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Bioorganic & Medicinal Chemistry 16 (2008) 2550–2557
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of imidazol-2-one derivatives as potential antitumor agents

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Received 14 October 2007; revised 16 November 2007; accepted 17 November 2007
Available online 22 November 2007

Abstract—A new series of aryl substituted imidazol-2-one derivatives structurally related to combretastatin A-4 (CA-4) were synthesized and evaluated for their cytotoxic activities in vitro against various human cancer cell lines including MDR cell line. The cytotoxic effects of compounds **7b** and **7i** proved to be similar to or greater than that of docetaxel. The highly active compound **7b** also exhibited excellent inhibitory activity on tumor growth in vivo.

1. Introduction

Combretastatin A-4 (CA-4), a *cis*-stilbene natural product isolated by Pettit et al. in 1989 from the South African willow tree *Combretum caffrum*,[1] strongly inhibits tubulin polymerization by binding to the colchicine site.[2] Recently, CA-4, which displayed its potent activity against a broad spectrum of human cancer cells including multidrug resistant cells (MDR),[3] has drawn the significant attention due to its potent and selective effect on the established tumor vasculature.[4] The vascular disrupting properties of CA-4 and related compounds represent a new approach to cancer therapy.[5,6] A water-soluble sodium phosphate prodrug (CA-4P) is currently in phase II and III clinical trials on advanced cancers based on the vascular shutdown mechanism of action.[7] Another CA-4 derivative AVE8062[8,9] is currently under clinical evaluation as tumor vascular targeting agent.[5,10]

To date, many CA-4 analogues were synthesized and their anticancer activities have been extensively studied. The structure–activity relationship demonstrated the *cis* configuration of double bond and 3,4,5-trimethyloxyphenyl group are fundamental.[11] The restricted rotation of rings A and B of CA-4 can be maintained by introducing suitable conformationally restricted heterocycles such as Imidazole,[12] Isoxazole,[13] and Indole[14] (Fig. 1). Many of them showed potent cytotoxicity against various cancer cell lines as compared to CA-4. According to the SAR, we designed and synthesized a series of *cis* restricted analogues with imidazol-2-one instead of the *cis* double bond in CA-4. In addition, we maintained 3,4,5-trimethoxyphenyl as ring A throughout the present investigation and examined several variation of substituents on ring B.

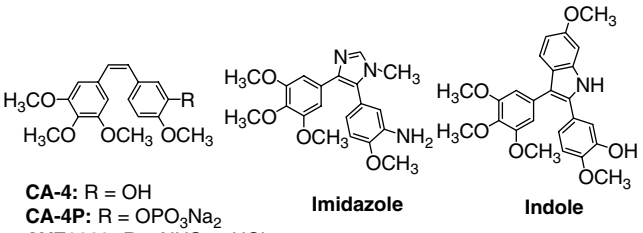

Figure 1. Structures of combretastatin A-4 and selected analogues of CA-4.

Keywords: Antitumor agents; Combretastatin A-4; Imidazol-2-one derivatives; Synthesis; Cytotoxicity.
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doi:10.1016/j.bmc.2007.11.048

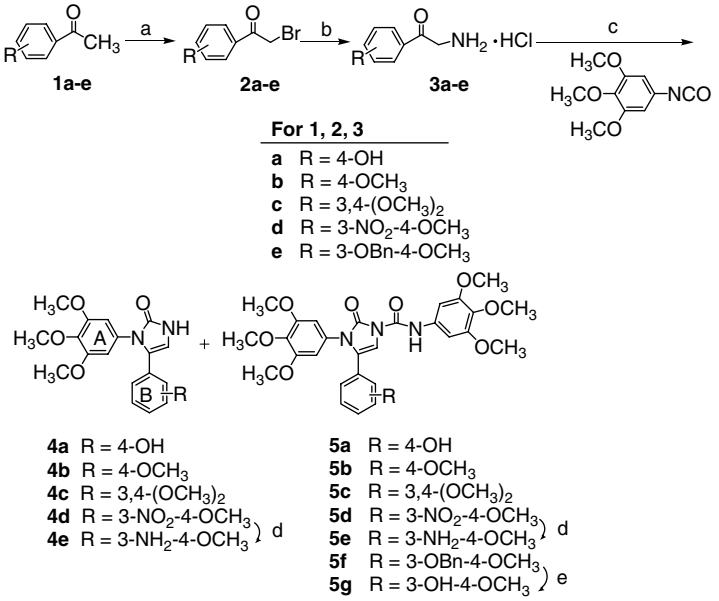
2. Results and discussion

2.1. Chemistry

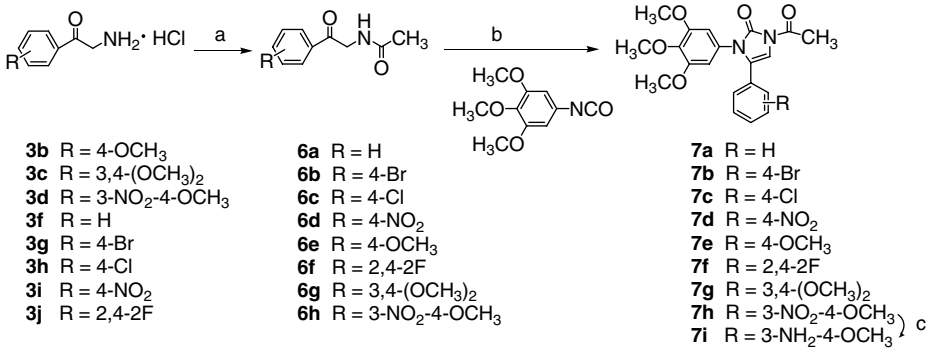
To prepare the imidazol-2-one derivatives, we utilized our recently developed synthetic protocol[15] which was summarized as follows. Bromination of substituted acetophenones **1a**–**e** gave substituted 2-bromo-1-phenylethanones **2a**–**e**, which were treated with hexamethylenetetramine and then hydrolyzed with concd HCl in EtOH to give the corresponding 2-amino-1-phenylethanone hydrochlorides **3a**–**e** (Scheme 1). Substituted 2-amino-1-phenylethanone hydrochlorides **3f**–**j** were prepared in the same manner.

