyphenyl)-1-(3-pyridyl) propen-1-one (2l). Pale yellow solid, yield 47.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.29 (t, J = 4.0 Hz, 1H, ArH), 8.80 (m, 1H, ArH), 8.43 (m, 1H, ArH), 7.86 (d, J = 12.0 Hz, 2H, ArH), 7.79 (d, J = 12.0 Hz, 2H, ─CH═), 7.59 (m, 1H, ArH), 7.01 (dd, J_1 = 12.0 Hz, J_2 = 4.0 Hz, 2H, ArH), 3.81 (s, 3H, ─OCH_3).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (3-pyridyl) methanone (3l). White solid, yield 58.2 %, mp 202–204 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.69 (s, 1H, pyrrole-NH), 8.82 (dd, J_1 = 4.0 Hz, J_2 = 1.2 Hz, 1H, pyridine H), 8.70 (dd, J_1 = 8.0 Hz, J_2 = 4.0 Hz, 1H, pyridine H), 8.05 (dt, J_1 = 12.0 Hz, J_2 = 4.0 Hz, 1H, pyridine H), 7.48 (ddd, J_1 = 12.0 Hz, J_2 = 4.0 Hz, J_3 = 1.2 Hz, 1H, pyridine H), 7.32 (d, J = 12.0 Hz, 2H, ArH), 7.30-7.28 (m, 1H, pyrrole H), 7.02 (t, J = 4.0 Hz, 1H, pyrrole H), 6.84 (d, J = 12.0 Hz, 2H, ArH), 3.74 (s, 3H, ─OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 188.56, 157.59, 151.70, 149.38, 136.20, 135.58, 129.61 (2C), 128.81, 127.32, 125.19, 123.33, 120.26, 119.39, 113.16 (2C), 54.95; ESI-HRMS *m/z*: calcd for $C_{17}H_{14}N_2O_2$ ([M + H]): 279.1134; found: 279.1043.

3-(4-Methoxyphenyl)-1-(2-thienyl) propen-1-one (2m). Yellow solid, yield 85.2%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.30 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, ArH), 8.04 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, ArH), 7.85 (d, J = 6.0 Hz, 2H, ArH), 7.75 (d, J = 18.0 Hz, 1H, ─CH=), 7.70 (d, J = 18.0 Hz, 1H, ─CH═), 7.31 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, ArH), 7.03 (d, J = 6.0 Hz, 2H, ArH), 3.83 (s, 3H, ─OCH_3).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (2-thienyl) methanone (3m). White solid, yield 67.5 %, mp 205–207 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.59 (s, 1H, pyrrole–NH), 7.92 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, thiophenyl H), 7.71 (d, J = 3.0 Hz, 1H, thiophenyl H), 7.49 (t, J = 3.0 Hz, 1H, pyrrole H), 7.30 (d, J = 9.0 Hz, 2H, ArH), 7.20 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, thiophenyl H), 7.01 (t, J = 3.0 Hz, 1H, pyrrole H), 6.85 (d, J = 9.0 Hz, 2H, ArH), 3.74 (s, 3H, ─OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 181.85, 157.51, 145.71, 133.16, 132.87, 129.26 (2C), 128.18, 127.50, 126.51, 124.80, 120.11, 118.91, 113.27 (2C), 54.95; ESI-HRMS *m/z*: calcd for $C_{16}H_{13}NO_2S$ ([M + H]): 284.0746; found: 284.0547.

3-(4-Methoxyphenyl)-1-(1-naphthyl) propen-1-one (2n). Pale yellow solid, yield 82.9%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.28 (m, 1H, ArH), 8.13 (d, J = 9.0 Hz, 1H, ArH), 8.04 (m, 1H, ArH), 7.93 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, ArH), 7.76 (d, J = 9.0 Hz, 2H, ArH), 7.77–7.75 (m, 4H, ArH + ─CH═), 7.43 (d, J = 15.0 Hz, 1H, ─CH═), 6.99 (d, J = 9.0 Hz, 2H, ArH), 3.80 (s, 3H, ─OCH_3).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (1-naphthyl) methanone (3n). White solid, yield 86.0 %, mp 209–211 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.56 (s, 1H, pyrrole-NH), 8.06-7.94 (m, 3H, ArH), 7.65-7.59 (m, 1H, ArH), 7.58–7.49 (m, 3H, ArH), 7.47 (d, J = 9.0 Hz, 2H, ArH), 6.99 (t, J = 3.0 Hz, 1H, pyrrole H), 6.95 (dd, J_1 = 3.0 Hz, J_2 = 0.9 Hz, 1H, pyrrole H), 6.87 (d, J = 9.0 Hz, 2H, ArH), 3.76 (s, 3H, ─OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 191.43, 157.68, 139.02, 133.14, 130.14, 129.98, 129.77 (2C), 129.61, 128.21, 127.49, 126.63, 126.10, 125.88, 125.33, 125.18, 124.67, 122.24, 119.67, 113.04 (2C), 54.98; ESI-HRMS *m/z*: calcd for $C_{22}H_{17}NO_2$ ([M + H]): 328.1338; found: 328.1064.

