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Zhenli Min, Yue Zhu, Xing Hong, Zhijun Yu, Min Ye, Qiong Yuan, Xiamin Hu

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Synthesis and Biological Evaluations of Monocarbonyl Curcumin Inspired Pyrazole Analogues as Potential Anti-Colon Cancer Agent

Purpose: The monocarbonyl analogs of curcumin (MCACs) have been widely studied for their promising antitumor activity. Pyrazole is a five-membered aromatic heterocyclic system with various bioactivities incorporated frequently in drugs. However, few of MCACs inspired pyrazole analogues were investigated. To search for more potent cytotoxic agents based on MCACs, a series of new 1,5-diaryl/heteroaryl-1,4-pentadien-3-ones inspired pyrazole moiety was synthesized and evaluated on their anti-colon cancer activities.

Methods: Fifteen new compounds were synthesized and characterized by spectral datum, and then they were tested preliminarily by MTT assay for their cytotoxic activities against a panel of four human cancer cell lines, namely, gastric (SGC-7901), liver (HepG2), lung (A549), and colon (SW620) cancer cells. Compound 7h exhibited excellent selectivity and outstanding anti-proliferation activity against SW620 cells among these 15 compounds. Further, the mechanisms were investigated by transwell migration and invasion assay, clonogenic assay, cell apoptosis analysis, cell cycle analysis, Western blot analysis.

Results: The IC_{50} value of 7h against SW620 cells was 12 nM, being more potent than curcumin (IC_{50} = 9.36 μM), adriamycin (IC_{50} = 3.28 μM) and oxaliplatin (IC_{50} = 13.33 μM). Further assays showed that 7h inhibited SW620 cell migration, invasion and colony formation obviously, which was due to its ability to induce cell cycle arrest in the G2/M and S phases and apoptosis. Western blot assay revealed that 7h decreased the protein expression of ATM gene, which may primarily contribute to its anticancer activity against SW620 cells.

Conclusion: A new MCACs 7h was synthesized and found to exhibit excellent anti-proliferation activity against SW620 cells. Further studies indicated that 7h exerted its anticancer activity against SW620 cells probably via decreasing the ATM protein expression. The present study suggested that 7h was a promising candidate as an anti-colon cancer drug for future development.

Keywords: 1, 5-diheteroarylpyrrola-1, 4-dien-3-one, colon cancer, cell proliferation, cell apoptosis, ATM gene

Introduction

Colon cancer is the third most common cancer and the second leading cause of cancer death worldwide. Although target therapies and immunotherapy have achieved progresses in recent years, their applications and efficacies are still far from satisfactory for an advanced-stage colon cancer. Chemotherapy is still considered the most effective for colon cancer. However, cytotoxic drugs, such as cisplatin and paclitaxel, can lead to side effects and chemoresistance, imposing
a financial burden on patients and impairing their quality of life. Therefore, development of a novel potent anti-colon cancer agent with a low toxicity is urgently required.

Natural products play an important role in a process of drug discovery, and many drugs used clinically are of natural origins. Curcumin is a primary bioactive compound isolated from the turmeric, a dietary spice made from the rhizome of *Curcuma longa* (Figure 1). It possesses versatile biological activities. However, curcumin has as yet achieved a limited success clinically although it had been studied in a number of clinical trials, and even there have been some controversies about its potential as a pharmaceutical agent recently. The nature of instability as well as pharmacokinetic deficiencies of curcumin resulted from an unstable β-diketone moiety are one of the reasons for the fails and controversies. In spite of these shortcomings, curcumin has still aroused interests of many scientists to overcome them as it is safe and a dietary spice in some countries. The problem can be addressed in part by a chemical structural modification of curcumin besides a pharmaceutical way. Indeed, great attempts have already been made by researchers to chemical modifications, and a large number of curcumin analogues have been synthesized. In this process, a major chemical class, namely the MCACs, evolves that is characterized by 1, 5-diaryl/heteroaryl penta-1, 4-dien-3-one and incorporating a range of alternative substituent groups into the terminal aryl rings. These MCACs display multiple biological activities, such as antitumor, anti-inflammatory, antioxidant and neuroprotection. Meanwhile, most of the MCACs show better stabilities and activities than curcumin does in both in vivo and in vitro model.

Among these derivatives, various aryl or heteroaryl rings were incorporated into the 1, 5-position of MCACs to explore bioactivities, including pyrazine, chromone, indole, imidazole, quinoline, quinazoline, and piperidone moieties, nevertheless, few of which were related to pyrazolyl group. Pyrazole, as a five-membered aromatic heterocyclic system, has attracted considerable attentions in development of pharmacological molecules, and many marketed drugs (Figure 1) bearing this moiety display a variety of biological activities, such as anti-tumor (ruxolitinib, crizotinib), anti-inflammatory (Celecoxib), and antiobesity (Rimonabant). Therefore, we envisioned that incorporating substituted pyrazole structure to replace the above-mentioned aryl or heteroaryl rings of MCACs may be beneficial to search for new anticancer drugs. In an effort to discover chemical entities active against colon cancer, this background motivated us to introduce pyrazole moiety to one terminal of MCACs and investigate their bioactivities (Figure 1). Presented here was a study on the synthesis and anti-cancer evaluations of a series of new MCACs which inspired a pyrazole moiety.
Materials and Methods

Chemistry

All reagents and solvents were obtained from commercially available sources and were used without further purification. Reaction progress was monitored using analytical thin-layer chromatography (TLC) on precoated silica gel GF254 (Qingdao Haiyang Chemical Plant, Qingdao, China) plates and spots were detected under UV light (254 nm). Melting points were determined on a WRS-2B digital melting point apparatus and uncorrected. IR spectra were recorded using a Nicolet 380 Fourier-transform infrared (FTIR) spectrophotometer (Thermo, USA) from KBr pellets. 1H NMR and 13C NMR spectra were recorded on a Bruker AVANCE III-600 NMR spectrometer (Bruker Biospin Co., Switzerland) with tetramethylsilane (TMS) as the internal standard and CDCl3 as solvent and known chemical shifts of residual proton signals of deuterated solvents (1H NMR δ: 7.26 for CDCl3 and δ: 3.33 for H2O) or carbon signals of deuterated solvents (13C NMR δ: 77.16 for CDCl3) as internal standard. MS (ESI) measurement was conducted on an Agilent 1100 LC-MS spectrometer (Agilent, Palo Alto, USA).

