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Synthesis and anticancer activity of 3-(substituted aroyl)-4-(3,4,5-trimethoxy phenyl)-1*H*-pyrrole derivatives

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Abstract:

A series of 3-(substituted aroyl)-4-(3,4,5-trimethoxy phenyl)-1*H*-pyrrole derivatives were synthesized and determined for their anticancer activity against eleven cancer cell lines and two normal tissue cell lines using MTT assay. Among the synthesized compounds, compound **3f** was the most potent compound against A375, CT-26, Hela, MGC80-3, NCI-H460 and SGC-7901 cells (IC_{50}s = 8.2－31.7 μM); **3g**, **3n** and **3a** were the most potent compounds against CHO (IC_{50} = 8.2 μM), HCT-15 (IC_{50} = 21 μM) and MCF-7 cells (IC_{50} = 18.7 μM), respectively. Importantly, all the target compounds showed no cytotoxicity towards the normal tissue cell (IC_{50} > 100 μM). Thus, these compounds with the potent anticancer activity and low toxicity have potential for the development of new anticancer chemotherapy agents.

Keywords: Pyrrole derivatives; Anticancer activity; Synthesis; MTT assay; Cytotoxicity

Introduction.- Cancer is considered a major public health problem worldwide because it is a major cause of death affecting millions of people worldwide. Chemotherapy has been widely employed for various cancer treatments. However traditional chemotherapeutic agents also kill normal tissue cells during killing tumor cells, chemotherapeutic agents in clinical use usually induce multidrug resistance, toxicity and mutation [1]. Hence, design and synthesis of novel anticancer agents with potent anticancer activity and minimum side effects is still one of the greatest medical challenges.

The *N*-containing heterocyclic unit is usually found in some natural anti-cancer products, thus it attracts many researchers' interest in the drug discovery. Neda *et al.* reported a series of 1,2,4-oxadiazole derivatives, these compounds exhibited excellent potency against many cancer cell lines and even the IC_{50} values (half maximal inhibitory concentration) of some compounds are less than 0.1 μM [2-5]. Pyrrole is another important five-membered *N*-containing heterocycle, the compounds bearing pyrrole moiety are found in many natural and artificial products with the broad-spectrum pharmacological effects including antifungal [6-8], antimicrobial [9-11], antivirus [12-15], anticancer [16-18], cyclooxygenase inhibitors [19-21], antidiabeti [22] and antianxiety [23]. Recently a new range of possible chemotherapeutic compounds have been studied using pyrrole derivatives, which have been demonstrated to have good anti-proliferative activity. Temburnikar *et al.* explored the pyrrolo[3,2-*d*]pyrimidines which showed significant antiproliferative activity against cancer cells [24]. Pérez *et al.* reported the synthesis of a series of ferrocene coupling with pyrrole complexes and their cytotoxic activity against MCF-7 breast cancer, MCF-10A normal breast and HT-29 colon cancer cell lines [25]. Ghorab *et al.* published a series of pyrrole and fused pyrrole derivatives including pyrrolopyrimidines, pyrazolopyrrolopyrimidine, triazolopyrrolopyrimidines, tetrazolopyrrolopyrimidine, triazinopyrrolopyrimidines and pyrrolopyrimidotriazepines, and their cytotoxic activity against MCF-7 cell [26]. Dyson *et al.* synthesized a series of the natural product oroidin analogues, which is a pyrrole alkaloid isolated from the marine sponge agelas oroides, and evaluated their cytotoxicity against twelve cancer cell lines [27].

In our previous work, we found that pyrrole derivatives (Fig. 1) showed potent and broad-spectrum anticancer activity against human gastric cancer, breast cancer, malignant melanoma, osteosarcoma and large cell lung cancer [28-30]. When the moieties at the 4th-position of the pyrrole ring is 4-methoxy phenyl, the IC_{50} values (half maximal inhibitory concentration) of some pyrrole derivatives are less than 20 μM, these compounds show good anti-proliferative activity [29]. When the moieties at the 4th-position is 4-methylthio phenyl, the IC_{50} values of some pyrrole derivatives achieve micromolar level, these compounds show potent anticancer activity [30].

