MedChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: Z. Wang, X. Deng, R. Xiong, S. Xiong, J. Liu, X. Cao, X. Lei, Y. Chen, G. Tang and X. Zheng, *Med. Chem. Commun.*, 2017, DOI: 10.1039/C7MD00578D.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/medchemcomm

View Article Online
DOI: 10.1039/C7MD00578D

Design, synthesis and biological evaluation of 3',4',5'-trimethoxy flavonoid benzimidazole derivatives as potential anti-tumor agents

Zhe Wang[a,1], Xiangping Deng[a,1], Runde Xiong[a], Shujuan Xiong[a], Juan Liu[a], Xuan Cao[a], Xiaoyong Lei[a], Yanming Chen[b], Xing Zheng [a,]*, Guotao Tang[a,]*

[a]Institute of Pharmacy and Pharmacology, Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study, University of South China, Hengyang, China

[b]Mu Dan Jiang You Bo Pharmacertical Co.Ltd, Mudanjiang, China

*Corresponding Author: Dr. Guotao Tang, Ph.D. Dr .Xing Zheng, Ph.D

Corresponding author at：

Guotao Tang, Xing Zheng, Institute of Pharmacy and Pharmacology, University of South China, Hengyang, Hunan, 421001,People's Republic of China.

E-mail adress: tgtzq@163.com (Guotao Tang), 2393628359@qq.com (Xing Zheng)

[1] These authors contributed equally to this work.

View Article Online
DOI: 10.1039/C7MD00578D

Abstract

A series of 3',4',5'-trimethoxy flavonoids with benzimidazole linked by different chain alkanes have been designed and synthesized. The potential activity of these compounds as anti-tumor agents was evaluated by cytotoxicity assay in MGC-803 (Human gastric cancer cell line), MCF-7 (Human breast cancer cell line), HepG-2 (Human hepatoma cell line) and MFC (Mouse gastric cancer cell line) tumor cell lines. Among them, compound **15** 7-(3-(2-chloro-1H-benzo[d]imidazol-1-yl)propoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one displayed the most potent antiproliferative activity with IC_{50} values of 20.47 ± 2.07, 43.42 ± 3.56, 35.45 ± 2.03 μM and 23.47 ± 3.59 μM, respectively. The flow cytometry (FCM) results showed that the compound **15** caused the cell cycle to be arrested in G1 phase and induced apoptosis of MFC cells in a dose dependent manner. In addition, compound **15** exhibited a significant inhibitory effect on tumor growth in vivo. All the results outlined the great potential of compound **15** for further exploitation as anti-tumor agent.

Keywords: Flavonoids; benzimidazoles; synthesis; anti-tumor

1. Introduction

Tumor is one of the most major threats to human life in the world. There is an urgent need for seeking safer and more effective chemotherapy drugs which will improve the survival rates of various types of tumor patients. It is well known that many anti-tumor drugs are derived from the structural of natural products at present [1]. Flavonoid is a class of natural products that are widely found in nature and exhibit a wide range of biological activities [2-5], and they tend to be used as dominant parental structure in drug discovery [6]. Over the past decade, a large number of natural, semi-synthetic, synthetic flavonoids have been exploited and evaluated in a variety of therapeutic activities, such as anti-inflammatory, anti-bacterial [7], anti-allergy, anti-oxidation [8], anti-tumor [9, 10]. Particularly, their potential therapeutic effects and reliable safety in the tumors treatment have received extensive attention [11-13].

Benzene trimethoxy has special value in the design of antineoplastic drugs, with particular concern in the design of vascular blockers. At present, the vascular disrupting agents in the clinical research stage are mainly targeted at the tubulin to inhibit the formation of microtubules, such as CAP4 and its analogues OXi4503, AVE8062 [14-17], colchicine analogs ZD6126 [18], BNC-105 [19] and CKD-516 [20] (Fig. 1). Interestingly, these compounds contain benzene trimethoxy. In addition, we also note that benzimidazoles have been widely used in the design and development of anticancer drugs [21-25], such as some benzimidazoles and their derivatives exhibited significant anti-tumor effect by inhibiting tumor cell mitosis and proliferation, mediating tumor cell apoptosis or inhibiting the expression of hypoxia inducible factor (HIF) [26-28].

View Article Online
DOI: 10.1039/C7MD00578D

In this study, we attempted to introduce the trimethoxy group into the B-ring of flavonoid and link benzimidazole and its derivatives to 7-OH with different chain alkanes. We are eager to discover new flavonoid derivatives with potential anti-tumor activity. In this study, we synthesized a series of 3',4',5'-trimethoxy flavonoid benzimidazole derivatives **5-28**, and described the anti-tumor activity screening results of these compounds. We successfully screened the compound **15** with notable anti-tumor activity in vitro, and further demonstrated in vivo. The inhibitory effect of compound **15** on expression of tubulin or hypoxia inducible factor (HIF) requires further investigation.

2. Results and discussion

2.1. Chemistry

In this study, we synthesized twenty four new 3',4',5'-trimethoxy flavonoid benzimidazole derivatives. The synthetic route was shown in Scheme 1. The key intermediate **3** was prepared in a three-step synthesis starting with resorcinol, 2-chloroacetonitrile and 3,4,5-trimethoxybenzaldehyde according to a literature procedure [29]. Houben-Hoesch reaction of resorcinol under the catalysis of zinc chloride in ether at 0 ℃ to provide 4-(2-chloro-1-iminoethyl)benzene-1,3-diol hydrochloride (1) and then be turned into 2-chloro-1-(2,4-dihydroxyphenyl)ethanone (2) by hydrolization. Base-catalyzed aldol condensation of **2** with 3, 4, 5-trimethoxybenzaldehyde afforded the chalcone. The chalone was cyclized in the solvent of 10% hydrochloric acid and ethanol at room temperature to provide the flavonoid **3** in high yield with recrystallization from 95% ethanol.

New analogues **4a-4c** were conveniently synthesized in high yields (83-92%) by etherification reaction of the key intermediate **3** with different chain length substituted alcohols in the presence of K_2CO_3 in acetone. Alkylation of the bromide intermediates **4a-4c** and corresponding benzimidazoles in the presence of K_2CO_3 in acetone introduced basic functionalities providing final compounds **5-28** in 50-80% yields. All of the synthetic compounds gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures.

2.2. Biological activity

2.2.1. In vitro antiproliferative assay

The in vitro antiproliferative activity of 3',4',5'-trimethoxy flavonoid benzimidazole derivatives **5-28** and the positive control 5-Fu on MGC-803, MCF-7, HepG-2 and MFC cells was studied by MTT colorimetric assay. The compounds were tested in a concentration range of 2 to 256 μM and the calculated IC_{50} values were reported in Table 1.

In general, some of the synthesized novel flavonoid derivatives exhibited excellent antiproliferative activity.

View Article Online
DOI: 10.1039/C7MD00578D

Among them, compounds with substitutions on the R_1-position of benzimidazole derivatives showed better antiproliferative activity than the R_2- and R_3- position ones. For the R_1- position substituted ones, replacement of H atom at R_1- position by electron-withdrawing groups (**7, 11, 15, 19, 23, 27**) resulted in the improving of their antiproliferative activity compared to the substitutions of electron-donation groups (**6, 14, 22**). For compounds **8, 16, 24** introduced nitro on the R_3- position, the antiproliferative activity was also decreasing compared to the electron-donation groups on R_3- position. Besides, since the number of carbon atoms on the three linkages is similar, the difference in antiproliferative activity of the three chain length derivatives is not significant.

It is clear that the synthesized compounds with chlorine atom substitutions in R_1-position on the benzimidazole derivatives (**7, 15, 23**) showed higher activity than others, Specially, compound **15** displayed the most potent inhibitory activity with IC_{50} of 20.47 ± 2.07 μM and 23.47 ± 3.59 μM on MGC-803 cells and MFC cells, respectively, which was better that of 5-Fu (IC_{50} = 74.39 ± 2.03 and 78.52 ± 3.92, respectively). Therefore, the IC_{50} value suggested that compound **15** may be a promising lead for the further development of novel anti-tumor agents, and we chose the MFC cell lines to carry out cell assay, apoptosis assay and anti-tumor efficacy assay in vivo.

In addition, it is worth noting that the three benzimidazole derivatives (6-nitrobenzimdazole, 5-methylbenzimidazole and 5-methoxybenzimidazole) we selected will produce isomers during the last step of the reaction [30]. Since we did not find any significant anti-tumor activity advantage among them during the previous active screening, we did not further disassemble the isomers of these compounds (compound 8,10,12,16,18,20,24,26,28). However, from the point of view of drug molecule synthesis design, we believe that isomers of the active compounds obtained by alkylation with 4-substituted or 5-substituted benzimidazoles may have to be isolated and purified prior to studying their further biological activity.

2.2.2. Apoptosis assay

Since compound **15** exhibited excellent activity against cell growth in MGC-803 cells and MFC cells, we plan to use MFC cells to establish mouse tumor models for preliminary study of the anti-tumor effect in vivo of the candidate compounds. Prior to this, in order to further verify the antitumor activity of candidate compounds in vitro and to explore its mechanism of action, MFC cells were also selected for apoptosis study. Induction of apoptosis is an effective anti-tumor strategy. The FCM assay determined that compound 15 could induce the apoptosis of MFC cells in a dose dependent manner. As shown in Fig.2, MFC cells were treated with 10, 20, 40 μM of compound **15** for 24 h. The compound increased the percentage of apoptosis by Annexin V-FITC/PI staining in a dose-dependent manner. The result indicated that compound **15** induced apoptosis of MFC cells.