The synthetic route of target molecules 7a-7o was depicted in Scheme 1. All the target compounds and some intermediates were synthesized according to the following procedures.

Synthesis of 1-phenyl-3-methyl-5-pyrazolone 9

Tungstophosphoric acid (1% mol) was dissolved in 40 mL water, and then phenylhydrazine (3 mmol) was added under stirring. To the mixture was slowly added ethyl acetoacetate (3 mmol) over a period of 20 min at room temperature. The mixture was further heated under reflux for 3 h. After completion of the reactions, the mixture was cooled to room temperature and solid was filtered off and washed with H2O (40 mL). The crude products were dried and purified by recrystallization from ethanol to give 9 as a yellow powder, m.p: 125.5–127.1°C, yield: 78%.

Synthesis of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde 10

To a solution of 0.52 g compound 9 (3 mmol, 1.0 equiv) in dry DMF (2 mL) was added dropwise POCl3 (0.42 mL, 4.5 mmol, 1.5 equiv) slowly while cooling in an ice-water bath. The mixture was stirred at 80°C for 2 h, and then cooled to room temperature, followed by pouring into ice-water (50 mL). The solution was neutralized by saturated NaHCO3 solution and kept stirring for 30 min. The solid precipitated, filtered. The residue was recrystallized from ethanol to generate 10 as a light yellow solid, m.p: 145.0–146.5°C, yield: 38.2%.

General Procedure for Synthesis of 11a–11c

To a solution of 2-methoxyphenol, 3, 4-methylenedioxyphenol or ethanol (27 mmol, 1.0 equiv) in dry DMF (20 mL) was added KOH solid (1.73g, 29.7 mmol, 1.1 equiv) while stirring. The mixture was stirred at 40°C for 1 h, to which 10 (5.96g, 27 mmol, 1.0 equiv) was added. The solution was stirred at 110°C for 5–6 h, and then cooled to ambient temperature, poured into ice-water. The mixture was stirred for 15 min, followed by extraction with ethyl acetate (3×20 mL). The combined organic layers were dried over Na2SO4 and concentrated and purified by silica gel column chromatography (hexanes/EtOAc = (10–6): 1) to give 11a-11c.

5-(2-methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (11a)

A yellow solid, m.p: 73.4–74.5°C, 68.1% yield. 1H NMR (600 MHz, CDCl3) δ: 9.84 (s, 1H, CHO), 6.76 (d, J = 7.8 Hz, 2H, H-2′, 6′), 7.35 (t, J = 7.9 Hz, 2H, H-3′, 5′), 7.24 (t, J = 7.4 Hz, 1H, H-4′), 7.10 (d, J = 8.3 Hz, 1H, H-6′), 7.05 (t, J = 7.4 Hz, 1H, H-4′), 7.02 (t, J = 7.8 Hz, 1H, H-5′), 6.95 (d, J = 8.2 Hz, 1H, H-3′), 3.83 (s, 3H, OCH3), 2.53 (s, 3H, CH3).

5-(benzo[d][1,3]dioxol-5-yloxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (11b)

A white solid, m.p:106.6–107.4°C, 48.6% yield. 1H NMR (600 MHz, CDCl3) δ: 9.58 (s, 1H, CHO), 7.63 (d, J = 7.7 Hz, 2H, H-2′, 6′), 7.43 (t, J = 7.9 Hz, 2H, H-3′, 5′), 7.34 (t, J = 7.4 Hz, 1H, H-4′), 6.69 (d, J = 8.5 Hz, 1H, H-5′), 6.59 (s, 1H, H-2′), 6.46 (d, J = 5.9 Hz, 1H, H-6′), 5.96 (s, 2H, OCH2O), 2.53 (s, 3H, CH3).

5-ethoxy-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (11c)

A light yellow oil, 40.1% yield. 1H-NMR (600 MHz, CDCl3) δ: 9.53 (s, 1H, CHO), 7.68 (d, J = 8.3 Hz, 2H, H-2′, 6′), 7.43 (t, J = 7.7 Hz, 2H, H-3′, 5′), 7.33 (t, J = 7.4 Hz, 1H, H-4′), 4.02 (q, J = 7.0 Hz, 2H, OCH2), 2.53 (s, 3H, CH3), 1.31 (t, J = 7.1 Hz, 3H, CH3).

General Procedure for Synthesis of Various (E)-4-phenylbut-3-en-2-ones 13a–13f

To a stirred solution of various benzaldehydes, 12 (3.6 mmol) in acetone (2 mL) was added 0.7 mL of 10%
aqueous NaOH solution at room temperature. The reaction was allowed to stir for 3–5 h till it was completed. The reaction mixture was poured into ice-water (30mL) and neutralized by 5% aqueous HCl while keeping stirring for 20 min. Then it was extracted with ethyl acetate (3×15 mL). The combined organic layers were dried over Na₂SO₄ and concentrated and purified by silica gel column chromatography (hexanes/EtOAc = (8–5): 1) to give 13a–13f.

**Scheme 1** Synthesis of target molecules 7a-7o; Reagents and conditions: (i) CH₃COCH₂COOC₂H₅, tungstophosphoric acid, H₂O, 100°C, 3h, 78%; (ii) POCl₃, DMF, 80°C, 3h, 42%; (iii) C₂H₅OH or substituted phenol, KOH, 100°C; (iv) CH₃COCH₃, 10%NaOH, rt, 2–4h, 47%; (v) 10 or 11a-11c, 10%NaOH, C₂H₅OH, rt, 12h, 20%; (vi) 1-methyl-1H-pyrazole-4-carbaldehyde, 10%NaOH, C₂H₅OH, rt, 12h, 17%.

**General Procedure for Synthesis of Target Compounds (7a–7o)**

To a stirred solution of 13a–13f (2.0 mmol) and 11a–11c (2.2 mmol) in ethanol (10 mL) was added 0.9 mL of 10% aqueous NaOH solution at room temperature. The reaction was allowed to stir for 12–18 h till it was completed. The reaction mixture was poured into ice-water (50mL) and neutralized by 5% aqueous HCl while keeping stirring for...
20 min. Then it was extracted with ethyl acetate (20×3 mL). The combined organic layers were dried over Na₂SO₄, concentrated and purified by silica gel column chromatography [hexanes/EtOAc = (7:3): 1] to give products, which were recrystallized from ethanol to afford 7a–7o.