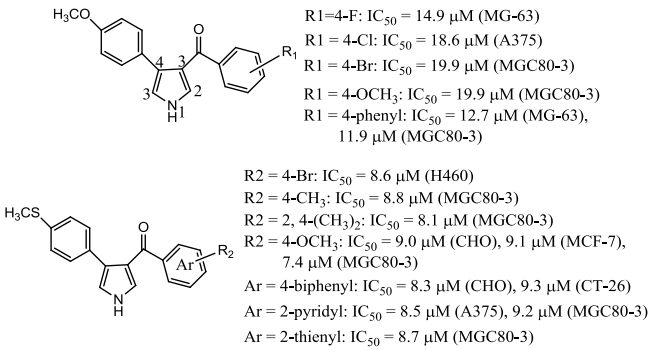


Figure 1.The structures and bioactivity of the pyrrole derivatives

Considering methoxy and methylthio belonging to electron-donating groups, we suppose that other electron-donating groups maybe increase the anticancer activity. Thus, 3, 4, 5-trimethoxy phenyl is condensed at the 4th-position of the pyrrole ring in this article, and the moieties at the 3rd-position of the pyrrole ring are still substituted aroyl or hetero aroyl referred to our previous work (Fig. 2). All the synthesized target compounds were confirmed by spectroscopic methods like ^{1}H NMR, ^{13}C NMR and HRMS spectrometry and screened for their anticancer activity *in vitro*.

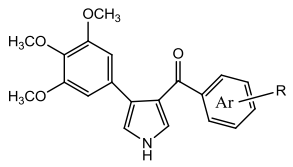
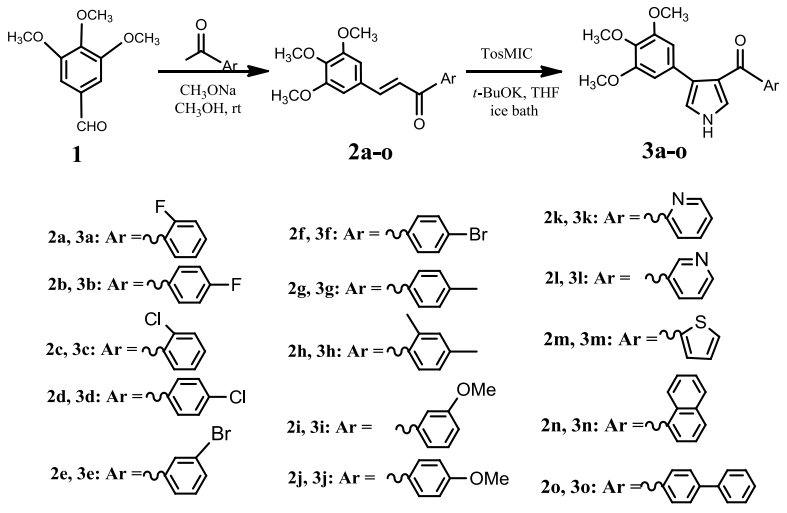


Figure 2. The structure of the target compounds

Results and Discussion

*Chemistry***.** The target compounds were synthesized according to the methods described in our previous work [28-30]. All target compounds were obtained through a facile two-steps reaction: (1) preparation of the intermediates, namely, 3,4,5-trimethoxy benzaldehyde **1** condensing with substituted benzoyl under base-catalyzed conditions to give the corresponding intermediates **2a-o**; (2) synthesis of the target compounds, namely, intermediates reacting with TosMIC reagent under ice-bath conditions to give the target compounds **3a-o** (Scheme 1).

Scheme 1. Synthetic route of the target compounds



Biological evaluation. The cytotoxicity activity of these target compounds was evaluated using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay against A375 (human malignant melanoma), CHO (Chinese hamster ovary), CT-26 (murine colon carcinoma), DU145 (human prostate cancer), HCT-15 (human colorectal adenocarcinoma), Hela (human cervical carcinoma), 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), NIH/3T3 (murine embryonic fibroblast) and HUVEC (human umbilical vein endothelial). The calculated IC_{50} values of tested compounds are presented in Table **1**.

