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ARTICLE

Synthesis and biological evaluation of novel hybrid compounds between chalcone and piperazine as potential antitumor agents†

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Chalcones play an important role in living organisms with a wide range of biological activities including potent antitumor activity. Previously, we reported that *N*-aryl piperazine compounds have excellent biological activity. To further explore the structure-activity relationships, a series of novel hybrid compounds between chalcone and piperazine have been synthesized, and their *in vitro* antitumor activity was evaluated against a panel of human tumor cell lines. The results demonstrated that compounds bearing acetophenone showed better anticancer activity than cisplatin and other hybrid compounds, and that substitution of the acetophenone with halogen atom, was vital for modulating cytotoxic activity. Among all synthetic derivatives, hybrid compound **7c** was found to be the most potent compound against A549, Hela and SGC7901 (IC_{50}=5.24μM, 0.19μM and 0.41μM, respectively) , importantly, **7c** exerted obvious inhibitory effect *in vivo*.

Introduction

Chalcones and their derivatives are a class of important organic compounds that play an important role in living organisms[1] with broad range of biological activities, including antitumor,[2] antioxidant,[3]anti-inflammatory,[4] and so on. Chalcones are widely present in nature, and a number of chalcones have been reported in recent years (Fig.1).[5-8]Present studies show that the antitumor activity of chalcones may result from multiple mechanisms, including inhibition of proliferation and promotion of apoptosis of tumor cells, inhibition of tyrosine kinases, as well as selective cytotoxic activity, which suggest these molecules may be used as potential antitumor agents and have attracted more pharmaceutical chemists into the exploration of novel chalcone antitumor drugs.[9-12]

Similarly, *N*-aryl piperazine moieties represent a series of important organic compounds that make up the core structures in medicines, such as Eperezolid[13]and Levodropropizine[14]. Recently, *N*-aryl piperazine derivatives have attracted considerable interests for their versatile properties in chemistry and pharmacology, and these moieties have been developed to drug molecular design widely.

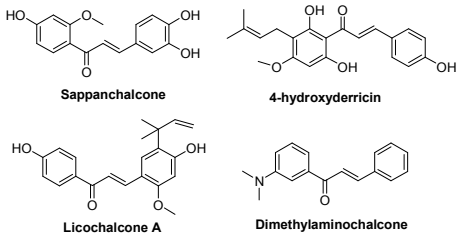

Fig. 1 Structures of natural chalcones with antitumor activities.

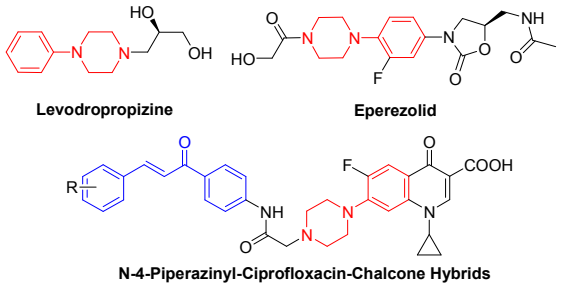

Fig.2 Structures of *N*-aryl piperazine agents.

Molecular hybridization has been one of the successfully applied strategies for the design and synthesis of novel and efficient biological agents. Molecular hybridization involves the combination of two distinct pharmacophores to form new hybrid molecules. For example, *N*-4-piperazinyl-ciprofloxacin-chalcone hybrids have been found to exhibit a broad-spectrum of antitumoractivities.[15]Moreover, in previous research, we have reported the synthesis of a series of novel *N*-aryl piperazine hybrid compounds and their potential antitumor activity.[16-17]In the present research, we have designed and synthesized new molecules towards the recombination of chalcone and piperazine in the same structure through medicinal chemistry hybridization (Fig.3). The potential cytotoxicity of derivatives was evaluated *in vitro* against a panel of human tumor cell lines and *in vivo*.

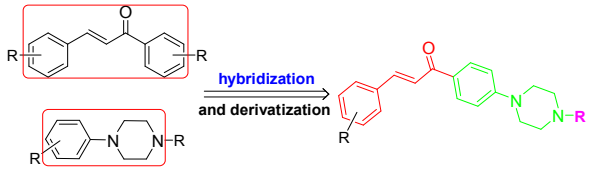

Fig. 3 Designed strategy of chalcone hybrids.