View Article Online
DOI: 10.1039/C7MD00578D

2.2.3. Cell cycle assay

Cell cycle regulation is a necessary process to maintain cell proliferation and development. However, the main feature of tumor cells is cell cycle regulation disorders leading to cell differentiation and apoptosis block, cell proliferation out of control [31]. We also chose MFC cell lines for cell cycle experiments. Studies were performed by flow cytometry analysis to determine the effects of the compound **15** on cell cycle progression. As shown from the cell cycle detection (Fig. 3), there was significant up-regulation of G1 phase in dose dependent manner after treatment with three different concentrations of compound **15**. Most of the tumor cells are often shortened in the G1 phase due to the abnormal expression of Cyclin, CDK (Cyclin-dependent kinase) and CKI (cyclin-dependent kinase inhibitor), thus rapidly entering the S phase and leading to excessive proliferation [32]. Thus, the experimental results showed that compound **15** could effectively upregulate G1 phase, thereby inducing apoptosis of tumor cells. However, which regulatory molecule was affected by the compound is not clear, further study is currently in progress.

2.2.4. In vivo anti-tumor efficacy

Gastric carcinoma was chosen as a model cancer to investigate the anti-tumor efficacy of the three different doses of compound **15**. The anti-tumor growth effect in vivo was evaluated by measuring tumor volume following treatment with different doses of compound **15** (10 mg/kg, 20 mg/kg, 40 mg/kg), 5-Fu (25 mg/kg) and saline. As illustrated in Fig. 4, different doses of compound **15** and the positive control 5-Fu inhibited the tumor growth remarkably as compared with the saline. The tumor growth inhibition of compound **15** was dose-independent. The middle-dose group and high-dose group exhibited stronger tumor grouth inhibition with TIR of 67.8% and 80.2%, respectively, compared with the 5-Fu (TIR of 60.8%) (Fig.5). Fig. 6 illustrated the growth in body weight during the 18 day experimental period. The gradual increase in body weight among all of the treat groups was consistent with that of the control group, which represented the natural growth in body weight of tumor-bearing mice. The animal experiment in vivo showed that the compound **15** was associated with stronger efficiency in suppressing tumor growth compared with 5-Fu and exhibited less toxic side effects. Based on the results of preliminary pharmacodynamic studies in mice above, we will conduct pharmacodynamic studies in nude mice in order to further validate the anti-tumor activity of this compound.

3. Conclusions

In summary, a series of 3',4',5'-trimethoxy flavonoid benzimidazole derivatives were firstly synthesized and evaluated for their antiproliferative activities against MGC-803, MCF-7, HepG-2 and MFC tumor cell lines. It is worth noting that compound **15** displayed the most outstanding antiproliferative activities in vitro with IC_{50} of

View Article Online
DOI: 10.1039/C7MD00578D

20.47 ± 2.07, 43.42 ± 3.56, 35.45 ± 2.03 μM and 23.47 ± 3.59 μM, respectively. In addition, the preliminary mechanism of compound **15** inhibition effects was detected by flow cytometry, and the compound exert antiproliferative activity via inducing the apoptosis of tumor cells in a dose dependent manner. Flow cytometry analysis also revealed that compound **15** arrested the cell cycle of MFC cells in the G_1 phase with a concentration-dependent effect. Furthermore, the animal experiment in vivo showed that compound **15** exhibited stronger efficiency in suppressing tumor growth compared with 5-Fu. All the results outlined the great potential of compound **15** for further exploitation as anti-tumor drug.

4. Experimental Section

4.1. Chemistry

All chemicals (reagent grade) used were purchased from commercial source and used without further purification unless otherwise stated. Separation of the compounds by column chromatography was carried out with silica gel (100-200 mesh size). TLC was run on the silica gel 60 F254 plates and visualized under UV light at 254 nm or iodine vapour. Melting points (uncorrected) were determined on a Thermo Scientific Electrothermal Digital Melting Point Apparatus. ESI mass spectra were obtained on a Waters GCT mass spectrometer, and ^{1}H NMR spectra were measured on a Bruker AV-400 model Spectrometer with TMS and solvent signals slotted as internal standards. Chemical shifts were reported in ppm (δ).

4.1.1. *7-hydroxy-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one (**3**)*

To a solution of resorcinol (2.5 g, 0.023 mol) and 2-chloroacetonitrile (2 mL, 0.032 mol) in absolute ether (30 mL) with catalytic amount of $ZnCl_2$ at 0 ℃ and freshly prepared hydrogen chloride gas was bubbled slowly and continuously to the reaction mixture for 2 h. After standing the reaction flask at low temperature for overnight, lots of yellow precipitate was prepared after filtrating, washing and drying. To reflux the yellow precipitate in water solvent, a precipitate was produced. The milky white precipitate2-chloro-1-(2,4-dihydroxyphenyl)ethanone was filtered off, washed with water, and dried in vacuo to give a milky white precipitate. 3,4,5-trimethoxybenzaldehyde (2.0 g, 0.01 mol) and 2-chloro-1-(2,4-dihydroxyphenyl)ethanone (2.2 g, 0.012 mol) were added in one portion to a stirred solution of 5 mL ethanol, the drop 10% NaOH aqueous solution into the solution. The reaction mixture was stirred at room temperature for 24 h, and acidification with 10% HCl (aq) gave a crude product, which was filtered off and purified by recrystallized from 95% ethanol to give the compound **3** as yellow powder.

4.1.2. General method of synthesis **4a-4c**

To a stirred solution of compound **3** (1.0 mol) in acetone (30 ml) with certain amount of K_2CO_3 at room

View Article Online
DOI: 10.1039/C7MD00578D

temperature and different chain length substituted alcohols (4.0 mol) was added to the reaction mixture. The mixture was refluxed for 5 h (the reaction was monitored by TLC). After cooling, the crude product was filtered off, washed with water and acetone, dried in vacuo. The crude product was purified by column chromatography on silica gel, eluting with dichloromethane/methanol (50:1) to give a viridescent powder.

4.1.2.1. *7-(2-bromoethoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one (**4a**)*. [33] Yellow powder, yield: 95%. Mp:142-145℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 20.0, 9.8 Hz, 1H), 7.07 (s, 2H), 6.84 – 6.55 (m, 3H), 4.30 (dt, *J* = 20.6, 4.8 Hz, 2H), 3.87 (t, *J* = 9.4 Hz, 9H), 3.65 (dt, *J* = 11.1, 8.1 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.76 (s), 168.05 (s), 165.61 (s), 153.32 (s), 147.21 (s), 127.71 (s), 126.08 (s), 115.51 (s), 112.49 (s), 112.18 (s), 108.81 (s), 97.62 (s), 68.43 (s), 61.03 (s), 56.25 (s), 29.71 (s), 28.21 (s). MS (ESI):434.0 ($C_{20}H_{19}BrO_6$, [M+H$^+$]).

4.1.2.2. *7-(3-bromopropoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one (**4b**).* [33]

Yellow powder, yield: 94%. Mp:168-170℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.3 Hz, 1H), 7.16 (s, 2H), 6.77 (d, *J* = 10.0 Hz, 3H), 4.25 (t, *J* = 5.4 Hz, 2H), 3.94 (d, *J* = 11.6 Hz, 9H), 3.63 (t, *J* = 6.0 Hz, 2H), 2.52 – 2.29 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.78 (s), 168.22 (s), 166.36 (s), 153.30 (s), 147.29 (s), 139.81 (s), 127.79 (s), 125.88 (s), 115.07 (s), 112.43 (s), 112.26 (s), 108.70 (s), 97.24 (s), 66.22 (s), 61.03 (s), 56.22 (s), 31.91 (s), 29.54 (s). MS (ESI):448.1 ($C_{21}H_{21}BrO_6$, [M+H$^+$]).

4.1.2.3. *7-(4-bromobutoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one (**4c**).*

Yellow powder, yield: 95%. Mp:159-160℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.5 Hz, 1H), 7.16 (s, 2H), 6.75 (t, *J* = 8.3 Hz, 3H), 4.13 (t, *J* = 5.2 Hz, 2H), 3.93 (d, *J* = 10.9 Hz, 9H), 3.51 (t, *J* = 6.0 Hz, 2H), 2.26 – 1.86 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.75 (s), 168.24 (s), 166.59 (s), 153.29 (s), 147.32 (s), 139.78 (s), 127.81 (s), 125.83 (s), 114.89 (s), 112.41 (s), 112.14 (s), 108.69 (s), 97.14 (s), 67.86 (s), 61.02 (s), 56.21 (s), 33.17 (s), 29.24 (s), 27.58 (s). MS (ESI):462.1 ($C_{22}H_{23}BrO_6$, [M+H$^+$]).

4.1.3. General method of synthesis **5-28**

Equimolar quantities of the bromide intermediates (**4a-4c**), corresponding benzimidazoles were dissolved in acetone (30 mL), and certain amount of K_2CO_3 was also added to the solution. The solution was then refluxed for approximately 12 h. The product was extracted by dichloromethane. Then evaporated of the solvent, the residue was purified by column chromatography on silica gel, eluting with dichloromethane/methanol (30:1) to give a yellow powder.

4.1.3.1.*7-(2-(1H-benzo[d]imidazol-1-yl)ethoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one (**5**)*. Yellow powder, yield: 64%. Mp:209-210℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.82 (d, *J* = 7.0 Hz, 1H), 7.68 (d, *J* = 8.6 Hz, 1H), 7.47 (d, *J* = 7.3 Hz, 1H), 7.32 (dq, *J* = 7.2, 6.0 Hz, 2H), 7.11 (s, 2H), 6.74 – 6.70 (m, 2H), 6.70 (d, *J* = 2.1 Hz, 1H), 6.65

View Article Online
DOI: 10.1039/C7MD00578D

(d, *J* = 2.0 Hz, 1H), 4.64 (t, *J* = 5.2 Hz, 2H), 4.41 (t, *J* = 5.1 Hz, 2H), 3.91 (d, *J* = 7.6 Hz, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.66 (s), 167.85 (s), 165.22 (s), 153.28 (s), 147.09 (s), 143.84 (s), 143.45 (s), 139.88 (s), 133.63 (s), 127.65 (s), 126.02 (s), 123.27 (s), 122.49 (s), 120.69 (s), 115.64 (s), 112.58 (s), 111.87 (s), 109.31 (s), 108.74 (s), 97.56 (s), 66.85 (s), 61.03 (s), 56.21 (s), 44.08 (s). MS (ESI):472.1 ($C_{27}H_{24}N_2O_6$, [M+H$^+$]).

