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2,4-Dihydroxychalcone derivatives as novel potent cell division cycle 25B phosphatase inhibitors and protein tyrosine phosphatase 1B inhibitors

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Eleven 2,4-dihydroxychalcone compounds were synthesized and identified as reversible and competitive cell division cycle 25 (CDC25) B and protein tyrosine phosphatase (PTP) 1B inhibitors with inhibition values in the micromolar range. The results showed that nine compounds significantly inhibited CDC25B phosphatase, whereas seven compounds inhibited the activity against PTP1B *in vitro*. Compound **8** had the greatest inhibition activity against CDC25B and PTP1B *in vitro*, with percentage inhibition values of 97.5% and 96.3% at a dose of 20 μg/mL, respectively. Cytotoxic activity assays revealed that compound **8** was the most potent against HCT116, HeLa, and A549 cells. Furthermore, compound **8** exhibited potent antitumor activity in a colo205 xenograft model.

1. Introduction

Cell division cycle 25B (Cdc25B) is a dual-specificity phosphatase that plays a pivotal role in controlling cell cycle progression by catalyzing removal of covalently attached contiguous phosphates and the subsequent activation of cyclin-dependent kinase 1 (Cdk1; Rudolph 2007). Among the CDC25 family members, CDC25B has been proposed to regulate reentry into mitosis after DNA damage (Boutros et al. 2005; Bugler et al. 2006; Vugt et al. 2004). Importantly, CDC25B is oncogenic (Galaktionov et al. 1995) and its over-expression has been documented in a variety of human cancers, including head and neck, colon, and non–small cell lung cancer (Gasparotto et al. 1997; Sasaki et al. 2001; Takemasa et al. 2000). CDC25B also interacts with the steroid receptors (Ma et al. 2001a), suggesting that some Cdk-independent sites might contribute to the oncogenic potential of CDC25B. In addition, transgenic mice that overexpress CDC25B in mammary epithelium exhibit mammary gland hyperplasia and increased susceptibility to 9,10-dimethyl-1,2-benzanthracene-induced mammary tumorigenesis (Ma et al. 1999b; Yao et al. 1999).

Protein tyrosine phosphatases (PTPs) play essential roles in intracellular signal transduction by regulating the cellular level of tyrosine phosphorylation. Cellular processes controlled by PTPs include growth, differentiation, metabolism, migration, gene transcription, ion channel activity, immune responses, apoptosis, and bone development (Neel and Tonks 1997; Hunter 2000; Tonks and Neel 2001; Lee and Wang 2007). Unregulated PTP activity is responsible for several human diseases including cancer, diabetes, obesity, and dysfunction of the immune system (Blume-Jensen and Hunter 2001; Cook and Unger 2002; Montalibet and Kennedy 2005). One member of the PTP family, PTP1B, activates c-Src in human breast cancer and also down-regulates insulin signaling by dephosphorylating the insulin receptor (IR), insulin receptor substrate-1 (IRS-1), and insulin receptor substrate-2 (IRS-2) (Wälchli et al. 2000; Goldstein et al. 2000; Cheng et al. 2002; Zabolotny et al. 2002; Bohm 1998).

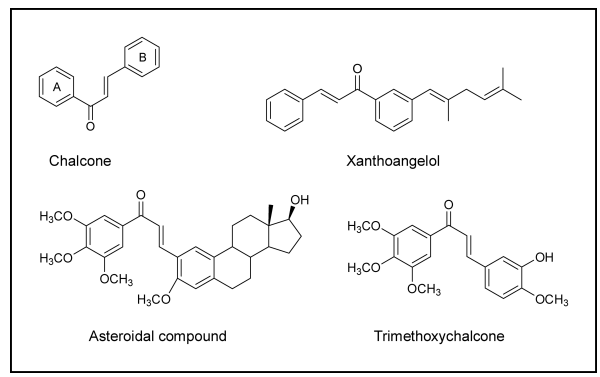
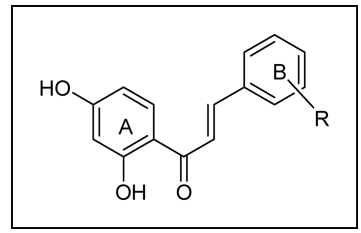


Fig. 1: Structures of chalcones and CDC25B inhibitors reported.