Among the tested target compounds, compound **3f** bearing 4-bromophenyl was the most potent against A375 (IC_{50} = 11.1 μM), CT-26 (IC_{50} = 14.7 μM), Hela (IC_{50} = 12.7 μM), MGC80-3 (IC_{50} = 8.2 μM), NCI-H460 (IC_{50} = 21.4 μM) and SGC-7901 (IC_{50} = 31.7 μM); **3g** bearing 4-methyl phenyl was the most potent against CHO (IC_{50} = 8.2 μM); **3n** bearing 2-naphthyl was the most potent against HCT-15 (IC_{50} = 21 μM); **3a** bearing 2-fluro phenyl was the most potent against MCF-7 (IC_{50} = 18.7 μM).

All the target compounds showed good anti-proliferative activity against MGC80-3 (IC_{50}s = 8.2－23.4 μM) except the compounds **3l** bearing 3-pyridyl and **3m** bearing 2-thienyl (IC_{50}s > 100 μM). However, all the target compounds showed poor anti-proliferative activity against DU145 (IC_{50}s > 100 μM) except **3f** (IC_{50} = 45.2 μM) and **3g** (IC_{50} = 43.5 μM) with moderate anticancer activity. And all the target compounds showed poor anticancer activity against MG-63 (IC_{50}s > 100 μM) except **3f** (IC_{50} = 64.7 μM), **3n** (IC_{50} = 49.9 μM), **3o** bearing 4-biphenyl (IC_{50} = 57.1 μM) with moderate anticancer activity.

There are only the IC_{50} values of the compounds **3f** inhibiting against CHO and MGC80-3 and **3g** inhibiting against CHO were micromolar level in the Table 1. However, many compounds in the series of 3-(substituted aroyl)-4-(methylthio phenyl) pyrrole derivatives could achieve this level in the publications [30]. In comparison with the compounds bearing 4-methylthio phenyl moiety, the anticancer activity of the target compounds bearing 3, 4, 5-trimethoxy phenyl moiety didn't significantly increase. But compared with the pyrrole derivatives bearing 4-methoxy phenyl moiety in the publications [29], the target compounds in this article showed better anticancer activity.

Compared with the *in vitro* activity of the chemotherapy reagents approved for cancer therapy, namely, taxol and 5-fluorouracil (5-Fu), no target compounds exhibited stronger anticancer activity against eleven cancer cell lines than taxol, but compound **3f** showed more potent anticancer activity against A375 (1.2-fold difference), CHO (1.2-fold difference), MGC80-3 (1.8-fold difference) and NCI-H460 (1.3-fold difference) than 5-Fu. Similar to the recent publications [28-30], compound **3f** showed no cytotoxicity towards normal tissue cells. It's very important to cancer therapy that the chemotherapy reagents have the properties of high effectiveness and low toxicity. Thus, compound **3f** has potential for the development of the new chemotherapy agent.

Table 1. The IC_{50} values of the target compounds

Comp.	IC50 (μM)												
	A375	CHO	CT-26	DU145	HCT-15	Hela	MCF-7	MG-63	MGC80-3	NCI-H460	SGC-7901	NIH/3T3	HUVEC
3a	>100	51.8	--[a)]	--	--	>100	**18.7**[b)]	>100	22.5	--	--	--	--
3b	--	72.7	--	--	--	>100	--	--	**14.4**	>100	--	--	--
3c	>100	20.7	>100	>100	>100	>100	--	--	23.4	--	--	--	--
3d	>100	67.3	>100	--	>100	>100	--	>100	**19.5**	--	--	--	--
3e	>100	67.3	>100	--	>100	>100	--	>100	**19.5**	--	--	--	--
3f	**11.1**	**9.4**	**14.7**	45.2	45	**12.7**	49.5	64.7	**8.2**	21.4	31.7	>100	>100
3g	50	**8.2**	>100	43.5	>100	44	>100	>100	**13.9**	>100	76	--	--

