MedChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: S. Lu, O. N. obianom and Y. Ai, *Med. Chem. Commun.*, 2018, DOI: 10.1039/C8MD00284C.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/medchemcomm

View Article Online
DOI: 10.1039/C8MD00284C

Novel hybrids derived from aspirin and chalcones potently suppress colorectal cancer in vitro and in vivo

Shan Lu, [a, *] Obinna N Obianom, [b] and Yong Ai [b, **]

[a]College of Pharmacy, Hubei University of Chinese Medicine, Hubei 430065, PR China

[b]Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD 21201, United States

* Corresponding author. College of Pharmacy, Hubei University of Chinese Medicine, Hubei 430065, PR China. E-mail address: lushan9805@163.com (S. Lu).

** Corresponding author. Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, Baltimore, MD 21201, United States. E-mail address: aiyong0508@126.com (Y. Ai).

View Article Online
DOI: 10.1039/C8MD00284C

Abstract

Colorectal cancer (CRC) remains the fourth leading cause of cancer deaths in the world despite the availability of many approved small molecules for treatments. The issues lie in the potency, selectivity and targeting of these compounds. Therefore, new strategies and targets are needed to optimize and develop novel treatments for CRC. Here, a group of novel hybrids derived from aspirin and chalcones were designed and synthesized based on recent reports of their individual benefits to CRC targeting and selectivity. The most active compound **7h** inhibited proliferation of CRC cell lines with better potency compared to 5-Fluorouracil, a current therapy for CRC. Importantly, **7h** had 8-fold less inhibitory activity against non-cancer CCD841 cells. In addition, **7h** inhibited CRC growth via the inhibition of the cell cycle in G1 phase. Furthermore, **7h** induced apoptosis by activating caspase 3 and PARP cleavage as well as increasing ROS in CRC cells. Finally, **7h** significantly retarded the CRC cells growth in a mouse xenograft model. These findings suggest that **7h** may have a potential to treat CRC.

View Article Online
DOI: 10.1039/C8MD00284C

Introduction

Colorectal cancer (CRC) originates from the inner walls of the colon and the rectum. It is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related death worldwide.[1,2] Common treatments for CRC include 5-Fluorouracil (5-FU), capecitabine, Irinotecan, and Oxaliplatin. The metastasis of CRC has fueled the discovery of many novel targets in recent years, and a number of groups have developed newer drugs to specifically alter these new targets. For example, the development of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) inhibitors.[3] However, in many cases, there has been reports of mild to grave side effects encountered with these new drugs due to their unselectivity towards cancer cells.[4-8] For this reason, novel therapeutics for CRC with reduced side effects is still urgently needed.

Acetylsalicylic acid, commonly known as Aspirin (ASA, **1**, Fig.1), is a widely used non-steroidal anti-inflammatory drug (NSAID) that has been shown to be effective for the prevention and remission of cancers, especially in CRC.[9-14] To improve its relatively low anticancer potency, many novel synthetic ASA derivatives have been developed including HS-releasing ASA,[15-17] HNO- and NO-releasing ASA,[18-22] dual nitric oxide- and hydrogen sulfide-releasing ASA,[23,24] phospho-ASA,[25] 6-gingerol-based ASA prodrug,[26] resveratrol-based ASA prodrug,[27] Se-ASA,[28] and Asplatin.[29,30] These ASA derivatives are more cytotoxic and chemo-preventive against CRC cells than ASA, hence, the advancement of new ASA derivatives with more favorable and safer profiles continues to be a welcome advancement.

Natural products and their derivatives provide a diverse source of biologically active lead compounds for drug discovery and development.[31-34] For example, chalcones (**2**, Fig. 1) are naturally occurring compounds, which bear an α,β-unsaturated ketone moiety and can act as Michael acceptors. Chalcones exert anticancer activity through a number of mechanisms including inhibition of thioredoxin reductases (TrxRs),[35-38] nuclear factor κB (NF-κB),[39-47] tubulin polymerization,[48-60] as well as inhibition of receptor tyrosine kinases such as EGFR[61]

View Article Online
DOI: 10.1039/C8MD00284C

and vascular endothelial growth factor receptor 2 (VEGFR-2).[62] More so, chalcones are recognized as a privileged scaffold for the incorporation of different molecules or pharmacophores using linkers such as amide, diol, esters or the use of a triazoles *via* click chemistry.[63-78] These hybrids typically retain or enhance the biological potency and selectivity of the parent chalcones.

The aforementioned investigations led us to hypothesize that a combination of ASA and chalcones can be an attractive strategy to selectively treat CRC. Accordingly, we synthesized a novel class of hybrids derived from aspirin and chalcones (**7a-p**, Fig.1), and evaluated their bioactivity *in vitro* and *in vivo* against CRC cells.

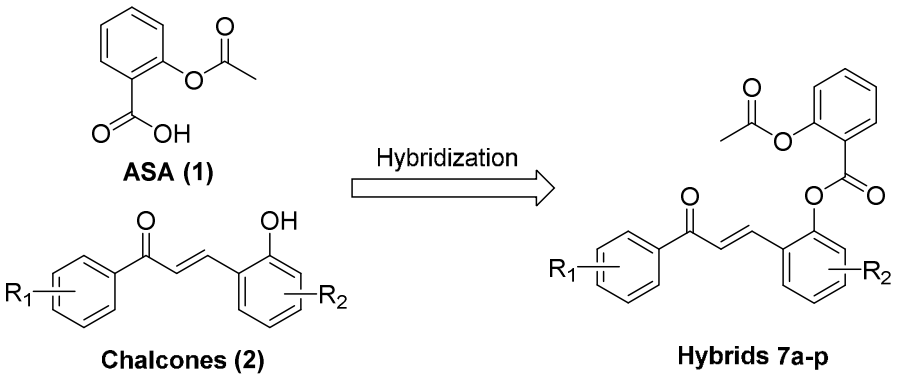


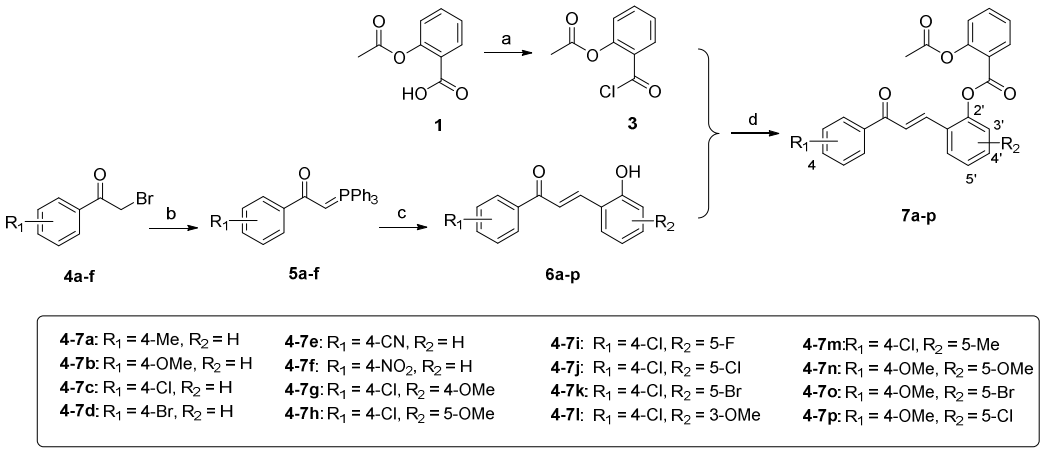
Fig. 1. Chemical structures of ASA, chalcones and hybrids **7a-p**.

Results and discussion

Chemistry

The target compounds **7a-p** were synthesized as depicted in Scheme 1. Initially, aspirin **1** was converted to the acid chloride **3** upon treatment with thionyl chloride. Then the corresponding chalcones **6a-p** were prepared by the Wittig Reaction starting from aldehydes **4a-f** in moderate to high yields. Next, reaction of acid chloride **3** with **6a-l** in dry CH_2Cl_2, in the presence of the non-nucleophilic base trimethylamine (TEA), furnished the respective target compounds **7a-p** in good yields. The structures of all hybrids were fully characterized by their ^1H and ^{13}C NMR spectroscopic data as well as by mass spectral analysis.

View Article Online
DOI: 10.1039/C8MD00284C



Scheme 1. Synthesis of hybrids **7a-p**. Reagents and conditions: (a) $SOCl_2$, anhydrous CH_2Cl_2, reflux, 2h; (b) i) PPh_3, anhydrous THF, rt, 16 h; ii) KOH, MeOH, rt, 12h; (c) aldehydes, anhydrous toluene, 60 ºC , 8h; (d) TEA, anhydrous CH_2Cl_2, 12h.