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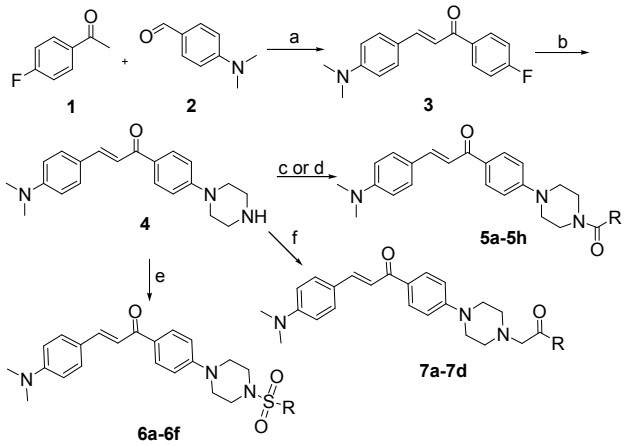


Scheme 1 Synthesis of hybrid derivatives. *Reagents and conditions*: (a) 20% KOH, EtOH, rt, 24h; (b) piperazine hexahydrate, Cs_2CO_3, DMF, 110°C, 12h; (c) RCOCl, Et_3N, DCM, 0°C, 2h; (d) RCOOH, DCC, DMAP, DCM, 24h; (e) RSO_2Cl, Pyridine, DCM, rt, 12h; (f) Cs_2CO_3, 2-bromoacetophenone, DCM, rt, 4h.

Results and discussion

Chemistry

The general route used for the synthesis of title compounds is shown in Scheme 1. Treatment of commercially available 4-fluoroacetophenone (**1**) with 4-dimethylaminobenzaldehyde (**2**)gave the 4-dimethylamino-4'-fluorochalcone (**3**) in the presence of KOH resulted by the Aldol condensation. Subsequently, the key intermediate (**4**) was prepared by substitution of fluorine atom of**3** with piperazine following the literature procedure.[18]Although in the product**4** preparation from chalcone intermediate (**3**) by nucleophilic substitution reaction with N-heterocycles, there is a possibility for Aza-Michael addition product,[19-20] no Michael-addition products were observed in our work.

Finally, acylation and sulfonylation of NH group with acyl chloride or carboxylic acid and sulfonyl chloride afforded hybrid compounds **5a-5h** and **6a-6f** in good yields (62-91%), respectively. For further insight toward the structure and activity relationship, a few tertiary amines (**7a-7d**) were synthesized by treatment of 2-bromoacetophenone as well. Structures of novel hybrid compounds are shown in Tables 1.

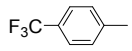
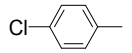
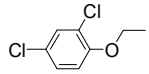
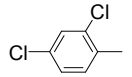
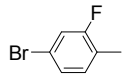
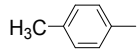
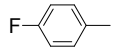
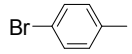
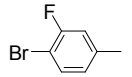
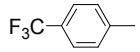
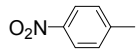
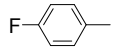
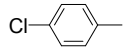
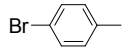
Biological assay

Cytotoxic activity of novel synthesized hybrid derivatives were evaluated against human lung cancer cell line A549, human cervical carcinoma Hela and human gastric carcinoma SGC7901 by MTT assay,[21] using cisplatin as the reference drug. The biological results of hybrid compounds are summarized in Tables 2.

As shown in Table 2, the structures of the hybrid compounds have an obvious influence on antitumor activities. Amide derivatives were more active in general, and the dichloro or trifluoromethyl substituent of the benzene ring displayed similar or more cytotoxic activity in vitro compared to cisplatin. Especially, compound **5d** showed similar antitumor activity against Hela and SGC7901 cells, similarly, compounds **5f** and **5g** displayed potent or similar cytotoxic activity against A549 and SGC7901 cells.

However, sulfonamide compounds were inactive against 3 tumor cell lines investigated at the concentration of 40μMexcept

Table 1. Structures of title compounds.

Compound	R or X	m. p(°C)	Yield (%)[a]
5a	H	231-233	87
5b	CH_3	231-234	91
5c	C_2H_5O	235-236	86
5d		240-242	83
5e		236-238	84
5f		247-249	78
5g		239-241	82
5h		237-239	70
6a		234-236	78
6b		238-239	85
6c		240-241	84
6d		240-242	86
6e		242-243	71
6f		238-240	62
7a		250-252	86
7b		252-254	84
7c		251-252	85
7d		254-256	79

[a] Yields represent isolated yields.

for hybrid **6e** against A549.It could be seen that chloro or trifluoromethyl substituent of the benzene ring might be attributed to the cytotoxic activity for amides and sulfonamides.