Chalcone moieties are common substructures in numerous natural products belonging to the flavonoid family (Nowakowska 2007; Akihisa et al. 2006; Zhang et al. 2006). Chalcone derivatives are very versatile as physiologically active compounds and substrates for the evaluation of various organic syntheses. Chalcones are highly distributed in nature and a large number has been synthesized in the laboratory. Many papers have been presented in the literature with references to structural modifications of the chalcone template (Nielsen et al. 1998). Chemically, they consist of open-chain flavonods in which the two aromatic rings are joined by a three carbon α,β-unsaturated carbonyl system (Fig. 1). The biological activities of chalcones are equally wide-ranging, such as anti-inflammatory, antimalarial, antifungal, antilipidemic, cytotoxicity, antioxidant, antiplasmodial, immunosuppression, anti-HIV, antiviral, antidepressant activities (Boeck et al. 2006; Cheenpracha et al. 2006; Lahtchev et al.2008; Lee et al. 2006; Sui et al. 2012; Trivedi et al. 2007).

A variety of chalcones have demonstrated cytotoxic activity toward several tumor cell lines. For example, Saxena et al. (2007)

Table 1: Percentage inhibition values of compounds 1–11 against CDC25B and PTP1B



Compd.	R	Dose (μg/mL)	Inhibition rates (%) (CDC25B)	Inhibition rates (%) (PTP1B)
1	*p*-CH_3	20	99.49 ± 0.42	71.19 ± 9.54
2	*p*-$N(CH_3)_2$	20	99.49 ± 0.41	$33.13 \pm 6.88^*$
3	*o*-F	20	$1.64 \pm 2.16^*$	$46.17 \pm 1.91^*$
4	*m*-F	20	55.30 ± 3.70	75.29 ± 7.02
5	*p*-F	20	78.02 ± 0.78	70.06 ± 2.63
6	*m*-Cl	20	70.00 ± 9.5	62.70 ± 3.51
7	*p*-Cl	20	$6.40 \pm 5.51^*$	$14.88 \pm 7.72^*$
8	*o*-Br	20	97.49 ± 0.48	96.31 ± 1.76
9	*m*-Br	20	85.13 ± 8.49	58.16 ± 6.10
10	*p*-Br	20	65.03 ± 2.31	56.89 ± 3.43
11	2,6-Cl_2	20	91.63 ± 2.98	$44.51 \pm 3.30^*$
Na_3VO_4	—	20	98.00 ± 0.06	–
Oleanolic acid			–	98.46 ± 0.11

* Compounds did not inhibit CDC25B and PTP1B.

reported the chalcone derivatives synthesized on a steroidal framework showed good anticancer activity against hormone dependent breast cancer cells and were found to be non-toxic to erythrocytes. Thus, steroidal chalcones may be developed as potent anticancer agents. Xanthoangelol has been reported to induce apoptosis and to inhibit tumor promotion and metastasis in several cancer cell lines (Kimura and Baba 2003; Tabata et al. 2005). The anticancer activity of various chalcone molecules is well studied and chalcone with a trimethoxyphenyl unit has been reported to be the most cytotoxic (trimethoxychalcone: $IC_{50} = 0.21$ nM) derivative synthesized so far (Lawrence et al. 2003) (Fig. 1). Having such varied pharmacological activities, these molecules have attracted medicinal chemists and therefore several strategies have been developed to synthesize them.

In addition, recently, several chalcones derived from natural products and their derivatives have been identified as PTP1B inhibitors (Fig. 2). For example, Chen et al. (2002) reported that brousso-chalcone A (I) isolated from *Broussonetia papyrifera* exhibited moderate inhibitory effects against PTP1B. Subsequently, Yoon et al. (2009) reported that several chalcones (II) isolated from *Glycyrrhiza inflata* and semisynthetic licochalcone A derivatives also exhibited moderate inhibitory effects against PTP1B. Sun et al. (2013) also reported that furan-chalcone compound (III) showed low micromolar IC_{50} ($IC_{50} = 2.49 \pm 0.23$ μM) values against PTP1B.

Although chalcones, which are widely expressed in plants, exert various biological activities, including CDC25B and PTP1B inhibition, the Structure-activity-relationships (SAR) of 2,4-dihydroxychalcones against CDC25B and PTP1B inhibition has not been fully examined because of an insufficient number of molecular compounds to test. Therefore, in the present study, we reported the evaluation of eleven 2,4-dihydroxychalcone compounds where the B ring was substituted with halogens (i.e., F, Cl, and Br) or an alkyl group, in terms of their inhibitory effects toward the CDC25B and PTP1B, and elucidated the SARs. These compounds displayed some structure activity relationships, and their mechanism of inhibition was studied.

