Accepted Manuscript

Design, synthesis and biological evaluation of novel benzimidazole-2-substituted phenyl or pyridine propyl ketenederivatives as antitumour agents

Lin-tao Wu, Zhi Jiang, Jia-jia Shen, Hong Yi, Yue-chen Zhan, Ming-quan Sha, Zhen Wang, Si-tu Xue, Zhuo-rong Li

PII: S0223-5234(16)30203-3

DOI: 10.1016/j.ejmech.2016.03.029

Reference: EJMECH 8454

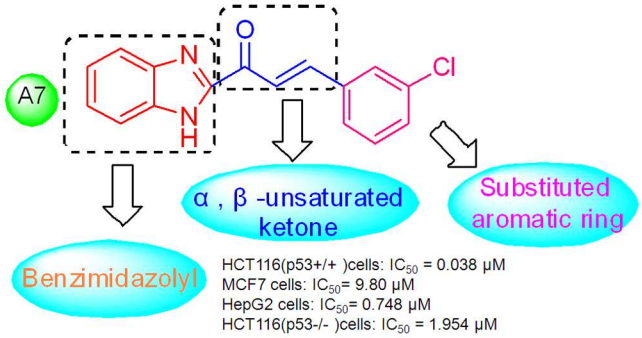
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 23 October 2015

Revised Date: 8 March 2016

Accepted Date: 10 March 2016

Please cite this article as: L.-t. Wu, Z. Jiang, J.-j. Shen, H. Yi, Y.-c. Zhan, M.-q. Sha, Z. Wang, S.-t. Xue, Z.-r. Li, Design, synthesis and biological evaluation of novel benzimidazole-2-substituted phenyl or pyridine propyl ketenederivatives as antitumour agents, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.03.029.



HCT116(p53+/+)cells: IC_{50} = 0.038 μM
MCF7 cells: IC_{50}= 9.80 μM
HepG2 cells: IC_{50}= 0.748 μM
HCT116(p53-/-)cells: IC_{50} = 1.954 μM

Design, synthesis and biological evaluation of novel benzimidazole-2-substituted phenyl or pyridine propyl ketenederivatives as antitumour agents

Lin-tao Wu[†], Zhi Jiang[†], Jia-jia Shen, Hong Yi, Yue-chen Zhan, Ming-quan Sha, Zhen Wang, Si-tu Xue[*], Zhuo-rong Li[*]

Institute of Medicinal Biotechnology, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050, People's Republic of China

[†]These authors made equal contributions to this work.

[*]Corresponding authors. E-mail addresses: l-z-r@263.net (Z.-r. Li); xuesitubear@sina.com (S.-t. Xue)

Abstract

A series of novel benzimidazole-2-subsituted phenyl or pyridine propyl ketenederivatives were designed and synthesized. The biological activities of these derivatives were then evaluated as potential antitumour agents. These compounds were assayed for growth-inhibitory activity against HCT116, MCF-7 and HepG2 cell lines in vitro. The IC_{50} values of compounds **A_1** and **A_7** against the cancer cells were 0.06-3.64 μM and 0.04-9.80 μM, respectively. Their antiproliferative activities were significantly better than that of 5-Fluorouracil (IC_{50}: 56.96-174.50 μM) and were close to that of Paclitaxel (IC_{50}: 0.026-1.53 μM). The activity of these derivatives was over 100 times more effective than other reported structures of chalcone analogues (licochalcone A). A preliminary mechanistic study suggested that these compounds inhibit p53-MDM2 binding. Compounds **A_1**, **A_7** and **A_9** effectively inhibited tumour growth in BALB/c mice with colon carcinoma HCT116 cells. The group administered 200 mg/kg of compound **A_7** showed a 74.6% tumour growth inhibition with no signs of toxicity at high doses that was similar to the inhibition achieved with the 12.5 mg/kg irinotecan positive control (70.2%). Therefore, this class of

benzimidazole-2-subsituted phenyl or pyridine propyl ketene derivatives represents a promising lead structure for the development of possible p53-MDM2 inhibitors as new antitumour agents.

Keywords: benzimidazole; antiproliferative activities; chalcone; p53-MDM2

1. Introduction

Malignant neoplasms are a major disease jeopardizing human life and health, and the morbidity and mortality associated with this disease is continuously rising[1]. Currently, over 70 anti-tumour drugs are being clinically applied, among which many are obtained from botanical sources, such as nitrogen mustard, vincaleucoblastine, taxoland flavonoids. Chalcones ubiquitously occur in medicinal plants, including *Glycyrrhizauralensis*[2], *Humuluslupulus*[3] and *Oxytropisfalcata*[4]. The flexibility of the chalcone molecule has made it possible to bind it to various receptors; thus, chalcones have shown diverse bio-activities[5-11]. In 2000, De Vincenzo found the anti-tumour activity of chalcones was a subtype of flavonoids; however, many reports have been published on the anti-tumour activity of chalcones bearing alkaline substituents[12]. The work by Yi Xia's group is the earliest record of the inhibitory effect of amino-chalcones on the proliferation of tumour cells[13]. Recent research suggests nitrogen-containing flavonoid derivatives significantly inhibit tumours[14].

Benzimidazoles are a group of hetero-cyclic organic compounds that show multiple bio-activities. Structurally, benzimidazole consists of two aromatic N-heterocycles that can bind to enzymes or receptors via hydrogen bonds coordinated with metal ions or hydrophobic interactions. Therefore, research on the medical use of benzimidazoles has been favoured and fruitful. As proton pump inhibitors (PPIs), anti-parasite reagents and anti-atherosclerosis reagents, benzimidazoles have been investigated for years. Many approved drugs consist of a benzimidazole skeleton, e.g., omeprazole, lansoprazole, albendazoleand oxfendazole. Gupta *et al*. have described the radiosensitization activity of benzimidazoles on the Chinese hamster cell line V-79[15]. However, the long-term application of benzimidazole has been plagued by

the reoccurrence of neoplasms during therapy and other restrictions. In recent years, a new series of benzimidazole derivatives was developed using a broad substitution pattern. A large number of derivatives were obtained, and the cellular cytotoxicity of these compounds was evaluated in vitro against different human tumour cell lines. The GI_{50} values reached the low micromolar level[16-18]. These promising results set the foundation for future investigations into the development of anticancer therapies.

Indeed, substructure combinations (chalcone and benzimidazolyl groups) that maintain their original effective characteristics may exhibit a synergistic effect and an improved anticancer activity.

A series of benzimidazole-2-subsituted phenyl or pyridine propyl ketenes (Fig 1) were synthesized and assayed for their antiproliferative activity against several cancer cell lines in vitro. In addition, preliminary structure-activity relationships (SAR) were determined. These compounds contained chalcone and benzimidazolyl structures.

Moreover, chalcone derivatives can act as anticancer agents through inhibition of MDM2-p53[19]. The anti-tumour mechanism of the novel compounds were preliminary investigated using Nutlin-3a as a positive control. Compounds that possessed consider ablepotency in HCT116 wild type ($p53^{+/+}$**)** cells were further evaluated for their activities in HCT116 deficient ($p53^{-/-}$) cells to determine if the antiproliferative activity was p53-dependent.The potential of these novel compounds to inhibit p53-MDM2 interactions were verified using an immunoprecipitation assay and cell cycle analysis.

2. Results and Discussion

2.1 Chemistry

Based on the analysis of the compound structures (Fig 1), an α, β-unsaturated ketone fragment was introduced to connect the benzimidazolyl ring and phenyl ring A. Compounds $\mathbf{A_1}$-$\mathbf{A_{13}}$ were designed with the benzimidazolyl ring and α, β-unsaturated ketone scaffold. The initial optimization of the scaffold began with the benzimidazolyl ring. By substituting the benzimidazolyl ring with a benzoxazolyl or benzothiazolyl group, compounds $\mathbf{B_1}$-$\mathbf{B_3}$ and $\mathbf{C_1}$-$\mathbf{C_3}$ were designed, respectively.

Modification of the α, β-unsaturated ketone fragment by reducing the carbonyl group or the double bond resulted in compounds **D_1-D_3** and **E_1-E_3**, respectively.

The synthesis of the target compounds was completed according to the procedures outlined in Scheme 1. A series of benzimidazolyl compounds **A_1-A_{13}** were synthesized by using commercially available phenylamine **1a** as the starting material. Compound **1a** was reacted with an equal equivalent of lactic acid to yield **1b.** The intermediate **1b** was then oxidized by an equalequivalent of chromium trioxide in acetic acid under reflux to afford **1c**[20]. Compound **1c** was further condensed with 1.2 equivalents of a variety of aromatic aldehydes in ethanol using NaOH as the base to produce the target compounds **A_1-A_{13}**. Benzoxazolyl or benzothiazolyl α, β-unsaturated ketone compounds **B_1-B_3** and **C_1-C_3** were obtained in good yields via the same procedure as described for the synthesis of compounds **A_1-A_{13}**, except for the use of **2a** or **3a** as the starting material, respectively[21, 22].Compounds **A_1**, **A_7** and **A_9** were reduced by $NaBH_4$ at -78 °C or by H_2 with 10% Pd/C to yield the hydroxyl compounds **D_1-D_3** and the α, β-saturated ketone compounds **E_1-E_3**, respectively.