Cyclization of 2-amino-1-(4-hydroxyphenyl)ethanone hydrochloride **3a** with 3,4,5-trimethoxyphenyl isocyanate in refluxing toluene provided an approximately 1:3 mixture of 1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-(4-hydroxyphenyl)-2*H*-imidazol-2-one **4a** and 2,3-dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-4-(4-hydroxyphenyl)-2-oxo-1*H*-imidazole-1-carboxamide **5a**, which were separated and assigned on the basis of its IR spectra, ^{1}H NMR, and MS data. Similarly, compounds **4b** and **5b**, **4c** and **5c**, **4d** and **5d** were achieved by reaction of the corresponding 2-amino-1-phenylethanone hydrochlorides **3b**–**d** with 3,4,5-trimethoxyphenyl isocyanate, respectively. However, when 2-amino-1-(3-benzyloxy-4-methoxyphenyl)ethanone hydrochloride **3e** was reacted with 3,4,5-trimethoxyphenyl isocyanate under the same condition, only compound **5f** was isolated in moderate yield. Reduction of the nitro groups of **4d** and **5d** using acetic acid and zinc powder yielded **4e** and **5e**, respectively. Deprotection of the phenol function of **5f** was carried out by hydrogenation to afford **5g** (Scheme 1).

On the other hand, acetylation of substituted 2-amino-1-phenylethanone hydrochlorides **3b**–**j** with acetic anhydride resulted in the corresponding *N*-(2-oxo-2-phenylethyl)acetamides **6a**–**h**[16] which were then reacted with 3,4,5-trimethoxyphenyl isocyanate to yield desired 1-acetyl-1,3-dihydro-3,4-diaryl-2*H*-imidazol-2-ones **7a**–**h**. Compound **7i** was prepared by using the same approach as that for **4e** (Scheme 2).



Scheme 1. Reagents and conditions: (a) pyridinium hydrobromide perbromide, THF, rt, 3 h; (b) i—hexamethylenetetramine, $CHCl_3$, rt, 1 h; ii—C_2H_5OH, HCl aq, rt, 1 h; (c) toluene, reflux, 4 h; (d) AcOH, Zn, rt, 2 h; (e) 10% Pd/C, H_2, rt, 2 h.



Scheme 2. Reagents and conditions: (a) $(Ac)_2O$, H_2O, $NaOAc/H_2O$, 0 °C to rt; (b) toluene, reflux, 4 h; (c) AcOH, Zn, rt, 2 h.

2.2. Biological activity

The synthesized aryl substituted imidazol-2-ones **4a**–**e**, **5a**–**e**, **5g**, **7a**–**i** were tested for their cytotoxic activities in vitro against several human cancer cell lines including human myeoloid leukemia cells HL-60, human myeoloid leukemia cells K562 and K562R (multidrug resistant cells), human prostate carcinoma cells PC-3, human breast carcinoma cells MCF-7, human esophageal carcinoma cells ECA-109, human hepatocarcinoma cells BEL-7402, and human non-small lung cancer cells A549. Docetaxel was employed as a positive control. The results are summarized in Table 1.

As shown in Table 1, compounds **7b** and **7i** displayed similar or more potent cytotoxic activities in comparison with docetaxel. Compound **7b**, with a 4-positioned bromine atom on the B ring, exhibited the most potent cytotoxic activity, with IC_{50} values ranging from 0.2 to 10.6 μM against the tested cancer cell lines. However, replacing 4-positioned bromine atom (**7b**) with chlorine or fluorine atom on the B ring (e.g., **7c**, **7f**), they showed a drastic loss of activities. Replacement of 4-bromine atom with 4-methoxy group on the B ring, **7e**, displayed a decrease in the cytotoxic activity by one order of magnitude.

Compound **5g** with 3-hydroxy-4-methoxy groups on the B ring, identical with B ring of CA-4, showed lower activity than **5b** with only 4-methoxy group on the B ring. The result implied that the introduction of hydroxyl group at the C-3 position of B ring failed to improve the cytotoxic activity profile in the tested compounds. This is in agreement with observation in other literature reports on CA-4 derivatives which suggest that 3-hydroxy group on the B ring is not essential for cytotoxicity.[7,17]

Consistent with previous reports,[18] increased cytotoxic activity was observed when the nitro groups of **4d**, **5d**, and **7h** were reduced to the amine compounds **4e**, **5e**, and **7i**. Besides, a comparison between cytotoxicities of **7e**, **7g**, **7h**, and **7i** revealed that the presence of an amino group at the C-3 position on the B ring was beneficial for potency especially against HL-60 and K562 cells.