Biological evaluation

Assessment of in vitro anti-proliferative activity

The anti-proliferative activity of compounds **7a-p** against HCT-8 and DLD-1 cells was initially evaluated using MTT assays with 5-FU as a positive control. As shown in Table 1, compounds **7c**, **7f**, **7h**, and **7i** at 10 μM displayed potent anti-proliferative activity (>50%) against both HCT-8 and DLD-1 cells, and this was more potent than that of 5-FU. It was obvious that R_1 and R_2 substituents on the benzene ring of the hybrids may be crucial for their anticancer activity. In general, the compounds with a Cl or NO_2 on the left benzene ring (**7c** and **7f**) showed more potent inhibitory activity than those bearing an Me, OMe, Br, or CN group (**7a**, **7b**, **7d**, or **7e**). Moreover, the compounds with an OMe or F at the 5-position of the beta benzene ring (**7h** and **7i**) generally showed more potent inhibitory activity than those bearing an 3-OMe, 4-OMe, 5-Cl, 5-Br, or 5-Me group (**7l**, **7g**, **7j**, **7k**, or **7m**).

The active compounds **7h** and **7i** were further assayed for their anti-proliferative activity against two CRC cell lines (Table 1). The IC_{50} values of **7h** were 2.4 and 2.7 μM against HCT-8 and DLD-1 cells, respectively, which is superior to that of 5FU, individual drugs **6h** (IC_{50} = 19.7, 14.7 μM) and ASA (IC_{50} > 100 μM), and even the

View Article Online
DOI: 10.1039/C8MD00284C

combination of **6h** and ASA (IC_{50} = 6.3, 6.9 μM, physical mixture, mole ratio, 1:1). Similarly, compound **7i** displays more potent anticancer activity than its individual moieties **6i** (IC_{50} = 25.6, 13.2 μM) and ASA (IC_{50} > 100 μM), and the combination of **6i** and ASA (IC_{50} = 9.3, 8.0 μM). These results confirm that the antitumor activity of **7h** and **7i** may be most likely attributed to the synergic effects derived from their chalcone and ASA moieties. Given the improved potency of **6h**+ASA and **6i**+ASA, it is probable that **7h** and **7i** may be acting as prodrugs and this will be tested in future studies. In non-tumorigenic cells, CCD841, **7h** had 8-fold less inhibitory activity (IC_{50} = 19.4 μM) than against the cancer cells tested. Hence, **7h** preferably inhibited the proliferation of CRC cells and was selected for further investigation.

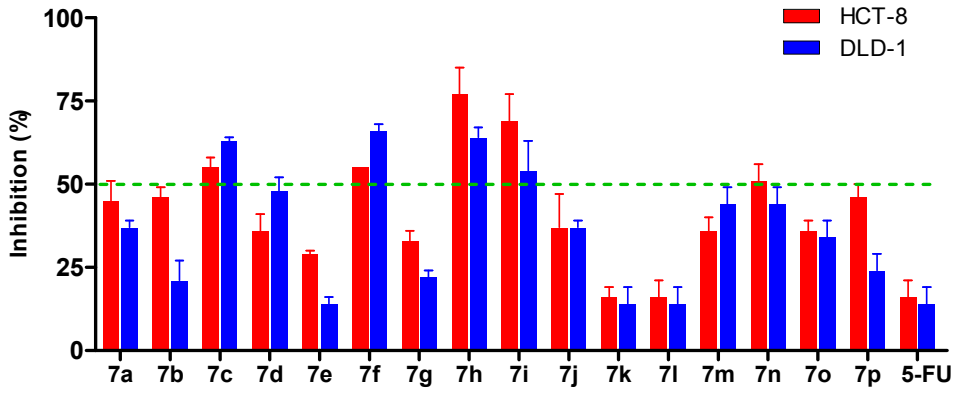


Fig. 2. Inhibition of CRC cell proliferation. Cells were treated with, or without, the indicated compounds at 10 μM for 72 h, and the cell proliferation was measured. The inhibition (%) of each compound was determined. Data are presented as the mean (%) ± SD of each compound from three independent experiments.

Table 1. The anti-proliferative activity of compounds **7h** and **7i**.

Compounds	IC_{50} (μM) [a]		
	HCT-8	DLD-1	CCD841
7h	2.4 ± 0.1	2.7 ± 0.2	19.4 ± 0.9
7i	2.7 ± 0.5	3.5 ± 0.4	ND

View Article Online
DOI: 10.1039/C8MD00284C

6h	19.7 ± 2.5	14.7 ± 1.8	ND
6h+ASA	6.3 ± 0.9	6.9 ± 0.9	ND
6i	25.6 ± 3.2	13.2 ± 1.4	ND
6i+ASA	9.3 ± 1.0	8.0 ± 0.6	ND
ASA	>100	>100	ND
5-FU	28.9 ± 2.7	33.0 ± 2.1	35.7 ± 3.2

[a]Data are expressed as mean IC_{50} (μM) ± SD of each compound from three independent experiments. IC_{50} is the drug concentration inhibiting 50% of the cell proliferation. ND, not determined.

Effect of 7h on cell cycle arrest in CRC cells

Since the anticancer agents can prevent the proliferation of cancer cells by blockade of the cell cycle at a specific checkpoints, we performed cell cycle analysis after treatment of HCT-8 cells with compound **7h**. In short, HCT-8 cells were treated with varying concentrations (0, 2.5, 5, and 10 μM) of **7h** for 24 h and then stained with propidium iodide (PI), followed by flow cytometry analysis. Figure 3 shows that treatment with **7h** induced HCT-8 cell cycle arrest at G_0/G_1 in a concentration dependent manner – 48.08% (2.5 μM), 51.03% (5 μM), and 56.46% (10 μM), higher than that of the vehicle-treated control. As a result, the fraction of CRC cells at the G2 phase was significantly lower in **7h**-treated than vehicle-treated control. This suggests that the anti-cancer mechanisms of **7h** may include its ability to induce cycle arrest in the CRC cells.

View Article Online
DOI: 10.1039/C8MD00284C

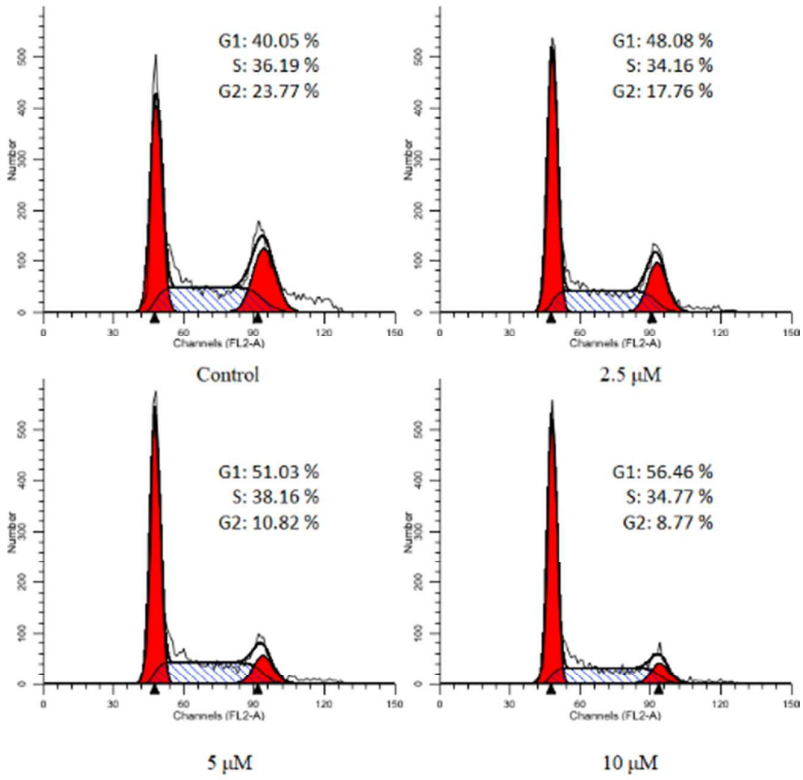


Fig. 3. Effects of **7h** on cell cycle distribution in HCT-8 cells. Cells were treated with the indicated concentrations of **7h** for 24 h and stained with PI, followed by flow cytometry analysis.

Effect of 7h on apoptosis in CRC cells

To determine whether the inhibitory effects of **7h** on CRC cell proliferation are accompanied by enhanced apoptosis, we next carried out Annexin V-FITC and PI staining on **7h** treated cells. HCT-8 cells were incubated with vehicle, or **7h** (2.5, 5, and 10 μM) for 24 h. Then flow cytometry analysis was used to determine the apoptotic status of the cells. We observed that treatment with **7h** induced apoptosis in HCT-8 cells in a concentration dependent manner (Fig. 4). This suggests that **7h** treatment significantly increases apoptosis in cancer cells as a result of its anti-cancer effects.