(1E, 4E)-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(5-phenylpenta-1,4-dien-3-one (7a)

A yellow solid, m.p: 123.8–124.5°C, 16.1% yield. ¹H NMR (600 MHz, CDCl₃) δ: 7.74 (d, J = 15.9 Hz, 1H, vinyl H), 7.69 (d, J = 16.1 Hz, 1H, vinyl H), 7.63 (td, J = 5.4, 1.8 Hz, 2H, H-2', 6'), 7.55 (dt, J = 7.3, 0.8 Hz, 2H, H-3', 5'), 7.50 (td, J = 6.7, 1.6 Hz, 2H, H-2", 6"), 7.45 (dt, J = 7.3, 1.3 Hz, 1H, H-4'), 7.43–7.41 (m, 3H, H-3", 4", 5"), 7.06 (d, J = 15.9 Hz, 1H, vinyl H), 7.05 (d, J = 16.1 Hz, 1H, vinyl H), 2.52 (s, 3H, CH₃). ¹³C NMR (600 MHz, CDCl₃) δ: 188.62, 150.01, 143.09, 137.62, 134.81, 132.15, 130.44, 129.13, 128.92, 128.66, 128.38, 125.69, 124.98, 124.32, 119.14, 14.34. IR (KBr, cm⁻¹): 1611, 1621. ESI-MS m/z: 349.11[M+H]⁺, caleed for C₂₁H₁₇ClN₂O: 348.10.

(1E, 4E)-1-(5-(3-methoxy-4-methylphenyl)-1H-pyrazol-4-yl)-5-(3-methoxy-4-methylphenyl) penta-1, 4-dien-3-one (7b)

A yellow solid, m.p: 144.9–146.1°C, 11.5% yield. ¹H NMR (600 MHz, CDCl₃) δ: 7.68 (d, J = 16.1 Hz, 1H, vinyl H), 7.67 (d, J = 15.8 Hz, 1H, vinyl H), 7.55 (d, J = 8.6 Hz, 2H, H-2', 6'), 7.50 (t, J = 7.8 Hz, 2H, H-3', 5'), 7.44 (t, J = 7.4 Hz, 1H, H-4'), 7.19 (d, J = 8.2, 1.7 Hz, 1H, H-6'), 7.12 (d, J = 1.7Hz, 1H, H-2"), 7.06 (d, J = 16.1 Hz, 1H, vinyl H), 6.95 (d, J = 8.2 Hz, 1H, H-5"), 6.89 (d, J = 15.8 Hz, 1H, vinyl H), 5.97 (s, 1H, Ar-OH), 3.96 (s, 3H, OCH₃), 2.51 (s, 3H, CH₃). ¹³C NMR (150 MHz, CDCl₃) δ: 188.53, 149.98, 148.30, 146.86, 143.43, 137.65, 134.13, 131.71, 129.12, 128.63, 127.35, 124.98, 124.27, 123.73, 123.45, 114.87, 114.22, 109.83, 55.99, 14.30. IR (KBr, cm⁻¹): 3068, 1643, 1574. ESI-MS m/z: 534.94[M+H]⁺, caleed for C₂₂H₁₇Br₂ClN₂O₂: 534.93.

(1E, 4E)-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(3, 4-dimethoxyphenyl) penta-1, 4-dien-3-one (7d)

A yellow solid, m.p: 137.9–139.8°C, 50.1% yield. ¹H-NMR (600 MHz, CDCl₃) δ: 7.68 (d, J = 16.0 Hz, 2H, vinyl H), 7.54 (d, J = 7.5 Hz, 2H, H-2', 6'), 7.49 (t, J = 7.8 Hz, 2H, H-3', 5'), 7.43 (t, J = 7.3 Hz, 1H, H-4'), 7.20 (dd, J = 8.2, 1.6 Hz, 1H, H-6"), 7.14 (d, J = 1.4 Hz, 1H, H-2"), 7.05 (d, J = 16.1 Hz, 1H, vinyl H), 6.90 (d, J = 15.9 Hz, 1H, vinyl H), 6.88 (d, J = 8.3 Hz, 1H, H-5"), 3.94 (s, 3H, OCH₃), 3.92 (s, 3H, OCH₃), 2.51 (s, 3H, CH₃).

(1E, 4E)-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(3, 4-dimethoxyphenyl) penta-1, 4-dien-3-one (7d)

A yellow solid, m.p: 198.2–200.9°C, 56.5% yield. ¹H-NMR (600 MHz, CDCl₃) δ: 7.72 (s, 2H, H-2", 6"), 7.69 (d, J = 16.1 Hz, 1H, vinyl H), 7.55–7.52 (m, 3H, vinyl H, H-2', 6'), 7.51–7.48 (m, 2H, H-3', 5'), 7.44 (tt, J = 7.3, 1.3 Hz, 1H, H-4'), 6.99 (d, J = 16.1 Hz, 1H, vinyl H), 6.91 (d, J = 15.8 Hz, 1H, vinyl H), 6.15 (s, 1H, Ar-Oh), 2.50 (s, 3H, CH₃). ¹³C-NMR (150 MHz, CDCl₃) δ: 187.86, 150.92, 150.09, 139.69, 137.57, 132.57, 131.85, 130.01, 129.15, 128.82, 128.71, 125.74, 124.98, 124.17, 114.07, 110.45, 14.33. IR (KBr, cm⁻¹): 3068, 1643, 1574. ESI-MS m/z: 534.94[M+H]⁺, caleed for C₂₂H₁₇Br₂ClN₂O₂: 534.93.