Compounds **7a**–**i** with acetyl group at N-1 position of imidazol-2-one ring showed more effective cytotoxic activities than those with hydrogen atom (**4a**–**e**) or trimethoxyphenyl carbomide group (**5a**–**e**, **5g**) in overall activity, suggesting that acetyl group at N-1 position of imidazol-2-one ring was favorable for cytotoxic activity. It is worthy to pinpoint that the cytotoxicities of imidazol-2-ones showed more sensitivity against leukemia cells HL-60 as compared to other cell lines over all activity.

To gain further insight into the mechanisms of action of these compounds, the most cytotoxic compound **7b** was further assayed for its effect on cell cycle (by flow cytometry). K562 cells were treated with **7b** at different concentrations for 48 h. In 0.01 μM **7b** treatment group, 31% cells were arrested in G_2/M phase and 54% cells were arrested in S phase, while in control group, 19% and 1% cells were observed in G_2/M phase and S phase, respectively, (Fig. 2). At higher concentrations of 0.1 μM and 1 μM, compound **7b** induced apoptosis in K562 cells, the percentages of apoptotic cells were 45.6% and 78.6%, respectively.

Table 1. In vitro cytotoxic activities of compounds **4a**–**e**, **5a**–**e**, **5g**, **7a**–**i** and docetaxel against eight human cancer cell lines

Compound	Cytotoxicity (IC_{50}, μM)[a,b]							
	HL-60	K562	K562R	PC-3	MCF-7	ECA-109	BEL-7402	A549
4a	20.0	>50	>50	30.2	>50	>50	>50	>50
4b	19.8	ND[c]	>50	>50	ND	>50	ND	>50
4c	38.8	ND	>50	>50	ND	>50	ND	>50
4d	14.3	ND	>50	>50	ND	>50	ND	>50
4e	10.3	49.2	>50	49.8	>50	>50	>50	>50
5a	6.3	10.4	>50	3.0	>50	>50	>50	2.5
5b	5.4	3.8	3.5	49.7	>50	>50	>50	>50
5c	6.5	>50	>50	>50	>50	>50	>50	>50
5d	19.7	7.2	>50	>50	ND	>50	ND	>50
5e	3.1	5.8	9.4	10.0	>50	>50	10.1	11.1
5g	15.4	>50	>50	>50	ND	>50	ND	>50
7a	5.6	>50	9.6	>50	>50	>50	>50	>50
7b	0.2	1.0	0.9	3.1	10.6	3.8	1.2	1.3
7c	4.9	>50	ND	ND	ND	>50	ND	>50
7d	7.2	26.6	49.6	32.9	>50	>50	>50	49.6
7e	5.5	0.2	9.5	19.7	>50	>50	9.5	17.8
7f	7.6	>50	>50	34.3	49.3	>50	36.2	24.1
7g	12.2	43.4	>50	>50	>50	>50	>50	>50
7h	5.6	>50	>50	30.3	>50	30.8	35.7	40.9
7i	0.4	0.5	49.3	21.4	43.4	>50	0.6	1.1
Doxetaxel	ND	8.5	ND	54.8	4.0	ND	ND	1.7

[a] IC_{50}, compound concentration required to inhibit tumor cell proliferation by 50%.
[b] Values are means of three experiments.
[c] ND, not determined.

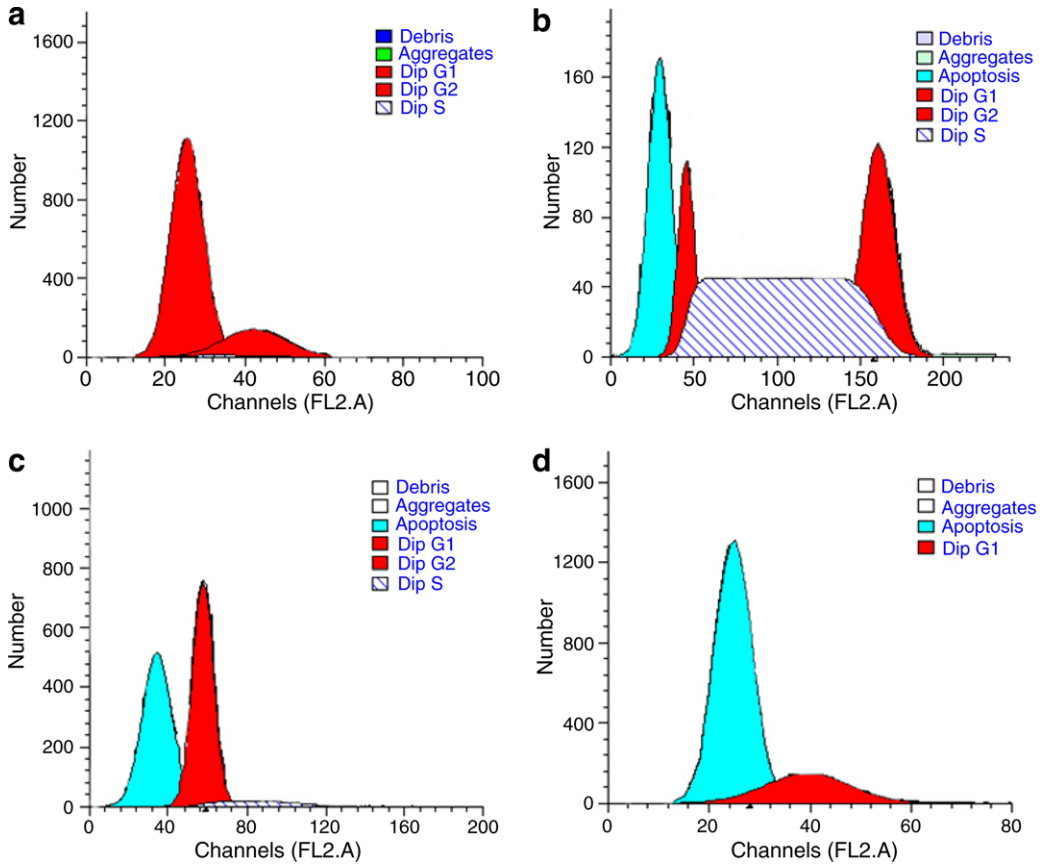


Figure 2. Effect of **7b** on the cell cycle as determined by flow cytometry. K562 Cells were treated with compound **7b** at the concentrations of 0 μM (control, a), 0.01 μM (b), 0.1 μM (c), and 1 μM (d) for 48 h.