3h	--	68.1	>100	--	>100	21.9	--	>100	**12.3**	>100	>100	>100	--
3i	46.6	**11.2**	37	>100	>100	**14.5**	>100	>100	**19.5**	--	66	>100	>100
3j	>100	22.1	45.5	--	>100	**19.1**	>100	>100	20.7	--	92.5	>100	>100
3k	>100	24.8	76.6	--	>100	47.1	>100	>100	**19.9**	>100	--	--	>100
3l	59.2	62.4	>100	>100	>100	89.9	>100	--	>100	--	>100	--	--
3m	>100	96.5	>100	--	>100	>100	--	>100	>100	--	--	--	--
3n	**18.4**	**11.4**	21.3	99	21	56.6	--	49.9	22.4	82.8	86.9	>100	80.1
3o	>100	27.6	**16.5**	>100	--	**14**	21.8	57.1	**14.3**	22.5	--	>100	>100
Taxol	**5.6**	**1.4**	**9.7**	**7.8**	**12.5**	**1.7**	**5.6**	20.5	**4.2**	**6.9**	**15.1**	>100	>100
5-Fu	**13.7**	**11.4**	**7.6**	**8.3**	**10.1**	**9.3**	**10.2**	27.8	**14.7**	28.7	**10.2**	83.2	92.3

[a]) "-- " meant IC_{50} > 300 μM. [b]) "bold type" meant IC_{50} < 20 μM

Conclusions

In conclusion, we report the synthesis of a series of 3-(substituted aroyl)-4-(3,4,5-trimethoxy phenyl) pyrrole derivatives. All the synthesized target compounds were evaluated for their cytotoxicity activity against eleven cancer cell lines and two normal cell lines. Among the target compounds, compound **3f** was the most potent compound against A375, CT-26, Hela, MGC80-3, NCI-H460 and SGC-7901; **3g**, **3n** and **3a** were the most potent compounds against CHO, HCT-15 and MCF-7 cells, respectively. Moreover, **3f** inhibiting against CHO and MGC80-3 and **3g** inhibiting against CHO achieved micromolar level.

Acknowledgements

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Experimental Part

Chemistry. All chemical reagents were purchased from commercial supplies 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 spectra (HRMS) data were obtained on an Agilent 6230 ToF 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.) and were uncorrected.

General procedure for the synthesis of the target compounds 3a-o. 10.0 mmoles of 3,4,5-trimethoxy benzaldehyde **1** was mixed with 12.0 mmoles of substituted benzoyl under base condition. The mixture was stirred at room temperature for 2—8 h. The end of the reaction was monitored by TLC. Afterwards, water (20 mL) and ethyl acetate (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 ethyl acetate/hexane as the eluent to get the pure intermediate **2a-o**.

The above intermediate (10.0 mmoles), toluenesulfonylmethyl isocyanide (TosMIC, 11.0 mmoles) and potassium *tert*-butoxide (12.0 mmoles) were added to an anhydrous tetrahydrofuran (20 mL) solution, and the mixture was stirred in an ice bath for 1 h. Ice water (50 mL) was poured into the mixture, tetrahydrofuran was removed under vacuum, and the solution was extracted by ethyl acetate (75 mL, 25 mL × 3). The combined organic layer solution was washed with saturated brine and dried over anhydrous sodium sulfate. The crude product was purified by aluminum oxide column chromatography with ethyl acetate/hexane as the eluent to get pure target compound.