2.2 In vitro antiproliferative activities and structure-activity relationships

The synthesized compounds **A_1-A_{13}** were examined for their antiproliferative activities after 48 h of exposure time using the MTT assay in three cell lines: HCT116, HepG2 and MCF-7.

To evaluate the tumour cell inhibition of the derivatives, 5-Fluorouracil and Paclitaxel, which exhibit a broad spectrum of anti-tumour activity, were selected as the positive controls for the in vitro experiment. The results are summarized in Table 1. Generally, all of the tested compounds **A_1-A_{13}** showed significantly more potent antiproliferative activities than 5-Fluorouracil against HCT116 cells (IC_{50} of **A_1-A_{13}**: 0.03-19.56 μM, 5-Fluorouracil IC_{50}: 56.96 μM) and HepG2 cells (IC_{50} of **A_1-A_{13}**: 0.34-29.17 μM, 5-Fluorouracil IC_{50}: 174.5 μM). The majority of the derivatives (except for compound **A_{12}**) showed higher absolute antiproliferative activities (IC_{50}: 3.64-62.49 μM) compared with 5-Fluorouracil(IC_{50}: 222.6 μM) in the MCF-7 cell line.

Compounds **A_1** and **A_7** displayed excellent activities against the three tested cell lines. For HCT116, HepG2 and MCF-7 cells, the IC_{50} values of **A_1** were 0.062, 3.64 and 0.34 μM, respectively. The IC_{50} values of **A_7** were 0.03, 9.80 and 0.75 μM, respectively. Among the derivatives, compound **A_7** showed a strong activity against HCT116 cells (IC_{50}: 0.03 μM) that was comparable to Paclitaxel (IC_{50}: 0.0672 μM).The antiproliferative activities of **A_1** and **A_7** were equivalent or more potent that the reported compounds being used in clinical trials. Under our experimental conditions, the activities of **A_1** and **A_7**showed an approximate 950-1400-fold increase against HCT116 cells compared with 5-Fluorouracil.

To our knowledge, a wide spectrum of biological activities, including antitumour and cytotoxic properties, has been reported for chalcones. However, benzimidazole-2-substituted phenyl or pyridine propyl ketenederivatives have never been reported as a series of potential antineoplastic active agents. Thus, this is the first publication that suggests that benzimidazole-2-subsituted phenyl or pyridine propyl ketenederivatives have antitumour activity. The antiproliferative activities of compounds **A_1**, **A_7** and **A_9** were significantly better (over 100-fold) than those reported for the chalconelicochalconeA[23].

With regard to antitumour activity, compounds with a substitution at the 3-position of the benzene ring (**A_7**-**A_9**) were superior to those with substitutions at the 2- and 4-positions (**A_4**-**A_6** and **A_{10}**-**A_{12}**) or no substitution on the phenyl group. Notably, compound **A_7** possesses a 3-Cl substitution on the phenyl ring and exhibited the best inhibitory activity (IC_{50}: 0.03 μM) against HCT116 cells; however, this compound showed a weak erantiproliferative activity against MCF-7 cells compared with HCT116 and HepG2 cells. When the phenyl group was replaced by a 2-pyridine ring, the compound obtained **A_1**, also showed a high inhibitory activity (IC_{50}: 0.06 μM) against HCT116 cells. However, replacing the phenyl ring with a 3- or 4-pyridine ring (**A_2**, **A_3**) led to a decreased antiproliferative activity.

2.3 In vitro antiproliferative activity for HCT116 (p53-/-)

Chalcone derivatives act as anticancer agentsvia inhibition of MDM2-p53[16].

Benzimidazole-2-subsituted phenyl or pyridine propyl ketenederivatives have a chalcone analogue structure. The antitumour mechanisms of the article compounds were preliminarily investigated. To evaluate cellular selectivity, we chose compounds **A_1**, **A_3**, **A_5**, **A_7**, **A_8**, **A_9** and **A_{13}** to confirm whether the antiproliferative activity of these compounds were p53-dependent *in vitro*. These compounds possessed considerable potency in HCT116 (p53+/+**)** cells. As predicted (Table 3), these compounds showed a superior potency in HCT116 (p53+/+**)** cells (IC_{50}: 0.04-0.78 μM) compared with p53 deficient HCT116 cells (IC_{50}: 1.45-5.61 μM).This result suggests that the target compounds act in a p53-dependent manner.

2.4 Cell cycle analysis

We further analyzed the antitumour mechanisms of the three compounds using FACS analysis to determine the alterations of cell cycle distribution in HCT116 cells. Nutlin-3abinds MDM2 in the p53-binding pocket and activates the p53 pathway in cancer cells, which leads to cell cycle arrest at G1 and apoptosis[24].The HCT116 cells were treated with compounds **A_1** (0.06 μM), **A_7** (0.04 μM) and **A_9** (0.58 μM) for 72 h, and Nutlin-3a was used as the positive control. Next, cells were stained with propidium iodide (PI) after fixation and detected using flow cytometry. As shown in Table 2, all of the tested compounds and Nutlin-3a delayed cell cycle progression by arresting the cell cycle in the G_1 phase after 72 h. Accordingly, HCT116 cell growth was slowed. These results indicate that the mechanism of action for the novel derivatives is likely via inhibition ofp53-MDM2 binding.

2.5 Immunoprecipitation analysis

Compounds **A_1**, **A_7** and **A_9** displayed excellent inhibitory activities. Thus, we further verified that these three compounds inhibited p53-MDM2 interactions using an immunoprecipitation assay. MCF-7 cells were used to assess the effects of compounds **A_1**, **A_7** and **A_9** after treatment with either the test compounds at the indicated concentration (μM)or 50 μM Nutlin-3a for 24 h. As shown in Figure 2, all three compounds at both test concentrations significantly inhibited the binding of p53

to MDM2 compared with the control group free of any drug treatment. The results were similar to the Nutlin-3a positive control.

2.6 Antitumour activities in vivo

We examined the antitumour activities of compounds **A_1**, **A_7** and **A_9** in BALB/c mice with colon carcinoma HCT116 cells. To determine the maximum administration dosage, acute toxicity tests were performed at the Institute of Cancer Research (ICR) with female mice. Compounds **A_1**, **A_7** and **A_9** were dissolved in 10% DMSO and administered via a single intraperitoneal injection to three groups of BALB/c mice (eight mice per group). Following the Bliss test protocol, the LD_{50} values were calculated (**A_1**: 634 mg/kg; **A_7**: 692 mg/kg; and **A_9**: 1221 mg/kg) and indicated that the three compounds were of low toxicity.

Next, **A_1**, **A_7** and **A_9**, were evaluated for their ability to inhibit tumour growth in a female BALB/c mice model. To evaluate the differences in activity levels between derivatives and first-line medicines used to treat bowel cancer, Irinotecan was selected as the positive control for the in vivo laboratory experiment. This study comprised nine groups (eight mice per group):a vehicle control group, a blank group and seven treatment groups that included a positive control (Irinotecan at 12.5 mg/kg)and two doses of **A_1**, **A_7** and **A_9** (100 and 200 mg/kg). HCT116 cells were injected subcutaneously into the flanks of mice (1×10^7 cells). The tumours were allowed to develop for eight days until they reached approximately 100 mm^3. Next, the compounds were administered intraperitoneally twice per week for four weeks. At the end of the experiment, the mice were killed, and the tumours were excised. Tumour sizes and body weights were measured for further biochemical analysis. As shown in Figure 3A, treatment with these three compounds showed a dose-dependent inhibition of tumour growth. The group treated with 200 mg/kg of compound **A_7** showed a 74.6% tumour growth inhibition that was similar to the positive control of 12.5 mg/kg Irinotecan (TGI: 70.2%)(Figure 3A).

There was no significant difference in body weight among the groups treated with the three test compounds. The body weight of the Irinotecan-treated group was

reduced by 3.66 g at a dose of 12.5 mg/kg (Figure 3B). These data show that the target compounds were well-tolerated in mice at doses of 100 and 200 mg/kg.

3. Conclusions

In this study, a series of benzimidazole-2-subsituted phenyl or pyridine propyl ketenederivatives were synthesized and evaluated for their biological activities. These compounds exhibited strong antiproliferative activities against HCT116, MCF-7 and HepG2 cells. Among these derivatives, compounds **A_1**, **A_7** and **A_9**, exhibited the most potent antitumour activity in the three tumour cell lines. The potency of their antiproliferative activities was significantly better than the positive control of 5-Fluorouracil, over 100-fold superior to the chalconelicochalcone A and similar to those of Paclitaxel (IC_{50}: 0.026–1.53 μM).