Based on the potent cytotoxic activities of the test compounds in vitro, we chose compound **7b** and cyclophosphamide (**CTX**) to investigate their growth inhibitory activities on murine S-180 and murine H-22 tumor bearing models. After daily administration with different doses for 10 days, animals were sacrificed and the tumors were excised and weighted. The results of experimental therapeutic efficacy of **7b** are shown in Tables 2, 3 and Figures 3, 4.

Compared to the control group, tumor weights of S-180 in 15 mg/kg **7b** group and 30 mg/kg **CTX** group reduced significantly ($P < 0.01$), and the inhibition rates were 55.3% and 63.5%, respectively. In H-22 bearing mice model, the inhibition rates of 20 mg/kg **7b** and 30 mg/kg MTX groups were 55.5% ($P < 0.01$) and 36.2%, respectively. Therefore, comparing with **CTX**, compound **7b** presented a similar inhibiting effect on murine S-180 tumor growth and more potent inhibiting

Table 2. Effect of compound **7b** and **CTX** on S-180 tumor bearing mice

Group	Dosage (mg/kg/day)	Ad.	Initial/end		Tumor weight (g) $X \pm SD$	Inhibition rate (%)
			Mice (n)	Body weight(g)		
NS	—	—	18/18	21.2/35.3	1.07 ± 0.59	—
CTX	30	ip	9/9	21.3/34.0	0.39 ± 0.14[a]	63.5
7b	15	im	9/9	21.0/35.1	0.48 ± 0.19[a]	55.3

[a] $p < 0.01$ versus NS group.

Table 3. Effect of compound **7b** and **CTX** on H-22 tumor bearing mice

Group	Dosage (mg/kg/day)	Ad.	Initial/end		Tumor weight (g) $X \pm SD$	Inhibition rate (%)
			Mice (n)	Body weight (g)		
NS	—	—	18/18	18.5/26.7	0.86 ± 0.55	—
CTX	30	ip	9/9	18.3/26.3	0.55 ± 0.23	36.2
7b	5	im	9/9	18.2/26.8	0.61 ± 0.41	29.4
7b	20	im	9/9	18.7/27.6	0.38 ± 0.45[a]	55.5

[a] $p < 0.01$ versus NS group.

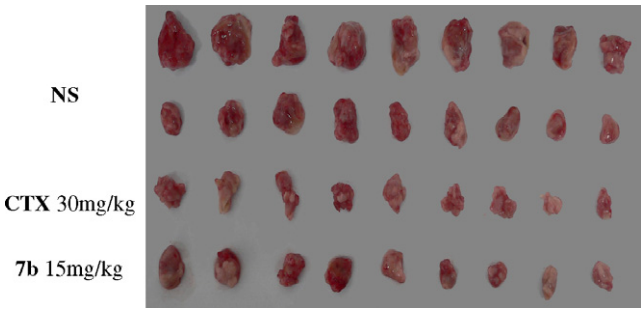


Figure 3. Antitumor effect of compound **7b** and **CTX** on S-180 tumor bearing mice.

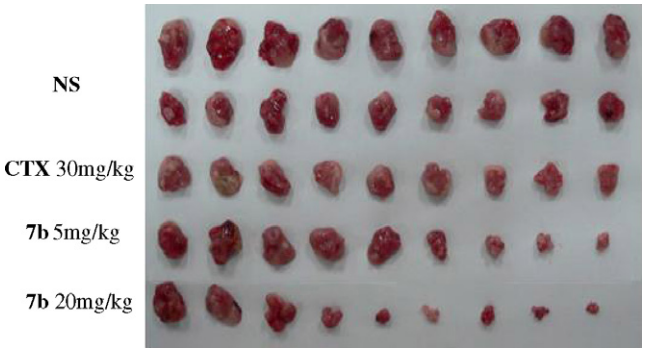


Figure 4. Antitumor effect of compound **7b** and **CTX** on H-22 tumor bearing mice.

effect on murine H-22 tumor growth (Tables 2, 3 and Figs. 3, 4).

3. Conclusions

We have synthesized a series of aryl substituted imidazol-2-one derivatives and evaluated their biological activities. Some of these compounds exhibited potent cytotoxic activities against the tested cancer cell lines including MDR cancer cell line in vitro. Compounds **7b** and **7i** displayed comparable cytotoxic activities with that of docetaxel against the tested human cancer cell lines. Cell cycle distribution analysis showed that **7b** acted on the S phase of the cell cycle arrest at 0.01 μM and induced cell apoptosis at 0.1–1.0 μM. Compound **7b** also showed significant anticancer activity in two murine tumor bearing models in vivo. Further studies of the mechanism of action for **7b** are underway.