*3-(2-fluorobezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3a**).* White solid, yield 87.5 %, mp 170-172 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.70 (s, 1H, pyrrole-NH), 7.50-7.44 (m, 2H, -CO-Ar-H), 7.22 (dd, *J*=8.0, 4.0 Hz, 2H, -CO-Ar-H), 7.17 (s, 1H, pyrrole-H), 7.08 (s, 1H, pyrrole-H), 6.75 (s, 2H, Ar-H), 3.74 (s, 6H, 3', 5'-OCH_3), 3.64 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 186.61, 158.77 (1C, J_{CF}=247.5 Hz), 152.05 (2C), 136.05, 131.80 (1C, J_{CF}=8.2 Hz), 130.31, 129.72, 129.66 (1C, J_{CF}=3.9 Hz), 129.53, 125.26, 124.03 (1C, J_{CF}=3.4 Hz), 121.70, 120.14, 115.77 (1C, J_{CF}=21.9 Hz), 106.39 (2C), 59.92, 55.70 (2C); ESI-HRMS *m/z*: calcd for $C_{20}H_{18}FNO_4$ ([M+H]): 356.1299; found: 356.1305.

*3-(4-fluorobezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3b**).* Pale yellow solid, yield 81.0 %, mp 209-211 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.66 (s, 1H, pyrrole-NH), 7.74 (dd, *J*=8.0, 4.0 Hz, 2H, -CO-Ar-H), 7.28-7.26 (m, 2H, -CO-Ar-H), 7.22 (s, 1H, pyrrole H), 7.12 (s, 1H, pyrrole H), 6.63 (s, 2H, Ar-H), 3.69 (s, 6H, 3', 5'-OCH_3), 3.62 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 189.18, 163.91 (1C, J_{CF}=249.1 Hz), 152.18 (2C), 136.59 (1C, J_{CF}=2.9 Hz), 135.89, 131.58 (2C, J_{CF}=9.0 Hz), 130.70, 127.67, 125.41, 120.62, 119.42, 114.89 (2C, J_{CF}=21.7 Hz), 106.15 (2C), 59.95, 55.70 (2C); ESI-HRMS *m/z*: calcd for $C_{20}H_{18}FNO_4$ ([M+H]): 356.1299; found: 356.1305.

*3-(2-chlorobezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3c**).* White solid, yield 89.9 %, mp 167-169 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.71 (s, 1H, pyrrole-NH), 7.48 (t, *J*=8.0 Hz, 1H, -CO-Ar-H), 7.43 (d, *J*=8.0 Hz, 2H, -CO-Ar-H), 7.38 (d, *J*=8.0 Hz, 1H, -CO-Ar-H), 7.10 (s, 1H, pyrrole H), 7.03 (s, 1H, pyrrole H), 6.82 (s, 2H, Ar-H), 3.78 (s, 6H, 3', 5'-OCH_3), 3.67 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 184.50, 152.06 (2C), 140.76, 136.10, 130.43, 130.24, 130.09, 129.55, 129.47, 128.56, 126.70, 125.25, 121.16, 120.45, 106.37 (2C), 59.94, 55.71 (2C); ESI-HRMS *m/z*: calcd for $C_{20}H_{18}ClNO_4$ ([M+H]): 372.1003, 374.0974; found: 372.1008, 374.0985.

*3-(4-chlorobezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3d**).* White solid, yield 77.4 %, mp 200-202 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.69 (s, 1H, pyrrole-NH), 7.69 (d, *J*=8.0 Hz, 2H, -CO-Ar-H), 7.47 (d, *J*=8.0 Hz, 2H, -CO-Ar-H), 7.29 (s, 1H, pyrrole H), 7.12 (s, 1H, pyrrole H), 6.64 (s, 2H, Ar-H), 3.70 (s, 6H, 3', 5'-OCH_3), 3.63 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 189.31, 152.17 (2C), 138.76, 136.09, 135.95, 130.71 (2C), 130.63, 128.04 (2C), 127.97, 125.45, 120.50, 119.53, 106.21 (2C), 59.98, 55.71 (2C); ESI-HRMS *m/z*: calcd for $C_{20}H_{18}ClNO_4$ ([M+H]): 372.1003, 374.0974; found: 372.1013, 374.0990.