2. Investigations and results

2.1. Inhibition of CDC25B in vitro using compounds 1-11

A high throughput screening (HTS) platform with recombinant CDC25B was developed to identify novel small-molecule inhibitors of this particular target (Feng et al. 2008). Eleven 2,4-dihydroxychalcone compounds were evaluated in terms of their ability to inhibit CDC25B activity at a dose of 20 μg/mL, and the results are shown in Table 1. The reference drug Na_3VO_4 was also screened against the same target at the same concentration. The results showed that 9 compounds out of 11 tested

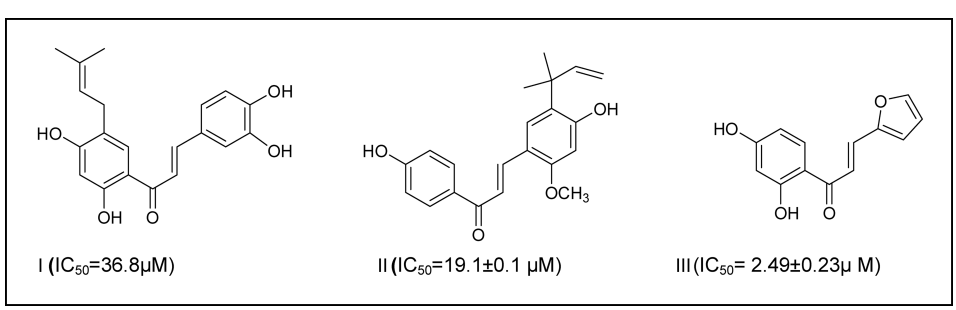


Fig. 2: Structures of PTP1B inhibitors reported.

Table 2: Antitumor activities of compound 8 *in vitro*[a]

Compd.	IC_{50} (μM)		
	HeLa	HCT116	A549
8	1.28	1.04	2.07
IRT	1.49	1.96	2.61

[a] Cancer cells: HeLa: cervical carcinoma cell, HCT116:colon cancer, A549:lung cancer. IRT: irinotecan

showed good CDC25B inhibitory activity at a concentration of 20 μg/mL, with percentage inhibition values in the range of 55.3% to 99.5%. Compounds **1**, **2** and **8** provided the highest levels of inhibitory activity towards CDC25B, with percentage inhibition values of 99.5%, 99.5%, and 97.5%, respectively, which were similar to that of the reference drug Na_3VO_4 (98.0%).

2.2. Inhibition of PTP1B in vitro using compounds 1-11

The inhibitory activities of the synthesized compounds towards PTP1B were measured using *p*-nitro phenyl phosphate (*p*NPP) as a substrate. The results are summarized in Table 1. Oleanolic acid, which is a known PTP1B inhibitor, was used as a positive control. Of the compounds tested, seven showed inhibitory activity towards PTP1B, with percentage inhibition values in the range of 56.9% to 96.3%. Compound **8**, in particular, showed the highest level of inhibitory activity of all of the compounds tested against PTP1B, with a percentage inhibition value of 96.3% at a dose of 20 μg/mL, which was similar to that of the reference drug oleanolic acid (98.5%).

2.3. Cytotoxicity assays and in vivo antitumor activity

Compound **8** was selected for further evaluation on the basis of it pronounced activity against the CDC25B phosphatases and PTP1B, and screened for its anti-proliferative activity in a number of different human tumor cell lines (i.e., HeLa, HCT116, and A549) using an MTT assay. As shown in Table 2, in the HCT116 cell line, the inhibitory activity of compound **8** was greater than that of the clinically validated anticancer drug irinotecan (IRT), which was used as a positive control, with an IC_{50} value of 1.04 μM. Compound **8** was also found to be cytotoxic towards the HeLa and A549 cell lines, with IC_{50} values of 1.28 and 2.07 μM, respectively, which were higher than those of IRT against the same cell lines (IC_{50}:1.49 and 2.61 μM, respectively).

Based on its favorable inhibitory and cytotoxicity properties, compound **8** was also evaluated for its antitumor activity in a colo205 human colon tumor xenograft model (Fig. 3.). Compound **8** was formulated in sodium carboxymethyl cellulose and water and given by gavage. In the colo205 xenograft model, compound **8** led to a tumor volume inhibition of approximately 50% when it was administered once daily for five consecutive days at a dose of 10 mg/kg. Furthermore, compound **8** was found to be well tolerated at a dose of 5 mg/kg, with no lethal toxicity observed.