4. Experimental

Melting points were obtained on a B-540 Buchi melting point apparatus and are uncorrected. IR spectra, KBr pellets, 400–4000 cm^{-1}, were recorded on a Bruker VECTOR 22 FTIR spectrophotometer. ^1H NMR spectra was recorded on a Bruker AM 400 instrument at 400 MHz (chemical shifts are expressed as δ values relative to TMS as internal standard). Mass spectra (MS), ESI (positive) were recorded on an Esquire-LC-00075 spectrometer.

4.1. Synthesis

4.1.1. 1,3-Dihydro-3-(3,4,5-trimethoxyphenyl)-4-(4-hydroxyphenyl)-2H-imidazol-2-one (4a) and 2,3-Dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-4-(4-hydroxyphenyl)-2-oxo-1H-imidazole-1-carboxamide (5a). A mixture of 2-amino-1-(4-hydroxyphenyl)ethanone hydrochloride **3a** (1.5 mmol) and 3,4,5-trimethoxyphenyl isocyanate (1.65 mmol) in dry toluene (10 mL) was refluxed for 5 h. After cooling to room temperature, the reaction mixture was diluted with water (10 mL) and extracted thoroughly with CH_2Cl_2 (3 × 20 mL). Then the CH_2Cl_2 layer was washed successively with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 1:1), yielding pure compounds **4a** and **5a**. Lower R_f compound **4a**, white solid (0.132 g, 23% yield), mp 207–209 °C; IR (KBr): 3369, 3272, 3163, 2939, 2839, 1782, 1616, 1572, 1513, 1458, 1422 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.84 (s, 3H, OCH_3), 3.88 (s, 6H, $OCH_3 \times 2$), 5.22 (s, 1H, OH), 6.85 (s, 2H, Ar–H), 6.90 (d, J = 8.4 Hz, 2H, Ar–H), 7.39 (s, 1H, imidazolone H), 7.45 (d, J = 8.4 Hz, 2H, Ar–H), 9.89 (s, 1H, N–H); MS (ESI): m/s = 385 [M+1]. Higher R_f compound **5a**, white solid (0.504 g, 61% yield), mp 186–188 °C; IR (KBr): 3306, 3146, 3089, 2935, 2841, 1730, 1680, 1608, 1564, 1507, 1458, 1416 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.72 (s, 6H, $OCH_3 \times 2$), 3.82 (s, 3H, OCH_3), 3.84 (s, 9H, $OCH_3 \times 3$), 5.52 (s, 1H, OH), 6.42 (s, 2H, Ar–H), 6.73 (d, J = 8.8 Hz, 2H, Ar–H), 6.88 (s, 2H, Ar–H), 7.00 (d, J = 8.8 Hz, 2H, Ar–H), 7.17 (s, 1H, imidazolone H), 10.87 (s, 1H, –O═C–N–H–); MS (ESI): m/s = 552 [M+1].

In the same manner, compounds **4b** and **5b**, **4c** and **5c**, **4d** and **5d**, **5f** were synthesized by reaction of the corresponding 2-amino-1-phenylethanone hydrochlorides **3b–e** with 3,4,5-trimethoxyphenyl isocyanate, respectively.

4.1.2. 1,3-Dihydro-3-(3,4,5-trimethoxyphenyl)-4-(4-methoxyphenyl)-2H-imidazol-2-one (4b) and 2,3-dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1H-imidazole-1-carboxamide (5b). Lower R_f compound **4b**, white solid (24% yield), mp 170–172 °C; IR (KBr): 3272, 3146, 2925, 2855, 1751, 1615, 1567, 1507, 1456, 1417 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.89 (s, 6H, $OCH_3 \times 2$), 6.85 (s, 2H, Ar–H), 6.96 (d, J = 8.8 Hz, 2H, Ar–H), 7.40 (s, 1H, imidazolone H), 7.49 (d, 2H, J = 8.8 Hz, Ar–H), 9.89 (s, 1H, N–H); MS (ESI): m/s = 399 [M+1]. Higher R_f compound **5b**, white solid (59% yield), mp 147–149 °C; IR (KBr): 3145, 3087, 2939, 2837, 1730, 1680, 1607, 1563, 1507, 1458, 1416 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.79 (s, 6H, $OCH_3 \times 2$), 3.84 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.91 (s, 6H, $OCH_3 \times 2$), 6.49 (s, 2H, Ar–H), 6.86 (d, J = 8.8 Hz, 2H, Ar–H), 6.94 (s, 2H, Ar–H), 7.12 (d, J = 8.8 Hz, 2H, Ar–H), 7.24 (s,

1H, imidazolone H), 10.93 (s, 1H, –O═C–N–H–); MS (ESI): *m/s* = 569 [M+1].