(1E, 4E)-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(3-bromo-4-hydroxyphenyl) penta-1, 4-dien-3-one (7f)

A yellow solid, m.p: 187.2–188.4°C, 50.5% yield. ¹H NMR (600 MHz, CDCl₃) δ: 7.75 (d, J = 1.9 Hz, 1H, H-1"), 7.68 (d, J = 16.1 Hz, 1H, vinyl H), 7.61 (d, J = 15.8 Hz, 1H, vinyl H), 7.55 (d, J = 7.9 Hz, 2H, H-2', 6'), 7.50 (td, J = 8.2, 1.9 Hz, 2H, H-3', 5'), 7.49 (d, J = 1.8 Hz, 1H, H-6"), 7.43 (t, J = 7.3 Hz, 1H, H-4"), 7.05 (d, J = 8.4 Hz, 1H, H-5"), 7.01 (d, J = 16.1 Hz, 1H, vinyl H), 6.91 (d, J = 15.8 Hz, 1H, vinyl H), 5.85 (s, 1H, Ar-Oh), 2.50 (s, 3H, CH₃). ¹³C NMR (150 MHz, CDCl₃) δ: 188.30, 154.22, 150.03, 141.30, 137.60, 132.20, 132.07, 129.56, 129.13, 129.07, 128.71, 128.68, 124.99, 124.63, 140.34, 137.61, 132.10, 130.27, 129.13, 128.66, 125.40, 124.96, 123.98, 114.17, 105.57, 60.98, 56.19, 14.33. ESI-MS m/z: 439.14[M+H]⁺, caleed for C₂₃H₂₃Cl₂O₄: 438.13.
(1E, 4E)-1-(5-(2-methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(3, 4-dimethoxyphenoxy)penta-1,4-dien-3-one (7g)

A yellow solid, mp: 159.7–160.2°C, 10.5% yield. 1H NMR (600 MHz, CDCl3) δ: 7.65 (d, J = 7.8 Hz, 2H, H-2′, 6′), 7.47 (d, J = 16.0 Hz, 1H, vinyl H), 7.44 (d, J = 15.9 Hz, 1H, vinyl H), 7.35 (t, J = 7.9 Hz, 2H, H-3′, 5′), 7.24 (t, J = 7.4 Hz, 1H, H-4′), 7.10 (dd, J = 8.3, 1.9 Hz, 1H, H-4″), 7.04 (d, J = 1.7 Hz, 1H, H-6″), 7.02 (dd, J = 8.4, 1.3 Hz, 1H, H-5″), 6.95 (dd, J = 8.2, 1.2 Hz, 1H, H-6″), 6.86 (d, J = 8.3 Hz, 1H, H-2″), 6.78 (dd, J = 8.3, 1.3 Hz, 1H, H-5″), 6.75 (d, J = 15.9 Hz, 1H, vinyl H), 6.70–6.68 (m, 2H, H-3″, vinyl H), 3.91 (s, 6H, OCH3), 3.90 (s, 3H, OCH3), 2.49 (s, 3H, CH3). 13C NMR (150 MHz, CDCl3) δ: 188.84, 151.62, 149.54, 149.20, 148.97, 148.29, 144.84, 142.48, 137.48, 131.44, 129.00, 127.92, 127.30, 124.69, 124.47, 123.29, 122.80, 122.35, 121.11, 115.83, 112.94, 111.06, 109.86, 105.40, 56.20, 55.96, 55.91, 14.30. IR (KBr, cm⁻¹): 3435, 1642, 1564. ESI-MS m/z: 483.17 [M+H]^+, caleed for C20H26N2O5: 482.16.

(1E, 4E)-1-(5-(benzo[d][1,3]dioxol-6-yloxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(3, 4-dimethoxyphenoxy)penta-1,4-dien-3-one (7h)

A yellow solid, mp: 151.4–153.6°C, 12.8% yield. 1H NMR (600 MHz, CDCl3) δ: 7.60 (dd, J = 8.6, 1.1 Hz, 2H, H-2′, 6′), 7.49 (d, J = 16.0 Hz, 1H, vinyl H), 7.43 (d, J = 15.9 Hz, 1H, vinyl H), 7.37 (td, J = 7.4, 1.6 Hz, 2H, H-3′, 5′), 7.27 (tt, J = 7.4, 1.0 Hz, 1H, H-4′), 7.11 (dd, J = 8.3, 1.9 Hz, 1H, H-6″), 7.06 (d, J = 1.9 Hz, 1H, H-2″), 6.86 (d, J = 8.3 Hz, 1H, H-5″), 6.74 (d, J = 15.2 Hz, 1H, vinyl H), 6.71 (d, J = 15.9 Hz, 1H, vinyl H), 6.65 (d, J = 8.5 Hz, 1H, H-5″), 6.51 (d, J = 2.6 Hz, 1H, H-6″), 6.35 (dd, J = 8.5, 2.6 Hz, 1H, H-6″), 5.89 (s, 2H, OCH2O), 3.91 (s, 3H, OCH3), 3.86 (s, 3H, OCH3), 2.49 (s, 3H, CH3). 13C NMR (150 MHz, CDCl3) δ: 188.63, 151.27, 150.80, 149.73, 149.22, 148.74, 147.60, 143.86, 142.69, 137.33, 131.06, 129.14, 127.81, 127.46, 124.58, 123.36, 122.90, 122.33, 111.05, 109.86, 108.28, 107.16, 105.79, 101.74, 98.20, 55.97, 55.92, 14.15. IR (KBr, cm⁻¹): 1668, 1616, 1506. ESI-MS m/z: 511.17 [M+H]^+, caleed for C30H28N2O6: 510.16.

(1E, 4E)-1-(5-(benzo[d][1,3]dioxol-6-yloxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(3,4,5-trimethoxyphenoxy)penta-1,4-dien-3-one (7i)

A yellow solid, mp: 116.4–118.1°C, 11.4% yield. 1H NMR (600 MHz, CDCl3) δ: 7.66 (d, J = 7.7 Hz, 2H, H-2′, 6′), 7.46 (d, J = 16.0 Hz, 1H, vinyl H), 7.42 (d, J = 15.9 Hz, 1H, vinyl H), 7.35 (t, J = 7.9 Hz, 2H, H-3′, 5′), 7.25 (d, J = 5.6 Hz, 1H, H-4′), 7.06 (d, J = 8.2 Hz, 1H, H-4″), 7.03 (t, J = 6.6 Hz, 2H, H-5″, 6″), 6.96 (d, J = 7.9 Hz, 1H, H-6″), 6.91 (d, J = 8.2 Hz, 1H, H-6″), 6.78 (t, J = 8.0 Hz, 2H, H-3″), 6.74 (d, J = 16.0 Hz, 1H, vinyl H), 6.70 (s, 1H, H-2″), 6.67 (d, J = 15.9 Hz, 1H, vinyl H), 5.91 (s, 1H, Ar-OH), 3.92 (s, 3H, OCH3), 3.90 (s, 3H, OCH3), 2.49 (s, 3H, CH3). 13C NMR (150 MHz, CDCl3) δ: 188.53, 153.41, 150.78, 149.75, 148.74, 147.65, 143.86, 142.65, 140.24, 137.28.