3. Discussion

The CDC25 phosphatases are considered potential targets for the development of new therapeutic agents against cancer, and considerable research efforts have been directed towards identifying novel CDC25 inhibitors that work *in vitro* as well as being active against human tumors *in viv*o (Brezak et al. 2005; Contour-Galcera et al. 2007). With this in mind, we recently developed a high throughput screening (HTS) platform with recombinant CDC25B to identify novel small-molecule inhibitors of this particular target (Feng et al. 2008).

We first examined the inhibitory effects of the synthesized compounds on CDC25B, which contained electron-donating or electron-withdrawing groups (Table 1). Nine compounds (**1**, **2**, **4-6**, and **8-11**) were confirmed as potent inhibitors of CDC25B at a dose of 20 μg/mL. The actual percentage inhibition values of ranging from 65% to 99.5%. Firstly, we analyzed the structure-activity relationships of compounds **1-11**. Generally, the activity of an organic compound may be influenced remarkably after the introduction of a halogen atom (Wermuth 2005). With this in mind, we designed and synthesized several halogen-substituted compounds and evaluated their CDC25B inhibitory activity. A structure-activity relationship was observed for the inhibition of the CDC25B phosphatase by compounds **3-11**. For compounds **3-10**, the introduction of a halogen atom on the B ring of chalcones led to changes in the observed activity. The introduction of a Br atom provided higher levels of inhibitory activity toward CDC25B than the corresponding F and Cl containing compounds. Furthermore, the position of the Br substituent on the phenyl ring had a significant influence on the inhibitory activity, with the order of activity being *o*-Br > *m*-Br > *p*-Br. Compound **8** bearing a 2-Br substituent on its phenyl ring had the greatest *in vitro* inhibition activity against CDC25B. In addition, compound **11**, which was 2,6-dichlorinated on B ring of chalcones, showed high levels of inhibitory activity against CDC25B. Next, compounds with electron-donating groups on the B ring appeared to have a positive influence of inhibitory activity of the compounds towards CDC25B, with

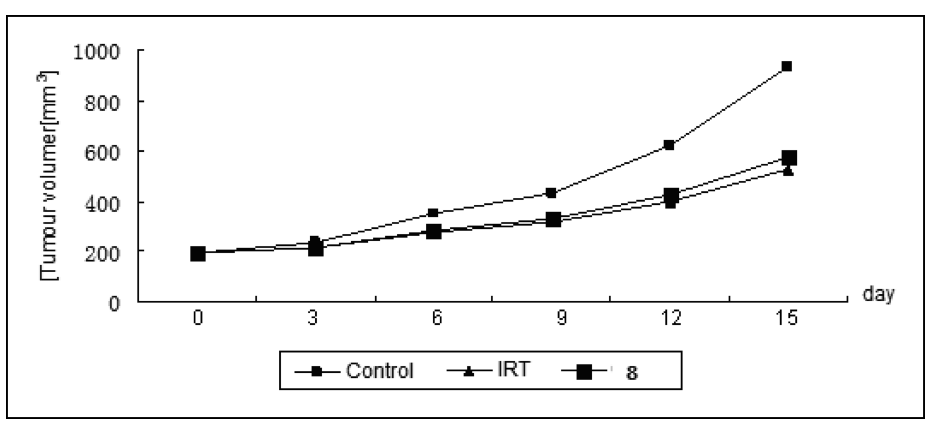


Fig. 3: Tumor growth and inhibition by compound **8** against colo205 xenografts in nude mice (Compound administered intragastrically).