4.1.3.2.*7-(2-(2-methyl-1H-benzo[d]imidazol-1-yl)ethoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(6)***. Orange yellow powder, yield: 53%. Mp:192-193℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.67 (m, 1H), 7.66 (s, 1H), 7.37 – 7.32 (m, 1H), 7.27 – 7.25 (m, 2H), 7.10 (s, 1H), 6.73 (s, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 6.66 (d, *J* = 2.1 Hz, 1H), 6.60 (d, *J* = 2.0 Hz, 1H), 4.59 (t, *J* = 5.3 Hz, 2H), 4.39 (t, *J* = 5.2 Hz, 2H), 3.92 (t, *J* = 8.8 Hz, 9H), 2.72 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.69 (s), 167.90 (s), 165.30 (s), 153.28 (s), 152.04 (s), 147.09 (s), 142.65 (s), 139.89 (s), 134.82 (s), 127.63 (s), 126.01 (s), 122.38 (s), 122.30 (s), 119.36 (s), 115.55 (s), 112.63 (s), 111.98 (s), 108.83 (s), 108.75 (s), 97.29 (s), 66.71 (s), 61.02 (s), 56.22 (s), 42.97 (s), 14.11 (s). MS (ESI):486.1 ($C_{28}H_{26}N_2O_6$, [M+H$^+$]).

4.1.3.3.*7-(2-(2-chloro-1H-benzo[d]imidazol-1-yl)ethoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(7)***. Yellow powder, yield: 78%. Mp:153-156℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.1 Hz, 1H), 7.66 (d, *J* = 8.5 Hz, 1H), 7.44 (d, *J* = 7.4 Hz, 1H), 7.37 – 7.26 (m, 2H), 7.11 (s, 1H), 6.73 (s, 1H), 6.68 (d, *J* = 2.1 Hz, 1H), 6.66 (d, *J* = 2.1 Hz, 1H), 6.63 (d, *J* = 2.0 Hz, 1H), 4.66 (t, *J* = 5.4 Hz, 2H), 4.41 (dd, *J* = 10.6, 5.1 Hz, 2H), 3.92 (t, *J* = 8.7 Hz, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.69 (s), 167.92 (s), 165.23 (s), 153.31 (s), 147.11 (s), 141.74 (s), 140.49 (s), 140.14 – 139.67 (m), 135.28 (s), 127.65 (s), 126.03 (s), 123.52 (s), 123.09 (s), 119.73 (s), 115.62 (s), 112.60 (s), 112.06 (s), 109.55 (s), 108.77 (s), 97.34 (s), 66.46 (s), 61.03 (s), 56.24 (s), 43.51 (s). MS (ESI):507.1 ($C_{27}H_{23}ClN_2O_6$, [M+H$^+$]).

4.1.3.4.*7-(2-(6(5)-nitro-1H-benzo[d]imidazol-1-yl)ethoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(8)***. Orange yellow powder, yield: 74%. Mp:207-209℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.54 (d, *J* = 2.0 Hz, 1H), 8.30 (d, *J* = 11.2 Hz, 1H), 8.27 – 8.20 (m, 1H), 7.88 (d, *J* = 9.0 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.11 (d, *J* = 2.1 Hz, 1H), 6.70 (dd, *J* = 16.3, 7.7 Hz, 2H), 4.72 (dt, *J* = 9.9, 4.7 Hz, 2H), 4.45 (dd, *J* = 9.8, 5.0 Hz, 2H), 3.92 (dd, *J* = 9.0, 7.4 Hz, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.58 (s), 167.78 (s), 164.84 (s), 153.30 (s), 147.87 (s), 147.01 (s), 146.72 (s), 144.05 (s), 143.92 (s), 127.56 (s), 126.14 (s), 120.78 (s), 119.05 (s), 118.25 (s), 117.34 (s), 115.89 (s), 112.76 (s), 111.74 (s), 109.64 (s), 108.81 (s), 108.76 (s), 107.00 (s), 97.67 (s), 67.02 (s), 66.88 (s), 61.03 (s), 56.22 (s), 44.82 (s), 44.62 (s). MS (ESI):517.1 ($C_{27}H_{23}N_3O_8$, [M+H$^+$]).

4.1.3.5.*7-(2-(5,6-dimethyl-1H-benzo[d]imidazol-1-yl)ethoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(9)***. Yellow powder, yield: 68%. Mp:235-237℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.57 (s, 1H), 7.21 (s, 1H), 7.11 (s, 2H), 6.73 (s, 1H), 6.70 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.65 (d, *J* = 2.0 Hz, 1H), 4.58 (t, *J* = 5.1 Hz, 2H), 4.38 (t, *J* = 5.2 Hz, 2H), 3.91 (d, *J* = 7.8 Hz, 9H), 2.38 (d, *J* = 13.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.47

View Article Online
DOI: 10.1039/C7MD00578D

(s), 167.68 (s), 165.32 (s), 153.32 (s), 147.15 (s), 142.69 (s), 139.89 (s), 132.47 (s), 131.42 (s), 127.68 (s), 120.67 (s), 115.63 (s), 112.59 (s), 111.91 (s), 109.45 (s), 108.76 (s), 97.62 (s), 66.81 (s), 61.04 (s), 56.23 (s), 44.04 (s), 20.67 (s), 20.25 (s), 0.01 (s). MS (ESI):500.1 ($C_{29}H_{28}N_2O_6$, [M+H$^+$]).

4.1.3.6.*7-(2-(5(6)-methyl-1H-benzo[d]imidazol-1-yl)ethoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(10)***. Yellow powder, yield: 62%. Mp:208-210℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 6.5 Hz, 1H), 7.73 (d, J = 8.5 Hz, 1H), 7.66 (d, J = 12.8 Hz, 1H), 7.39 (d, J = 8.5 Hz, 1H), 7.24 – 7.10 (m, 3H), 6.80 – 6.72 (m, 2H), 6.70 (s, 1H), 4.64 (s, 2H), 4.44 (s, 2H), 3.96 (t, J = 9.0 Hz, 9H), 2.54 (d, J = 13.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.68 (s), 167.87 (s), 165.27 (s), 153.28 (s), 147.11 (s), 144.20 (s), 143.39 (s), 143.03 (s), 141.95 (s), 139.88 (s), 133.79 (s), 133.29 (s), 132.21 (s), 131.71 (s), 127.65 (s), 126.02 (s), 124.73 (s), 124.08 (s), 120.41 (s), 120.17 (s), 115.61 (s), 112.57 (s), 111.89 (s), 109.18 (s), 108.72 (s), 97.56 (s), 66.82 (d, *J* = 9.0 Hz), 61.02 (s), 56.20 (s), 43.95 (s), 21.88 (s), 21.51 (s). MS (ESI):486.1 ($C_{28}H_{26}N_2O_6$, [M+H$^+$]).

4.1.3.7.*7-(2-(2-(trifluoromethyl)-1H-benzo[d]imidazol-1-yl)ethoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(11)***. Yellow powder, yield: 77%. Mp:207-208℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.0 Hz, 1H), 7.67 (t, *J* = 8.3 Hz, 2H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.12 (s, 2H), 6.74 (s, 1H), 6.71 – 6.58 (m, 2H), 4.82 (s, 2H), 4.47 (s, 2H), 3.94 (t, *J* = 8.2 Hz, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.70 (s), 167.93 (s), 165.11 (s), 153.32 (s), 147.08 (s), 141.08 (s), 139.97 (s), 135.82 (s), 127.62 (s), 126.04 (s), 125.71 (s), 124.07 (s), 121.84 (s), 115.66 (s), 112.69 (s), 112.11 (s), 110.89 (s), 108.81 (s), 97.20 (s), 66.95 (s), 61.03 (s), 56.25 (s), 44.08 (s). MS (ESI):540.1 ($C_{28}H_{23}F_3N_2O_6$, [M+H$^+$]).

4.1.3.8.*7-(2-(5(6)-methoxy-1H-benzo[d]imidazol-1-yl)ethoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(12)***. Yellow powder, yield: 71%. Mp:204-206℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 17.8 Hz, 1H), 7.69 (dd, *J* = 16.8, 9.5 Hz, 2H), 7.12 (s, 2H), 6.97 (dd, *J* = 22.2, 11.3 Hz, 2H), 6.81 – 6.61 (m, 3H), 4.60 (d, *J* = 4.8 Hz, 2H), 4.41 (s, 2H), 4.04 – 3.79 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.67 (s), 167.87 (s), 165.24 (s), 157.03 (s), 156.37 (s), 153.28 (s), 147.10 (s), 144.68 (s), 143.57 (s), 142.67 (s), 139.88 (s), 138.29 (s), 134.31 (s), 128.22 (s), 127.65 (s), 126.03 (s), 121.10 (s), 115.63 (s), 113.51 (s), 112.60 (s), 111.92 (s), 111.42 (s), 109.74 (s), 108.73 (s), 102.54 (s), 97.54 (s), 93.22 (s), 66.94 (s), 66.84 (s), 61.03 (s), 56.21 (s), 55.97 (s), 55.82 (s), 44.22 (s), 43.99 (s). MS (ESI):502.1 ($C_{28}H_{26}N_2O_7$, [M+H$^+$]).