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131.41, 130.32, 129.15, 127.50, 125.94, 123.11, 122.32, 108.26, 107.15, 105.77, 105.45, 101.76, 98.17, 60.97, 56.18, 14.18. IR (KBr, cm⁻¹): 1667, 1622. ESI-MS m/z: 541.19 [M+H]⁺, calef for C₃H₂₃N₂O₂: 540.19.

(1E, 4E)-1-(5-benzo[d][1.3]dioxol-6-yl)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(4-hydroxy-3-methoxyphenyl) penta-1, 4-dien-3-one (7l)

A yellow solid, m.p:152.2–153.9°C, 10.7% yield. ¹H NMR (600 MHz, CDCl₃): δ: 7.60 (d, J = 7.9 Hz, 2H, H-2”, 6’), 7.49 (d, J = 16.0 Hz, 1H, vinyl H), 7.41 (d, J = 16.0 Hz, 1H, vinyl H), 7.38 (t, J = 8.0 Hz, 2H, H-3’, 5’), 7.28 (t, J = 7.4 Hz, 1H, H-4’), 7.08 (dd, J = 8.1, 1.6 Hz, 1H, H-6”), 7.04 (s, 1H, H-2”), 6.92 (d, J = 8.2 Hz, 1H, H-5”), 6.74 (d, J = 16.0 Hz, 1H, vinyl H), 6.69 (d, J = 15.9 Hz, 1H, vinyl H), 6.66 (d, J = 8.5 Hz, 1H, H-5”), 6.51 (d, J = 2.5 Hz, 1H, H-2”), 6.35 (dd, J = 8.5, 2.6 Hz, 1H, H-6”), 5.90 (s, 2H, OCH₃), 5.88 (s, 1H, Ar-OH), 3.93 (s, 3H, OCH₃), 2.50 (s, 3H, CH₃). ¹³C NMR (150 MHz, CDCl₃): δ: 188.72, 150.77, 149.76, 148.74, 148.15, 147.58, 146.81, 143.87, 142.94, 137.31, 131.05, 129.15, 127.48, 127.37, 124.28, 123.34, 123.26, 122.34, 114.77, 109.64, 108.29, 107.13, 105.78, 98.20, 55.95, 14.14. IR (KBr, cm⁻¹): 3334, 1647, 1592. ESI-MS m/z: 597.17 [M+H]⁺, calef for C₂₉H₂₃N₂O₆: 496.16.

(1E, 4E)-1-(5-ethoxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(4-hydroxy-3-methoxyphenyl) penta-1, 4-dien-3-one (7m)

A yellow solid, m.p:153.3–153.5°C, 14.1% yield. ¹H NMR (600 MHz, CDCl₃): δ: 7.77: (s, 1H, Pyrazole-H), 7.63 (d, J = 15.8 Hz, 1H, vinyl H), 7.61 (d, J = 15.9 Hz, 1H, vinyl H), 7.59 (s, 1H,Pyrazole-H), 7.15 (dd, J = 8.2, 1.7 Hz, 1H, H-6’), 7.09 (d, J = 1.7 Hz, 1H, H-2’), 6.93 (d, J = 8.2 Hz, 1H, H-5’), 6.85 (d, J = 15.8 Hz, 1H, vinyl H), 6.82 (d, J = 15.8 Hz, 1H, vinyl H), 5.91 (s, 1H, Ar-OH), 3.94 (s, 3H, NCH₃), 3.92 (s, 3H, OCH₃). ¹³C NMR (150 MHz, CDCl₃): δ: 188.67, 148.19, 146.84, 143.07, 138.81, 133.40, 130.83, 127.38, 123.42, 123.34, 119.02, 114.84, 109.69, 55.94, 39.19. IR (KBr, cm⁻¹): 1667, 1617. ESI-MS m/z: 285.12 [M+H]⁺, calef for C₁₈H₁₈N₂O₃: 284.11.

Biology

Cell Culture and Reagents

The human cancer cell lines SGC-7901, HepG2, A549, SW620 and human normal colon epithelial cells HCoEpiC were purchased from the Cell Collection Center of Wuhan University and maintained in Gibco RPMI medium 1640 (Gibco Company, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco Company, Grand Island, NY, USA), 1% penicillin and streptomycin in a 5% CO₂ atmosphere at 37°C. Curcumin was purchased from Sigma Company (C1386, Sigma-Aldrich Chemical Company, Louis, Missouri, USA). Compound 7h and curcumin were, respectively, dissolved in DMSO at 20 mM, which were then diluted to different concentrations in the prepared medium. Primary antibodies against ATM (2873, Cell Signaling Technology, Trask Lane Danvers, MA, USA), PARP (9542, Cell Signaling Technology, Trask Lane Danvers, MA, USA), cyclin B1 (sc-7393, Santa Cruz Biotechnology, Delaware Ave Santa Cruz, CA, USA), cyclin D1 (sc-6283, Santa Cruz Biotechnology, Delaware Ave Santa Cruz, CA, USA), CC3 (9662, Cell Signaling Technology, Trask Lane Danvers, MA, USA), Bcl-2 (ab196495, Abcam Inc., Cambridge, MA, USA), Bcl-2 (ab196495, Abcam Inc., Cambridge, MA, USA), Bax (ab134953, Abcam Inc., Cambridge, MA, USA), Bcl-xL (ab32124, Abcam Inc., Cambridge, MA, USA), P53 (ab12410, Abcam Inc., Cambridge, MA, USA), P21 (ab7581, Abcam Inc., Cambridge, MA, USA), and Mcl-1 (ab13469, Abcam Inc., Cambridge, MA, USA).
plus all secondary antibodies, were obtained from Thermo (Thermo Fisher Scientific, Waltham, MA, USA).