3-(4-Methoxyphenyl)-1-(4-biphenyl) propen-1-one (2o). Yellow solid, yield 79.7%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.12 (d, J = 9.0 Hz, 2H, ArH), 7.9-7.55 (m, 6H, ArH + ─CH═), 7.55–7.35 (m, 3H, ArH + ─CH═), 7.27 (s, 2H, ArH), 6.96 (d, J = 9.0 Hz, 2H, ArH), 3.88 (s, 3H, ─OCH_3).

[4-(4-Methoxyphenyl)-1*H*-pyrrol-3-yl] (4-biphenyl) methanone (3o). Yellow solid, yield 40.2 %, mp 252–254 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 11.59 (s, 1H, pyrrole-NH), 7.90–7.65 (m, 6H, ArH), 7.50 (t, J = 6.0 Hz, 2H, ArH), 7.41 (t, J = 6.0 Hz, 1H, ArH), 7.33 (d, J = 9.0 Hz, 2H, ArH), 7.25 (t, J = 3.0 Hz, 1H, pyrrole H), 7.01 (t, J = 3.0 Hz, 1H, pyrrole H), 6.85 (d, J = 9.0 Hz, 2H, ArH), 3.73 (s, 3H, ─OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 189.83, 157.52, 142.93, 139.19, 138.91, 129.68 (2C), 129.44 (2C), 129.03 (2C), 128.05, 127.90, 127.65, 126.83 (2C), 126.35 (2C), 125.19, 120.42, 118.99, 113.18 (2C), 54.95; ESI-HRMS *m/z*: calcd for $C_{24}H_{19}NO_2$ ([M + H]): 354.1495; found: 354.1321.

***In vitro* Cytotoxicity Studies**

Cell Culture. All cell lines, namely A375 (human malignant melanoma), A549 (human lung cancer), CHO (Chinese hamster ovary), CT-26 (murine colon carcinoma), DU145 (human prostate cancer), HCT-15 (human colorectal adenocarcinoma), HCT-116 (human colon cancer), HeLa (human cervical carcinoma), Hep G2 (human hepatoma), K-562 (human chronic myeloid leukemia), L1210 (murine leukemia), MCF-7 (human breast cancer), MG-63 (human

osteosarcoma), MGC80-3 (human gastric cancer), NCI-H460 (human large cell lung cancer), SGC-7901 (human gastric adenocarcinoma), HUVEC (human umbilical vein endothelial), and NIH/3T3 (murine embryonic fibroblast) were purchased from Type Culture Collection of the Chinese Academy of Science (Shanghai, China). The cells were cultured in RPMI-1640 (Hyclon) or high glucose DMEM (Hyclon) medium with 10% heat-inactivated fetal bovine serum (Hyclon) 1% penicillin–streptomycin (Hyclon), and incubated at standard culture conditions (37 °C, 5% CO_2 in air) (Thermo Fisher Scientific, Wisconsin, USA). The culture medium was refreshed every 2 days.

MTT Assay. The logarithmic growth phase cells were collected and seeded into 96-well plates at a seeding density 5000 cells/well and incubated at 37 °C for 24 h. The cells were treated with different concentrations of drugs, and four parallel wells were arranged for each concentration. The blank control groups were prepared by the same procedure without sample treatment. After 24 h of treatment, the treatment medium was removed, and then 150 μL culture medium and 20 μL MTT solution (5 mg/mL in PBS) were added to each well. After 4 h of incubation, the medium was discarded, 100 μL DMSO was added to each well, and then the plate was shaken for 10 min for dissolving the formazan crystals. The percentage of cell viability was determined by measuring the absorption at 570 nm using a Multiskan MK3 microplate reader (Thermo, USA).