Several experiments were completed to explore the possible mechanism of these compounds. The compounds showed a significant reduction in the antiproliferative activity against HCT116 (p53-/-) cells compared with their antiproliferative activities against HCT116 cells. A cell cycle analysis revealed a similar action as the positive control Nutlin-3a as evidence by cell cycle inhibition at the G_1 phase. Once again similar to Nutlin-3a, immunoprecipitation data showed that the compounds significantly inhibited the binding of p53 to MDM2.These results indicate that the mechanism of action for these compounds likely occurs via an inhibition of p53-MDM2 binding. In addition, these three compounds demonstrated *in vivo* antitumour activities in BALB/c mice with the colon carcinoma HCT116 cells and no sign of toxicity at high doses in the efficacy study.

Taken together, this class ofbenzimidazole-2-subsituted phenyl or pyridine propyl ketenederivatives represents a promising lead structure for the development of new antitumour agents.

4. Experimental Section

4.1 *Synthesis and characterization*

The ^{1}H NMR and ^{13}C NMR spectra were recorded using TMS as the internal

standard in DMSO-d_6 or $CDCl_3$ with a BrukerBioSpin GmbH spectrometer at 300, 400 and 600 MHz, respectively. The mass spectra (MS) were recorded on a Thermo Scientific LTQ ORBITRAP instrument with an ESI mass selective detector. The melting points (m.p.) were determined using an SRS-OptiMelt automated melting point instrument without correction. Flash column chromatography was performed with silica gel (200-300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd.

General procedure A for the preparation of A_1-A_{13}, B_1-B_3 and C_1-C_3

Various *o*-substituted anilines (0.05 mol) and lactic acids (3.75 mL, 0.05 mol) were dissolved in 20 mL of HCl (4 M). The mixture was heated under reflux for 1.5 h. After cooling to room temperature, the reaction mixture was poured into water. Next, the pH was adjusted to 10-11 with ammonia. The mixture was filtered, and the residue was recrystallized from water to give intermediates **1b**-**3b**. Next, **1b**-**3b** (0.02 mol) were each dissolved in 15 mL of acetic acid. When the mixture was heated to 90°C, chromium trioxide solution was added. The reaction mixture was stirred at 105°C for 30 min. After cooling to room temperature, the reaction mixture was poured into water (1 L) and then extracted by ethyl acetate (EtOAc). The organic layers were combined, dried with $MgSO_4$, filtered and then concentrated under reduced pressure. The residue was recrystallized from toluene to give the intermediates **1c**-**3c**. Last, **1c**-**3c** (0.02 mol) and 8 mL of a 10% NaOH aqueous solution were dissolved in 20 mL of ethanol. The reaction mixture became a clear red solution. Next, various substituted aromatic aldehydes (0.02 mol) were added. After stirring for 5 h, the mixture was filtered, and the residue was purified by column chromatography (EtOAc : hexane = 1:5) to give the target compounds **A_1-A_{13}**, **B_1-B_3** and **C_1-C_3**.

General procedure B for the preparation of D_1-D_3

$CeCl_3$ was added to a solution of compounds **I-a**(0.02 mol) in dichloromethane(10 mL). The mixture was cooled at -78°C for 1.5 h. After sodium borohydride was added, the reaction mixture was stirred at room temperature for 3 h. The mixture was washed with water and extracted with dichloromethane. The organic

layers were combined, dried with $MgSO_4$, filtered and then concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:hexane = 1:2) to give the target compounds **D_1-D_3**.

General procedure C for the preparation of E_1-E_3

A 10% Pd/C (0.002 mol) mixture was added as a catalyst to a solution of compounds **I-a** (0.02 mol) in methanol. The reaction mixture was stirred under a H_2 atmosphere for 4 h. The catalyst was filtered, and the combined organic solutions were concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc ： hexane = 1:6) to give the target compounds **E_1-E_3**.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(pyridin-2-yl)prop-2-en-1-one (A_1)

Following general procedure A, compound **1a** was reacted with 2-pyridinecarboxaldehyde to afford compound **A_1** as a bright yellow solid (yield 56%), m.p. 193-195°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.43-7.46 (dd, *J* = 6.0 Hz, 6.0 Hz, 2H), 7.71-7.73 (t, *J* = 6.0 Hz, 1H), 7.77-7.79 (dd, *J* = 6.0 Hz, 6.0 Hz, 2H), 7.99-8.02 (d, *J* = 18.0 Hz, 1H), 8.16-8.22 (m, 2H), 8.53-8.56 (d, *J* = 18.0 Hz, 1H)，8.85-8.86 (d, *J* = 6.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.93, 125.24, 126.19, 126.31, 126.44, 126.83, 137.98, 139.84, 140.72, 139.83, 147.80, 148.02, 180.11; HRMS (ESI) m/z calcd for $[C_{15}H_{12}ON_3+H]^+$, 250.0975; found, 250.0973; IR (KBr, cm^{-1}) v: 3275.6, 1667.9, 1614.7, 1567.3, 1472.8, 1438.5, 1327.4, 1231.1, 1088.9, 1051.9, 969.2, 783.9, 734.6.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(pyridin-3-yl)prop-2-en-1-one (A_2)

Following general procedure A, compound **1a** was reacted with 3-pyridinecarboxaldehyde to afford compound **A_2** as a bright yellow solid (yield 48%), m.p. 217-220°C. ^{1}H NMR (600 MHz, DMSO): δ 7.34-7.38 (d, *J* = 24.0 Hz, 3H), 7.54 (d, *J* = 6.0 Hz, 1H), 7.74-7.77(m, 4H), 7.97 (d, *J* = 18.0 Hz, 1H), 8.16 (d, *J* = 18.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 122.91, 123.60, 128.12, 131.64, 131.89, 133.96, 137.30, 142.88, 149.40,181.38; ESI-MS (m/z): 250.2$[M+H]^+$; IR (KBr, cm^{-1})

v: 3275.6, 1668.3, 1614.4, 1495.8, 1438.6, 1324.7, 1230.3, 1090.1, 1004.0, 967.7, 823.8.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(pyridin-4-yl)prop-2-en-1-one (A_3)

Following general procedure A, compound **1a** was reacted with 4-pyridinecarboxaldehyd to afford compound **A_3** as a light grey solid(yield 50%), m.p. 200-203°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.06-7.08 (dd, *J* = 6.0 Hz, 6.0 Hz, 1H), 7.38-7.46 (m, 3H), 7.62-7.68 (m, 2H), 7.97-8.04 (dd, *J* =12.0 Hz, 6.0 Hz, 2H), 8.25-8.29 (dd, *J* =12.0 Hz, 6.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 113.33, 113.44, 126.16, 126.21, 126.55, 141.83, 143.22, 143.52, 148.28, 149.10, 150.00, 151.04, 153.22, 192.32; HRMS (ESI) m/z calcd for $[C_{15}H_{12}ON_3+H]^+$, 250.0975; found, 250.0975; IR (KBr, cm^{-1}) v: 2988.3, 1693.0, 1669.5, 1417.0, 1322.3, 1230.3, 1076.8, 952.2, 822.6, 747.3.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(2-bromo-phenyl)prop-2-en-1-one (A_4)

Following general procedure A, compound **1a** was reacted with 2-bromobenzaldehyde to afford compound **A_4** as a light yellow solid (yield 67%), m.p.147-148°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.37-7.38 (m, 2H), 7.43-7.44 (d, *J* = 6.0 Hz, 1H), 7.52-7.54 (t, *J* = 6.0 Hz, 1H), 7.74 (s, 2H), 7.78-7.79 (d, *J* = 6.0 Hz, 1H), 8.10-8.16 (m, 2H), 8.21-8.23 (d, *J* = 12.0 Hz, 1H); ^{13}C NMR (100MHz, DMSO-d_6): δ 115.48, 118.12, 123.17, 123.62, 126.56, 126.66, 127.74, 129.33, 130.01, 134.02, 137.10, 137.50, 137.69, 138.79, 142.62, 179.61; HRMS (ESI) m/z calcd for $[C_{16}H_{12}ON_2+H]^+$, 327.0128; found, 327.0128; IR (KBr, cm^{-1}) v: 3271.6, 1660.6, 1595.8, 1424.9, 1328.9, 1213.7, 1094.9, 977.6, 711.0, 737.3.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(o-tolyl)prop-2-en-1-one (A_5)

Following general procedure A, compound **1a** was reacted with 2-methylbenzaldehyde to afford compound **A_5** as a light grey solid (yield 56%), m.p. 245-246°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.50 (s, 3H), 7.33-7.34 (dd, *J* = 6.0 Hz, 6.0 Hz, 2H), 7.74 (s, 2H), 7.37-7.41 (m, 3H), 7.91 (d, *J* = 6.0 Hz, 1H), 8.05 (d, *J* =