View Article Online
DOI: 10.1039/C8MD00284C

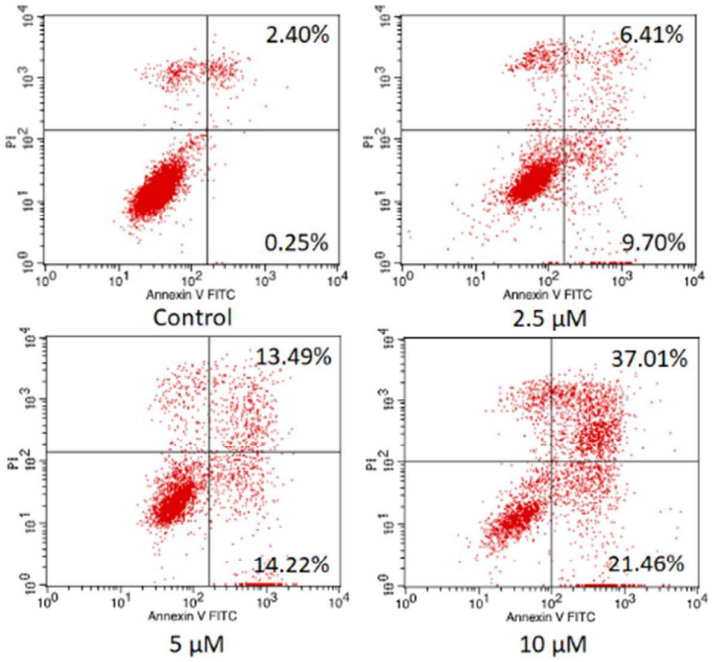


Fig. **4**. Effects of **7h** on the induction of apoptosis. HCT-8 cells were treated with the indicated concentrations of **7h** or vehicle (control) for 24 h. The cells were stained with Annexin V-FITC and propidium iodide (PI), followed by flow cytometry analysis.

To understand the mechanism of **7h**-induced apoptosis, we further examined the changes of the intracellular proteins related to apoptosis such as caspase-3, and poly(ADP-ribose)polymerase (PARP) in HCT-8 cells treated with **7h** (0, 2.5, 5, and 10 μM). As shown in Figure 5, **7h** significantly induced the cleavage of both caspase 3 and PARP in a dose-dependent manner in the cells. Overall, our results confirm that compound **7h** inhibits the growth of CRC cells by inducing apoptosis in addition to its effects on cell cycle.

View Article Online
DOI: 10.1039/C8MD00284C

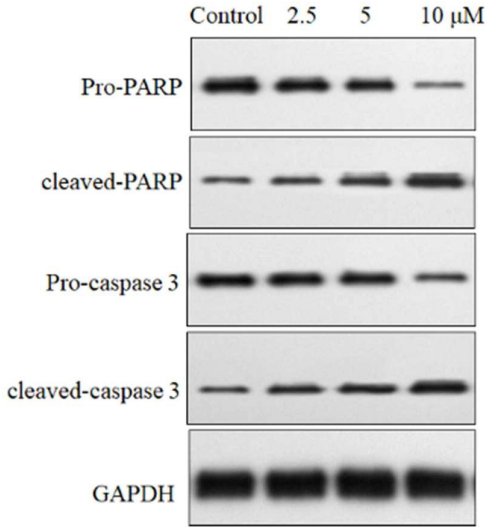


Fig. 5. Effect of **7h** on the expression of apoptotic markers. The relative protein expression levels of PARP and Caspase 3 were measured following **7h** treatment in HCT-8 cells. Cells were treated with increasing concentrations of **7h** or control (0.1% DMSO) for 24 h and immunoblotting of the whole cell lysate was carried out to determine the protein levels.

Compound 7h increased ROS levels in CRC cells

Previous studies have demonstrated that the anticancer effect of chalcones is associated with increase in ROS levels.[35, 79, 80] To investigate whether induced cytotoxic effect of **7h** is related to ROS production, we measured ROS levels in **7h**-treated CRC cells by flow cytometry analysis. Again, we treated HCT-8 with different concentrations of **7h**, and measured the ROS levels. As shown in Figure 6, a dramatic increase (from 3.67 to 53.64) in the levels of ROS generation in HCT-8 cells was observed after treatment with **7h**. These results indicate that the induction of cell death by **7h** may be due to its effect on ROS production.

View Article Online
DOI: 10.1039/C8MD00284C

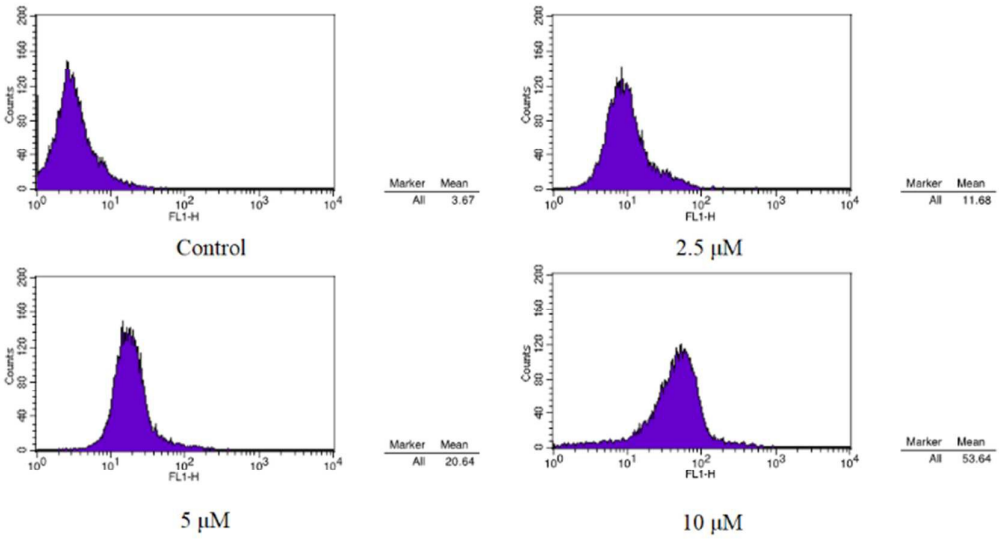


Fig. 6. Effect of compound **7h** on ROS levels in CRC cells. Flow cytometry analysis of HCT-8 cells treated with the indicated concentrations of **7h** for 24 h and stained with the fluorogenic dye 2',7'-dichlorofluorescin diacetate (DCFH-DA).

In vivo anticancer activity of 7h

To evaluate the *in vivo* anticancer activity of **7h**, BALB/c nude mice were inoculated subcutaneously with HCT-8 cells. After the establishment of solid tumor, the mice were randomly treated intraperitoneally with **7h** (60 mg/kg) or the vehicle consisting of Tween 80/PBS (5:95) daily for 32 consecutive days, respectively. Treatment with 60 mg/kg of **7h** significantly reduced the volume of implanted colon tumors ($P < 0.001$ vs. the vehicle-treated control, Figs. 7A and 7B). More so, the tumor weights in the mice treated with **7h** at 60 mg/kg were 42% (w/w) less than that in the vehicle-treated controls (1.95 ± 0.42 g vs 1.13 ± 0.07 g, $P < 0.001$, Fig. 7C) while the body weights of the mice were not significantly changed by treatment with **7h** (Fig. 7D). Together, our data clearly demonstrated that **7h** inhibited the growth of implanted tumors *in vivo* with little off-target cell toxicity.

View Article Online
DOI: 10.1039/C8MD00284C

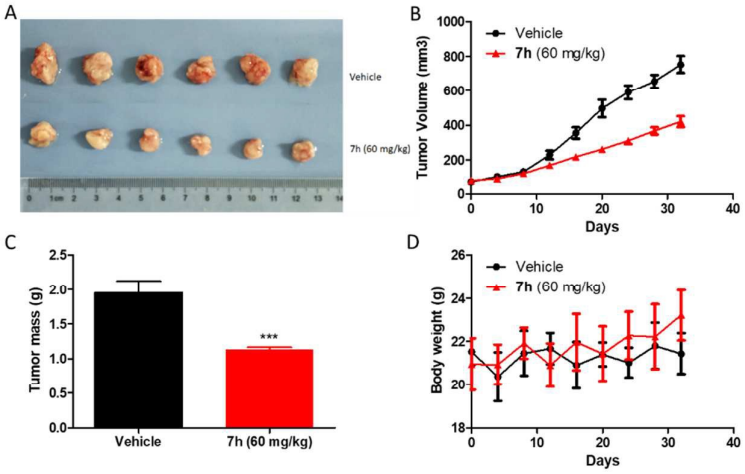


Fig. 7. Anticancer effects of **7h** in mice inoculated with HCT-8 cells. (A) Images of the tumors. The effect of intraperitoneal **7h** (60 mg/kg) on tumor volumes (B), tumor weight (C) and body weight (D) in a HCT-8 xenograft model. Data are expressed as the mean ± SD from each group of mice (n = 6 per group). (***) $P < 0.001$ vs the vehicle-treated.