Results and Discussion

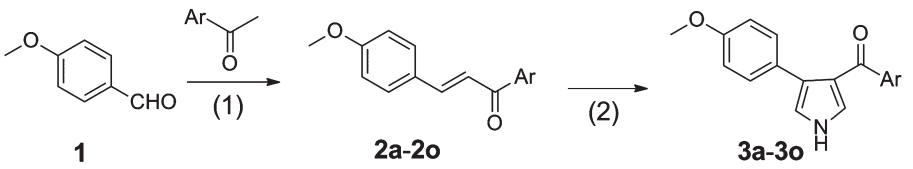
Chemistry. The target compounds, namely 3-substituted-4-(4-methyloxy phenyl)-1*H*-pyrrole derivatives **3a–3o**, were synthesized according to the methods described in Scheme 1.

As shown in Scheme 1, a simple synthetic route of 3,4-disubstituted pyrroles includes a two-step reactions: (1) α,β-unsaturated carbonyl compounds (**2a–3o**) synthesized by Aldol condensation reaction under base-catalyzed conditions, and (2) 3,4-disubstituted pyrroles (**3a–3o**) synthesized by Van Leusen pyrrole methodology under ice-bath conditions.

Biological Evaluation. The results of the proliferation inhibitory activity tests are shown in Table 1. It was found that all targeted compounds showed no obvious cytotoxicity toward normal cell lines (HUVEC and NIH/3T3) but potent proliferation inhibitory activity against some cancer cell lines. Among the tested derivatives, compounds **3b** and **3o** showed better or similar cytotoxic effect toward MG-63 cell compared with positive controls (paclitaxel and 5-fluorouracil) with an IC_{50} value of 14.9, 12.7, 20.5, and 27.8 μM, respectively. Other compounds also showed promising anticancer activity; for example, the IC_{50} value of compound **3d** inhibiting against A375 was 18.6 μM, the IC_{50} of compound **3f** and **3j** against MGC80-3 was 19.9 μM, and the IC_{50} of compound **3o** against MGC80-3 was 11.9 μM.

Although paclitaxel and 5-fluorouracil showed stronger proliferation inhibitory activity against cancer cells in comparison with most pyrrole derivatives, they also showed medium cytotoxicity toward normal cells. The IC_{50} values of paclitaxel inhibiting against HUVEC and NIH/3T3 were 57.9 and 54.3 μM, respectively. The IC_{50} values of 5-fluorouracil inhibiting against HUVEC and NIH/3T3 were 83.2 and 92.3 μM, respectively. However, the tested pyrrole derivatives hardly showed cytotoxicity toward normal cells. Thus, 3,4-disubstitued pyrrole derivatives had higher selectivity compared with other anticancer agents in clinical use.

Based on the activity data in Table 1 and the results of the previously reported compounds,[23,24] some preliminary structure–activity relationship of 3,4-disubstituted pyrrole compounds could be summarized as follows: (i) When substituted groups were benzoyl analogs at the 3-position of the pyrrole ring, the type of substituents on the benzoyl ring affected the anticancer activity. The structure–activity relationship was usually in the order: methoxy > bromo > chloro > fluoro > methyl, such as compounds **3j**, **3f**, **3d**, **3b,** and **3g**. (ii) When the substituents on the benzoyl ring were the same, the position of the substituents on the benzoyl ring also affected the anticancer activity. The structure–activity relationship was in the order: para- > (meta-, ortho-), such as in compounds **3j** and **3i**, compounds **3b** and **3a**, compounds **3f** and **3e**. (iii) When the benzene ring on the benzoyl was replaced by pyridine ring, the anticancer activity of compounds decreased, such as compounds **3k** and **3l**.



1 **2a-2o** **3a-3o**

2a, 3a: Ar = 2-F-Phenyl;
2b, 3b: Ar = 4-F-Phenyl;
2c, 3c: Ar = 2-Cl-Phenyl;
2d, 3d: Ar = 4-Cl-Phenyl;
2e, 3e: Ar = 3-Br-Phenyl;
2f, 3f: Ar = 4-Br-Phenyl;
2g, 3g: Ar = 4-CH_3-Phenyl;
2h, 3h: Ar = 2,4-$(CH_3)_2$-Phenyl;
2i, 3i: Ar = 3-OCH_3-Phenyl;
2j, 3j: Ar = 4-OCH_3-Phenyl;
2k, 3k: Ar = 2-Pyridyl;
2l, 3l: Ar = 3-Pyridyl;
2m, 3m: Ar = 2-Thienyl;
2n, 3n: Ar = 1-Naphthyl;
2o, 3o: Ar = 4-Biphenyl

Scheme 1. Synthetic route of 3-substituted-4-(4-methyloxy phenyl)-1*H*-pyrrole **3a–3o**. Reagents and conditions: (1) CH_3ONa, CH_3OH; (2) 1.1 equiv TosMIC, 1.2 equiv *t*-BuOK, THF, or DMSO, ice bath.