12.0 Hz, 1H), 8.23 (d, *J* = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.05, 115.07, 118.23, 123.33, 123.62, 127.66, 128.35, 129.45, 130.07, 130.52, 133.77, 135.23, 136.03, 137.50, 137.69, 138.79, 179.23; HRMS (ESI) m/z calcd for $[C_{17}H_{15}ON_2 + H]^+$, 263.1179; found, 263.1181; IR (KBr, cm^{-1}) v: 3257.5, 1656.2, 1587.3, 1424.8, 1329.8, 1220.9, 1091.9, 977.9, 733.9, 714.4.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(2-methoxyphenyl)prop-2-en-1-one (A_6)

Following general procedure A, compound **1a** was reacted with 2-methoxylbenzaldehyde to afford compound **A_6** as a yellow solid (yield 62%), m.p. 221-224°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 3.95 (s, 3H), 7.07 (t, *J* = 12.0 Hz, 1H), 7.17 (s, 1H), 7.37 (dd, *J* = 6.0 Hz, 6.0 Hz, 2H), 7.50 (t, *J* = 12.0 Hz, 1H), 7.73 (s, 2H),7.89 (d, *J* = 6.0 Hz, 1H), 8.15 (d, *J* = 18.0, 1H), 8.23 (d, *J* = 18.0, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.79, 113.50, 115.23, 118.49, 120.84, 123.33, 123.62, 125.62, 127.84, 129.59, 130.57, 134.08, 137.34, 137.69, 138.79, 157.37, 179.57; HRMS (ESI) m/z calcd for $[C_{17}H_{15}O_2N_2+H]^+$, 279.1128; found, 279.1130; IR (KBr, cm^{-1}) v: 3224.6, 1652.1, 1576.1, 1512.6, 1426.1, 1324.6, 1243.9, 1205.5, 1164.1, 1088.3, 1045.2, 1022.5, 861.9, 824.2, 742.2.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(3-chlorophenyl)prop-2-en-1-one (A_7)

Following general procedure A, compound **1a** was reacted with 3-chlorobenzaldehyde to afford compound **A_7** as a white solid (yield 63%), m.p.172-175°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.47-7.49 (dd, *J* = 6.0 Hz, 6.0 Hz, 2H), 7.53-7.58 (m,2H), 7.80-7.82 (dd, *J* = 6.0 Hz, 6.0 Hz,2H), 7.87 (d, *J* = 6.0 Hz, 1H), 7.97-7.99 (t, *J* = 12.0 Hz, 2H), 8.12-8.15 (d, *J* = 18.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.07, 123.26, 125.99, 127.98, 128.99, 131.32, 131.46, 134.40, 136.88, 137.32, 143.77, 147.96, 179.82; HRMS (ESI) m/z calcd for $[C_{16}H_{12}ON_2Cl + H]^+$, 283.0633; found, 283.0638; IR (KBr, cm^{-1}) v: 2972.5, 1675.3, 1608.7, 1212.6, 1138.8, 1037.0, 982.4, 750.3, 677.6, 619.1.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(3-bromophenyl)prop-2-en-1-one (A_8)

Following general procedure A, compound **1a** was reacted with 3-bromobenzaldehyde to afford compound **A_8** as a light yellow solid (yield 54%), m.p.189-193°C.^{1}H NMR (600 MHz, DMSO-d_6): δ 7.38 (dd, *J* = 6.0 Hz, 6.0 Hz,2H), 7.45 (t, *J* = 18.0 Hz, 1H), 7.68-7.74 (m, 3H), 7.90-7.96 (m, 2H), 8.10 (s, 1H), 8.14-8.17 (d, *J* = 18.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 122.91, 123.60, 125.01, 128.12, 128.40, 131.64, 131.89, 133.96, 137.30, 142.88, 149.40, 181.38; HRMS (ESI) m/z calcd for $[C_{16}H_{12}ON_2Br+H]^+$, 327.0128; found, 327.0134; IR (KBr, cm^{-1}) v: 3229.4, 1661.1, 1593.6, 1556.8, 1507.9, 1484.2, 1408.2, 1322.7, 1214.9, 1069.0, 985.9, 899.4, 786.9, 746.2, 669.6, 622.4.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (A_9)

Following general procedure A, compound **1a** was reacted with 3-methoxybenzaldehyde to afford compound **A_9** as a white solid (yield 57%), m.p. 214-216°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.86 (s, 3H), 7.09-7.11 (dd, *J* = 6.0 Hz, 6.0 Hz, 1H), 7.42-7.52 (m, 5H), 7.81-7.85 (m, 2H), 7.99 (d, *J* = 12.0 Hz, 1H), 8.07-8.10 (dd, *J* =6.0 Hz, 6.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.04, 113.59, 115.12, 115.57, 118.49, 121.08, 123.03, 123.62, 127.00, 129.18, 137.50, 137.69, 137.97, 138.79, 143.18, 160.77, 179.23; HRMS (ESI) m/z calcd for $[C_{17}H_{15}O_2N_2+H]^+$, 279.1128; found, 279.1131; IR (KBr, cm^{-1}) v: 3224.6, 1671.1, 1610.7, 1487.5, 1435.8, 1322.3, 1219.3, 1140.7, 1038.1, 981.7, 898.5, 749.6, 682.9, 619.2.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(4-bromophenyl)prop-2-en-1-one (A_{10})

Following general procedure A, compound **1a** was reacted with 4-bromobenzaldehyde to afford compound **A_{10}** as a dark grey solid (yield 69%), m.p. 200-205°C.^{1}H NMR (600 MHz, DMSO-d_6): δ 7.37 (dd, *J* = 6.0 Hz, 6.0 Hz, 2H), 7.68 (d, *J* = 6.0 Hz, 2H), 7.73-7.74 (m, 2H), 7.84 (d, *J* = 6.0 Hz, 2H), 7.94 (d, *J* = 18.0 Hz, 1H), 8.15 (d, *J* = 12.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 115.18, 118.49, 123.33, 123.62, 124.52, 126.05, 129.18, 132.42, 135.77, 137.50, 137.69, 138.79, 143.27, 179.03; HRMS (ESI) m/z calcd for $[C_{16}H_{12}ON_2Br+H]^+$, 327.0128; found,

327.0133; IR (KBr, cm^{-1}) v: 3253.4, 1661.7, 1599.6, 1583.3, 1558.3, 1509.5, 1486.8, 1424.7, 1399.3, 1326.9, 1214.8, 1088.1, 1068.9, 1009.0, 974.7, 883.3, 875.4, 816.9, 764.9, 738.8, 721.9, 698.9, 618.1.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one (A_{11})

Following general procedure A, compound **1a** was reacted with 4-chlorobenzaldehyde to afford compound **A_{11}** as a white solid (yield 70%), m.p. 206-210°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.36-7.39 (m, 2H), 7.54 (d, *J* = 12.0 Hz, 2H), 7.73-7.74 (m, 2H), 7.90 (d, *J* = 6.0 Hz, 2H), 7.96 (d, *J* = 18.0 Hz, 1H), 8.14 (d, *J* = 18.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 122.80, 124.93, 128.40, 128.86, 129.35, 129.64, 129.98, 131.07, 133.75, 136.03, 143.14, 149.52, 181.40; HRMS (ESI) m/z calcd for $[C_{16}H_{12}ON_2Cl+H]^+$, 283.0636; found, 283.0636; IR (KBr, cm^{-1}) v: 3252.3, 1661.4, 1599.7, 1486.8, 1326.8, 1214.9, 1068.9, 983.2, 816.7, 738.7, 618.1.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (A_{12})

Following general procedure A, compound **1a** was reacted with 4-methoxybenzaldehyde to afford compound **A_{12}** as a white solid (yield 62%), m.p.193-195°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.83 (s, 3H), 7.05 (d, *J* = 12.0 Hz, 2H), 7.35 (dd, *J* = 6.0 Hz, 6.0 Hz, 2H), 7.72-7.74 (m, 2H), 7.85 (t, *J* = 12.0 Hz, 2H), 7.94 (d, *J* = 12.0 Hz, 1H), 8.00 (d, *J* = 18.0 Hz, 1H); ^{13}C NMR (100MHz, DMSO-d_6): δ 55.89, 114.96, 115.13, 119.57, 124.72, 127.43, 131.40, 144.67, 149.90, 162.25, 181.38; ESI-MS (m/z): 279.2 $[M+H]^+$; IR (KBr, cm^{-1}) v: 3261.6, 1654.7, 1590.8, 1326.6, 979.4, 807.0, 743.5.