4.1.3. 1,3-Dihydro-3-(3,4,5-trimethoxyphenyl)-4-(3,4-dimethoxyphenyl)-2H-imidazol-2-one (4c) and 2,3-dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-4-(3,4-dimethoxyphenyl)-2-oxo-1H-imidazole-1-carboxamide (5c). Lower R_f compound **4c**, pale yellow solid (21% yield), mp 174–176 °C; IR (KBr): 3280, 3142, 2942, 2838, 1773, 1604, 1557, 1511, 1456, 1417 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.84 (s, 3H, OCH_3), 3.89 (s, 6H, $OCH_3 \times 2$), 3.92 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.85 (s, 2H, Ar–H), 6.92 (d, J = 8.8 Hz, 1H, Ar–H), 7.02 (d, J = 2.4 Hz, 1H, Ar–H), 7.13 (dd, 1H,J = 8.8, 2.4 Hz, Ar–H), 7.42 (s, 1H, imidazolone H), 9.87 (s, 1H, N–H); MS (ESI): *m/s* = 387 [M+1]. Higher R_f compound **5c**, pale yellow solid (58% yield), mp 178–180 °C; IR (KBr): 3144, 3091, 2943, 2836, 1729, 1680, 1608, 1509, 1459, 1417 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 3.64 (s, 3H, OCH_3), 3.72 (s, 6H, $OCH_3 \times 2$), 3.80 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.84 (s, 9H, $OCH_3 \times 3$), 6.46 (s, 2H, Ar–H), 6.59 (s, 1H, imidazolone H), 6.73 (dd, J = 8.0, 2.0 Hz, 2H, Ar–H), 6.87 (s, 2H, Ar–H), 7.22 (d, J = 8.0 Hz, 1H, Ar–H), 10.85 (s, 1H, –O═C–N–H–); MS (ESI): *m/s* = 596 [M+1].

4.1.4. 1,3-Dihydro-3-(3,4,5-trimethoxyphenyl)-4-(3-nitro-4-methoxyphenyl)-2H-imidazol-2-one (4d) and 2,3-dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-4-(3-nitro-4-methoxyphenyl)-2-oxo-1H-imidazole-1-carboxamide (5d). Lower R_f compound **4d**, yellow solid (20% yield), mp 199–201 °C; IR (KBr): 3286, 3153, 2930, 2848, 1768, 1612, 1547, 1456, 1417 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 6H, $OCH_3 \times 2$), 3.83 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 6.84 (s, 2H, Ar–H), 7.18 (d, J = 8.8 Hz, 1H, Ar–H), 7.54 (s, 1H, imidazolone H), 7.71 (dd, J = 8.8, 2.4 Hz, 1H, Ar–H), 8.04 (d, J = 2.4 Hz, 1H, Ar–H), 9.80 (s, 1H, N–H); MS (ESI): *m/s* = 402 [M+1]. Higher R_f compound **5d**, yellow solid (61% yield), mp 162–164 °C; IR (KBr): 3153, 3089, 2930, 2847, 1735, 1693, 1609, 1567, 1507, 1458, 1416 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.75 (s, 6H, $OCH_3 \times 2$), 3.80 (s, 3H, OCH_3), 3.83 (s, 6H, $OCH_3 \times 2$), 3.84 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.44 (s, 2H, Ar–H), 6.85 (s, 2H, Ar–H), 6.96 (d, J = 8.8 Hz, 1H, Ar–H), 7.22 (dd, J = 8.8, 1.6 Hz, 1H, Ar–H), 7.29 (s, 1H, imidazolone H), 7.69 (d, J = 1.6 Hz, 1H, Ar–H), 10.75 (s, 1H, –O═C–N–H–); MS (ESI): *m/s* = 611 [M+1].

4.1.5. 1,3-Dihydro-3-(3,4,5-trimethoxyphenyl)-4-(3-amino-4-methoxyphenyl)-2H-imidazol-2-one (4e). To a solution of nitro compound **4d** (0.2 mmol) in AcOH (4 mL) was added zinc powder (0.5 g). The reaction mixture was stirred at room temperature for 2 h. The mixture was filtered over Celite, and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 1:1), yielding pure compound **4e** as a white solid (60 mg, 80%), mp 175–177 °C; IR (KBr): 3378, 3286, 3160, 2923, 2853, 1762, 1612, 1564, 1508, 1458, 1417 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.82 (s, 3H, OCH_3), 3.88 (s, 6H, $OCH_3 \times 2$), 3.89 (s, 3H, OCH_3), 6.81 (d, J = 8.8 Hz, 1H, Ar–H), 6.85 (s, 2H, Ar–H), 6.86 (d, J = 1.6 Hz, 1H, Ar–H), 6.94 (dd, J = 8.8, 1.6 Hz, 1H, Ar–H), 7.34 (s, 1H, imidazolone H), 9.90 (s, 1H, N–H); MS (ESI): *m/s* = 372 [M+1].

4.1.6. 2,3-Dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-4-(3-amino-4-methoxyphenyl)-2-oxo-1H-imidazole-1-carboxamide (5e). The same procedure as described above was performed with **5d** and gave pure compound **5e** as a white solid (75% yield), mp 179–181 °C; IR (KBr): 3370, 3160, 3093, 2937, 2838, 1727, 1686, 1608, 1569, 1509, 1457, 1417 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.80 (s, 6H, $OCH_3 \times 2$), 3.87 (s, 6H, $OCH_3 \times 2$), 3.90 (s, 9H, $OCH_3 \times 3$), 6.49 (m, 3H, Ar–H), 6.58 (d, J = 1.6 Hz, 1H, Ar–H), 6.69 (d, 1H, J = 8.4 Hz, Ar–H), 6.94 (s, 2H, Ar–H), 7.19 (s, 1H, imidazolone H), 10.94 (s, 1H, –O═C–N–H–); MS (ESI): *m/s* = 581 [M+1].

4.1.7. 2,3-Dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-4-(3-benzyloxy-4-methoxyphenyl)-2-oxo-1H-imidazole-1-carboxamide (5f). Light yellow solid (48% yield), mp 159–161 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 3.81 (s, 6H, $OCH_3 \times 2$), 3.94 (m, 15H, $OCH_3 \times 5$), 5.00 (s, 2H, CH_2), 6.50 (s, 2H, Ar–H), 6.70 (s, 1H, Ar–H), 6.82 (d, J = 8.4 Hz, 1H, Ar–H), 6.88 (d, J = 8.4 Hz, 1H, Ar–H), 6.96 (s, 2H, Ar–H), 7.23 (s, 1H, imidazolone H), 7.40 (m, 5H, Ar–H), 10.94 (s, 1H, –O═C–N–H–); MS (ESI): *m/s* = 672 [M+1].