Table 1. The IC_{50} of the targeted compounds

Compound	IC_{50} (μmol/L)								
	A375	A549	CHO	CT-26	DU-145	HCT-15	HCT-116	Hela	Hep G2
3a	>100	—[a]	76.3	>100	>100	>100	>100	67.8	—
3b	56.9	>100	78.3	57.6	>100	41.7	—	20.8	>100
3c	—	—	—	>100	—	>100	—	—	—
3d	18.6	>100	40.1	27.9	>100	60.4	>100	26	>100
3e	>100	—	>100	>100	>100	87.6	—	>100	—
3f	25	>100	24.2	25.3	>100	38.8	20.5	>100	61
3g	>100	>100	78	>100	>100	>100	—	>100	91.1
3h	>100	—	>100	>100	>100	54.8	—	>100	>100
3i	—	—	>100	—	—	—	—	>100	—
3j	25.1	>100	24.2	25.3	>100	38.8	20.2	>100	61
3k	>100	—	>100	>100	—	—	>100	>100	—
3l	>100	—	>100	—	—	—	>100	>100	—
3m	43.5	>100	94.3	35.7	>100	86.6	45.6	74.9	>100
3n	56.9	—	82.6	27.2	—	57.8	>100	>100	56
3o	—	—	34.6	22.9	—	23.5	>100	>100	77.3
Paclitaxel	5.6	3.8	3.4	6.7	7.8	5.5	16.5	1.7	13.8
5-Fluorouracil	13.7	8.2	11.4	7.6	8.3	10.1	13.6	9.3	10.4

Compound	*IC_{50} (μmol/L)*								
	K-562	*L1210*	*MCF-7*	*MG-63*	*MGC80-3*	*NCI-H460*	*SGC-7901*	*NIHn3 T3*	*HUVEC*
3a	>100	>100	>100	>100	22	>100	>100	—	—
3b	94.7	24.4	>100	14.9	26.4	23.4	40	>100	>100
3c	—	—	—	—	—	—	—	—	—
3d	65.2	>100	>100	31.8	22.2	25	>100	—	>100
3e	>100	>100	>100	>100	22.5	>100	>100	—	—
3f	>100	>100	>100	30.6	19.9	25.8	>100	—	>100
3g	90	>100	>100	>100	28.9	67.7	—	—	—
3h	>100	>100	—	59.7	62.6	>100	>100	—	>100
3i	>100	>100	—	—	69.7	—	—	—	—
3j	>100	>100	>100	30.6	19.9	25.8	>100	—	>100
3k	—	>100	—	—	—	—	—	—	—
3l	—	>100	—	—	—	—	—	—	—
3m	—	>100	—	>100	45.9	64.7	>100	—	>100
3n	—	>100	—	96.3	43.4	56.9	—	—	—
3o	>100	>100	>100	12.7	11.9	>100	—	—	—
Paclitaxel	5.1	7.9	5.6	20.5	4.2	6.9	15.1	54.3	57.9
5-Fluorouracil	10.3	23.2	10.2	27.8	14.7	28.7	10.2	83.2	92.3

[a] When IC_{50} > 300 μmol/L, the compound is considered to have no cytotoxicity.

Conclusion

A series of novel 3,4-disubstituted pyrrole derivatives (**3a–3o**) were synthesized and their *in vitro* anticancer activities evaluated. These pyrrole derivatives exhibited broad-spectrum anticancer activity against the screened cancer cell lines; especially, compounds **3b** and **3o** showed stronger cytotoxicity toward MG-63 than positive controls. Structure–activity studies revealed that the type and position of the substituents on the benzoyl ring at the 3-position of the pyrrole ring seriously affected their proliferation inhibitory activities. These results contributed to the design and development of lead compounds with potent anticancer activity and high selectivity.

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Supporting Information. Additional supporting information is available in the online version of this article.

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