To our delight, hybrid compounds bearing acetophenone all showed a higher potency anticancer activity than cisplatin and the above hybrid compounds. Interestingly, the halogen atom substituent atposition-4 of the benzene ring was more sensitive to cytotoxic activity. Notably, derivatives **7c** displayed the best IC_{50} value against 3 tumor cell lines. In comparison to compounds **7b-7d**, compound **7a** was less potent against all tumor cell lines.

Derivatives **7c** showed best anti-tumoral activity *in vitro* as described above. In order to further evaluate *in vivo* anti-tumoral effect of **7c**, S180 tumor-bearing mice was established for detecting its *in vivo* anti-tumoral effect. The result indicated that daily administration of 25mg/kg **7c** significantly inhibited the growth of tumor cell, implying 7c may be a potential therapeutic agent for tumor (Fig. 4).

The results suggest that the existence of halogen atom has played an important role in the antitumor activity of hybrids. Generally, the chlorine atom substituent atposition-4 of benzene ring, as well as the fluorine group exhibit higher cytotoxic activity. The structure-activity relationship (SAR) results were summarized in Scheme 2.

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Table 2. *In vitro* cytotoxic activities of hybrid compounds.

Compound	Cell lines (IC_{50}, μM) [a, b]		
	A549	Hela	SGC7901
5a	>40	>40	>40
5b	>40	>40	>40
5c	>40	>40	>40
5d	>40	16.32±2.02	17.29±1.25
5e	>40	>40	>40
5f	10.36±2.35	>40	14.16±2.68
5g	15.13±2.97	35.76±4.13	8.72±2.10
5h	>40	>40	>40
6a	>40	>40	>40
6b	>40	>40	>40
6c	>40	>40	>40
6d	>40	>40	>40
6e	16.59±2.73	>40	34.18±3.71
6f	>40	>40	>40
7a	11.12±2.13	2.08±0.75	8.84±1.59
7b	7.17±0.88	1.02±0.49	0.16±0.11
7c	5.24±1.01	0.19±0.13	0.41±0.26
7d	9.43±1.27	0.26±0.18	4.04±1.03
cisplatin	11.54±1.15	20.52±1.84	12.44±2.12

[a] Cytotoxicity as IC_{50} values for each cell line, the concentration of compound that inhibit 50% of the cell growth measured by MTT assay.
[b] Each value was reproduced in triplicate.

On the whole, although compounds 7a-7d werefound toexhibit broad-spectrum of antitumor activities, they also showed no pronounced selectivity. Amide hybrids displayed similar active and better selectivity compared to 7a-7d. These hybrids were to be lead compounds for further structural modification and activity research.

Conclusions

In summary, a series of novel hybrid compounds between chalcone and piperazine have been synthesized and proved to be potent antitumor agents. The chalcone hybrids **7a-7d** bearing acetophenone were found to be the most potent compounds, and halogen atom substituent atposition-4 of benzene ring were vital for modulating cytotoxic activity. Among all synthetic derivatives, hybrid compound **7c** was found to be the most potent compound in treatment of tumor *in vitro* and i*n vivo*. Further molecular mechanism is currently underway and the results will be reported in due course.

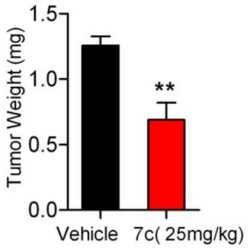
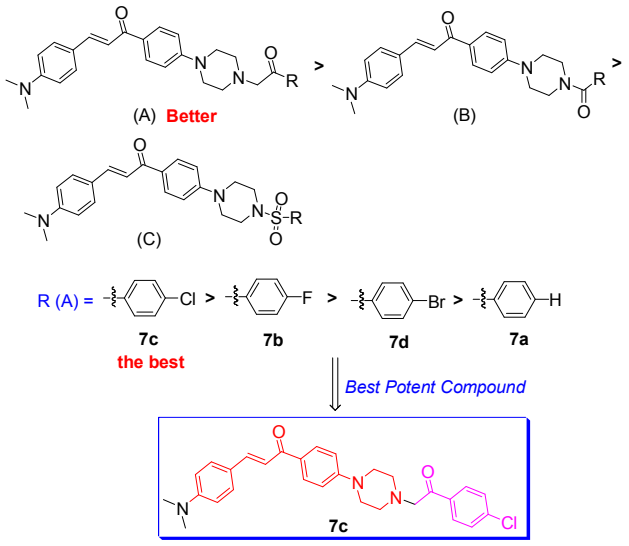