Cell Viability Assay
A MTT assay was performed to assess cell viability according to the instructions of the manufacturer (Sigma-Aldrich Chemical Company, Louis, Missouri, USA). Briefly, 1×10⁴ SW620 cells were plated onto 96-well plates. After cultured overnight, the cells were treated with different concentrations of 7h and curcumin (0, 0.5, 1.25, 2.5, 5, 10 and 20 μM) for 24 h, 48 h and 72 h. At the end of each treatment, 20 μL of MTT (5 mg/mL) was added. After incubation for 4 h, the medium was carefully discarded and dimethyl sulfoxide (100 μL) was added. Absorbance was recorded at a wavelength of 490 nm by a Universal Microplate Reader (Bio-Tek Instruments, Winooski, USA), using a blank well as control. Cell viability and IC₅₀ values of compounds for the four cancer cell lines were determined by comparison with control. Each experiment was repeated at least 3 independent times.

Transfection
SW620 cells were seeded into 6-well plates and transfected with Amt CRISPR Activation Plasmid (sc-400,192-ACT, Santa Cruz Biotechnology, Delaware Ave Santa Cruz, California, USA) and lipofectamine 2000 (Invitrogen Inc., Carlsbad, CA, USA) by following the manufacturer’s instruction. After the indicated periods of incubation, the cells were subjected to further analysis.

Transwell Migration and Invasion Assay
For transwell migration assays, 1×10⁶ cells were plated into the top chamber of a transwell (Corning, Acton, MA, USA) with a porous membrane (8.0-μm pore size). Then, the cells were plated in medium with minimal serum (0.5% FBS), and medium supplemented with additional serum (10% FBS) was used to produce a chemoattractant effect in the lower chamber. Compound 7h at different concentrations was added. The cells were incubated for 24 h at 37°C and cells that did not migrate through the pores were removed using a cotton swab. Cells on the lower surface of the membrane were stained with crystal violet (Sigma-Aldrich Chemical Company, Louis, Missouri, USA). The migrated cells were manually quantified. The inhibition rates of migrated cells were calculated using the untreated group as 100%.

As for in vitro cellular invasion analysis, briefly, 1×10⁶ cells in serum-free medium were seeded into the upper chamber of an insert coated with Matrigel. Medium supplemented with 10% FBS was placed over the lower chamber and then 7h at different concentrations were added. After incubation for 24 h, the upper layer of cells was removed by cotton wool. The cells on the lower surface were fixed in methanol and stained with 0.1% crystal violet, and then imaged using an IX71 inverted microscope (Olympus, Tokyo, Japan). The number of migrating cells in a total of five randomly selected fields was counted. All experiments were performed in triplicate.

Clonogenic Assay
In order to examine the survival of cells treated with 7h, SW620 cells were plated (1×10⁶ per well) in a 6-well plate and incubated overnight. After exposure to different concentrations of 7h for 48 h, the viable cells were counted and then seeded into 6-well plate in a density of 5,000 cells per well. The cells were then incubated for 14 days at 37°C in a humidified 5% CO₂ atmosphere. All the colonies were stained with 2% crystal violet and cells were counted manually.

Cell Apoptosis Analysis
The apoptotic cells were detected with an Annexin V-FITC/PI apoptosis detection kit (Biouniqure, Beijing, China) according to the manufacturers’ instructions. Briefly, 1×10⁶ SW620 cells were incubated in 6-well plate overnight and treated with various concentrations of 7h and curcumin for 48 h. Cells were harvested by centrifugation, washed with PBS, then resuspended in 500 μL of binding buffer with 5 μL propidium iodide (PI) and kept in the dark for 5 min at room temperature. Next, 5 μL FITC-conjugated anti-Annexin V antibody was added, which was kept in the dark for 10 min at room temperature. Apoptosis was analyzed by a flow cytometer (Becton Dickinson, Mountain View, CA, USA).

Cell Cycle Analysis
Exponentially growing SW620 cells were seeded in a 6-well plate and incubated overnight. The cells were treated, respectively, with curcumin and 7h and then cultured for 48 h. At the end of treatment period, cells were collected and fixed with ice-cold 75% (v/v) ethanol and kept at 4°C overnight. The cells were collected and washed with ice-cold PBS. Then, the cell pellets were re-suspended at 1×10⁶ cells/mL in PBS and incubated with 0.1 mg/mL RNase I and 50 mg/mL Propidium iodide (PI) at 37°C for 30 min. DNA contents were determined with a flow cytometer (Becton Dickinson, Mountain View, CA, USA).
Figure 2 The inhibitions of cell viability for the synthesized compounds (7a–7o) on human cancer cell lines SGC-7901 (A), HepG2 (B), A549 (C) and SW620 (D). The cells were treated with 7a-7o (20 μM) for 72 h, and then the cell viability was determined by MTT assay, curcumin as positive control. Results were presented as Mean±S.D (n=3) and analyzed by GraphPad Prism 6.0 followed by the Student’s t-test. *p < 0.05, **p < 0.01 or ***p < 0.001 vs curcumin.
Western Blot Analysis

The expressions of several proteins were detected by Western blot analysis. The SW620 cells were treated, respectively, with \(7h\), Atm CRISPR Activation Plasmid, \(7h^+\) Atm CRISPR Activation Plasmid. The harvested SW620 cells were washed by PBS and lysed with cell lysis buffer (Cell Signaling, Danvers, MA, USA). The protein concentrations were tested by a BCA Protein Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Equal amounts of protein samples were prepared and fractionated by electrophoresis in a sodium dodecyl sulfonate-polyacrylamide gel and transferred onto a polyvinylidene fluoride membrane. Skimmed milk powder was added, and the samples were incubated for 2 h. Next, appropriate primary antibodies were added, and the sample was finally incubated at 4°C overnight. The membranes were incubated for 1 h with secondary antibodies at room temperature and then washed three times with tris-buffered saline and Tween 20. Protein bands were subsequently detected by electrogenerated chemiluminescence assay.

Statistical Analysis

All data analyses were conducted using IBM SPSS, Version 19.0 (IBM Corp., Armonk, NY, USA). Statistical comparisons were performed using the Student \(t\)-test. \(P\)-values < 0.05 were considered statistically significant. Results were expressed as means ± standard deviations.