4.1.3.9.*7-(3-(1H-benzo[d]imidazol-1-yl)propoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(13)***. Yellow powder, yield: 70%. Mp:185-187℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 17.7 Hz, 1H), 7.82 (d, *J* = 3.3 Hz, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.41 (s, 1H), 7.36 – 7.21 (m, 4H), 6.78 (d, *J* = 9.3 Hz, 2H), 6.66 (s, 1H), 4.49 (t, *J* = 6.3 Hz, 2H), 4.00 (dd, *J* = 13.4, 8.4 Hz, 2H), 3.92 (d, *J* = 7.2 Hz, 9H), 2.52 – 2.32 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.76 (s),

View Article Online
DOI: 10.1039/C7MD00578D

168.15 (s), 165.93 (s), 153.31 (s), 147.21 (s), 143.93 (s), 143.10 (s), 139.87 (s), 133.67 (s), 127.73 (s), 126.02 (s), 123.19 (s), 122.36 (s), 120.61 (s), 115.35 (s), 112.45 (s), 112.31 (s), 109.43 (s), 108.74 (s), 97.23 (s), 64.79 (s), 61.03 (s), 56.23 (s), 41.29 (s), 29.18 (s). MS (ESI):486.1 ($C_{28}H_{26}N_2O_6$, [M+H$^+$]).

4.1.3.10.*7-(3-(2-methyl-1H-benzo[d]imidazol-1-yl)propoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(14)***. Yellow powder, yield: 57%. Mp:169-171℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (t, *J* = 8.8 Hz, 2H), 7.19 (t, *J* = 12.3 Hz, 2H), 7.11 (td, *J* = 14.2, 6.7 Hz, 3H), 6.67 (d, *J* = 11.4 Hz, 2H), 6.56 (s, 1H), 4.30 (t, *J* = 6.3 Hz, 2H), 3.90 (dd, *J* = 12.8, 7.5 Hz, 2H), 3.83 (d, *J* = 3.5 Hz, 9H), 2.44 (d, *J* = 42.5 Hz, 2H), 2.28 (dd, *J* = 11.8, 5.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.74 (s), 168.14 (s), 165.86 (s), 153.29 (s), 151.52 (s), 147.19 (s), 142.44 (s), 139.89 (s), 134.88 (s), 127.69 (s), 126.02 (s), 122.32 (s), 122.15 (s), 119.12 (s), 115.32 (s), 112.50 (s), 112.32 (s), 109.01 (s), 108.76 (s), 97.19 (s), 64.76 (s), 61.00 (s), 56.21 (s), 40.07 (s), 28.84 (s). MS (ESI):500.1 ($C_{29}H_{28}N_2O_6$, [M+H$^+$]).

4.1.3.11.*7-(3-(2-chloro-1H-benzo[d]imidazol-1-yl)propoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(15)***. Yellow powder, yield: 66%. Mp:140-142℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (t, *J* = 7.8 Hz, 1H), 7.56 (s, 1H), 7.37 – 7.29 (m, 2H), 7.14 (s, 2H), 7.07 (s, 1H), 6.76 (d, *J* = 6.8 Hz, 1H), 6.65 (s, 1H), 4.49 (t, *J* = 5.7 Hz, 2H), 4.09 (d, *J* = 20.6 Hz, 2H), 3.93 (d, *J* = 6.5 Hz, 9H), 2.40 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.89 (s), 168.18 (s), 165.98 (s), 153.31 (s), 147.24 (s), 141.59 (s), 140.51 (s), 139.87 (s), 135.03 (s), 128.92 (s), 127.73 (s), 126.04 (s), 123.52 (s), 123.10 (s), 123.03 (s), 119.53 (s), 115.29 (s), 112.59 (s), 112.36 (s), 109.27 (s), 108.77 (s), 97.22 (s), 64.90 (s), 61.05 (s), 56.25 (s), 41.00 (s), 29.28 (s), 28.76 (s). MS (ESI):521.1 ($C_{28}H_{25}ClN_2O_6$, [M+H$^+$]).

4.1.3.12.*7-(3-(6(5)-nitro-1H-benzo[d]imidazol-1-yl)propoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(16)***. Yellow powder, yield: 54%. Mp:225-228℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 113.5 Hz, 1H), 8.23 – 8.01 (m, 2H), 7.62 (ddd, *J* = 88.9, 65.5, 8.9 Hz, 2H), 7.05 (s, 2H), 6.76 – 6.54 (m, 3H), 4.61 – 4.38 (m, 2H), 4.01 (dd, *J* = 11.2, 5.5 Hz, 2H), 3.86 (t, *J* = 10.2 Hz, 9H), 2.40 (dt, *J* = 11.4, 5.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.66 (s), 168.06 (s), 165.60 (s), 153.31 (s), 148.18 (s), 147.54 (s), 147.13 (s), 146.46 (s), 143.96 (s), 143.85 (s), 143.23 (s), 139.95 (s), 137.82 (s), 133.07 (s), 127.65 (s), 126.11 (s), 120.78 (s), 118.99 (s), 118.16 (s), 117.34 (s), 115.55 (s), 112.60 (s), 112.53 (s), 112.08 (s), 109.48 (s), 108.79 (s), 106.62 (s), 97.28 (s), 64.68 (s), 61.03 (s), 56.23 (s), 53.47 (s), 41.97 (s), 29.23 (s). MS (ESI):531.1 ($C_{28}H_{25}N_3O_8$, [M+H$^+$]).

4.1.3.13.*7-(3-(5,6-dimethyl-1H-benzo[d]imidazol-1-yl)propoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(17)***. Yellow powder, yield: 63%. Mp:222-224℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 23.4, 14.8 Hz, 2H), 7.56 (s, 1H), 7.14 (s, 3H), 6.78 (d, *J* = 12.1 Hz, 2H), 6.65 (s, 1H), 4.42 (s, 2H), 3.99 (s, 2H), 3.92 (d, *J* = 6.9 Hz, 9H), 2.38 (d, *J* = 14.7 Hz, 6H), 2.31 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.74 (s), 168.17 (s), 166.01 (s), 153.30 (s), 147.22 (s), 142.35 (s), 139.84 (s), 132.36 (s), 132.25 (s), 131.23 (s), 127.73 (s), 125.96 (s), 120.47 (s), 115.25 (s), 112.41 (s),

View Article Online
DOI: 10.1039/C7MD00578D

109.66 (s), 108.71 (s), 97.21 (s), 64.77 (s), 61.02 (s), 56.21 (s), 41.12 (s), 29.26 (s), 20.52 (s), 20.24 (s). MS (ESI):514.1 ($C_{30}H_{30}N_2O_6$, [M+H$^+$]).

4.1.3.14.*7-(3-(5(6)-methyl-1H-benzo[d]imidazol-1-yl)propoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(18)***. Yellow powder, yield: 58%. Mp:154-156℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 6.4 Hz, 1H), 7.74 (t, *J* = 9.0 Hz, 1H), 7.70 (d, *J* = 8.3 Hz, 1H), 7.62 (s, 1H), 7.22 – 7.09 (m, 3H), 6.85 – 6.75 (m, 2H), 6.67 (s, 1H), 4.46 (s, 2H), 4.01 (d, *J* = 4.7 Hz, 2H), 3.94 (d, *J* = 7.3 Hz, 9H), 2.50 (s, 2H), 2.46 – 2.38 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.78 (s), 168.17 (s), 165.98 (s), 153.30 (s), 147.22 (s), 144.21 (s), 143.03 (s), 142.63 (s), 141.91 (s), 139.85 (s), 133.92 (s), 133.22 (s), 132.10 (s), 131.74 (s), 127.73 (s), 126.01 (s), 124.69 (s), 123.97 (s), 120.26 (s), 120.00 (s), 115.28 (s), 112.70 – 112.18 (m), 109.36 (s), 108.96 (s), 108.73 (s), 97.21 (s), 64.78 (d, *J* = 3.4 Hz), 61.02 (s), 56.22 (s), 41.29 (s), 41.09 (s), 29.24 (s), 21.76 (s), 21.50 (s). MS (ESI):500.1 ($C_{29}H_{28}N_2O_6$, [M+H$^+$]).

4.1.3.15.*7-(3-(2-(trifluoromethyl)-1H-benzo[d]imidazol-1-yl)propoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(19)***. Yellow powder, yield: 72%. Mp:175-178℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 6.4 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.40 (dd, *J* = 15.9, 8.3 Hz, 3H), 7.15 (s, 2H), 6.78 (d, *J* = 6.8 Hz, 2H), 6.68 (s, 1H), 4.62 (t, *J* = 6.8 Hz, 2H), 4.14 (s, 2H), 3.95 (t, *J* = 10.9 Hz, 9H), 2.45 (d, *J* = 5.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.89 (s), 168.17 (s), 165.94 (s), 153.31 (s), 147.23 (s), 141.00 (s), 139.88 (s), 135.45 (s), 127.72 (s), 126.03 (s), 125.69 (s), 123.99 (s), 123.64 (s), 121.72 (s), 115.32 (s), 112.64 (s), 112.31 (s), 110.30 (s), 108.78 (s), 97.22 (s), 65.17 (s), 61.04 (s), 56.21 (s), 41.80 (s), 29.57 (s). MS (ESI):554.1 ($C_{29}H_{25}F_3N_2O_6$, [M+H$^+$]).

4.1.3.16.*7-(3-(5(6)-methoxy-1H-benzo[d]imidazol-1-yl)propoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(20)***. Yellow powder, yield: 67%. Mp:129-131℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 15.8 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.69 (d, *J* = 8.9 Hz, 1H), 7.28 (s, 1H), 7.14 (s, 2H), 6.93 (t, *J* = 8.6 Hz, 1H), 6.86 – 6.74 (m, 2H), 6.67 (s, 1H), 4.43 (d, *J* = 6.0 Hz, 2H), 4.07 – 3.98 (m, 2H), 3.98 – 3.69 (m, 12H), 2.51 – 2.29 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.77 (s), 168.17 (s), 165.95 (s), 156.98 (s), 156.30 (s), 153.31 (s), 147.20 (s), 142.27 (s), 139.90 (s), 138.30 (s), 134.39 (s), 127.70 (s), 126.05 (s), 121.04 (s), 115.34 (s), 113.51 (s), 112.57 (s), 112.38 (s), 111.64 (s), 109.82 (s), 108.76 (s), 102.42 (s), 97.21 (s), 92.91 (s), 64.73 (s), 61.03 (s), 56.24 (s), 55.82 (s), 55.77 (s), 41.40 (s), 41.05 (s), 29.24 (s). MS (ESI):516.1 ($C_{29}H_{28}N_2O_7$, [M+H$^+$]).