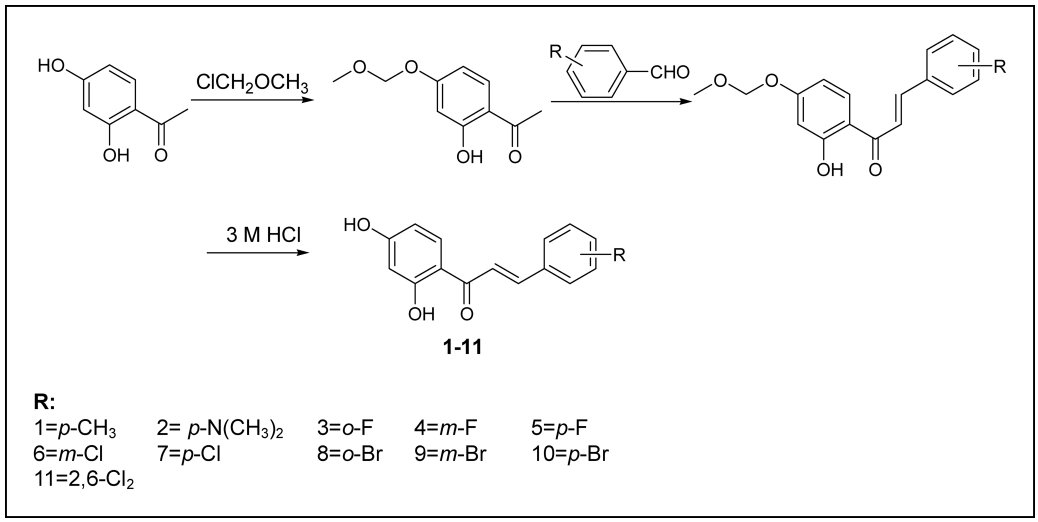


Fig. 4: Chemical structures of compounds **1–11**.

compounds **1** and **2** showing excellent inhibition (99.5% inhibition) similar to that of the reference drug Na_3VO_4 (98.00%). CDC25B is a dual-specificity phosphatase, and represents an attractive therapeutic antitumor target for small molecule intervention because of its central role in controlling malignant cell proliferation by regulating cyclin-dependent kinases, and its high level of expression in many human tumors (Ducruet et al. 2005).

PTP1B is one member of the PTP family, and several groups have demonstrated that the overexpression of PTP1B is sufficient to drive tumorigenesis in mice, providing additional support for the use of PTP1B inhibitors as therapeutic agents against cancer (Easty et al. 2006; Tonks et al. 2007). The inhibitory activity of the synthesized compounds (**1-11**) against PTP1B was measured using *p*NPP as a substrate. The results are summarized in Table 1. Seven compounds showed potent PTP1B inhibitory activity at a concentration of 20 μg/mL, with percentage inhibition values in the range from 56.9% to 96.3%. After analyzing the activities of the synthesized compounds **1–11**, the following SARs were obtained. The substitutions (R) with electron-withdrawing groups on the B ring in **3-11**, except compound **3**, **7** and **11** showed better or comparative activity. Furthermore, the position of the substituent (Br, F and Cl) on the B ring significantly influenced the PTP1B inhibitory activities, with an order of activity of *o*-Br > *m*-Br > *p*-Br for the brominated compounds, *m*-F > *p*-F > *o*-F for the fluorinated compounds, and *m*-Cl > 2,6-Cl_2 > *p*-Cl for the chlorinated compounds. In all cases, the Br containing analogues provided higher levels of inhibitory activity toward PTP1B than the corresponding F and Cl containing compounds. The presence of an electron-donating group at the 4-position on the B ring led to moderate levels of activity against PTP1B. Compound **1** bearing a methyl group provided a high level of inhibitory activity toward PTP1B (71.2%), whereas compound **3** bearing a dimethylamino group at the same position have not active (33.1%). Compound **8** showed the highest level of inhibition of all of compounds tested toward PTP1B, with a percentage inhibition of 96.31% at a dose of 20 μg/mL, which was similar to that of the reference drug oleanolic acid (98.49%).

To determine the antiproliferative activity of compound **8** towards the proliferation of several different cancer cell lines, including HeLa, HCT116 and A549 cells. The results are shown in Table 2. Compound **8** showed a higher level of inhibitory activity than IRT, with IC_{50} values of 1.28 and 2.07 μM against HeLa and A549 cells, respectively (IRT showed IC_{50} values of 1.49 and 2.61 μM, respectively). These results showed that compound **8** was potently cytotoxic towards the three cancer cell lines tested, and exhibited higher levels of cytotoxicity than the reference drug IRT.

Based on its favorable *in vitro* data, compound **8** was also evaluated for its antitumor activity in the colo205 human colon tumor xenograft model (Fig. 3). The results revealed that compound **8** was active against colon tumor xenografts in nude mice.

In summary, eleven 2,4-dihydroxychalcone compounds synthesized were evaluated as reversible and competitive CDC25B and PTP1B inhibitors. Nine compounds significantly inhibited CDC25B phosphatase, whereas seven compounds inhibited the activity against PTP1B *in vitro*. Compound **8** had the greatest inhibition activity against CDC25B and PTP1B *in vitro*, with percentage inhibition values of 97.5% and 96.3% at a dose of 20 μg/mL, respectively, which was similar to those of the reference drugs Na_3VO_4 (98.0%) and oleanolic acid (98.5%). Cytotoxic activity assays revealed that compound **8** was the most potent against HCT116, HeLa, and A549 cells. Furthermore, compound **8** potently inhibited tumor activity in a colo205 xenograft model.