Results and Discussion

Synthesis of MCACs

The condensation of phenylhydrazine with ethyl acetoacetate afforded 1-phenyl-3-methyl-5-pyrazolone 9, which was followed by the Vilsmeier reaction to produce 5-chloro-3-methyl-1-phenyl-1\(H\)-pyrazole-4-carbaldehyde 10. Compound 10 was treated with ethanol or substituted phenols in the presence of KOH to give substituted \(1H\)-pyrazole-4-carbaldehydes 11a–11c. Various substituted benzaldehydes conducted the Claisen-Schmid condensation with acetone in the presence of 10%NaOH to furnish \(\alpha,\beta\)-unsaturated ketones 13a–13f. Using the same NaOH-catalysed Claisen-Schmidt condensations of 13a–13f with 10, 11a–11c or 1-methyl-1\(H\)-pyrazole-4-carbaldehyde, the target compounds 7a–7o were finally obtained. All the target compounds 7a–7o were well characterized by spectroscopic techniques such as IR, NMR and ESI-MS, which were in full accordance with the depicted structures. The
Figure 3 Compound 7h inhibited clone formation (A and B), migration (C and D) and invasion (E and F) on SW620 cells. SW620 cells were treated with 7h at the concentration of 1.25 μM, 2.5 μM and 5 μM for 48h respectively, the cells treated with DMSO as control and with curcumin (5 μM) as positive control. Results were presented as Mean ± SD (n = 3) and analyzed by GraphPad Prism 6.0 followed by the Student t-test. *P < 0.05 or **P < 0.01 or ***P < 0.001 vs control, ++P < 0.01 or +++P < 0.001 vs curcumin.
proton NMR of 7a–7o exhibited two pair of doublets in the aromatic region with \( J \) values between 15.5 and 16.1 Hz for the alkene protons present in the penta-1, 4-dien-3-one skeleton, providing evidence of \((E)\)-stereochemistry. Before used for biological assays, all synthesized compounds 7a–7o were determined their purity (all >97%) by HPLC.

**Effect of the Synthesized Compounds on Cell Viability**

The inhibitory effects of the synthesized compounds on the proliferation of human cancer cell lines GC-7901, HepG2, A549 and SW620 were assessed by MTT assay at a concentration of 20\( \mu \)M respectively, and curcumin was used as positive control. The results indicated that most of the synthesized compounds decreased cell viability significantly as compared to curcumin, especially after 72 h when HepG2, A549 and SW620 cells were treated with these synthesized compounds (Figure 2). Among them, compounds 7a, 7h and 7i exhibited excellent inhibitory activities, especially in SW620 cells with the ratio of cell viability <50%. Further, the \( IC_{50} \) values of compounds 7a, 7h, 7i were determined at the time of 24h, 48h and 72h, respectively, and curcumin, adriamycin and oxaliplatin were used as positive control. The results are shown in Table 1. Compound 7h exhibited more potent inhibitory activity against SW620 cells with \( IC_{50} \) value of 0.012 \( \mu \)M than the three positive controls. Furthermore, in order to evaluate its cytotoxicity to normal cells, 7h was chosen to test its inhibitory effects on the proliferation of human normal colon epithelial cells HCoEpiC at the time of 72 h, and the \( IC_{50} \) value was more than 100\( \mu \)M, implying that 7h exhibited selective cytotoxicity to cancer cells. The preliminary study on structure–activity relationship (SAR), taking that in
SW620 cells as an example, indicated that on the terminal containing pyrazolyl ring of these MCACs ethoxy or phenoxo groups resulted in better activity than chlorine atom. Excepted for 7g, 2-methoxyphenoxy group was introduced into 7h, 7i to replace the chlorine atom, which showed the most potent activity. On another terminal of these MCACs, trimethoxyphenoxy (3, 4, 5-OCH$_3$) group together with both 3-methoxy and 4-hydroxyl groups might contribute to good activities.

**Effect of 7h on Migration and Colony Formation in SW620 Cells**

The migration and invasion of cancer cells due to their promoted penetration into the lymphatic system and blood vessels can lead to metastasis dissemination, and then cancer cells undergo extravasation into a newly metastatic site where they proliferate. Thus, an ability to inhibit the migration and invasion of cancer cells is very important for an anticancer compound. As 7h exhibited excellent inhibitory activities against the proliferation of SW620 cancer cells, further, its effects on inhibitions of the migration and colony formation of SW620 cells were evaluated at the various concentrations of 1.25 μM, 2.5 μM and 5 μM after the cells were treated with 7h for 48 h. The cells treated with DMSO and curcumin (5 μM) were used as blank and positive control correspondingly. The results indicated that 7h decreased the number of migration and invasion in a dose-dependent manner (Figure 3A–D), and there was a significant reduction in the percentages of cell migration and invasion especially at the concentration of 5 μM as compared with both control groups. As shown in Figure 3E and F, the clonogenic quantity of

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**Figure 5** The effect of 7h on cell cycle. (A and B) SW620 cells were treated with 7h (1.25, 2.5 or 5 μM) for 48 h, and then the cell cycle distribution was detected by PI staining, the cells with DMSO as control and with curcumin (5 μM) as positive control. (C) Western blotting was used to detect the expression of cyclin B1 and cyclin D1 protein. Data were presented as Mean ± SD (n = 3) and analyzed by GraphPad Prism 6.0 followed by the Student t-test. *P < 0.01 or **P < 0.001 vs control, +P < 0.05 or +++P < 0.001 vs curcumin.
SW620 cells treated with 7h (5μM) decreased nearly by thirteen-fold as compared to the positive control. These results indicated that 7h could significantly inhibit cell proliferation.

**Effect of 7h on SW620 Cell Apoptosis**

In order to evaluate the effect of 7h on cell apoptosis, SW620 cells were treated with 7h at the concentrations of 1.25μM, 2.5μM and 5μM for 48 h, respectively, and then the apoptosis was analyzed by flow cytometry, using the cells treated with DMSO and curcumin (5μM) as blank and positive control correspondingly. The results (Figure 4A and B) showed that the treatment with 7h increased the apoptotic rate from 18.64% of vehicle control to 19.69% (1.25μM), 47.4% (2.5μM) and 87.3% (5μM) in a concentration-dependent manner. Compared to curcumin group, 7h significantly increased the cell apoptotic rate at the concentration of 2.5μM and 5μM. On the other hand, Western blotting result (Figure 4C) showed that 7h markedly increased the protein expression of cleaved-caspase 3 and PARP and decreased Bcl-2 expression at the concentration of 5μM in SW620 cells as compared with curcumin (5μM) group, which suggested that 7h promoted SW620 cell apoptosis.