4.1.8. 2,3-Dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-4-(3-hydroxy-4-methoxyphenyl)-2-oxo-1H-imidazole-1-carboxamide (5g). A mixture of **5f** (0.2 mmol) and 10% Pd/C (20 mg) in THF (5 mL) was stirred at room temperature under H_2 for 12 h. Then the mixture was filtered, and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 2:1) to give pure compound **5g** as a white solid (45 mg, 39%), mp 175–177 °C; IR (KBr): 3396, 3160, 3092, 2935, 2841, 1722, 1680, 1605, 1563, 1511, 1457, 1417 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.79 (s, 6H, $OCH_3 \times 2$), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.89 (s, 6H, $OCH_3 \times 2$), 3.90 (s, 3H, OCH_3), 5.72 (s, 1H, OH), 6.57 (s, 2H, Ar–H), 6.79 (m, 2H, Ar–H), 6.87 (m, 2H, Ar–H), 6.93 (s, 1H, Ar–H), 7.29 (s, 1H, imidazolone H), 10.40 (s, 1H, –O═C–N–H–); MS (ESI): *m/s* = 582 [M+1].

4.2. General procedure for the preparation of imidazol-2-ones 7a–h

A mixture of substituted *N*-(2-oxo-2-phenylethyl)acetamide (1.5 mmol) and 3,4,5-trimethoxyphenyl isocyanate (1.65 mmol) in dry toluene (10 mL) was refluxed for 5 h. After cooling to room temperature, the reaction mixture was diluted with water (10 mL) and extracted thoroughly with CH_2Cl_2 (20 mL × 3). Then the CH_2Cl_2 layer was washed successively with brine (20 mL × 2), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Usual workup afforded the crude product which was further purified by a silica gel column chromatography.

4.2.1. 1-Acetyl-1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-phenyl-2H-imidazol-2-one (7a). Yellow solid (78% yield), mp 177–179 °C; IR (KBr): 3144, 2936, 2836, 1720, 1594, 1506, 1458, 1419 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.74 (s, 3H, O=C–CH_3), 3.70 (s, 6H, $OCH_3 \times 2$), 3.84 (s, 3H, OCH_3), 6.40 (s, 2H, Ar–H), 7.14 (m, 2H, Ar–H), 7.20 (s, 1H, imidazolone H), 7.28 (m, 3H, Ar–H); MS (ESI): *m*/*s* = 369 [M+1].

4.2.2. 1-Acetyl-1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-(4-bromophenyl)-2H-imidazol-2-one (7b). White solid (86% yield), mp 175–177 °C; IR (KBr): 3144, 2935, 2841, 1718, 1594, 1557, 1507, 1458, 1416 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.72 (s, 3H, O=C–CH_3), 3.72 (s, 6H, $OCH_3 \times 2$), 3.79 (s, 3H, OCH_3), 6.39 (s, 2H, Ar–H), 7.00 (d, J = 8.8 Hz, 2H, Ar–H), 7.20 (s, 1H, imidazolone H), 7.40 (d, J = 8.8 Hz, 2H, Ar–H); MS (ESI): *m*/*s* = 448 [M+1].

4.2.3. 1-Acetyl-1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-(4-chlorophenyl)-2H-imidazol-2-one (7c). White solid (76% yield), mp 147–149 °C; IR (KBr): 3144, 2937, 2841, 1725, 1596, 1503, 1459, 1419 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.72 (s, 3H, O=C–CH_3), 3.72 (s, 6H, $OCH_3 \times 2$), 3.85 (s, 3H, OCH_3), 6.39 (s, 2H, Ar–H), 7.06 (d, J = 8.8 Hz, 2H, Ar–H), 7.20 (s, 1H, imidazolone H), 7.26 (m, 2H, Ar–H); MS (ESI): *m*/*s* = 403 [M+1].

4.2.4. 1-Acetyl-1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-(4-nitrophenyl)-2H-imidazol-2-one (7d). Yellow solid (73% yield), mp 107–109 °C; IR (KBr): 3141, 2925, 2852, 1730, 1598, 1514, 1456, 1416 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.75 (s, 3H, O=C–CH_3), 3.75 (s, 6H, $OCH_3 \times 2$), 3.88 (s, 3H, OCH_3), 6.43 (s, 2H, Ar–H), 7.32 (d, J = 8.8 Hz, 2H, Ar–H), 7.40 (s, 1H, imidazolone H), 8.15 (d, J = 8.8 Hz, 2H, Ar–H); MS (ESI): *m*/*s* = 414 [M+1].

4.2.5. 1-Acetyl-1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-(4-methoxyphenyl)-2H-imidazol-2-one (7e). White solid (80% yield), mp 177–179 °C; IR (KBr): 3186, 2935, 2841, 1729, 1601, 1509, 1458, 1417 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.71 (s, 3H, O=C–CH_3), 3.71 (s, 6H, $OCH_3 \times 2$), 3.78 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.40 (s, 2H, Ar–H), 6.79 (d, J = 8.8 Hz, 2H, Ar–H), 7.05 (d, J = 8.8 Hz, 2H, Ar–H), 7.10 (s, 1H, imidazolone H); MS (ESI): *m*/*s* = 390 [M+1].