Conclusion

In summary, we have designed and synthesized a novel group of hybrids derived from aspirin and chalcones **7a-p**. We found that compound **7h** had potent and selective anti-proliferative activity against CRC cells *in vitro*. In addition, treatment with **7h** induced CRC cell cycle arrest at G1 phase and apoptosis *in vitro*, increased the relative levels of cleaved caspase 3 and PARP as well as the ROS in HCT-8 cells. Finally, treatment with **7h** significantly inhibited the growth of implanted CRC cancer in mice. These findings may provide a proof of principle that **7h** may be potential chemotherapeutic agents for the intervention of CRC.

Experimental protocols

Chemical analysis

All chemicals were obtained from commercial suppliers and used without further purification unless noted specifically. Analytical thin layer chromatography was visualized by ultraviolet light at 254 nM. ^{1}H and ^{13}C NMR spectra were recorded on

View Article Online
DOI: 10.1039/C8MD00284C

Bruker Avance 400, and tetramethylsilane (TMS) was used as a reference. Data for ^{1}H are reported as follows: chemical shift (ppm), and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Data for ^{13}C NMR are reported as ppm. High resolution mass spectrometry (HRMS) spectra were recorded on an Agilent Technologies LC/MSD TOF instrument. Aspirin **2** and α-bromoacetophenones **4a-f** were commercially available.

Synthesis of intermediate 3

Intermediate **3** were prepared according to the method described previously[81] wtih minor modification. To a solution of aspirin (180 mg, 1 mmol) in anhydrous CH_2Cl_2 (2 mL), $SOCl_2$ (1 mL) was added, and the mixture was refluxed under nitrogen for 2h. The reaction solution was evaporated in vacuo to give corresponding crude intermediate **3**, which was used in the next without further purification.

Synthesis of intermediates 5a-f

Intermediates **5a-f** were prepared according to the method described previously [82-85] wtih minor modification. Triphenyl phosphine (PPh_3, 1 mmol) was dissolved in anhydrous THF (2 mL). To this mixture was added α-bromoacetophenones **4a-f** (1 mmol). The mixture was stirred at room temperature for 16h. The resulting white precipitate was collected by filtration, washed with *n*-hexane (3 × 20 mL). The white solid was dissolved in MeOH (5 mL). To this solution was added KOH (10 mmol) dissolved in H_2O (5 mL). The mixture was allowed to stir at room temperature (rt) for 12h. The MeOH was removed under reduced pressure and the crude reaction mixture extracted with DCM (3 × 20 mL). The combined organic layers were washed with water (3 × 5 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated in vacuo to give corresponding crude intermediate **5a-f**, which was used in the next without further purification.

Synthesis of 6a-p

Chalcones **6a-p** were prepared using a known synthetic method procedure.[85] A

View Article Online
DOI: 10.1039/C8MD00284C

mixture of a corresponding aldehyde (1.0 mmol) with intermediates **5a-f** (1.0 mmol) in anhydrous toluene (2.0 mL) was stirred at 60 ºC under nitrogen for 8 h. The solvent was evaporated in vacuo to give corresponding crude intermediate **6a-p**, which were directly used in the next without further purification. (data in Supplementary Materials)

General procedures for the preparation of 7a-p

A mixture of **6a-p** (1 mmol) and TEA (2 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at 0 ℃. Intermediate **3** (1 mmol) in anhydrous CH_2Cl_2 (2 mL) was sequentially added dropwise, and the mixture was stirred at room temperature for 12 h. The reaction solution was quenched by adding water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The resulting crude product was purified by flash chromatography on silica gel column to provide the compounds **7a-p** (53-88%), respectively.

(*E*)-2-(3-oxo-3-(p-tolyl)prop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7a**). The title compound was prepared according to the general procedure, as described above in 66% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 6.8 Hz, 1H), 8.09 (d, *J* = 15.6 Hz, 1H), 8.01-7.99 (m, 3H), 7.88 (t, *J* = 7.2, 7.6 Hz, 1H), 7.75-7.52 (m, 4H), 7.45-7.36 (m, 4H), 2.57 (s, 3H), 2.48 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.9, 169.7, 162.6, 151.4, 149.5, 143.6, 137.7, 135.3, 134.9, 132.2, 131.3, 129.3, 128.7, 128.5, 127.9, 126.6, 126.4, 124.6, 124.2, 123.4, 122.0, 21.7, 21.0; HRMS (ESI): calcd for $C_{25}H_{21}O_5$ $[M + H]^+$ 401.1389, found 401.1395.

(*E*)-2-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7b**). The title compound was prepared according to the general procedure, as described above in 77% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.30 (d, *J* = 8.0 Hz, 1H), 7.94-7.90 (m, 3H), 7.81 (d, *J* = 6.8 Hz, 1H), 7.69 (t, *J* = 8.0, 7.2 Hz, 1H), 7.57 (d, *J* = 16.0 Hz, 1H, CO<u>CH</u>=CH), 7.50-7.41 (m, 2H), 7.35 (6.8, 8.0 Hz, 1H), 7.28-7.23 (m, 2H), 6.88 (d, *J* = 8.0 Hz, 2H), 3.83 (s, 3H, Ar-OCH_3), 2.31 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.6, 169.7, 163.4, 162.7, 151.4, 149.4, 137.2, 135.0, 132.2,

View Article Online
DOI: 10.1039/C8MD00284C

131.2, 130.9, 130.7, 128.5, 128.0, 126.7, 126.4, 124.4, 124.3, 123.4, 122.1, 113.8, 55.4, 21.0; HRMS (ESI): calcd for $C_{25}H_{21}O_6$ $[M + H]^+$ 417.1338, found 417.1344.

(*E*)-2-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7c**). The title compound was prepared according to the general procedure, as described above in 80% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J* = 7.6 Hz, 1H), 8.20 (d, *J* = 15.6 Hz, 1H), 8.15-8.11 (m, 3H), 8.05 (t, *J* = 7.6, 8.0 Hz, 1H), 7.85-7.65 (m, 6H), 7.61-7.56 (m, 2H), 2.63 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.4, 169.6, 162.6, 151.4, 149.5, 139.1, 138.8, 136.1, 135.1, 132.2, 131.6, 130.0, 128.8, 128.7, 127.7, 126.7, 126.4, 124.3, 124.2, 123.4, 121.9, 21.0; HRMS (ESI): calcd for $C_{24}H_{18}ClO_5$ $[M + H]^+$ 421.0843, found 421.0849.

(*E*)-2-(3-(4-bromophenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7d**). The title compound was prepared according to the general procedure, as described above in 60% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.43 (d, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 16.0 Hz, 1H, COCH=CH), 7.95 (d, *J* = 7.6 Hz, 1H), 7.90-7.88 (m, 3H), 7.68-7.50 (m, 6H), 7.42 (t, *J* = 8.0 Hz, 2H), 2.46 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.7, 169.7, 162.6, 151.4, 149.5, 138.9, 136.5, 135.1, 132.2, 131.8, 131.6, 130.1, 128.7, 127.8, 127.6, 126.8, 126.4, 126.3, 124.3, 124.2, 123.4, 121.9, 21.0; HRMS (ESI): calcd for $C_{24}H_{18}BrO_5$ $[M + H]^+$ 465.0338, found 465.0345.

(*E*)-2-(3-(4-cyanophenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7e**). The title compound was prepared according to the general procedure, as described above in 53% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 7.6 Hz, 1H), 7.88-7.73 (m, 5H), 7.60 (d, *J* = 7.6 Hz, 2H), 7.52 (t, *J* = 8.0 Hz, 1H), 7.44-7.36 (m, 3H), 7.26 (d, *J* = 7.6 Hz, 2H), 2.29 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 190.0, 169.6, 162.5, 151.5, 149.6, 141.1,149.2, 135.3, 132.3, 132.1, 132.0, 129.4, 128.9, 128.8, 127.3, 126.9, 126.4, 124.4, 124.2, 123.5, 121.8, 118.0, 115.6, 21.0; HRMS (ESI): calcd for $C_{25}H_{18}NO_5$ $[M + H]^+$ 412.1185, found 412.1198.