4.1.3.17.*7-(4-(1H-benzo[d]imidazol-1-yl)butoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(21)***. Yellow powder, yield: 59%. Mp:177-179℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.83 (d, *J* = 6.8 Hz, 1H), 7.70 (t, *J* = 9.8 Hz, 1H), 7.43 (d, *J* = 7.1 Hz, 1H), 7.31 (dd, *J* = 19.0, 13.9 Hz, 4H), 6.72 (dd, *J* = 19.2, 10.4 Hz, 3H), 4.31 (t, *J* = 6.7 Hz, 2H), 4.09 (t, *J* = 5.5 Hz, 2H), 4.02 – 3.84 (m, 9H), 2.22 – 2.07 (m, 2H), 2.00 – 1.83 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.77 (s), 168.19 (s), 166.38 (s), 153.30 (s), 147.28 (s), 139.83 (s), 127.78 (s), 125.92 (s), 123.02 (s),

View Article Online
DOI: 10.1039/C7MD00578D

122.24 (s), 120.56 (s), 115.04 (s), 112.26 (s), 109.58 (s), 108.73 (s), 97.20 (s), 68.07 (s), 61.03 (s), 56.23 (s), 44.75 (s), 26.70 (s), 26.33 (s). MS (ESI):500.1 ($C_{29}H_{28}N_2O_6$, [M+H$^+$]).

4.1.3.18.*7-(4-(2-methyl-1H-benzo[d]imidazol-1-yl)butoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(22)***.

Yellow powder, yield: 61%. Mp:160-163℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.2 Hz, 2H), 7.31 (s, 1H), 7.25 (d, *J* = 3.2 Hz, 2H), 7.15 (s, 2H), 6.77 – 6.65 (m, 3H), 4.23 (t, *J* = 6.9 Hz, 2H), 4.10 (dd, *J* = 14.1, 8.6 Hz, 2H), 3.94 (t, *J* = 10.4 Hz, 9H), 2.64 (s, 3H), 2.15 – 1.99 (m, 2H), 1.89 (d, *J* = 6.3 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.77 (s), 168.20 (s), 166.39 (s), 153.32 (s), 151.32 (s), 147.29 (s), 142.71 (s), 135.06 (s), 127.78 (s), 125.95 (s), 122.10 (s), 121.93 (s), 119.23 (s), 115.07 (s), 112.27 (s), 112.20 (s), 109.04 (s), 108.76 (s), 97.21 (s), 68.17 (s), 61.04 (s), 56.25 (s), 43.49 (s), 26.60 (s), 26.45 (s), 14.05 (s). MS (ESI):514.1 ($C_{30}H_{30}N_2O_6$, [M+H$^+$]).

4.1.3.19.*7-(4-(2-chloro-1H-benzo[d]imidazol-1-yl)butoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(23)***.

Yellow powder, yield: 60%. Mp:163-166℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (q, *J* = 8.0 Hz, 3H), 7.33 – 7.12 (m, 4H), 6.75 – 6.50 (m, 3H), 4.24 (t, *J* = 6.7 Hz, 2H), 4.01 (t, *J* = 5.5 Hz, 2H), 3.85 (t, *J* = 9.3 Hz, 9H), 2.13 – 1.92 (m,2H), 1.89 – 1.74 (m,2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.78 (s), 168.19 (s), 166.40 (s), 153.31 (s), 147.29 (s), 141.79 (s), 140.45 (s), 139.85 (s), 134.94 (s), 127.78 (s), 125.93 (s), 123.27 (s), 122.80 (s), 119.63 (s), 115.04 (s), 112.26 (s), 112.23 (s), 109.35 (s), 108.75 (s), 97.21 (s), 69.53 (s), 68.06 (s), 61.03 (s), 56.23 (s), 53.80 (s), 53.47 (s), 44.11 (s), 31.76 (s), 29.28 (s), 26.24 (s), 26.18 (s). MS (ESI):534.1($C_{29}H_{27}ClN_2O_6$, [M+H$^+$]).

4.1.3.20.*7-(4-(6(5)-nitro-1H-benzo[d]imidazol-1-yl)butoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(24)***.

Yellow powder, yield: 62%. Mp:140-142℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, *J* = 113.5 Hz, 1H), 8.21 (dd, *J* = 23.4, 12.4 Hz, 2H), 7.98 – 7.38 (m, 2H), 7.12 (s, 2H), 6.70 (dd, *J* = 8.7, 5.1 Hz, 3H), 4.39 (dt, *J* = 14.0, 7.1 Hz, 2H), 4.22 – 4.03 (m, 2H), 3.92 (d, *J* = 6.2 Hz, 9H), 2.36 – 2.06 (m, 2H), 1.91 (dd, *J* = 14.0, 7.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.69 (s), 168.12 (s), 166.22 (s), 153.28 (s), 148.18 (s), 147.37 (s), 147.20 (s), 146.31 (s), 143.86 (s), 143.73 (s), 143.17 (s), 139.85 (s), 137.83 (s), 133.06 (s), 127.70 (s), 125.91 (s), 120.66 (s), 118.84 (s), 118.05 (s), 117.23 (s), 115.08 (s), 112.33 (s), 112.18 (s), 112.11 (s), 109.66 (s), 108.73 (s), 106.74 (s), 97.28 (s), 97.17 (s), 67.93 (s), 67.87 (s), 61.01 (s), 56.21 (s), 45.27 (s), 26.84 (s), 26.25 (s), 26.19 (s). MS (ESI):545.2 ($C_{29}H_{27}N_3O_8$, [M+H$^+$]).

4.1.3.21.*7-(4-(5,6-dimethyl-1H-benzo[d]imidazol-1-yl)butoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(25)***.

Yellow powder, yield: 73%. Mp:204-207℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.69 (t, *J* = 8.7 Hz, 1H), 7.57 (s, 1H), 7.16 (d, *J* = 11.1 Hz, 3H), 6.82 – 6.61 (m, 3H), 4.25 (t, *J* = 6.7 Hz, 2H), 4.12 – 3.99 (m, 2H), 3.94 (t, *J* = 10.1 Hz, 9H), 2.38 (d, *J* = 7.1 Hz, 6H), 2.22 – 2.02 (m, 2H), 1.83 (d, *J* = 19.3 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.74 (s), 168.18 (s), 166.42 (s), 153.29 (s), 147.28 (s), 142.58 (s), 142.17 (s), 139.80 (s), 132.15 (s), 131.10 (s), 127.78 (s), 125.85 (s), 120.45 (s), 114.96 (s), 112.21 (s), 109.77 (s), 108.70 (s), 97.16 (s), 68.08 (s), 61.02 (s), 56.21 (s), 44.67

View Article Online
DOI: 10.1039/C7MD00578D

(s), 26.54 (s), 26.29 (s), 20.64 (s), 20.26 (s). MS (ESI):528.1 ($C_{31}H_{32}N_2O_6$, [M+H$^+$]).

4.1.3.22.*7-(4-(5(6)-methyl-1H-benzo[d]imidazol-1-yl)butoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(26)***. Yellow powder, yield: 54%. Mp:159-163℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 7.1 Hz, 1H), 7.75 – 7.58 (m, 2H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.22 – 7.08 (m, 3H), 6.78 – 6.64 (m, 3H), 4.27 (d, *J* = 3.1 Hz, 2H), 4.08 (d, *J* = 5.2 Hz, 2H), 3.93 (d, *J* = 9.0 Hz, 9H), 2.50 (d, *J* = 6.4 Hz, 3H), 2.11 (dd, *J* = 23.2, 16.2 Hz, 2H), 1.84 (t, *J* = 19.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.76 (s), 168.19 (s), 166.40 (s), 153.30 (s), 147.29 (s), 142.84 (s), 142.47 (s), 127.79 (s), 125.92 (s), 124.51 (s), 123.85 (s), 120.27 (s), 115.02 (s), 112.24 (s), 109.45 (s), 109.09 (s), 108.73 (s), 97.19 (s), 68.08 (s), 61.03 (s), 56.23 (s), 44.76 (s), 44.62 (s), 26.65 (s), 26.32 (s), 21.88 (s), 21.53 (s). MS (ESI):514.1 ($C_{30}H_{30}N_2O_6$, [M+H$^+$]).

4.1.3.23.*7-(4-(2-(trifluoromethyl)-1H-benzo[d]imidazol-1-yl)butoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-on e* ***(27)***. Yellow powder, yield: 63%. Mp:153-156℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 7.8 Hz, 1H), 7.72 (t, *J* = 8.4 Hz, 1H), 7.48 (q, *J* = 8.0 Hz, 2H), 7.42 (dd, *J* = 14.8, 7.5 Hz, 1H), 7.17 (s, 2H), 6.81 – 6.68 (m, 2H), 5.32 (s, 1H), 4.47 (t, *J* = 7.3 Hz, 2H), 4.15 (t, *J* = 5.1 Hz, 2H), 3.96 (t, *J* = 10.3 Hz, 9H), 2.25 – 2.09 (m, 2H), 1.98 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.79 (s), 168.20 (s), 166.33 (s), 153.32 (s), 147.28 (s), 141.21 (s), 139.87 (s), 135.31 (s), 127.78 (s), 125.96 (s), 125.46 (s), 123.78 (s), 121.87 (s), 115.13 (s), 112.29 (s), 112.22 (s), 110.42 (s), 108.76 (s), 97.22 (s), 68.03 (s), 61.03 (s), 56.23 (s), 53.45 (s), 44.77 (s), 26.86 (s), 26.26 (s). MS (ESI):568.2 ($C_{30}H_{27}F_3N_2O_6$, [M+H$^+$]).