(E)-1-(1H-benzo[d]imidazol-2-yl)-3-phenylprop-2-en-1-one (A_{13})

Following general procedure A, compound **1a** was reacted with benzaldehyde to afford compound **A_{13}** as a white solid (yield 72%), m.p. 235-238°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.38 (d, *J* = 36.0 Hz, 2H), 7.51 (m, 3H), 7.61 (d, *J* = 6.0 Hz, 1H), 7.88 (m, 3H), 7.99 (d, *J* = 12.0 Hz, 1H), 8.14 (d, *J* = 18.0 Hz, 1H),13.48 (s, 1H); ^{13}C

NMR (100 MHz, DMSO-d_6): δ 115.12, 118.49, 123.33, 123.62, 126.05, 128.06, 129.02, 129.46, 135.88, 137.50, 137.69, 138.79, 143.42, 179.61; HRMS (ESI) m/z calcd for $[C_{16}H_{13}ON_2+H]^+$, 249.1022; found, 249.1024; IR (KBr, cm^{-1}) v: 3242.1, 1660.4, 1592.6, 1510.9, 1484.9, 1424.9, 1329.0, 1215.4, 1138.0, 1087.8, 973.9, 871.1, 779.1, 739.7, 718.1, 686.7, 621.6.

(E)-1-(benzo[d]oxazole-2-yl)-3-(pyridin-2-yl)prop-2-en-1-one (B_1)

Following general procedure A, compound **2a** was reacted with 2-pyridinecarboxaldehyd to afford compound **B_1** as a dark yellow solid(yield 43%), m.p. 209-214°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (dd, *J* = 6.8, 4.8 Hz ,1H), 7.54-7.46 (m, 1H), 7.54-7.62(m, 1H), 7.66 (d, *J* = 3.0 Hz, 1H), 7.72(d, *J* = 6.0 Hz, 1H), 7.83 (dd, *J* = 6.0Hz, 6.0 Hz, 1H), 7.97 (d, *J* = 6.0 Hz, 1H), 8.12 (d, *J* = 12.0Hz, 1H), 8.44 (d, *J* = 12.0 Hz, 1H), 8.78 (d, *J* = 3.0 Hz, 1H); ESI-MS: 251.0 $[M+H]^+$; IR (KBr, cm^{-1}) v: 1682.5, 1553.5, 1475.2, 1443.8, 1280.3, 1154.4, 1138.2, 1009.2, 935.3, 795.8, 886.3, 743.8.

(E)-1-(benzo[d]oxazole-2-yl)-3-(3-chlorophenyl)prop-2-en-1-one (B_2)

Following general procedure A, compound **2a** was reacted with 3-chlorobenzaldehyde to afford compound **B_2** as a light yellow solid (yield 40%), m.p.172-175°C.^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34-7.52 (m, 3H), 7.58 (m, 2H), 7.67-7.76 (m, 2H), 7.89-8.07 (m, 3H); ESI-MS: 283.9 $[M+H]^+$; IR (KBr, cm^{-1}) v: 2988.0, 1696.9, 1594.4, 1529.1, 1477.6, 1454.9, 1244.9, 1201.3, 1155.9, 1079.4, 872.0, 783.2, 745.2, 690.4.

(E)-1-(benzo[d]oxazole-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one(B_3)

Following general procedure A, compound **2a** was reacted with 3-methoxybenzaldehyde to afford compound **B_3** as a light yellow solid (yield 32%), m.p. 214-216°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H), 7.02 (m, 1H), 7.25 (s, 1H), 7.31-7.40 (m, 2H), 7.51 (m, 2H), 7.69 (s, 1H), 7.89-7.98 (m, 2H), 8.08 (d, *J* = 15.0 Hz, 1H); ESI-MS: 280.1 $[M+H]^+$; IR (KBr, cm^{-1}) v: 2973.4, 1695.5, 1687.5,

1153.1, 1046.3, 879.5, 779.4, 748.6, 693.7, 684.4.

(E)-1-(benzo[d]thiazol-2-yl)-3-(pyridin-2-yl)prop-2-en-1-one (C_1)

Following general procedure A, compound **3a** was reacted with 2-pyridinecarboxaldehyd to afford compound **C_1** as a light yellow solid (yield 32%), m.p. 214-216°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52-7.57 (m, 2H), 7.58-7.76 (m, 3H), 7.86-7.97 (m, 2H), 8.25 (d, *J* = 6.0 Hz, 1H), 8.56 (d, *J* = 18.0 Hz, 1H), 8.77 (d, *J* = 3.0 Hz, 1H). ESI-MS: 266.9 $[M+H]^+$; IR (KBr, cm^{-1}) v: 3427.9, 1690.9, 1476.4, 1367.6, 1313.4, 1203.5, 1113.9, 1019.2, 881.5, 790.6, 755.2, 618.0.

(E)-1-(benzo[d]thiazol -2-yl)-3-(3-chlorophenyl)prop-2-en-1-one (C_2)

Following general procedure A, compound **3a** was reacted with 3-chlorobenzaldehyde to afford compound **C_2** as a light yellow solid (yield 50%), m.p.172-175°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34-7.44 (m, 2H), 7.48-7.65 (m, 3H), 7.75 (d, *J* = 3.0 Hz, 1H), 7.94-8.03 (m, 3H), 8.23-8.26 (m, 1H); ESI-MS: 300.0 $[M+H]^+$; IR (KBr, cm^{-1}) v: 3419.0, 1695.1, 1476.3, 1367.6, 1313.8, 1203.0, 1019.3, 881.5, 788.3, 755.5, 731.3.

(E)-1-(benzo[d]thiazol-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-one (C_3)

Following general procedure A, compound **3a** was reacted with 3-methoxybenzaldehyde to afford compound **C_3** as a light yellow solid (yield 52%), m.p. 214-216°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 3H), 6.97-7.04 (m, 1H), 7.23-7.29 (m, 2H), 7.33-7.40 (m, 2H), 7.50-7.64 (m, 2H), 7.98-8.08 (m, 3H), 8.24 (dd, *J* = 3.0 Hz, 3.0 Hz, 1H); ESI-MS: 296.0 $[M+H]^+$; IR (KBr, cm^{-1}) v: 3270.7, 1698.3, 1667.6, 1613.5, 1498.3, 1431.8, 1382.9, 1368.5, 1315.4, 1157.8, 1116.9, 1047.4, 944.2, 880.6, 795.5, 763.1, 754.8, 734.1, 706.3, 617.9.

1-(1H-benzo[d]imidazol-2-yl)-3-(pyridin-2-yl)prop-2-en-1-ol (D_1)

Following general procedure B, compound **A_1** was reacted with H_2 to afford compound **D_1** as a light yellow solid (yield 73%), m.p.177-180°C. ^{1}H NMR (600

MHz, DMSO-d_6): δ 5.56 (d, *J* = 6.0 Hz, 1H), 5.84 (dd, *J* = 12.0, 6.0 Hz, 1H), 7.20 (dd, *J* = 6.0, 6.0 Hz, 1H), 7.35 (td, *J* = 6.0, 12.0 Hz, 3H), 7.56 (d, *J* = 12.0 Hz, 1H), 7.71 (td, *J* = 12.0, 12.0 Hz, 1H), 7.82 (d, *J* = 12.0 Hz, 1H), 8.44 (d, *J* = 12.0 Hz, 1H), 13.28 (s, 1H); ^{13}C NMR (100 MHz, DMSO): δ 31.66, 37.21, 113.28, 121.79, 123.35, 125.95, 149.30, 160.38, 193.47; HRMS (ESI^+): 252.11308, Calcd for $C_{15}H_{14}ON_3$: 252.11314 $[M+H]^+$; IR (KBr, cm^{-1}) v: 3348.7, 2925.0, 2600.2, 1890.3, 1689.8, 1598.9, 1522.6, 1483.6, 1442.6, 1412.9, 1372.2, 1287.1, 1230.7, 1143.4, 1109.9, 1076.9, 1039.5, 952.9, 793.9, 733.9, 634.6.