(*E*)-2-(3-(4-nitrophenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7f**). The

View Article Online
DOI: 10.1039/C8MD00284C

title compound was prepared according to the general procedure, as described above in 67% yield. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.27-8.20 (m, 6H), 7.92 (d, *J* = 15.6 Hz, 1H, COCH=CH), 7.83 (t, *J* = 6.8, 8.0 Hz, 1H), 7.73 (d, *J* = 15.6 Hz, 1H, COCH=CH), 7.61 (t, *J* = 6.8, 8.0 Hz, 1H), 7.53 (t, *J* = 8.0, 6.8 Hz, 1H), 7.46 (t, *J* = 7.6, 7.2 Hz, 1H), 7.40-7.35 (m, 2H), 2.22 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 189.0, 169.6, 162.8, 151.2, 150.2, 149.9, 142.2, 138.3, 136.0, 132.8, 132.2, 130.2, 128.9, 127.4, 127.3, 127.1, 124.9, 124.6, 124.2, 123.9, 121.9, 21.1; HRMS (ESI): calcd for $C_{24}H_{18}NO_7$ $[M + H]^+$ 432.1083, found 432.1094.

(*E*)-2-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-5-methoxyphenyl 2-acetoxybenzoate (**7g**). The title compound was prepared according to the general procedure, as described above in 88% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 8.0 Hz, 1H), 7.96 (m, 5H), 7.56-7.35 (m, 5H), 7.02 (d, *J* = 8.0 Hz, 1H), 6.90 (s, 1H), 3.98 (s, 3H, Ar-OCH_3), 2.42 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.5, 169.7, 162.4, 151.5, 150.9, 138.9, 136.4, 135.2, 132.2, 129.9, 128.7, 126.4, 124.3, 121.8, 121.6, 120.1, 113.3, 108.6, 55.8, 21.0; HRMS (ESI): calcd for $C_{25}H_{20}ClO_6$ $[M + H]^+$ 451.0948, found 451.0955.

(*E*)-2-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-4-methoxyphenyl 2-acetoxybenzoate (**7h**). The title compound was prepared according to the general procedure, as described above in 78% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 8.0 Hz, 1H), 8.06-8.04 (m, 3H), 7.96 (t, *J* = 8.0 Hz, 1H), 7.70-7.66 (m, 2H), 7.59-7.57 (m, 2H), 7.50-7.48 (m, 2H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 4.13 (s, 3H, Ar-OCH_3), 2.55 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.5, 169.7, 163.0, 157.6, 151.4, 143.1, 139.1, 138.9, 136.0, 135.0, 132.1, 130.0, 128.8, 128.3, 126.4, 124.4, 124.3, 124.2, 122.0, 117.2, 112.9, 55.8, 21.0; HRMS (ESI): calcd for $C_{25}H_{20}ClO_6$ $[M + H]^+$ 451.0948, found 451.0953.

(*E*)-2-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-4-fluorophenyl 2-acetoxybenzoate (**7i**). The title compound was prepared according to the general procedure, as

View Article Online
DOI: 10.1039/C8MD00284C

described above in 59% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 7.2 Hz, 1H), 7.72-7.67 (m, 3H), 7.60 (t, *J* = 7.2, 8.0 Hz, 1H), 7.37-7.29 (m, 3H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.13-7.07 (m, 3H), 2.19 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.9, 169.6, 162.6, 160.4 (d, *J* = 245 Hz), 151.4, 145.4, 139.3, 137.4, 135.8, 135.2, 132.1, 130.4, 130.0, 129.3, 128.9, 126.4, 125.0, 124.9, 124.3, 121.7, 118.3 (d, *J* = 24.4 Hz), 114.4 (d, *J* = 24.4 Hz), 21.0; HRMS (ESI): calcd for $C_{24}H_{17}ClFO_5$ $[M + H]^+$ 439.0749, found 439.0757.

(*E*)-4-chloro-2-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7j**). The title compound was prepared according to the general procedure, as described above in 70% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.39 (d, *J* = 8.0 Hz, 1H), 7.99-7.84 (m, 5H), 7.63-7.55 (m, 3H), 7.50 (d, *J* = 7.6 Hz, 2H), 7.37 (t, *J* = 8.0, 7.6 Hz, 2H), 2.44 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.8, 169.6, 162.4, 151.5, 147.9, 139.4, 137.1, 135.8, 135.3, 132.2, 132.1, 131.2, 130.0, 129.3, 128.9, 128.0, 126.5, 125.0, 124.8, 124.3, 121.6, 21.0; HRMS (ESI): calcd for $C_{24}H_{17}Cl_2O_5$ $[M + H]^+$ 455.0453, found 455.0459.

(*E*)-4-bromo-2-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7k**). The title compound was prepared according to the general procedure, as described above in 83% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.0 Hz, 1H), 7.79 (s, 1H), 7.73-7.66 (m, 3H), 7.60 (t, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.37-7.29 (m, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 1H), 2.18 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.7, 169.6, 162.3, 151.5, 148.4, 139.4, 137.0, 135.8, 135.3, 134.2, 132.1, 131.0, 130.0, 129.7, 128.9, 126.5, 125.1, 125.0, 124.3, 121.6, 119.9, 21.0; HRMS (ESI): calcd for $C_{24}H_{17}BrClO_5$ $[M + H]^+$ 498.9948, found 498.9959.

(*E*)-2-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-6-methoxyphenyl 2-acetoxybenzoate (**7l**). The title compound was prepared according to the general procedure, as

View Article Online
DOI: 10.1039/C8MD00284C

described above in 65% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 7.2 Hz, 1H), 7.85-7.78 (m, 3H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 15.6 Hz, 1H, COCH=CH), 7.38 (t, *J* = 8.0, 7.2 Hz, 1H), 7.31-7.19 (m, 5H), 7.01 (d, *J* = 8.0 Hz, 1H), 3.78 (s, 3H, Ar-OCH_3), 2.25 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.3, 169.5, 161.9, 151.8, 151.4, 139.1, 139.0, 138.8, 136.1, 134.8, 132.4, 130.0, 128.8, 127.0, 126.3, 124.4, 124.3, 122.0, 119.8, 114.1, 56.1, 21.0; HRMS (ESI): calcd for $C_{25}H_{20}ClO_6$ $[M + H]^+$ 451.0948, found 451.0953.

(*E*)-2-(3-(4-chlorophenyl)-3-oxoprop-1-en-1-yl)-4-methylphenyl 2-acetoxybenzoate (**7m**). The title compound was prepared according to the general procedure, as described above in 75% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.36 (d, *J* = 7.6 Hz, 1H), 7.95-7.90 (m, 3H), 7.81 (t, *J* = 6.8, 8.0 Hz, 1H), 7.68 (s, 1H), 7.57-7.50 (m, 2H), 7.44-7.37 (m, 3H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 2.53 (s, 3H, Ar-CH_3), 2.39 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 189.5, 169.7, 162.8, 151.4, 147.4, 139.0, 136.5, 136.1, 135.0, 132.4, 132.2, 130.0, 129.0, 128.8, 127.2, 126.4, 124.3, 123.9, 123.1, 122.0, 21.0; HRMS (ESI): calcd for $C_{25}H_{20}ClO_5$ $[M + H]^+$ 435.0999, found 435.1006.

(*E*)-4-methoxy-2-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7n**). The title compound was prepared according to the general procedure, as described above in 79% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 16.0 Hz, 1H, COCH=CH), 7.30 (t, *J* = 7.6, 8.0 Hz, 1H), 7.13 (d, *J* = 16.0 Hz, 1H, COCH=CH), 7.03 (t, *J* = 7.6, 8.0 Hz, 1H), 6.89 (s, 1H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.63 (dd, *J* = 8.0 Hz, 1H), 6.49 (d, *J* = 8.0 Hz, 2H), 3.49 (s, 3H, Ar-CH_3), 3.45 (s, 3H, Ar-CH_3), 1.92 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 188.7, 169.7, 163.4, 163.1, 157.6, 151.3, 143.0, 137.3, 134.9, 132.2, 130.9, 130.6, 128.7, 126.4, 124.6, 124.2, 124.1, 122.1, 116.8, 113.8, 112.7, 55.7, 55.4, 21.0; HRMS (ESI): calcd for $C_{26}H_{23}O_7$ $[M + H]^+$ 447.1444, found 447.1453.

View Article Online
DOI: 10.1039/C8MD00284C

(*E*)-4-bromo-2-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7o**). The title compound was prepared according to the general procedure, as described above in 85% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.0 Hz, 1H), 7.92-7.90 (m, 3H), 7.78 (d, *J* = 15.6 Hz, 1H, COCH=CH), 7.65 (t, *J* = 7.6, 8.0 Hz, 1H), 7.53-7.49 (m, 2H), 7.38 (t, *J* = 6.8, 8.0 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 2H), 3.80 (s, 3H, Ar-CH_3), 2.27 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 187.9, 169.6, 163.6, 162.4, 151.4, 148.3, 135.5, 135.2, 133.8, 132.2, 130.9, 130.8, 130.4, 130.1, 126.4, 125.3, 125.1, 124.3, 121.7, 119.8, 113.8, 55.5, 21.0; HRMS (ESI): calcd for $C_{25}H_{20}BrO_6$ $[M + H]^+$ 495.0443, found 495.0449.