*3-(3-bromobezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3e**).* Yellow solid, yield 71.5 %, mp 202-204 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.71 (s, 1H, pyrrole-NH), 7.69-7.53 (m, 3H, -CO-Ar-H), 7.44-7.35 (m, 1H, -CO-Ar-H), 7.32 (s, 1H, pyrrole H), 7.10 (s, 1H, pyrrole H), 6.60 (s, 2H, Ar-H), 3.68 (s, 6H, 3', 5'-OCH_3), 3.61 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 188.93, 152.18 (2C), 142.09, 135.88, 133.85, 131.46, 130.59, 130.19, 127.98, 127.65, 125.53, 121.25, 120.48, 119.48, 106.23 (2C), 59.86, 55.68 (2C); ESI-HRMS *m/z*: calcd for $C_{20}H_{18}BrNO_4$ ([M+H]): 416.0498, 418.0478; found: 416.0513, 418.0496.

*3-(4-bromobezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3f**).* Pale yellow solid, yield 91.3 %, mp 200-202 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.69 (s, 1H, pyrrole-NH), 7.59 (s, 4H, -CO-Ar-H), 7.28 (s, 1H, pyrrole H), 7.11 (s, 1H, pyrrole H), 6.63 (s, 2H, Ar-H), 3.69 (s, 6H, 3', 5'-OCH_3), 3.62 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ

(ppm): 189.44, 152.17 (2C), 139.10, 135.94, 130.98 (2C), 130.89 (2C), 130.62, 125.45, 125.06, 120.43, 119.55, 106.21 (2C), 60.00, 55.71 (2C); ESI-HRMS *m/z*: calcd for $C_{20}H_{18}BrNO_4$ ([M+H]): 416.0498, 418.0478; found: 416.0518, 418.0500.

*3-(4-methylbezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3g**).* White solid, yield 76.0 %, mp 214-216 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.59 (s, 1H, pyrrole-NH), 7.61 (d, *J*=8.0 Hz, 2H, -CO-Ar-H), 7.23 (s, 1H, pyrrole H), 7.20 (s, 2H, -CO-Ar-H), 7.11 (s, 1H, pyrrole H), 6.65 (s, 2H, Ar-H), 3.68 (s, 6H, 3', 5'-OCH_3), 3.62 (s, 3H, 4'-OCH_3), 2.33 (s, 3H, $PhCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 190.31, 152.17 (2C), 141.46, 137.38, 135.85, 130.85, 129.12 (2C), 128.54 (2C), 127.38, 125.31, 120.83, 119.27, 106.06 (2C), 59.96, 55.69 (2C), 20.97; ESI-HRMS *m/z*: calcd for $C_{21}H_{21}NO_4$ ([M+H]): 352.1550; found: 352.1576.

*3-(2,4-dimethylbezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3h**).* White solid, yield 69.1 %, mp 164-166 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.57 (s, 1H, pyrrole-NH), 7.22 (d, *J*=8.0 Hz, 1H, -CO-Ar-H), 7.06 (s, 1H, pyrrole H), 7.04 (s, 1H, pyrrole-H), 6.97 (s, 2H, -CO-Ar-H), 6.75 (s, 2H, Ar-H), 3.73 (s, 6H, 3', 5'-OCH_3), 3.64 (s, 3H, 4'-OCH_3), 2.27 (s, 3H, $PhCH_3$), 2.22 (s, 3H, $PhCH_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 192.25, 152.06 (2C), 138.62, 138.49, 135.97, 135.17, 131.03, 130.66, 129.09, 128.02, 125.39, 125.22, 122.15, 119.93, 106.27 (2C), 59.96, 55.69 (2C), 20.77, 19.36; ESI-HRMS *m/z*: calcd for $C_{22}H_{23}NO_4$ ([M+H]): 366.1701; found: 366.1736.

*3-(3-methoxybezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3i**).* White solid, yield 79.1 %, mp 166-168 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.64 (s, 1H, pyrrole-NH), 7.32 (t, *J*=8.0 Hz, 1H, pyrrole H), 7.28-7.22 (m, 2H, -CO-Ar-H), 7.15 (s, 1H, pyrrole H), 7.11-7.05 (m, 2H, -CO-Ar-H), 6.63 (s, 2H, Ar-H), 3.72 (s, 3H, -CO-Ar-OCH_3), 3.68 (s, 6H, 3', 5'-OCH_3), 3.61 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 190.22, 158.79, 152.15 (2C), 141.47, 135.86, 130.80, 129.09, 127.85, 125.41, 121.33, 120.73, 119.42, 117.45, 113.63, 106.15 (2C), 59.93, 55.68 (2C), 55.09; ESI-HRMS *m/z*: calcd for $C_{21}H_{21}NO_5$ ([M+H]): 368.1499; found: 368.1533.