1-(1H-benzo[d]imidazol-2-yl)-3-(3-chlorophenyl)prop-2-en-1-ol (D_2)

Following general procedure B, compound **A_7** was reacted with H_2 to afford compound **D_2** as a light yellow solid (yield 64%), m.p.179-183°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 5.56 (d, *J* = 12.0 Hz, 1H), 6.00 (dd, *J* = 6.0 Hz, 6.0 Hz, 1H), 6.65 (d, *J* = 18.0 Hz, 1H), 7.18 (td, *J* = 6.0 Hz, 6.0 Hz, 2H), 7.29-7.37 (m, 3H), 7.43-7.49 (m, 2H) 7.57 (d, *J* = 12.0 Hz, 1H); ESI-MS: 285.3$[M+H]^+$; IR (KBr, cm^{-1}) v: 3389.9, 2977.1, 1490.3, 1424.3, 1313.9, 1274.7, 1088.8, 1012.5, 966.2, 807.5, 740.7, 604.2;

1-(1H-benzo[d]imidazol-2-yl)-3-(3-methoxyphenyl)prop-2-en-1-ol (D_3)

Following general procedure B, compound **A_9** was reacted with H_2 to afford compound **D_1** as a light yellow solid (yield 79%), m.p.170-173°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.84 (s, 3H), 5.56 (d, *J* = 12.0 Hz, 1H), 6.00 (dd, *J* = 6.0 Hz, 6.0 Hz, 1H), 6.69 (d, *J* = 18.0 Hz, 1H), 6.81 (d, *J* = 6.0 Hz, 1H), 7.03 (s, 1H), 7.14-7.22 (m, 3H), 7.38-7.48 (m, 3H); ESI-MS: 281.2$[M+H]^+$; IR (KBr, cm^{-1}) v: 2988.5, 1620.9, 1428.6, 1314.0, 1272.9, 1048.5, 964.4, 876.4, 739.4, 690.5, 606.5;

1-(1H-benzo[d]imidazol-2-yl)-3-(pyridin-2-yl)propanl-1-one (E_1)

Following general procedure C, compound **A_1** was reacted with $NaBH_4$to afford compound **E_1** as a white solid (yield 73%), m.p.177-180°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.06 (t, *J* = 5.0 Hz, 2H), 3.22 (t, *J* = 5.0 Hz, 2H), 7.10 (td, *J* = 5.0 Hz, 10.0 Hz, 10.0 Hz, 1H), 7.16 (d, *J* = 10.0 Hz, 1H), 7.25 (d, *J* = 5.0 Hz, 2H), 7.53 (d, *J* =

15.0 Hz, 2H), 7.60 (d, *J* = 5.0 Hz, 1H), 7.80 (s, 1H), 8.31 (d, *J* = 5.0 Hz, 1H); ^{13}C NMR (100MHz, DMSO-d_6): δ 38.44, 39.56, 104.75, 104.89, 107.53, 107.59, 107.56, 113.33, 116.41,116.69, 121.55, 123.49, 126.03, 130.87, 131.62, 134.99, 143.28, 148.35, 158.32; ESI-MS: 252.1$[M+H]^+$; IR (KBr, cm^{-1}) v: 3675.8, 3280.4, 2988.2, 2901.2, 1678.4, 1578.1, 1509.2, 1487.2, 1422.9, 1347.0, 1313.3, 1216.5, 1076.1, 1049.0, 1023.1, 898.9, 883.3, 790.9, 735.3, 701.4, 627.0, 603.9.

1-(1H-benzo[d]imidazol-2-yl)-3-(3-chlorophenyl)propanl-1-one (E_2)

Following general procedure C, compound **A_7** was reacted with $NaBH_4$ to afford compound **E_2** as a light grey solid (yield 84%), m.p.179-183°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.04 (t, *J* = 6.0Hz, 2H), 3.53-3.58 (m, 2H), 7.03-7.08 (m, 2H), 7.43-7.49 (m, 1H), 7.53 (dd, *J* = 6.0 Hz, 6.0 Hz, 2H), 7.99 (t, *J* = 6.0 Hz, 2H), 8.26 (s, 1H); ESI-MS: 284.9$[M+H]^+$; IR (KBr, cm^{-1}) v: 3280.9, 2901.2, 1677.3, 1619.1, 1509.4, 1489.5, 1398.3, 1312.4, 1227.7, 1077.7, 1015.6, 834.5, 806.9, 742.9, 674.7, 621.1.

1-(1H-benzo[d]imidazol-2-yl)-3-(3-methoxyphenyl)propan1-1-one (E_3)

Following general procedure C, compound **A_9** was reacted with $NaBH_4$ to afford compound **E_3** as a white solid (yield 77%), m.p.206-209°C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.98 (t, *J* = 6.0 Hz, 2H), 3.52 (dd, *J* = 6.0 Hz, 6.0 Hz, 2H), 3.86 (s, 3H), 7.83 (ddd, *J* = 12.0 Hz, 6.0 Hz, 6.0 Hz, 4H), 7.98 (s, 1H), 8.00 (s, 1H), 8.07 (s, 1H), 8.10 (s, 1H); ESI-MS: 281.3$[M+H]^+$; IR (KBr, cm^{-1}) v: 3278.7, 2900.8, 1679.3, 1614.3, 1509.5, 1426.2, 1398.8, 1311.7, 1231.2, 1210.9, 1067.2, 935.5, 742.9, 695.4, 674.7.

4.2 *In vitro antiproliferative assay*

The cellular growth inhibitory activities of the compounds were determined with MTT assays and HCT116, HepG2 and MCF-7 cell lines (purchased from the American Type Culture Collection). The cells were seeded at 5000 (100 μL)/well into a 96-well plate. After culturing in growth media at 37 °C for 24 h, the test compound dissolved in 100 μL of DMSO was added. Nutlin-3a was used as the positive control

(purchased from MedChem express). After 48 h of incubation, 20 μL of MTT solution (5 mg/ml) was added to each well, and the plates were incubated for 4 h at 37°C with 5% CO_2. After removing the culture medium, 150 μL of DMSO was added. The concentration of the compound that inhibited cell growth by 50% (IC_{50}) was calculated. The assays were repeated in triplicate.

4.3 *In vivo antitumour assay*

Animal Experiments. BALB/c mice of sanitary degree were obtained from the Peking University Health Science Center. Animals were treated according to the institutional guidelines of the Chinese Academy of Medical Science and housed in an air-conditioned room with a 12 h light and 12 h dark cycle.

The *in vivo* antitumour activities of compounds **A_1**, **A_7** and **A_9** were evaluated. Irinotecan (IRT) was used as the reference drug. BALB nude female mice were obtained from Vital River Laboratories and housed in the animal care facility at Vital River Laboratories. HCT116 cancer cell suspensions were subcutaneously implanted into the right axilla region of the mice. The tumours were allowed to develop for eight days until they reached approximately 100 mm^3. The BALB/c mice were randomized into nine groups (eight mice per group) that received the following treatments: blank control; vehicle group; 100 mg/kg or 200 mg/kg of **A_1**, **A_7** and **A_9**; and 12.5 mg/kg of Irinotecan. The treatments were administered twice per week for four weeks. Twenty-four hours after the final administration, the mice were killed, and the tumours were excised, weighed and recorded for analysis. Tumour length and width was measured using callipers, and the tumour volume was calculated using the formula $TV = 1/2 \times a \times b^2$, where a is the tumour length and b is the tumour width.

4.4 *Cell cycle analysis*

HCT116 cells were seeded at 2×10^5 cells/well in 6-well plates and cultured for 48 h. Next, the cells were incubated with the test compounds (**A_1**, **A_7** and **A_9**) for 48 h. The cells were then treated with cold PBS. After harvest, the cells were fixed in 70% ice-cold ethanol overnight. Subsequently, the cells were centrifuged, the supernatant

was discarded and the pellet was treated with RNase A (100 mg/ml) for 30 min at room temperature. After the addition of PI at a final concentration of 50 mg/mL, the stained cells were analyzed for cell cycle distribution using flow cytometry (BECKMAN). Changes in the cell cycle profiles were analyzed using CELL QUEST PRO.

4.5 *Immunoprecipitation analysis*

HCT116 cells with wild type p53 were treated with varying concentrations of the test compound for 48 h. Next, the cells were harvested by centrifugation at 1000 g for 5 min. The cell pellets were washed with PBS, resuspended in RIPA buffer (Cell Signaling Technology), supplemented with a protease inhibitor cocktail (Thermo Scientific) and centrifuged at 12,000 g for 10 min. The protein concentration was determined using the Bio-Rad protein assay. Next, 1 mg of total protein was incubated with the indicated antibodies at 4°C with gentle agitation. Samples were then incubated with Protein A/G PLUS-Agarose (Santa Cruz, CA, USA) for 4-6 h at 4°C. For western blot analysis, the proteins were resolved by SDS-PAGE and transferred to polyvinylidenedifluoride membranes (Millipore Corp., Bedford, MA, USA). The membranes were incubated with the indicated primary antibody for 2 h and then incubated with the HRP-conjugated secondary antibody for 1 h at room temperature. Last, the membranes were developed using an ECL detection system (Millipore, Billerica, MA, USA).

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant 81402800) and the PUMC Youth Fund (NO.3332013027).

References

[1] M. Ferrari, Cancer nanotechnology: opportunities and challenges, Nat. Rev. Cancer. 5 (2005) 161-171.

[2] H. Haraguchi, H. Ishikawa, K. Mizutani, Antioxidative and superoxide

scavenging activities of retrochalcones in Glycyrrhiza inflate, Bioorg. Med. Chem. 6 (1998) 339-347.