(*E*)-4-chloro-2-(3-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)phenyl 2-acetoxybenzoate (**7p**). The title compound was prepared according to the general procedure, as described above in 74% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 2H), 7.81-7.75 (m, 2H), 7.67 (t, *J* = 6.8, 8.0 Hz, 1H), 7.52 (d, *J* = 15.6, 1H, COCH=CH), 7.41-7.40 (m, 2H), 7.19 (t, *J* = 6.4, 8.0 Hz, 2H), 6.87 (d, *J* = 8.0 Hz, 2H), 3.83 (s, 3H, Ar-OCH_3), 2.27 (s, 3H, CH_3CO); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 183.3, 164.9, 158.8, 157.7, 146.7, 143.0, 130.9, 130.4, 127.4, 127.4, 126.2, 126.1, 125.7, 124.9, 123.1, 121.7, 120.6, 120.0, 119.5, 116.9, 109.1, 50.7, 16.2; HRMS (ESI): calcd for $C_{25}H_{20}ClO_6$ $[M + H]^+$ 451.0948, found 451.0956.

Biological assays

MTT assay

HCT-8, DLD-1, and CCD841 cell lines were purchased from American Tissue Culture Collection (ATCC, Rockville, MD, USA). HCT-8, DLD-1 and CCD841 cells were seeded in 96-well plates, then treated with vehicle alone or tested compounds for 72 h. Then 20 μL of MTT (5 mg/mL, in PBS) was added to each well and further incubated for another 4 h. The MTT formazan formed by viable cells was dissolved in DMSO (150 μL), and absorbance was measured using a microplate reader (570 nm).

View Article Online
DOI: 10.1039/C8MD00284C

Cell cycle analysis

HCT-8 cells were treated with **7h** at concentrations of 0, 2.5, 5 and 10 μM for 24 h, respectively. The cells were harvested, fixed with 70% ethanol for 2 h, and incubated with PI/RNase staining buffer for 15 min at room temperature. The DNA content in the different groups of cells was assessed by flow cytometry.

Apoptosis Analysis

HCT-8 cells were incubated in six-well plates (1×10^5/well) and treated with DMSO (1%), and compound **7h** at concentrations of 2.5, 5 and 10 μM for 24 h, respectively. The cells were collected, washed with PBS, and stained with FITC-Annexin-V and PI. Apoptosis was determined by flow cytometry.

Western Blotting

The assay was performed according to the method described previously wtih minor modification.[86] HCT-8 cells were incubated in six-well plates (1×10^6/well) overnight and treated with vehicle DMSO (0.1%, v/v) alone or **7h** for 24 h. The cells were harvested and lysed at 4 °C for 30 min in a lysis buffer. The cell lysates were centrifuged at 15000 rpm for 15 min at 4 °C, and the supernatants were collected. The protein concentration in the cell lysates was determined using the bicinchoninic acid protein assay kit. Protein samples were separated by SDS-polyacrylamide gel electrophoresis (7.5% gel, 20 μg per lane) and then transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with skim milk (5%) in Tris-buffered saline containing 0.05% Tween 20 and sequentially incubated with primary antibodies [anti-caspase 3, anticleaved caspase 3, anti-PARP, anticleaved PARP, anti-GAPDH (Cell Signaling, Boston, MA)] and followed by enhanced chemiluminescence detection.

Determination of ROS generation

The assay was performed according to the method described previously wtih minor modification.[87] HCT-8 cells treated with different concentrations of **7h** (0, 2.5,

View Article Online
DOI: 10.1039/C8MD00284C

5, 10 μM) for 24 h. After being loaded with 10 μM DCFH-DA at 37 C for 20 min and washed three times with PBS buffer to remove excess dye. The fluorescence intensity, used as a fluorescent indicator of intracellular ROS, was recorded in a plate reader and measured with the flow cytometer with an excitation/emission (Ex/Em) frequency of 488/530 nm.

In vivo tumor growth inhibition experiment

The assay was performed according to the method described previously wtih minor modification.[88] Individual male BALB/c nude mice (5-6 weeks, 18-20 g, Experimental Animal Research Center, Chinese Academy of Military Medical Sciences) were inoculated subcutaneously with HCT-8 cells (1×10^7/mouse, American Tissue Culture Collection, ATCC, Rockville, MD, USA). After the solid tumors were established and allowed to reach 180-200 mm^3, the mice were randomized and treated intraperitoneally with **7h** (60 mg/kg) or the same volume of vehicle consisting of Tween 80/PBS (5:95) daily for 32 consecutive days. The tumor growth was recorded every 4 days from the measurement of length and width using a vernier caliper and calculated as tumor volumes (TV) with the following formula: TV (mm^3) = $width^2$ (length/2). The tumor growth inhibition rate (weight per unit weight, w/w, %) was calculated as described previously.[88] The experimental protocols were evaluated and approved by the Ethics Committee of the Hubei University of Chinese Medicine. All animal experiments were complied with the International Guiding Principles for Animals Research (A CIOMS Ethical Code for Animal Experimentation, WHO Chronicle, 39 (2): 51-56, 1985).

Conflicts of Interest

There are no conflicts to declare.

Acknowledgments

This work was supported by grants from Qing Miao Plan of Hubei University of Chinese Medicine (2016ZZX013).

View Article Online
DOI: 10.1039/C8MD00284C

References

1 R. L. Siegel, K. D. Miller and A. Jemal, *CA Cancer J. Clin.*, 2018, **68**, 7-30.

2 M. Arnold, M. S. Sierra, M. Laversanne, I. Soerjomataram, A. Jemal and F. Bray, *Gut*, 2016, **66**, 683-691.

3 K. Jin, H. Lan, F. Cao, N. Han, Z. Xu, G. Li, K. He and L. Teng, *Int. J. Oncol.*, 2012, **41**, 583-588.

4 D. B. Longley, D. P. Harkin and P. G. Johnston, *Nat. Rev. Cancer.*, 2003, **3**, 330-338.

5 B. R. Hirsch and S. Y. Zafar, *Cancer Manag. Res.*, 2011, **3**, 79-89.

6 K. Fujita, Y. Kubota, H. Ishida and Y. Sasaki, *World J. Gastroenterol.*, 2015, **21**, 12234-12248.

7 P. Comella, R. Casaretti, C. Sandomenico, A. Avallone and L. Franco, *Ther. Clin. Risk Manag.*, 2009, **5**, 229-238.

8 T. Kish and P. Uppal, *P T.*, 2016, **41**, 314-325.

9 C. Sostres, C. J. Gargallo and A. Lanas, *World J. Gastrointest. Pharmacol. Ther.*, 2014, **5**, 40-49.

10 G. J. Tsioulias, M. F. Go and B. Rigas, *Curr. Pharmacol. Rep.*, 2015, **1**, 295-301.

11 P. C. Elwood, A. M. Gallagher, G. G. Duthie, L. A. Mur and G. Morgan, *Lancet*, 2009, **373**, 1301-1309.

12 M. A. Thorat and J. Cuzick, Curr. *Oncol. Rep.*, 2013, **15**, 533-540.

13 M. J. Thun, E. J. Jacobs and C. Patrono, *Nat. Rev. Clin. Oncol.*, 2012, **9**, 259-267.

14 J. Burn, A. M. Gerdes, F. Macrae, J. P. Mecklin, G. Moeslein, S. Olschwang, D. Eccles, D. G. Evans, E. R. Maher, L. Bertario, M. L. Bisgaard, M. G. Dunlop, J. W. Ho, S. V. Hodgson, A. Lindblom, J. Lubinski, P. J. Morrison, V. Murday, R. Ramesar, L. Side, R. J. Scott, H. J. Thomas, H. F. Vasen, G. Barker, G. Crawford, F. Elliott, M. Movahedi, K. Pylvanainen, J. T. Wijnen, R. Fodde, H. T. Lynch, J. C. Mathers and D. T. Bishop, CAPP2 Investigators. *Lancet*, 378 (2011) 2081-2087.

15 M. Chattopadhyay, R. Kodela, N. Nath, A. Barsegian, D. Boring and K. Kashfi, *Biochem. Pharmacol.*, 2012, **83**, 723-732.

View Article Online
DOI: 10.1039/C8MD00284C

16 M. Chattopadhyay, R. Kodela, N. Nath, Y. M. Dastagirzada, C. A. Velazquez-Martinez, D. Boring and K. Kashfi, *Biochem. Pharmacol.*, 2012, **83**, 715-722.