Fig. 4 *In vivo* anti-tumoral effect of **7c** in S180 tumor-bearing mice. Female BALB/c mice was injected subcutaneously into the right forelimb armpit with 1×10^6 S180 tumor cells, 24 h after injected tumor, 7c or vehicle was administered via intragastric administration, the experiment was terminated after 21 days and the mice were sacrificed, tumors were harvest and weighed. Results presented are mean± s.e.m, n = 8. *P<0.05, **P<0.01 versus vehicle group.



Scheme 2 Structure-activity relationship of hybrid compounds.

Experimental

General procedure for the preparation of chalcone derivatives 5a-5h.

Method A: To a stirred solution of compound**4** (0.5mmol) and pyridine (0.5mL) in dried DCM (10mL), acylchloride (1.0mmol) was added and reaction mixture was stirred for 2 h at 0°C. After completion of the reaction as indicated by TLC, the reaction was quenched by the addition of saturated $NaHCO_3$ (20mL) and was extracted with $CHCl_3$ (3×10mL). The organic layer was dried using anhydrous sodium sulfate, concentrated in vacuo and purified by column chromatography (DCM) to afford products.

Method B: To a stirred solution of DCC (1.0mmol) and carboxylic acid(1.0mmol)in dried DCM (10mL), compound**4**(0.5mmol) was added and reaction mixture was stirred for 24h at rt. After completion of the reaction as indicated by TLC, the mixture was filted and the filtrate was concentrated in vacuo and purified by column chromatography (DCM) to afford compounds.

Compound **5d**:Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.99(d, *J*=8.7Hz, 2H), 7.68-7.79 (m, 3H), 7.54-7.60(m, 2H), 7.53(d, *J*=8.7Hz, 2H), 7.37(d, *J*=15.3Hz, 1H), 6.90(d, *J*=8.7Hz, 2H), 6.67(d, *J*=8.7Hz, 2H), 3.90(s, 2H), 3.57(s, 2H), 3.35(s, 4H), 2.99(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 188.2, 168.8, 153.2, 151.8, 144.4, 136.2, 130.4, 130.3, 130.2, 130.1, 129.2, 126.8, 124.2, 122.8, 116.5, 114.27, 111.8, 47.7, 40.1.HRMS: m/z calcd for $C_{29}H_{29}F_3N_3O_2$ $(M+H)^+$508.2206, found 508.2204.

Compound **5f**: Brown solid; ^{1}H NMR (300MHz, $CDCl_3$)δ: 8.00(d, *J*=8.7Hz, 2H), 7.79(d, *J*=15.3Hz, 1H), 7.55(d, *J*=8.7Hz, 2H), 7.38(s, 1H), 7.38(d, *J*=15.3Hz, 1H), 7.16-7.20(dd, *J*=2.7Hz, 2.4Hz, 1H), 6.98(d, *J*=8.7Hz, 1H), 6.91(d, *J*=9.0Hz, 2H), 6.70(d, *J*=9.0Hz, 2H), 4.80(s, 2H), 3.75-3.81(m, 4H), 3.31-3.37(m, 4H), 3.03(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$)δ: 188.5, 165.8, 153.3, 152.1, 152.0, 144.7, 130.5, 130.3, 128.0, 127.1, 123.7, 123.1, 116.8, 114.4, 114.4, 112.0, 68.9, 40.3, 34.1, 25.1; HRMS: m/z calcd for $C_{29}H_{30}Cl_2N_3O_3$ $(M+H)^+$538.1659, found 538.1653.

Compound **5g**: Pale yellow solid; ^{1}H NMR (300MHz, $CDCl_3$)δ: 8.01(d, *J*=8.7Hz, 2H), 7.80(d, *J*=15.3Hz, 1H), 7.50-7.56(m, 4H), 7.38(d, *J*=15.3Hz, 1H), 7.26-7.30(dd, *J*=2.1Hz, 2.4Hz, 1H), 6.93(d, *J*= 9.0Hz, 2H), 6.70(d, *J*=8.7Hz, 2H), 3.87(s, 2H), 3.63(s, 2H), 3.37(s, 4H), 3.03(s, 6H); ^{13}C NMR (75MHz, $CDCl_3$)δ: 188.4, 168.1, 153.3, 152.0, 144.7, 135.2, 134.5, 133.2, 130.8, 130.4, 130.3, 129.4,

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126.6, 123.0, 116.7, 114.4, 111.9, 40.2, 34.0, 25.0; HRMS: m/z calcd for $C_{28}H_{28}Cl_2N_3O_2$ $(M+H)^+$508.1553, found 508.1550.