4.1.3.24.*7-(4-(5(6)-methoxy-1H-benzo[d]imidazol-1-yl)butoxy)-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one* ***(28)***. Yellow powder, yield: 56%. Mp:87-90℃ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 17.0 Hz, 1H), 7.76 – 7.64 (m, 2H), 7.15 (s, 2H), 6.95 (t, *J* = 10.7 Hz, 1H), 6.85 (s, 1H), 6.79 – 6.63 (m, 3H), 4.25 (t, *J* = 6.7 Hz, 2H), 4.08 (d, *J* = 4.7 Hz, 2H), 3.93 (dd, *J* = 23.4, 14.4 Hz, 12H), 2.20 – 2.04 (m, 2H), 1.88 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.79 (s), 168.19 (s), 166.39 (s), 156.83 (s), 153.31 (s), 147.28 (s), 144.72 (s), 142.99 (s), 142.16 (s), 139.85 (s), 138.41 (s), 134.32 (s), 127.78 (s), 125.95 (s), 120.96 (s), 115.04 (s), 113.35 (s), 112.31 (s), 112.22 (s), 111.27 (s), 109.97 (s), 108.75 (s), 108.52 (s), 102.37 (s), 97.20 (s), 96.29 (s), 93.35 (s), 68.09 (s), 61.03 (s), 56.24 (s), 55.94 (s), 55.82 (s), 44.89 (s), 44.64 (s), 26.67 (s), 26.49 (s), 26.30 (s). MS (ESI):530.1 ($C_{30}H_{30}N_2O_7$, [M+H$^+$]).

4.2. Biological assay

4.2.1. Cell culture

Human gastric tumor cell line MGC-803, breast tumor cell line MCF-7, liver tumor cell line HepG-2 and murine gastric tumor cell line MFC were obtained from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China and cells were cultured in DMEM medium (Gibco, NY, USA) which was supplemented

View Article Online
DOI: 10.1039/C7MD00578D

with 10% fetal bovine serum (FBS) (sigma). The cultures were incubated at 37 □ in humidified 5% CO_2 incubator. When experiments were performed in the absence of PBS, PBS was eliminated 24 h before initiating the experiments.

4.2.2. In vitro antiproliferative assay

The antiproliferative activities of compounds **5-28** were determined using a standard (MTT)-based colorimetric assay (Sigma). The cells were seeded at a density of approximately 8×10^3 cells/well in 96-well microtiter plates (Costar) and maintained at 37 ℃ in 95% humidity and 5% CO_2 for 48 h. Different concentration (256, 128, 64, 32, 16, 8, 4, 2 μM) of compounds were treated. After 48 h, the cells washed twice with PBS, and 20 μL of the MTT solution (5 mg/mL in PBS) added to each well and plate was incubated at 37 ℃. After 4 h, 100 μL of DMSO was added to each well to dissolve the formazan crystals and absorbance was measured at 570 nm on aWellscanMK-2 microplate reader. The results are compared with the standard drug 5-Fu as positive control drug against all tested cell lines. IC_{50} values were determined form replicates of 96-well from three independent experiments.

4.2.3. Apoptosis assay

The MFC cells were incubated for 48 h after different concentration of compound **15** were added and then stained with both Annexin V-FITC (fluorescein isothiocyanate) and propidium iodide (PI). Then samples were analyzed by FACSCalibur flow cytometer.

4.2.4. Cell cycle assay

The MFC cells were incubated with different concentrations of compound **15** (0,10, 20, 40 μM) in a six-well plate. After 48 h the cells were harvested by trypsinization, washed in PBS, and fixed in 70% ice-cold (4°C) ethanol for 12 h. Then added 500 of proppidium staining to each tube. Gently vortex the cells and incubated for 30 min at 37°C in the dark. Analyzed by flow cytometry.

4.2.5. In vivo anti-tumor efficacy

All experiments with animals were performed in compliance with the relevant laws and institutional guidelines of China animal experiments, including Laboratory Animal Management Regulations and Guidance on the Treatment of Experimental Animals. The Institutional Animal Care and Committee of University of South China [permit number: SYXK (Xiang) 2015-0001] have approved the experiments.

Gastric tumor cell line MFC was chosen as a model tumor to investigate the anti-tumor efficacy of the three doses of compound **15** (10 mg/kg, 20 mg/kg, 40 mg/kg). Gastric tumor-bearing mice were established by subcutaneous injection of MFC cells in the ventral part that suspended in saline to 1×10^7/0.2 ml/mouse (defined

View Article Online
DOI: 10.1039/C7MD00578D

as day 0). On day 1, the mice were randomly divided into five groups (n = 6) and treated with three doses of compound **15**, 5-Fu (positive control 25 mg/kg) and saline (control) via a intraperitoneal injection on days 2, 5, 8, 11 and 14. From the 6th day on, tumor volumes were monitored every other day by measuring two perpendicular diameters using Vernier caliper and calculated using the formula: $\text{Volume} = 0.5 \times \text{Length} \times (\text{Width})^2$. Body weights were recorded every other day. On day 18, the animals were sacrificed and the tumor mass was dissected, weight, and photographed. The tumor inhibition ratio (TIR) was calculated using the formula: $\text{TIR}(\%) = \left(1 - \frac{Wt}{Ws}\right) \times 100\%$, where Wt and Ws represent the average tumor weight of the treatment and saline groups, respectively.

Acknowledgements

This work was financially supported the Construct Program of the key Discipline in Hunan province and Hun an Province Cooperative Innovation Center for Molecular Target New Drug Study.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. P. G. G. Gordon M. Cragg, and David J. Newman, *Chem. Rev.*, 2009, **109**, 3012-3043.
2. M. Singh, M. Kaur and O. Silakari, *Eur. J. Med. Chem.*, 2014, **84**, 206-239.
3. S. K. Katiyar and M. Athar, *Cancer Prev. Res.*, 2013, **6**, 617-621.
4. V. Izzi, L. Masuelli, I. Tresoldi, P. Sacchetti, A. Modesti, F. Galvano and R. Bei, *Front. Biosci.*, 2012, **17**, 2396-2418.
5. G. R. Beecher, *Am. Soci. Nutr. Sci.*, 2003, **133**, 3248-3254.
6. W. Huang, Q. Chen, W. C. Yang and G. F. Yang, *Eur. J. Med. Chem.*, 2013, **66**, 161-170.
7. T. P. T. Cushnie and A. J. Lamb, *Int. J. Antimicrob. Agents*, 2005, **26**, 343-356.
8. B. Havsteen, *Biochem. Pharmacol.*, 1983, **32**, 1141-1148.
9. M. Lopez-Lazaro, *Curr. Med. Chem. Anti-Cancer Agents*, 2002, **2**, 691-714.
10. Y. Li, H. Fang and W. Xu, *Mini-Rev. Med. Chem.*, 2007, **7**, 663-678.
11. W. Y. Ren, Z. H. Qiao, H. W. Wang, L. Zhu and L. Zhang, *Med. Res. Rev.*, 2003, **23**, 519-534.
12. S. Ramos, *Mol. Nutr. & Food Res.*, 2008, **52**, 507-526.
13. S. Rahmani-Nezhad, M. Safavi, M. Pordeli, S. K. Ardestani, L. Khosravani, Y. Pourshojaei, M. Mandavi, S. Emami, A. Foroumadi and A. Shafiee, *Eur. J. Med. Chem.*, 2014, **86**, 562-569.

View Article Online
DOI: 10.1039/C7MD00578D

14. G. R. Pettit, J. W. Lippert, D. L. Herald, E. Hamel and R. K. Pettit, *J Nat. Prod.*, 2000, **63**, 969-974.

15. G. J. S. Rustin, S. M. Galbraith, H. Anderson, M. Stratford, L. K. Folkes, L. Sena, L. Gumbrell and P. M. Price, *J. Clin. Oncol.*, 2003, **21**, 2815-2822.

16. G. R. Pettit, C. Temple, Jr., V. L. Narayanan, R. Varma, M. J. Simpson, M. R. Boyd, G. A. Rener and N. Bansal, *Anti-cancer Drug Des.*, 1995, **10**, 299-309.

17. G. R. Pettit and J. W. Lippert, *Anti-Cancer Drug Des.*, 2000, **15**, 203-216.

18. G. Micheletti, M. Poli, P. Borsotti, M. Martinelli, B. Imberti, G. Taraboletti and R. Giavazzi, *Cancer Res.*, 2003, **63**, 1534-1537.

19. G. Kremmidiotis, A. F. Leske, T. C. Lavranos, D. Beaumont, J. Gasic, A. Hall, M. O'Callaghan, C. A. Matthews and B. Flynn, *Mol. Cancer Ther.*, 2010, **9**, 1562-1573.

20. J. Lee, S. J. Kim, H. Choi, Y. H. Kim, I. T. Lim, H.-m. Yang, C. S. Lee, H. R. Kang, S. K. Ahn, S. K. Moon, D.-H. Kim, S. Lee, N. S. Choi and K. J. Lee, *J. Med. Chem.*, 2010, **53**, 6337-6354.

21. S. Dey, S. Sarkar, H. Paul, E. Zangrando and P. Chattopadhyay, *Polyhedron*, 2010, **29**, 1583-1587.

22. M. Devereux, O. S. D, A. Kellett, M. McCann, M. Walsh, D. Egan, C. Deegan, K. Kedziora, G. Rosair and H. Muller-Bunz, *J. Inorg. Biochem.*, 2007, **101**, 881-892.

23. Z. M. Nofal, E. A. Soliman, S. S. Abd El-Karim, M. I. El-Zahar, A. M. Srour, S. Sethumadhavan and T. J. Maher, *Acta Pol. Pharm.*, 2011, **68**, 519-534.