4. Experimental

4.1. Drug and reagents

The synthetic pathways to the 2,4-dihydroxychalcone derivatives **1-11** are illustrated in Fig. 4. The synthesis procedure and spectral data of the compounds **1-11** were previously described (Guan et al. 2004; Zhang et al. 2010). All compounds were confirmed by IR spectra (FT-IR1730, Bruker, Switzerland), ^{1}H NMR and ^{13}C-NMR spectra (AV-300, Bruker, Switzerland), Mass spectra (HP1100LC/MS, Agilent Technologies, USA). 3-*O*-Methylfluorescein phosphate (OMFP), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliom bromide (MTT), HCT116, HeLa, and A549 cells were purchased from Sigma Aldrich (St Louis, MO, USA). All other reagents and solvents used in experiments were of analytical grade.

4.2. CDC25B activity assay

The enzymatic activity of the CDC25B catalytic domain was determined by monitoring the dephosphorylation of OMFP. Dephosphorylation of OMFP generates product OMF, which was detected at a 485 nm excitation/535 nm emission. In a typical 100 μL assay mixture containing 50 mmol/L Tris-HCl, 50 mmol/L NaCl, pH 8.0, 5 μmol/L OMFP, 20 nmol/L recombinant CDC25B, 1% glycerin, 1 mmol/L DTT, in presence or absence of 2 μL inhibitor in dimethyl sulfoxide (DMSO), activities were continuously monitored and the initial rate of the dephosphorylation was determined using the early linear region of the enzymatic reaction kinetic curve.

4.3. Biological assays for PTP1B

The enzymatic assays for PTP1B were performed as described elsewhere (Shi et al. 2008). Briefly, the enzymatic activity of the PTP1B catalytic domain was determined at 30 °C by monitoring the hydrolysis of *p*NPP. Dephosphorylation of *p*NPP generates the product *p*NP, which was monitored at an absorbance of 405 nm using an EnVision multilabel plate reader (PerkinElmer Life Sciences, Boston, MA, USA). Assays were performed in a total volume of 100 μL containing 50 mM 3-[*N*-morpholino]propanesulfonic acid (MOPs), pH 6.5, 2 mM *p*NPP, and 30 nM recombinant PTP1B, as well as the indicated concentrations of the inhibitor. The production of *p*NP was continuously monitored, and the initial rate of the hydrolysis was determined from the early linear region of the enzymatic reaction kinetic curve.

4.4. Cell culture

HCT116, HeLa, and A549 cells were kept at logarithmic growth in 5% CO_2 at 37 °C with HG-DMEM, McCoy's 5A and F12 medium, respectively, supplemented with 10% FBS and 100 units/mL each of penicillin G and streptomycin.

4.5. Cytotoxicity

Cytotoxicity assays were performed on human colon cancer (HCT116), lung cancer (A549), and cervical carcinoma cell lines. Cells (6000-10000) in 100 μL culture medium per well were seeded onto a 96-well micrrotest plate (Falcon, CA). Cells were treated in trplicate with a gradient concentration of tested compounds were added and the plate was incubated at 37 °C for 72 h. For three cell lines, MTT assay was performed to measure the cytotoxic effects. The compounds concentration required for IC_{50} of tumor cells was determined from the dose-response curve.

4.6. In vivo antitumour activities

The antitumor activity of compound h was evaluated and IRT was used as a reference drug *in vivo*. BALB/C nude male mice (weighing 18-20 g) were obtained from Shanghai weasley grams of laboratory animal co., LTD. Colo205 cancer cell suspensions were implanted subcutaneously into the right axilla region of the mice. Treatment began when the implanted tumor had reached a volume of ~100–300 mm^3(after 17 days). The animals were randomized into appropriate groups (6 animals per treatment and 8 animals for the control group) and administered by gavage once daily for five consecutive days from day 17 after implantation of the cells. Tumor volumes were monitored by caliper measurement of the length and width and calculated using the formula of $TV = 1/2 \times a \times b^2$, where a is the tumor length and b is the width. Tumor volumes and body weights were monitored every 3 days over the course of treatments. Mice were sacrificed on day 35 after implantation of cells and tumors were removed and recorded for analysis.

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