*3-(4-methoxybezoyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3j**).* White solid, yield 82.8 %, mp 210-212 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.57 (s, 1H, pyrrole-NH), 7.72 (d, *J*=8.0 Hz, 2H, -CO-Ar-H), 7.21 (s, 1H, pyrrole H), 7.12 (s, 1H, pyrrole H), 6.96 (d, *J*=8.0 Hz, 2H, -CO-Ar-H), 6.64 (s, 2H, Ar-H), 3.80 (s, 3H, -CO-Ar-OCH_3), 3.68 (s, 6H, 3', 5'-OCH_3), 3.62 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 189.54, 162.00, 152.20 (2C), 135.79, 132.46, 131.24 (2C), 130.90, 126.64, 125.18, 120.93, 119.03, 113.27 (2C), 105.96 (2C), 59.95, 55.68 (2C), 55.34; ESI-HRMS *m/z*: calcd for $C_{21}H_{21}NO_5$ ([M+H]): 368.1499; found: 368.1538.

*3-(2-pyridylformyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3k**).* Pale yellow solid, yield 68.3 %, mp 155-157 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.65 (s, 1H, pyrrole-NH), 8.62 (d, *J*=8.0 Hz, 1H, pyridine H), 7.95 (td, J_1=8.0 Hz, J_2=2.0 Hz, 1H, pyridine H), 7.80 (td, *J*=8.0 Hz, J_2=0.8 Hz, 1H, pyridine H), 7.70 (dd, J_1=4.0 Hz, J_2=2.0 Hz, 1H, pyrrole H), 7.54 (m, 1H, pyridine H), 7.06 (t, *J*=4.0 Hz, 1H, pyrrole H), 6.72 (s, 2H, Ar-H), 3.75 (s, 6H, 3', 5'-OCH_3), 3.67 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 187.75, 156.93, 152.04 (2C), 148.21, 137.00, 135.92, 131.08, 130.38, 126.07, 125.60, 123.04, 119.38, 119.25, 106.36 (2C), 59.95, 55.73 (2C); ESI-HRMS *m/z*: calcd for $C_{19}H_{18}N_2O_4$ ([M+H]): 339.1346; found: 339.1387.

*3-(3-pyridylformyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3l**).* Pale yellow solid, yield 50.0 %, mp 163-165 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.77 (s, 1H, pyrrole-NH), 8.80 (s, 1H, pyridine H), 8.68 (dd, J_1=4.0 Hz, J_2=2.0 Hz, 1H, pyridine H), 8.01 (dt, J_1=8.0 Hz, J_2=2.0 Hz, 1H, pyridine H), 7.48-7.41 (m, 1H, pyridine H), 7.36 (t, *J*=4.0 Hz, 1H, pyrrole H), 7.15 (t, *J*=4.0 Hz, 1H, pyrrole H), 6.68 (s, 2H, Ar-H), 3.71 (s, 6H, 3', 5'-OCH_3), 3.64 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 188.78, 152.18 (2C), 151.65, 149.36, 136.16, 135.96, 135.58, 130.46, 128.61, 125.46, 123.21, 120.59, 119.84, 106.30 (2C), 59.94, 55.73 (2C); ESI-HRMS *m/z*: calcd for $C_{19}H_{18}N_2O_4$ ([M+H]): 339.1346; found: 339.1390.