17 M. Chattopadhyay, R. Kodela, N. Nath, C. R. Street, C. A. Velazquez-Martinez, D. Boring and K. Kashfi, *Biochem. Pharmacol.*, 2012, **83**, 733-740.

18 D. Basudhar, G. Bharadwaj, R. Y. Cheng, S. Jain, S. Shi, J. L. Heinecke, R. J. Holland, L. A. Ridnour, V. M. Caceres, R. C. SpadariBratfisch, N. Paolocci, C. A. Velazquez-Martinez, D. A. Wink and K. M. Miranda, *J. Med. Chem.*, 2013, **56**, 7804-7820.

19 V. Kozoni, T. Rosenberg and B. Rigas, *Acta Pharmacol. Sin.*, 2007, **28**, 1429-1433.

20 C. V. Rao, B. S. Reddy, V. E. Steele, C. X. Wang, X. Liu, N. Ouyang, J. M. Patlolla, B. Simi, L. Kopelovich and B. Rigas, *Mol. Cancer Ther.*, 2006, **5**, 1530-1538.

21 J. L. Williams, S. Borgo, I. Hasan, E. Castillo, F. Traganos and B. Rigas, *Cancer Res.*, 2001, **61**, 3285-3289.

22 J. L. Williams, N. Nath, J. Chen, T. R. Hundley, J. Gao, L. Kopelovich, K. Kashfi and B. Rigas, *Cancer Res.*, 2003, **63**, 7613-7618.

23 M. Chattopadhyay, R. Kodela, K. R. Olson and K. Kashfi, *Biochem. Biophys. Res. Commun.*, 2012, **419**, 523-528.

24 R. Kodela, M. Chattopadhyay and K. Kashfi, *ACS Med. Chem. Lett.*, 2012, **3**, 257-262.

25 L. Huang, G. G. Mackenzie, Y. Sun, N. Ouyang, G. Xie, K. Vrankova, D. Komninou and B. Rigas, *Cancer Res.*, 2011, **71**, 7617-7627.

26 Y. Zhu, F. Wang, Y. Zhao, P. Wang and S. Sang, *Sci. Rep.*, 2017, **7**, 40119.

27 Y. Zhu, J. Fu, K. L. Shurlknight, D. N. Soroka, Y. Hu, X. Chen and S. Sang, *J. Med. Chem.*, 2015, **58**, 6494-6506.

28 D. Plano, D. N. Karelia, M. K. Pandey, J. E. Spallholz, S. Amin and A. K. Sharma, *J. Med. Chem.*, 2016, **59**, 1946-1959.

29 Q. Cheng, H. Shi, H. Wang, Y. Min, J. Wang and Y. Liu, *Chem. Commun.*, 2014, **50**, 7427-7430.

30 Q. Cheng, H. Shi, H. Wang, J. Wang and Y. Liu, *Metallomics*, 2016, **8**, 672-678.

View Article Online
DOI: 10.1039/C8MD00284C

31 D. G. I. Kingston, *J. Nat. Prod.*, 2011, **74**, 496-511.

32 F. E. Koehn and G. T. Carter, *Nat. Rev. Drug Discov.*, 2005, **4**, 206-220.

33 A. Rejhová, A. Opattová, A. Čumová, D. Slíva and P. Vodička, *Eur. J. Med. Chem.*, 2018, **144**, 582-594.

34 B. B. Mishra and V. K. Tiwari, *Eur. J. Med. Chem.*, 2011, **46**, 4769-807.

35 B. Zhang, D. Duan, C. Ge, J. Yao, Y. Liu, X. Li and J. Fang, *J. Med. Chem.*, 2015, **58**, 1795-1805.

36 H. L. Ng, X. Ma, E. H. Chew and W. K. Chui, *J. Med. Chem.*, 2017, **60**, 1734-1745.

37 H. L. Ng, S. Chen, E. H. Chew and W. K. Chui, *Eur. J. Med. Chem.*, 2016, **115**, 63-74.

38 F. F. Gan, K. K. Kaminska, H. Yang, C. Y. Liew, P. C. Leow, C. L. So, L. N. Tu, A. Roy, C. W. Yap, T. S. Kang, W. K. Chui and E. H. Chew, *Antioxid. Redox Signal.*, 2013, **19**, 1149-1165.

39 F. F. Gan, R. Zhang, H. L. Ng, M. Karuppasamy, W. Seah, W. H. Yeap, S. M. Ong, E. Hadadi, S. C. Wong, W. K. Chui and E. H. Chew, *Food Chem. Toxicol.*, 2018, **116**, 238-248.

40 Y. Zuo, Y. Yu, S. Wang, W. Shao, B. Zhou, L. Lin, Z. Luo, R. Huang, J. Du and X. Bu, *Eur. J. Med. Chem.*, 2012, **50**, 393-404.

41 B. Orlikova, D, Tasdemir, F. Golais, M. Dicato and M, Diederich, *Biochem. Pharmacol.*, 2011, **82**, 620-631.

42 B. Srinivasan, T. E. Johnson, R. Lad and C. Xing, *J. Med. Chem.*, 2009, **52**, 7228-7235.

43 B. Orlikova, M. Schnekenburger, M. Zloh, F. Golais, M. Diederich and D. Tasdemir, *Oncol. Reports*, 2012, **28**, 797-805.

44 K. B. Harikumar, A. B. Kunnumakkara, K. S. Ahn, P. Anand, S. Krishnan, S. Guha and B. B. Aggarwal, *Blood*, 2009, **113**, 2003-2013.

45 M. V. B. Reddy, Y. Shen, J. Yang, T. Hwang, K. F. Bastow, K. Qian, K. Lee and T. Wu, *Bioorg. Med. Chem.*, 2011, **19**, 1895-1906.

46 M. K. Pandey, S. K. Sandur, B. Sung, G. Sethi, A. B. Kunnumakkara and B. B.

View Article Online
DOI: 10.1039/C8MD00284C

Aggarwal, *J. Biol. Chem.*, 2007, **282**, 17340-17350.

47 V. R. Yadav, S. Prasad, B. Sung and B. B. Aggarwal, *Int. Immunopharmacol.*, 2011, **11**, 295-309.

48 M. T. Konieczny, A. Bułakowska, D. Pirska, W. Konieczny, A. Skladanowski, M. Sabisz, M. Wojciechowski, K. Lemke, A. Pieczykolan and W. Strozek, *Eur. J. Med. Chem.*, 2015, **89**, 733-742.

49 M. D. Vitorović-Todorović, A. Erić-Nikolić, B. Kolundžija, E. Hamel, S. Ristić, I. O. Juranić and B. J. Drakulić, *Eur. J. Med. Chem.*, 2013, **62**, 40-50.

50 L. B. Salum, W. F. Altei, L. D. Chiaradia, M. N. S. Cordeiro, R. R. Canevarolo, C. P. S. Melo, E. Winter, B. Mattei, H. N. Daghestani, M. C. Santos-Silva, T. B. Creczynski-Pasa, R. A. Yunes, J. A. Yunes, A. D. Andricopulo, B. W. Day, R. J. Nunes and A. Vogt, *Eur. J. Med. Chem.*, 2013, **63**, 501-510.

51 G. Wang, C. Li, L. He, K. Lei, F. Wang, Y. Pu, Z. Yang, D. Cao, L. Ma, J. Chen, Y. Sang, X. Liang, M. Xiang, A. Peng, Y. Wei and L. Chen, *Bioorg. Med. Chem.*, 2014, **22**, 2060-2079.

52 S. Ducki, G. Mackenzie, B. Greedy, S. Armitage, J. F. D. Chabert, E. Bennett, J. Nettles, J. P. Snyder and N. J. Lawrence, *Bioorg. Med. Chem.*, 2009, **17**, 7711-7722.

53 Y. Kong, K. Wang, M. C. Edler, E. Hamel, S. L. Mooberry, M. A. Paige and M. L. Brown, *Bioorg. Med. Chem.*, 2010, **18**, 971-977.

54 B. F. Ruan, X. Lu, J. F. Tang, Y. Wei, X. L. Wang, Y. B. Zhang, L. S. Wang and H. L. Zhu, *Bioorg. Med. Chem.*, 2011, **19**, 2688-2695.

55 O. Bueno, G. Tobajas, E. Quesada, J. Estévez-Gallego, S. Noppen, M. J. Camarasa, J. F. Díaz, S. Liekens, E. M. Priego and M. J. Pérez-Pérez, *Eur. J. Med. Chem.*, 2018, **148**, 337-348.

56 X. Huang, R. Huang, Z. Wang, L. Li, S. Gou, Z. Liao and H. Wang, *Eur. J. Med. Chem.*, 2018, **146**, 435-450.

57 X. Huang, R. Huang, L. Li, S. Gou and H. Wang, *Eur. J. Med. Chem.*, 2017, **132**, 11-25.

58 S. Sharma, C. Kaur, A. Budhiraja, K. Nepali, M. K. Gupta, A. K. Saxena and P. M.

View Article Online
DOI: 10.1039/C8MD00284C

Bedi, *Eur. J. Med. Chem.*, 2014, **85**, 648-660.