**Effect of 7h on SW620 Cell Cycle**

SW620 cells were treated with 7h at the concentrations of 1.25μM, 2.5μM and 5μM for 48h, respectively, and then the cell cycle was determined, the cells treated with DMSO as control and curcumin (5μM) as positive control. As shown in Figure 5A and B, compound 7h at the concentration of 5μM increased the percentages of G2/M phase cells from 10% to 21% and that of S phase cells from 43% to 66%, while decreased the percentages of G0/G1 phase cells from 46% to 11%. Compared to the control and the positive control group, compound 7h significantly increased the percentages of G2/M phase cells at the concentration of 5μM. The results indicated that 7h inhibited SW620 cell proliferation by inducing cell cycle arrest at G2/M and S phase. Meanwhile, the expressions of cyclin B1 and cyclin D1 were also determined by Western blot assay, and the results (Figure 5C) showed that 7h increased cyclin B1 protein expression and decreased cyclin D1 protein expression at the concentration of 5μM in SW620 cells.

**Compound 7h Regulated ATM Expression on SW620 Cells**

The ATM gene is a DNA repair gene and is located on the chromosome 11q22-q23. Studies have shown that the ATM gene may become a new potential target for the treatment of colon cancer, and then we hypothesized that the inhibitory effect of 7h on the proliferations of SW620 cells might be related to ATM pathway. Therefore, SW620 cells were treated with 7h (5μM) and curcumin (5μM) for 48 h, and then ATM protein expressions were determined, the cells treated with DMSO as control. The
Figure 7 The effects of 7h on apoptosis, cell cycle, migration and invasion were partly dependent on ATM pathway in SW620 cells. SW620 cells were transfected with ATM plasmid (Atm CRISPR Activation) for 24 h and then combined with or without 7h for 48 h, and the cells were treated with negative plasmid as control. The clone formation (A and B) and cell migration and invasion (C-F) were detected; The apoptotic rate and apoptotic relative protein expression (G-I) were detected by FCM and Western blot assay; The cell cycle arrest, cell cycle arrest-related protein expression (J-L) were also detected. Results were presented as Mean ± SD (n = 3) and analyzed by GraphPad Prism 6.0 followed by the Student t-test. *P < 0.05, **P < 0.01 or ***P < 0.001 vs control, +P < 0.05, ++P < 0.01 or +++P < 0.001 vs compound 7h, ##P < 0.01 or ###P < 0.001 vs ATM.
Western blotting results revealed that 7h markedly decreased ATM protein expression as compared to both control and curcumin (5μM) groups (Figure 6A). Further, in order to confirm whether 7h could really down-regulated ATM signal, SW620 cells were transfected with the ATM activated plasmids that over-expressed ATM protein for 24 h. The cells were treated with 7h for 48 h, using the cells treated with negative plasmids as control. The result showed that the ATM activated plasmids reversed the down-regulation of ATM expression induced by 7h (Figure 6B), which also proved that 7h decreases the ATM protein expression in SW620 cells.

Effects of 7h on Cell Apoptosis, Cycle, Migration and Invasion in SW620 Cells Were Partly via ATM Pathway
As 7h decreased the ATM protein expression, in order to investigate whether its effects on SW620 cells migration, invasion, cell cycle and apoptosis were via ATM pathway, the related assays were performed, and SW620 cells were transfected with or without ATM activated plasmids were used for comparison. The cells were treated with 7h (5μM), using the cells with negative plasmids as control. The result demonstrated that the transfection with ATM activated plasmids could reverse the up-regulation of the apoptotic rate induced by 7h (Figure 7G and H). Similarly, the Western blotting result indicated that the expressions of cleaved-caspase 3, PARP and Bcl-2 induced by 7h in SW620 cells were all decreased by 7h combined with ATM activated plasmid (Figure 7I). Meanwhile, the treatment with 7h+ATM significantly restored cell cycles that were obviously different from the ones induced only by 7h (Figure 7J and K). Correspondingly, 7h+ATM clearly reduced the up-regulation of cyclin B1 protein expression while enhanced the protein expression of cyclin D1 as compared to treatment only with 7h (Figure 7L). Finally, the present study also demonstrated that 7h+ATM significantly enhanced the decline of the migration and invasion number induced only by 7h (Figure 7C–F). Although 7h decreased the amount of clone formation, the amount was drastically increased from 1.33 ± 1.01 to 8.22 ± 2.22 after treatment with 7h+ATM for 48 h (Figure 7A and B). In brief, the treatment combined 7h with ATM protein could almost reverse all the effects resulted only from 7h, which suggested that 7h exerted its activities, such as induction of cell apoptosis, cycle arrest, migration and clone formation inhibitions, possibly due to its ability to decrease the ATM expression in SW620 cells.

Conclusion
A serial of monocarbonyl analogs of curcumin containing pyrazole moiety was designed and synthesized whose structures were confirmed by spectroscopic techniques. Their inhibitory activities against four human cancer lines, SGC-7901, HepG2, A549 and SW620, were evaluated. Of these 15 newly synthesized compounds, (1E,4E)-1-(3-(2-methoxyphenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(3, 4, 5-trimethoxyphenyl) penta-1, 4-dien-3-one 7h exhibited excellent selectivity and outstanding anti-proliferation activity against SW620 cells with a IC_{50} value of 12 nM, which was more potent than curcumin (IC_{50} = 9.36 μM), adriamycin (IC_{50} = 3.28 μM) and oxaliplatin (IC_{50} = 13.33 μM).

In order to investigate the mechanisms that 7h exerted its inhibitory effect on SW620 cells proliferation, further assays were performed. The results showed that 7h inhibited cell migration, invasion and colony formation of SW620 colon cancer cells obviously, which was due to its ability to induce cell cycle arrest in the G2/M and S phases and apoptosis. Ultimately, 7h decreased the expression of ATM protein, which may primarily contribute to its anticancer activity against SW620 cells. All the data indicated that 7h could be identified and developed as a novel potential anti-colon cancer agent in the future.

Abbreviations
MCACs, monocarbonyl analogs of curcumin; MTT, methyl thiazolyl tetrazolium; IC_{50}, 50% inhibitory concentration; ATM, ataxia-telangiectasia mutated; PARP, poly ADP-ribose polymerase; CRISPR, clustered regularly interspaced short palindromic repeats; FBS, fetal calf serum.

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Disclosure
The authors report no conflicts of interest in this work.

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