*3-(2-thienylformyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3m**).* White solid, yield 79.0 %, mp 161-163 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.66 (s, 1H, pyrrole-NH), 7.92 (dd, J_1=4.0 Hz, J_2=1.2 Hz, 1H, thiophene H), 7.65 (dd, J_1=4.0 Hz, J_2=1.2 Hz, 1H, thiophene H), 7.50 (t, *J*=4.0 Hz, 1H, pyrrole H), 7.18 (dd, J_1=4.0 Hz, J_2=1.2 Hz, 1H, thiophene H), 7.14 (t, *J*=4.0 Hz, 1H, pyrrole H), 6.70 (s, 2H, Ar-H), 3.73 (s, 6H, 3', 5'-OCH_3), 3.65 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 182.09, 152.25 (2C), 145.65, 135.92, 133.12, 132.98, 130.70, 128.11, 126.50, 125.03, 120.42, 119.47, 105.98 (2C), 59.97, 55.73 (2C); ESI-HRMS *m/z*: calcd for $C_{18}H_{17}NO_4S$ ([M+H]): 344.0957; found: 344.1008.

*3-(2-naphthylformyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3n**).* Brown solid, yield 89.1 %, mp 157-159 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.51 (s, 1H, pyrrole-NH), 7.97 (dd, J_1=8.0 Hz, J_2=2.8 Hz, 1H, naphthalene H), 7.91 (d, *J*=8.0 Hz, 2H, naphthalene H), 7.51-7.46 (m, 3H, naphthalene H), 7.40 (t, *J*=8.0 Hz, 1H, naphthalene H), 7.14 (t, *J*=4.0 Hz, 1H, pyrrole H), 7.03 (t, *J*=4.0 Hz, 1H, pyrrole H), 6.57 (s, 2H, ArH), 3.68 (s, 6H, 3', 5'-OCH_3), 3.63 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 192.13, 152.47 (2C), 139.19, 136.38, 133.60, 131.11, 130.64, 130.26, 129.81, 128.70, 127.14, 126.82, 126.52, 126.09, 125.80, 125.05, 123.14, 120.46, 106.83 (2C), 60.36, 56.05 (2C); ESI-HRMS *m/z*: calcd for $C_{24}H_{21}NO_4$ ([M+H]): 388.1550; found: 388.1609.

*3-(4-biphenylformyl)-4-(3,4,5-trimethoxy phenyl)-1H-pyrrole (**3o**).* Pale yellow solid, yield 61.7 %, mp 211-213 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.66 (s, 1H, pyrrole-NH), 7.79 (d, *J*=6.0 Hz, 2H, Ar-H), 7.72 (d, *J*=6.0 Hz, 2H, Ar-H), 7.70 (d, *J*=6.0 Hz, 2H, Ar-H), 7.48 (t, *J*=6.0 Hz, 2H, Ar-H), 7.40 (t, *J*=6.0 Hz, 1H, Ar-H), 7.30 (t, *J*=6.0 Hz, 1H, pyrrole H), 7.13 (t, *J*=6.0 Hz, 1H, pyrrole H), 6.67 (s, 2H, Ar-H), 3.69 (s, 6H, 3', 5'-OCH_3), 3.59 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 190.06, 152.19 (2C), 142.94, 139.20, 138.89, 135.91, 130.79, 129.70 (2C), 129.01 (2C), 128.04, 127.80, 126.81 (2C), 126.25 (2C), 125.43, 120.76, 119.47, 106.16 (2C), 59.94, 55.72 (2C); ESI-HRMS *m/z*: calcd for $C_{26}H_{23}NO_4$ ([M+H]): 414.1706; found: 414.1769.

In vitro cytotoxicity studies. Cell culture. All cell lines: A375, CHO, CT-26, DU145, HCT-15, Hela, MCF-7, MG-63, MGC80-3, NCI-H460, SGC-7901, NIH/3T3 and HUVEC were purchased from Type Culture Collection of 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) and 1% penicillin-streptomycin (Hyclon), and incubated at a standard culture condition (37 °C, 5% CO_2 in air) (Thermo Fisher Scientific, 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 concentration 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 treatment, remove the treatment medium, and then add 150 μL of culture medium and 20 μL of MTT solution (5 mg/mL in PBS) to each well. After 4 h incubation, discard the medium and add 100 μL of DMSO to each well, and then shake plate 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).

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