59 D. K. Mahapatra, S. K. Bharti and V. Asati, *Eur. J. Med. Chem.*, 2015, **98**, 69-114.

60 P. Singh, A. Anand and V. Kumar. *Eur. J. Med. Chem.*, 2014, **85**, 758-777.

61 S. Fortin, B. Bouchon, C. Chambon, J. Lacroix, E. Moreau, J. M. Chezal, F. Degoul and R. C-Gaudreault, *J. Pharmacol. Exp. Ther.*, 2011, **336**, 460-467.

62 B. Bouchon, C. Chambon, E. Mounetou, J. Papon, E. Miot-Noirault, R. C. Gaudreault, J. C. Madelmont and F. Degoul, *Mol. Pharmacol.*, 2005, **68**, 1415-1422.

63 C. Zhuang, W. Zhang, C. Sheng, W. Zhang, C. Xing and Z. Miao, *Chem. Rev.*, 2017, **117**, 7762-7810.

64 G. Loch-Neckel, M. A. Bicca, P. C. Leal, A. Mascarello, J. M. Siqueira and J. B. Calixto, *Eur. J. Med. Chem.*, 2015, **90**, 93-100.

65 T. R. Mielcke, A. Mascarello, E. Filippi-Chiela, R. F. Zanin, G. Lenz, P. C. Leal, L. D. Chiaradia, R. A. Yunes, R. J. Nunes, A. M. Battastini, F. B. Morrone and M. M. Campos, *Eur. J. Med. Chem.*, 2012, **48**, 255-264.

66 R. Gaur, A. S. Pathania, F. A. Malik, R. S. Bhakuni and R. K. Verma, *Eur. J. Med. Chem.*, 2016, **122**, 232-246.

67 V. Marković, N. Debeljak, T. Stanojković, B. Kolundžija, D. Sladić, M. Vujčić, B. Janović, N. Tanić, M. Perović, V. Tešić, J. Antić and M. D. Joksović, *Eur. J. Med. Chem.*, 2015, **89**, 401-410.

68 A. Kamal, S. Prabhakar, M. Janaki Ramaiah, P. Venkat Reddy, Ch. Ratna Reddy, A. Mallareddy, N. Shankaraiah, T. Lakshmi Narayan Reddy, S. N. Pushpavalli and M. Pal-Bhadra, *Eur. J. Med. Chem.*, 2011, **46**, 3820-3831.

69 R. Romagnoli, P. G. Baraldi, M. D. Carrion, O. Cruz-Lopez, C. L. Cara, J. Balzarini, E. Hamel, A. Canella, E. Fabbri, R. Gambari, G. Basso and G. Viola, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 2022-2028.

70 R. Pingaew, A. Saekee, P. Mandi, C. Nantasenamat, S. Prachayasittikul, S. Ruchirawat and V. Prachayasittikul, *Eur. J. Med. Chem.*, 2014, **85**, 65-76.

71 S. Gupta, P. Maurya, A. Upadhyay, P. Kushwaha, S. Krishna, M. I. Siddiqi, K. V. Sashidhara and D. Banerjee, *Eur. J. Med. Chem.*, 2018, **143**, 1981-1996.

View Article Online
DOI: 10.1039/C8MD00284C

72 S. Park, E. H. Kim, J. Kim, S. H. Kim and I. Kim, *Eur. J. Med. Chem.*, 2018, **144**, 435-443.

73 C. Bagul, G. K. Rao, V. K. K. Makani, J. R. Tamboli, M. Pal-Bhadra, and A. Kamal, M*ed. Chem. Commun*., 2017, **8**, 1810-1816.

74 M. Cabrera, H. Cerecetto, and M. González, *Med. Chem. Commun*., 2016, **7**, 2395-2409.

75 D.J. Fu, S.Y. Zhang, Y.C. Liu, X.X. Yue, J.J. Liu, J. Song, R.H. Zhao, F. Li, H.H. Sun, Y.B. Zhang, and H.M. Liu, *Med. Chem. Commun*., 2016, **7**, 1664-1671.

76 J. Hu, J. Yan, J. Chen, Y. Pang, L. Huang, and X. Li, *Med. Chem. Commun*., 2015, **6**, 1318-1327.

77 G. A. M. Jardim, T. T. Guimarães, M. do C. F. R. Pinto, B. C. Cavalcanti, K. M. de Farias, C. Pessoa, C. C. Gatto, D. K. Nair, I. N. N. Namboothiri, and E. N. da Silva Júnior, *Med. Chem. Commun*., 2015, **6**, 120-130.

78 A. H. Banday, V. V. Kulkarni, and V. J. Hruby, *Med. Chem. Commun.*, 2015, **6**, 94-104.

79 D. Coskun, M. Erkisa, E. Ulukaya, M. F. Coskun and F. Ari, *Eur. J. Med. Chem.*, 2017, **136**, 212-222.

80 L. Raj, T. Ide, A. U. Gurkar, M. Foley, M. Schenone, X. Li, N. J. Tolliday, T. R. Golub, S. A. Carr, A. F. Shamji, A. M. Stern, A. Mandinova, S. L. Schreiber and S. W. Lee, *Nature*, 2011, **475**, 231-234.

81 I. H. Eissa, H. Mohammad, O. A. Qassem, W. Younis, T. M. Abdelghany, A. Elshafeey, M. M. Abd Rabo Moustafa, M. N. Seleem and A. S. Mayhoub, *Eur. J. Med. Chem.*, 2017, **130**, 73-85.

82 H. Kuroda, E. Hanaki, H. Izawa, M. Kano and H. Itahashi, *Tetrahedron*, 2004, **60**, 1913-1920.

View Article Online
DOI: 10.1039/C8MD00284C

83 S. Lopes, C. M. Nunes, A. Gomez-Zavaglia, T. M. V. D. Pinho e Melo and R. Fausto, *Tetrahedron*, 2011, **67**, 7794-7804.

84 E. Venkateswararao, M. S. Kim, V. K. Sharma, K. C. Lee, S. Subramanian, E. Roh, Y. Kim and S. H. Jung, *Eur. J. Med. Chem.*, 2013, **59**, 31-38.

85 A. Cullen, A. J. Mullera and D. B. G. Williams, RSC Adv. 2017, **7**, 42168-42171.

86 Y. Ai, B. Zhu, C. Ren, F. Kang, J. Li, Z Huang, Y. Lai, S. Peng, K. Ding, J. Tian and Y. Zhang, *J. Med. Chem.*, 2016, **59**, 1747-1760.

87 F. Kang, Y. Ai, Y. Zhang and Z. Huang, *Eur. J. Med. Chem.* 2018, **149**, 269-280.

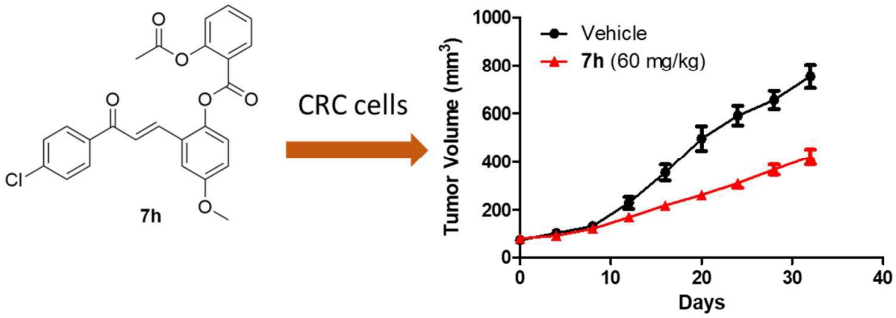
88 Y. Ai, Y. Hu, F. Kang, Y. Lai, Y. Jia, Z. Huang, S. Peng, H. Ji, J. Tian and Y. Zhang, *J. Med. Chem.*, 2015, **58**, 4506-4520.

View Article Online
DOI: 10.1039/C8MD00284C

Table of contents entry

Novel hybrids derived from aspirin and chalcones potently suppress colorectal cancer in vitro and in vivo

Shan Lu, [a, *] Obinna N Obianom, [b] and Yong Ai [b, **]



A novel group of hybrids derived from aspirin and chalcones were designed and synthesized. The most active compound **7h** had potent and selective anti-proliferative activity against CRC cells *in vitro*. **7h** induced CRC cell cycle arrest at G1 phase and apoptosis in vitro, increased the relative levels of cleaved caspase 3 and PARP as well as the ROS in HCT-8 cells. **7h** significantly inhibited the growth of implanted CRC cancer in mice.