[3] S. Vogel, J. Heilmann, Synthesis, cytotoxicity, and antioxidative activity of minor prenylated chalcones from humulus lupulus, J. Nat. Prod. 71 (2008) 1237-1241.

[4] C.S. Yang, J.M. Landau, M.T. Huang, H.L Newmark, Inhibition of carcinogenesis by dietary polyphenolic compounds, Annu. Rev. Nutr. 21 (2001) 381-406.

[5] E.J. Park, H.R. Park, J.S. Lee, J. Kim, Licochalcone A: an inducer of cell differentiation and cytotoxic agent from Pogostemon cablin, Planta. Med. 64 (1998) 464-466.

[6] C.A. Claude, J.C. Le Bail, P. Trouillas, G. Habrioux, A.J. Chulia, J.L. Duroux, Chalcones: structural requirements for antioxidant, estrogenic and antiproliferative activities, Anticancer. Res. 21 (2001) 3949-3956.

[7] S.K. Kumar, H. Erin, P. Catherine, G. Halluru, N. E. Davidson, S. R, Khan, Design, synthesis, and evaluation of novel boronic-chalcone derivatives as anti-tumor agents, J. Med. Chem. 46 (2003) 2813-2815.

[8] L.H. Huang, L.W. Fu, Mechanisms of resistance to EGFR tyrosine kinase inhibitors, Acta Pharm Sinica B. 5(2015) 390-401.

[9] H. Matsuda, T. Morikawa, S. Ando, T. Iwao, Y. Mas-ayuki, Structural requirements of flavonoids for nitric oxide production inhibitory activity and mechanism of action, Bioorg. Med. Chem. 11 (2003) 1995-2000.

[10] Y. Qian, G.Y. Ma, Y. Yang, K. Cheng, Q.Z. Zheng, W.J. Mao, L. Shi, J. Zhao, H.L. Zhu, Synthesis, molecular modeling and biological evaluation of dithiocarbamates as novel antitubulin agents, Bioorg. Med. Chem. 18 (2010) 4310-4316.

[11] K.L. Lahtchev, D.I. Batovska, St.P. Parushev, V.M. Ubiyvovk, A.A. Sibirny, Antifungal activity of chalcones: A mechanistic study using various yeast strains, Eur. J. Med. Chem. 43 (2008) 2220-2228.

[12] R.De Vincenzo, C. Ferlini, M. Distefano, C. Gaggini, In vitro evaluation of newly developed chalcone analogues in human cancer cell, Cancer. Chemother. Pharmacol. 46 (2000) 305-312.

[13] Y. Xia, Z.Y. Yang, P. Xia, K.F. Bastow, Anti-tumor agents. Part 202: Novel 2’-amino chalcones: design, synthesis and biological evaluation, Bioorg.

Med. Chem. Lett. 10 (2000) 699-701.

[14] R. Stoll, C. Renner, S. Hansen, Chalcone derivatives antagonize interactions between the human oncoprotein MDM2 and p53, Biochemistry. 40 (2001) 336-344.

[15] R.P. Gupta, C.A. Larroquette. K.C. Agrawal, Potential radiosensitizing agents. 5.2-substituted benzimidazole derivatives, J. Med. Chem. 25 (1982) 1342-1346.

[16] B. Parrino, A. Carbone, M. Muscarella, V. Spano, A. Montalbano, P. Barraja, A. Salvador, D. Vedaldi, G. Cirrincione, P. Diana, 11H-Pyrido[3′,2′:4,5] pyrrolo[3,2-c]cinnoline and Pyrido[3′,2′:4,5] pyrrolo[1,2-c][1,2,3] benzotriazine: Two New Ring Systems with Anti-tumor Activity, J. Med. Chem. 57 (2014) 9495-9511.

[17] P. Diana, A. Carbone, P. Barraja, A. Montalbano, B. Parrino, A. Lopergolo, M. Pennati, N. Zaffaroni, G. Cirrincione, Synthesis and Antitumor Activity of 3-(2-Phenyl-1,3- thiazol-4-yl)-1H- indoles and 3-(2-Phenyl-1,3-thiazol-4-yl)-1H-7-azaindoles, ChemMedChem. 6 (2011) 1300-1309.

[18] V. Spano, A. Montalbano, A. Carbone, B. Parrino, P. Diana, G. Cirrincione, I. Castagliuolo, P. Brun, O. Issinger, S. Tisi, Irina Primac, D. Vedaldi, A. Salvador, P. Barraja, Synthesis of a new class of pyrrolo[3,4-h]quinazolines with antimitotic activity, Eur. J. Med. Chem. 74 (2014) 340-357.

[19] S.K. Kumar, E. Hager, C. Pettit, H. Gurulingappa, NE. Davidson, SR. Khan, Design, synthesis and evaluation of novel boronic-chalcone derivatives as antitumor agents, J. Med. Chem. 46 (2003) 2813-2815.

[20] E.S. Charles, V.K. Agrawal, S. Sharma, R.N. Iyer, O.P. Srivastava, Synthesis of 2,5-disubstituted benzimidazoles as potential antihookworm and antimicrobial agents, Eur. J. Med. Chem. 14 (1979) 435-438.

[21] S. Ohta, S. Hayakawa, H. Moriwaki, S. Tsuboi, M. Okamoto, Synthesis of 2-acyl-1-methyl-1H-imidazoles and reactivity of the acyl group, Heterocycles. 23(1985) 1759-1764.

[22] A.W.E. Chan, J.M.C. Golec, Prediction of relative potency of ketone protease inhibitors using molecular orbital theory, Bioorg. Med. Chem. Lett. 4 (1996) 1673-1677.

[23] E.J. Park, H.R. Park, J.S. Lee, J. Kim, Licochalcone A: an inducer of cell

differentiation and cytotoxic agent from Pogostemon cablin, Planta. Med. 64 (1998) 464-466.

[24] L.T. Vassilev, B.T. Vu, B Graves, D. Carvajal, F. Podlaski1, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi, E.A. Liu, In vivo activation of the p53 pathway by small-molecule antagonists of MDM2, Science. 303 (2004) 844-848.

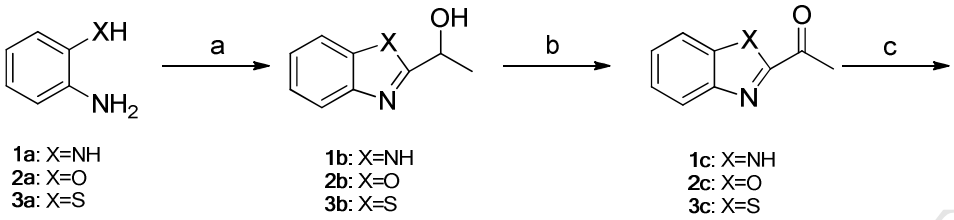
Figure captions:

Figure 1. Chemical structures ofthe target benzimidazolylα, β-unsaturated ketones.

Figure2. The effects of compounds A_1, A_7, $A_9$and Nutlin-3a on HCT116 cells as shown by immunoprecipitation analysis.

Figure 3. Theantitumour activities of compounds **A_1**, **A_7** and **A_9** in mice. The data are represented as the mean. (A) tumour volume; (B) change in mouse body weight.

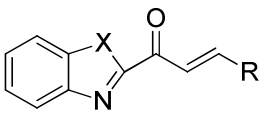
Scheme 1. Reagents and conditions: (a) lactic acid, 4 M HCl, reflux; (b) CrO_3, acetic acid, 90-105°C; (c) aromatic aldehyde, 10% NaOH aqueous, ethanol; (d) $NaBH_4$, $CeCl_3$, CH_2Cl_2, -78°C; (e) H_2, 10% Pd/C, methanol, rt.