4.2.6. 1-Acetyl-1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-(2,4-difluorophenyl)-2H-imidazol-2-one (7f). White solid (77% yield), mp 153–154 °C; IR (KBr): 3177, 2942, 2841, 1723, 1598, 1505, 1456, 1428 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.73 (s, 3H, O=C–CH_3), 3.71 (s, 6H, $OCH_3 \times 2$), 3.82 (s, 3H, OCH_3), 6.37 (s, 2H, Ar–H), 6.80 (m, 2H, Ar–H), 7.23 (s, 1H, imidazolone H), 7.54 (s, 1H, Ar–H); MS (ESI): *m*/*s* = 405 [M+1].

4.2.7. 1-Acetyl-1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-(3,4-dimethoxyphenyl)-2H-imidazol-2-one (7g). Pale yellow solid (78% yield), mp 174–176 °C; IR (KBr): 3144, 2934, 2841, 1719, 1597, 1513, 1460, 1419 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.75 (s, 3H, O=C–CH_3), 3.67 (s, 3H, OCH_3), 3.76 (s, 6H, $OCH_3 \times 2$), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.47 (s, 2H, Ar–H), 6.62 (s, 1H, Ar–H), 6.77 (m, 2H, Ar–H), 7.17 (s, 1H, imidazolone H); MS (ESI): *m*/*s* = 429 [M+1].

4.2.8. 1-Acetyl-1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-(3-nitro-4-methoxyphenyl)-2H-imidazol-2-one (7h). Yellow solid (75% yield), mp 231–233 °C; IR (KBr): 3137, 2935, 2841, 1718, 1594, 1530, 1511, 1458, 1416 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.80 (s, 3H, O=C–CH_3), 3.83 (s, 6H, $OCH_3 \times 2$), 3.93 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.50 (s, 2H, Ar–H), 7.04 (d, J = 8.0 Hz, 1H, Ar–H), 7.27 (d, J = 8.0 Hz, 1H, Ar–H), 7.31 (s, 1H, imidazolone H, Ar–H), 7.80 (s, 1H, Ar–H); MS (ESI): *m*/*s* = 444 [M+1].

4.2.9. 1-Acetyl-1,3-dihydro-3-(3,4,5-trimethoxyphenyl)-4-(3-amino-4-methoxyphenyl)-2H-imidazol-2-one (7i). The same procedure as described above was performed with **7h** and gave pure compound **7i** as a white solid (78% yield), mp 230–232 °C; IR (KBr): 3377, 3131, 2946, 2838, 1719, 1602, 1557, 1510, 1457, 1416 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.68 (s, 3H, O=C–CH_3), 3.69 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.39 (s, 2H, Ar–H), 6.49 (d, J = 8.4 Hz, 1H, Ar–H), 6.60 (d, J = 8.4 Hz, 1H, Ar–H), 6.77 (s, 1H, Ar–H), 7.03 (s, 1H, imidazolone H); MS (ESI): *m*/*s* = 414 [M+1].

4.3. Biology

The human tumor cell lines (HL-60, K562, K562R, PC-3, MCF-7, ECA-109, BEL-7402, and A549) were obtained from Shanghai Institute of Pharmaceutical Industry.

4.3.1. Cytotoxicity assay. The cytotoxic activity in vitro was measured using the MTT assay.[19] MTT solution (10.0 μl/well) in RPMI-1640 (Sigma, St. Louis, MO) was added after cells were treated with drug for 48 h, and cells were incubated for a further 4 h at 37 °C. The purple formazan crystals were dissolved in 100.0 μl DMSO. After 5 min, the plates were read on an automated microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT) at 570 nm. Assays were performed in triplicate in three independent experiments. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated using the software 'Dose–Effect Analysis with Microcomputers'. The tumor cell line panel consisted of HL-60, K562, K562R, PC-3, MCF-7, ECA-109, BEL-7402, and A549. In all of these experiments, three replicate wells were used to determine each point.

4.3.2. Antitumor activity in S-180 and H-22 tumor bearing mice. Tumor cells of S-180 and H-22 were inoculated to mice. After 10 days, tumors were taken out and cells were harvested. Viable tumor cells (2.5×10^6 cells per mouse) were inoculated to the armpit of mice by subcutaneous injection and were divided into several groups: negative control group containing 18 female mice, treatment groups containing 9 female mice. Twenty-four hours later, treatment groups were admin-

istered with various doses of **7b** (im) or CTX (ip), respectively, once a day for consecutive 10 days. CTX at 30.0 mg/kg was used as a positive control and physiological saline as negative control. Tumors were dissected and weighed, and inhibition rates were calculated on day 10. The inhibition rate was calculated as follows: $C - T/C \times 100$, T, average tumor weight of treated group; C, average tumor weight of negative control group.

4.3.3. Flow cytometry analysis of apoptosis. For flow cytometry analysis of DNA content, K562 cells in exponential growth were treated with graded concentrations of **7b** (0.01–1 μM) for 48 h. Cells were washed twice with PBS and fixed in 70% ethanol at −20 °C. The cell pellet was resuspended in 100.0 μl of PBS containing 50.0 mg/ml RNase (Amersco, Solon, OH), then incubated at 37 °C for 1 h. After incubation, the cells were stained with 200.0 mg/ml propidium iodide (PI, Sigma, St. Louis, MO) at 4 °C for 30 min. The fluorescence of 2×10^4 cell was measured with FACSCalibur (Becton–Dickinson, Lincoln Park, NJ).[20]

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