24. N. S. El-Gohary and M. I. Shaaban, *Eur. J. Med. Chem.*, 2017, **131**, 255-262.

25. M. Rashid, A. Husain, R. Mishra, S. Karim, S. Khan, M. Ahmad, N. Al-wabel, A. Husain, A. Ahmad and S. A. Khan, *Arab. J. Chem.*, 2015, DOI: 10.1016/j.arabjc.2015.08.019.

26. J. Sasaki, R. Ramesh, S. Chada, Y. Gomyo, J. A. Roth and T. Mukhopadhyay, *Mol. Cancer Ther.*, 2002, **1**, 1201-1209.

27. N. Doudican, A. Rodriguez, I. Osman and S. J. Orlow, *Mol. Cancer Res.*, 2008, **6**, 1308-1315.

28. L. Nardinocchi, V. Pantisano, R. Puca, M. Porru, A. Aiello, A. Grasselli, C. Leonetti, M. Safran, G. Rechavi, D. Givol, A. Farsetti and G. D'Orazi, *Plos One*, 2010, **5**, e15048.

29. J. J. Chen, Y. L. Li, *Journal of Xiamen University (Natural Science)*, (In Chinese), 1992, **31**, 651-656.

30. J. Howell and M. Rasmussen, *Australian Journal of Chemistry*, 1993, **46**, 1177-1191.

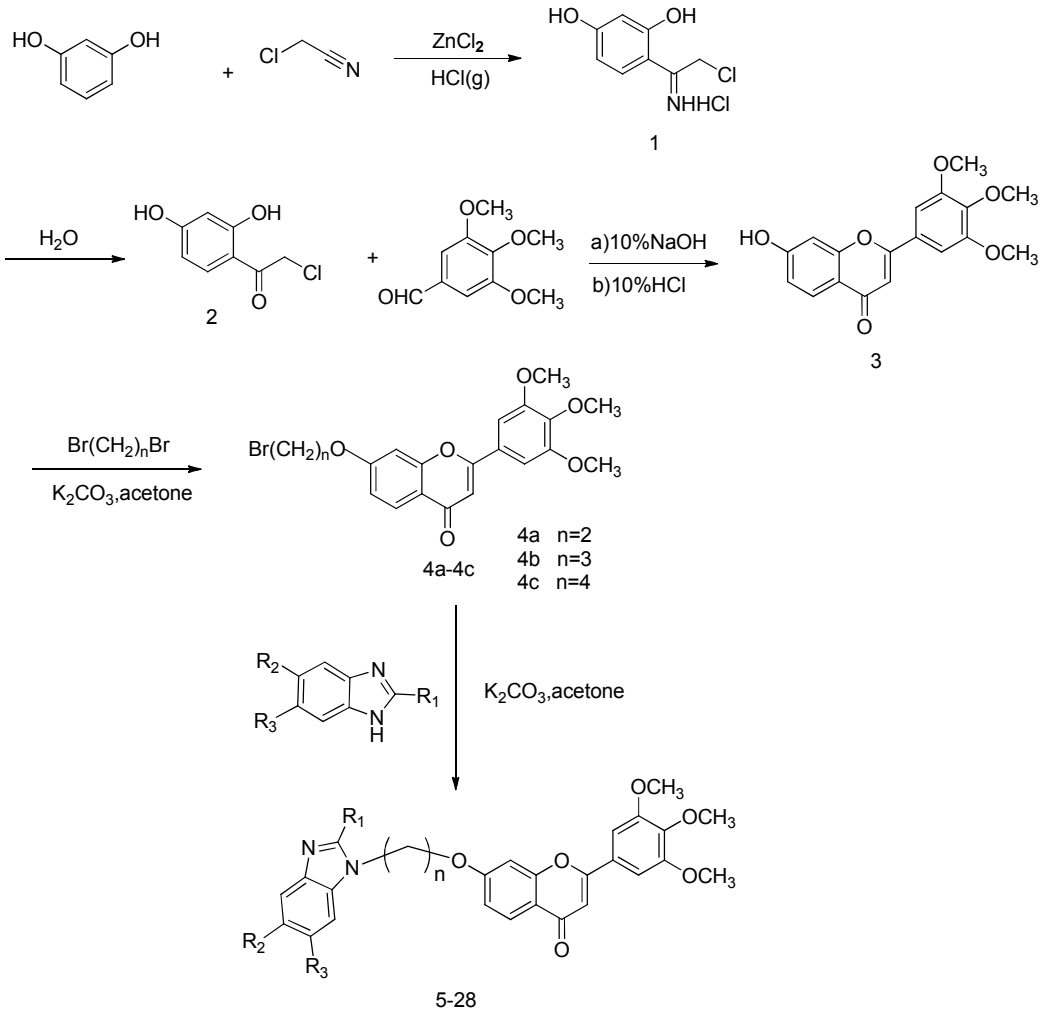
View Article Online
DOI: 10.1039/C7MD00578D

31. C. J. Sherr, *Science* (New York, N.Y.), 1996, **274**, 1672-1677.

32. T. Sandal, *Oncologist*, 2002, **7**, 73-81.

33. R. Pratap, M. Satyanarayana, C. Nath, R. Raghubir, A. Puri, R. Chander, P. Tiwari, B. K. Tripathi, A. K. Srivastava, WO 2006040621 A1, 2006.

View Article Online
DOI: 10.1039/C7MD00578D



5	n=2 R_1=H,R_2=H,R_3=H	13	n=3 R_1=H,R_2=H,R_3=H	21	n=4 R_1=H,R_2=H,R_3=H
6	n=2 R_1=CH_3,R_2=H,R_3=H	14	n=3 R_1=CH_3,R_2=H,R_3=H	22	n=4 R_1=CH_3,R_2=H,R_3=H
7	n=2 R_1=Cl,R_2=H,R_3=H	15	n=3 R_1=Cl,R_2=H,R_3=H	23	n=4 R_1=Cl,R_2=H,R_3=H
8	n=2 R_1=H,R_2=H,R_3=NO_2	16	n=3 R_1=H,R_2=H,R_3=NO_2	24	n=4 R_1=H,R_2=H,R_3=NO_2
9	n=2 R_1=H,R_2=CH_3,R_3=CH_3	17	n=3 R_1=H,R_2=CH_3,R_3=CH_3	25	n=4 R_1=H,R_2=CH_3,R_3=CH_3
10	n=2 R_1=H,R_2=CH_3,R_3=H	18	n=3 R_1=H,R_2=CH_3,R_3=H	26	n=4 R_1=H,R_2=CH_3,R_3=H
11	n=2 R_1=CF_3,R_2=H,R_3=H	19	n=3 R_1=CF_3,R_2=H,R_3=H	27	n=4 R_1=CF_3,R_2=H,R_3=H
12	n=2 R_1=H,R_2=OCH_3,R_3=H	20	n=3 R_1=H,R_2=OCH_3,R_3=H	28	n=4 R_1=H,R_2=OCH_3,R_3=H

Scheme 1 General synthesis of compounds 5-28.

View Article Online
DOI: 10.1039/C7MD00578D

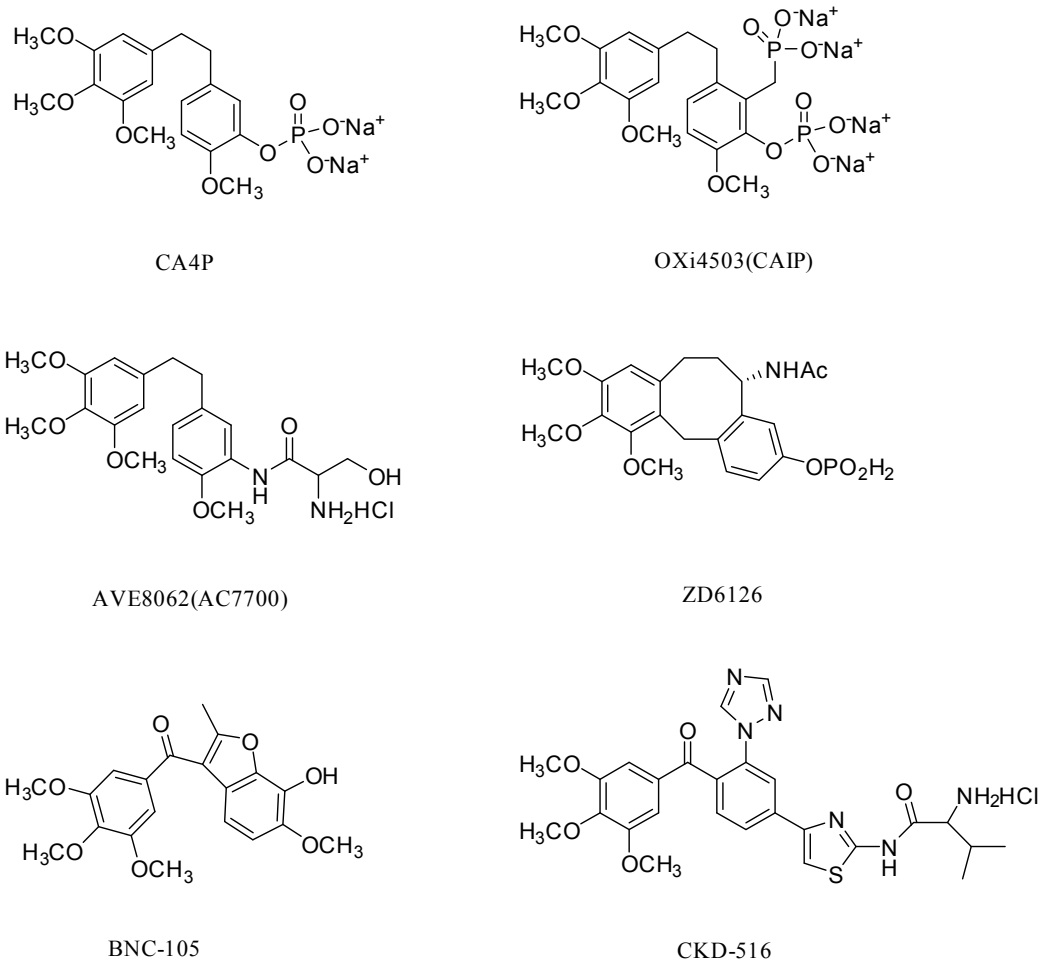


Fig. 1 The structures of CA4P, OXi4503, AVE8062, ZD6126, BNC-105 and CKD-516.