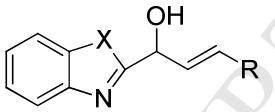
1a: X=NH
2a: X=O
3a: X=S

1b: X=NH
2b: X=O
3b: X=S

1c: X=NH
2c: X=O
3c: X=S

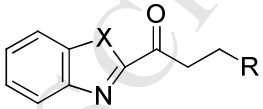


d



D_1: X=NH, R=2-pyridine
D_2: X=NH, R=3-Cl-Ph
D_3: X=NH, R=3-OMe-Ph

e



E_1: X=NH, R=2-pyridine
E_2: X=NH, R=3-Cl-Ph
E_3: X=NH, R=3-OMe-Ph

A_1: X=NH, R=2-pyridine
A_2: X=NH, R=3-pyridine
A_3: X=NH, R=4-pyridine
A_4: X=NH, R= 2-Br-Ph
A_5: X=NH, R=2-Me-Ph
A_6: X=NH, R=2-OMe-Ph
A_7: X=NH, R=3-Cl-Ph
A_8: X=NH, R=3-Br-Ph
A_9: X=NH, R=3-OMe-Ph
A_{10}: X=NH, R=4-Br-Ph
A_{11}: X=NH, R=4-Cl-Ph
A_{12}: X=NH, R=4-OMe-Ph
A_{13}: X=NH, R=-Ph
B_1: X=O, R=2-pyridine
B_2: X=O, R=3-Cl-Ph
B_3: X=O, R=3-OMe-Ph
C_1: X=S, R=2-pyridine
C_2: X=S, R=3-Cl-Ph
C_3: X=S, R=3-OMe-Ph

Table 1. The *in vitro* antiproliferative activities of the synthesized compounds

Compound	IC_{50} (μM)			Compound	IC_{50} (μM)		
	HCT116	MCF7	HepG2		HCT116	MCF7	HepG2
A_1	0.062±1.45	3.64 ± 0.39	0.34 ± 0.68	**A_{13}**	0.20± 0.11	39.79± 16.73	6.30± 3.10
A_2	15.33±2.42	21.70± 3.18	8.48 ± 0.86	**B_1**	>100	>100	>100
A_3	0.71 ±1.22	20.87±1.40	6.94 ± 2.04	**B_2**	>100	>100	>100
A_4	16.37± 3.66	50.94± 7.40	29.17 ± 2.47	**B_3**	>100	>100	>100
A_5	0.86± 0.19	62.49 ± 4.62	6.71 ± 0.92	**C_1**	>100	>100	>100
A_6	4.79 ± 0.49	41.26± 2.88	7.99 ± 0.38	**C_2**	>100	>100	>100
A_7	0.03 ± 0.02	9.80± 1.35	0.75 ± 5.41	**C_3**	>100	>100	>100
A_8	0.79 ± 0.28	19.32± 1.62	1.59 ± 6.62	**D_1**	>100	>100	>50
A_9	0.58 ± 0.11	21.68± 0.76	5.68 ± 1.37	**D_2**	>100	>100	>100
A_{10}	19.56± 4.31	52.71± 8.27	10.87 ± 0.31	**D_3**	>100	>100	>100
A_{11}	6.79± 0.36	24.77± 1.97	12.46 ± 3.49	**E_1**	>100	>100	>100
A_{12}	13.06± 0.85	>100	7.41 ± 0.95	**E_2**	>100	>100	>100
5-Fuorouracil	56.96	222.6	174.5	**Paclitaxel**	0.06727	0.02581	0.02171

[a] The antitumor activity of each compound was tested by a MTT assay. The IC_{50} values are the mean values calculated from three independent experiments.

Table 2. Compositions of G1, S and G2 cells in HCT116 treated for 72 h with **A_1**, **A_7** and **A_9**

Compd	G_1%	S%	G_2%
ctrl	31.72± 2.21	40.55± 1.36	27.73± 3.56
A_1	64.48± 5.94	18.88± 1.51	16.63± 7.45
A_7	64.97± 2.86	17.24± 2.34	17.78± 5.20
A_9	60.46± 3.03	19.03± 2.90	20.50± 5.93
Nutlin-3a	62.50± 2.75	24.80± 1.83	12.70± 0.76

Table 3. The antiproliferative activities of selected compounds for HCT116 (p53-/-) cells

Compd	IC_{50} (μM)	
	HCT116 p53+/+	HCT116 p53-/-
A_1	0.06 ± 1.45	1.45 ± 0.22
A_3	0.71 ± 1.22	5.61 ± 2.19
A_5	0.86 ± 0.19	4.39 ± 1.37
A_7	0.04 ± 0.02	1.95 ± 0.50
A_8	0.79 ± 0.28	2.94 ± 0.94
A_9	0.58 ± 0.11	4.75 ± 1.81
A_{13}	0.20 ± 0.11	2.07 ± 0.40

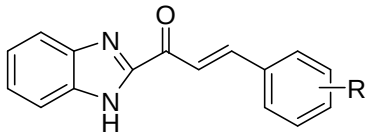


Figure 1 Chemical structures of the target benzimidazole-2-subsituted phenyl or pyridin propyl ketene

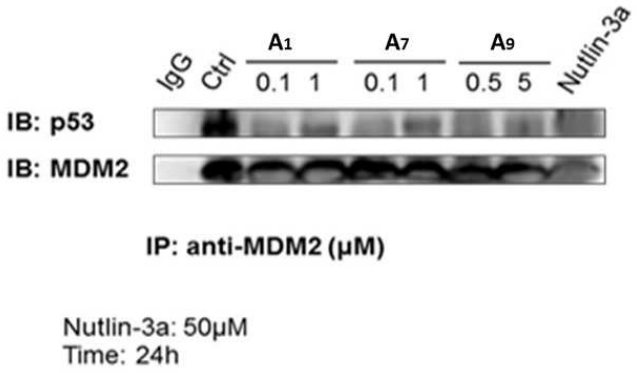


Figure 2. The effects of compounds A_1, A_7, A_9 or Nutlin-3a in HCT116 cells by immunoprecipitation analysis

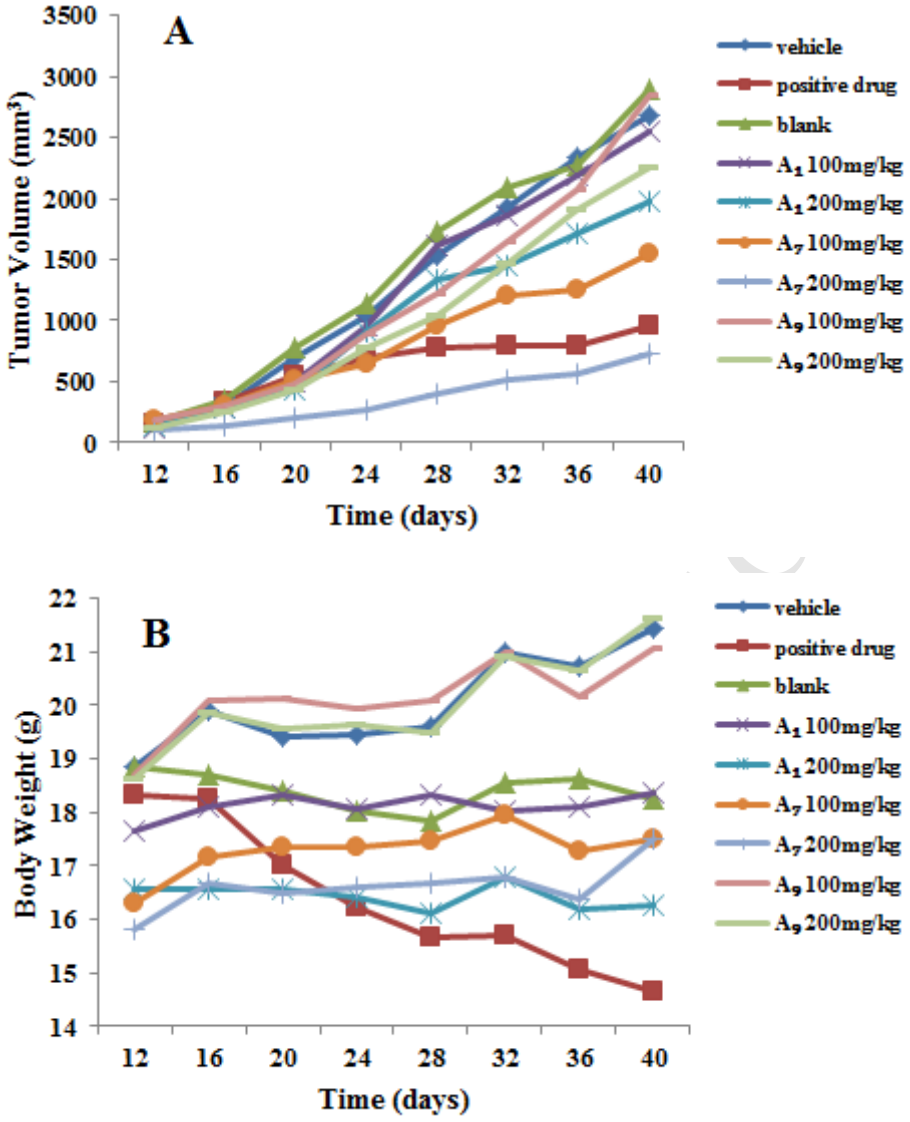


Figure 3. Antitumor activities of compounds **A_1**, **A_7** and **A_9** in mice. The data are represented as the mean. (A) tumor volume; (B) mice body weight change.

Highlights

1. A series of benzimidazolyl chalcone derivatives was synthesized and characterized.
2. Derivatives were evaluated for their antitumor activities and SAR
3. $\mathbf{A_1}$ and $\mathbf{A_7}$ indicated strong antitumor activity both in vivo and vitro.
4. $\mathbf{A_1}$ and $\mathbf{A_7}$ were considerably safe in vivo.
5. These compounds possible inhibited the p53-MDM2 binding.