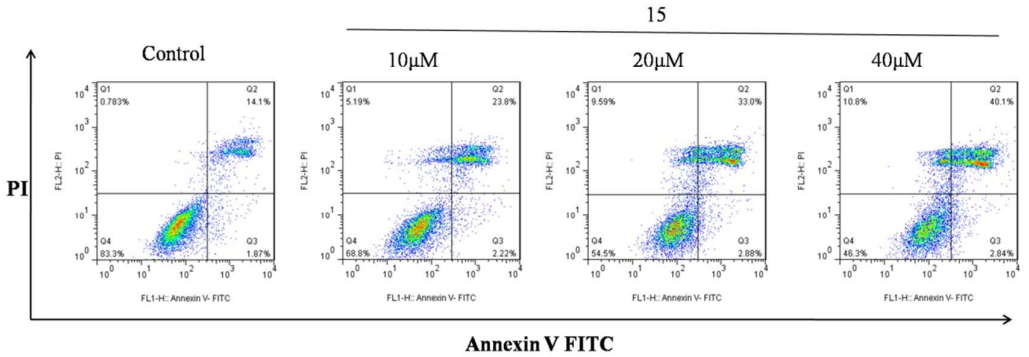


Fig. 2 MFC cells were cultured with various concentration of compound 15 for 24 h. Cells were stained by Annexin V-FICT/PI and apoptosis was analyzed by flow cytometry.

View Article Online
DOI: 10.1039/C7MD00578D

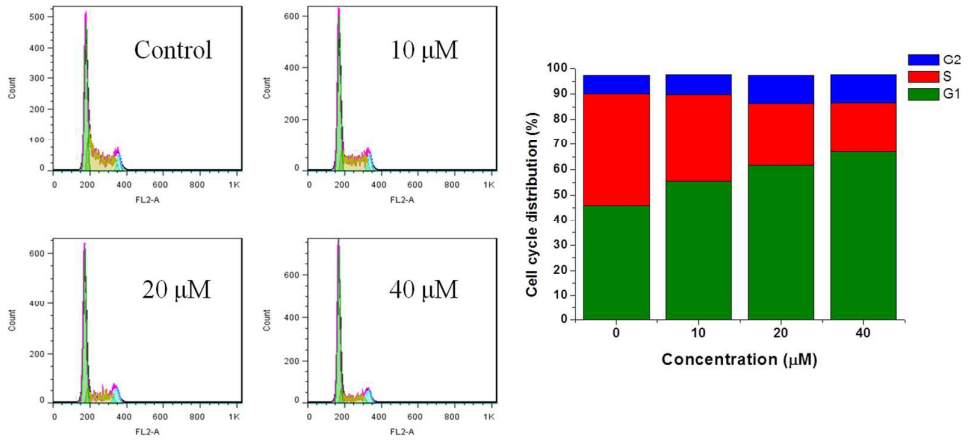


Fig. 3 Flow cytometry analysis of cell cycle distribution for MFC cells treated with compound 15 (0, 10, 20, 40 μmol/L) for 24 h.

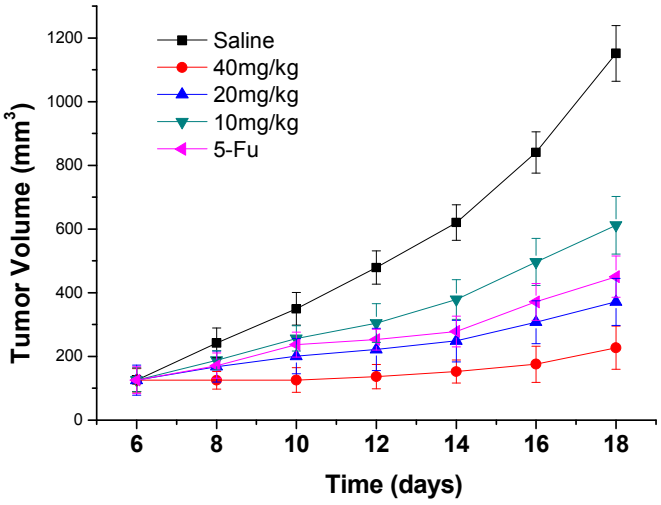


Fig. 4 Tumor volume growth curves of the mice after treatment with saline, different concentrations of compound 15 (10, 20, 40 mg/kg) and 5-Fu. Data were showed as a mean ± SD (n = 6). The difference of tumor volume growth among five groups was statistically significant ($P<0.05$).

View Article Online
DOI: 10.1039/C7MD00578D

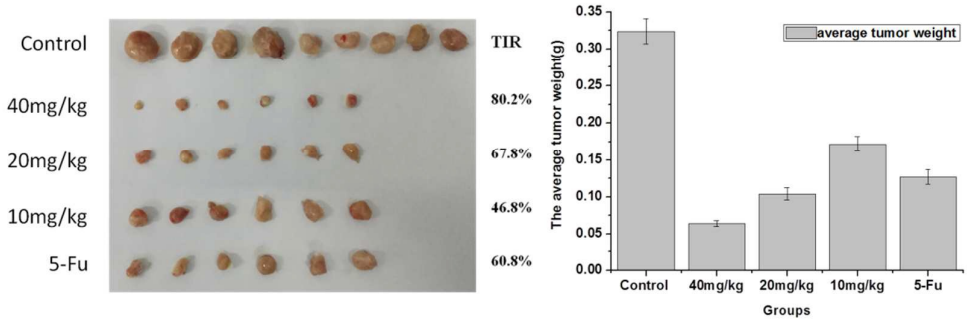


Fig. 5 Photographs of the harvested tumors from the mice of 18 days (left); Average tumor weight of the mice of 18 days (right).

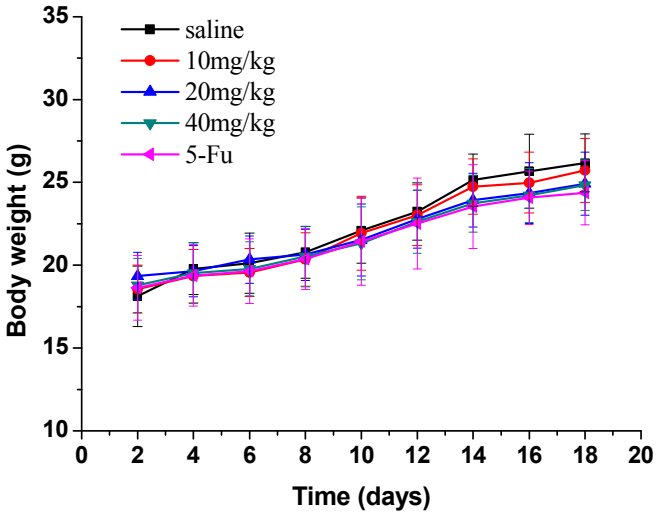


Fig. 6 Body weight evolution curves of the mice after treatment with saline, different concentrations of compound 15 (10, 20, 40 mg/kg) and 5-Fu. Data were showed as a mean ± SD (n = 6). No significant body weight loss was observed compared with the saline group.

View Article Online
DOI: 10.1039/C7MD00578D

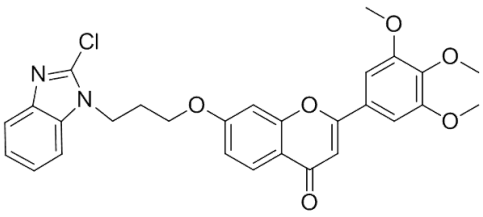
Table 1 Anti-proliferative activity of compounds against the tumor cell lines

compounds	IC_{50} (μM) ±SD			
	MGC-803	MCF-7	HepG-2	MFC
5	>100	>100	>100	>100
6	94.47±5.21	>100	84.32±3.54	87.68±5.47
7	75.12±3.47	87.34±4.39	82.53±3.28	72.56±2.83
8	91.54±4.26	95.17±3.21	87.53±3.28	90.73±5.14
9	>100	N.D	N.D	N.D
10	84.58±3.42	>100	>100	>100
11	75.43±3.12	84.71±4.17	>100	80.37±3.62
12	>100	N.D	>100	>100
13	N.D	N.D	N.D	>100
14	77.52±3.71	91.46±4.36	>100	88.46±3.16
15	20.47±2.07	43.42±3.56	35.45±2.03	23.47±3.59
16	83.14±2.52	90.47±5.32	>100	88.43±3.63
17	N.D	>100	N.D	N.D
18	97.38±5.54	>100	N.D	>100
19	77.27±4.76	92.37±3.62	86.83±4.73	82.24±3.81
20	>100	N.D	N.D	90.32±2.17
21	94.38±4.76	N.D	N.D	>100
22	73.62±4.73	81.43±2.57	>100	77.42±4.37
23	63.51±2.64	78.34±3.53	76.82±5.39	67.15±4.26
24	54.86±4.28	>100	72.48±3.51	62.37±5.14
25	N.D	N.D	>100	>100
26	95.62±5.34	87.46±4.17	>100	90.01±3.23
27	76.27±3.74	62.37±2.35	>100	58.43±4.43
28	84.73±5.12	>100	N.D	>100
5-Fu	74.39±2.03	57.09±3.17	63.37±2.52	78.52±3.92

SD=standard deviation

N.D=not detected

View Article Online
DOI: 10.1039/C7MD00578D



Compound 15

IC_{50}: 23.47 ± 3.59 μM
(against MFC cells)