General procedure for the preparation of chalcone derivatives 6a-6f: To a stirred solution of compound**4** (0.5mmol) and pyridine (0.5mL) in dried DCM (10mL), sulfonyl chloride (1.0mmol) was added and reaction mixture was stirred for 12h at room temperature. After completion of the reaction as indicated by TLC, the reaction was quenched by the addition of 10%NaOH(20mL) and was extracted with $CHCl_3$ (3×10mL). The organic layer was dried using anhydrous sodium sulfate, concentrated in vacuo and purified by column chromatography (DCM) to afford products.

Compound **6e**: Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$)δ: 7.97(d, *J*=8.7Hz, 2H), 7.94(d, *J*=9.0Hz, 2H), 74-7.84(m, 3H), 7.54(d, *J*=8.4Hz, 2H), 7.35(d, *J*=15.6Hz, 1H), 6.88(d, *J*=8.4Hz, 2H), 6.70(d, *J*=8.4Hz, 2H), 3.43(d, *J*=5.1Hz, 4H), 3.21(s, 4H), 3.03(s, 6H); HRMS: m/z calcd for $C_{28}H_{29}F_3N_3O_3S$ $(M+H)^+$544.1876, found 544.1875.

General procedure for the preparation of chalcone derivatives 7a-7d. To a stirred solution of compound**4** (0.5mmol) and Cs_2CO_3 (1.0g) in dried DCM (10mL), 2-bromoacetophenone (0.6mmol) was added and reaction mixture was stirred for 4h at rt. After completion of the reaction as indicated by TLC, the reaction was quenched by the addition of 10% NaOH(20mL) and was extracted with $CHCl_3$ (3×10mL). The organic layer was dried using anhydrous sodium sulfate, concentrated in vacuo and purified by column chromatography (1% Et_3N/DCM) to afford products.

Compound **7a**: Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$)δ: 7.99(d, *J*=8.1Hz, 4H), 7.79(d, *J*=15.3Hz, 1H), 7.50-7.54(m, 3H), 7.46(d, *J*=7.5Hz, 2H), 7.39(d, *J*=15.6Hz, 1H), 6.89(d, *J*=8.7Hz, 2H), 6.66(d, *J*=8.7Hz, 2H), 3.84(s, 2H), 3.41(t, *J*=4.2Hz, 4H), 2.97(s, 6H), 2.74(t, *J*=4.5Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$)δ: 196.1, 188.2, 153.7, 151.7, 144.1, 135.9, 133.4, 130.3, 130.1, 129.1, 128.6, 128.1, 123.0, 116.7, 113.6, 111.8, 64.2, 53.1, 47.3, 40.1; HRMS: m/z calcd for $C_{29}H_{32}N_3O_2$ $(M+H)^+$454.2489, found 454.2488.

Compound **7b**: Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$)δ: 8.02-8.07(dd, *J*=5.7Hz, 5.4Hz, 2H), 7.99(d, *J*= 8.7Hz, 2H), 7.79(d, *J*=15.6Hz, 1H), 7.54(d, *J*=8.7Hz, 2H), 7.38(d, *J*=15.3Hz, 1H), 7.14(t, *J*=8.4Hz, 2H), 6.90(d, *J*=8.7Hz, 2H), 6.68(d, *J*=8.7Hz, 2H), 3.81(s, 2H), 3.42(t, *J*=4.2Hz, 4H), 3.00(s, 6H), 2.74(t, *J*=4.8Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$)δ: 194.8, 188.4, 164.2, 153.8, 151.9, 144.2, 132.4, 131.1, 131.0, 130.4, 130.2, 129.3, 123.1, 116.9, 115.0, 116.66, 113.8, 111.9, 64.5, 53.2, 47.4, 40.2; HRMS: m/z calcd for $C_{29}H_{31}FN_3O_2$ $(M+H)^+$472.2395, found 472.2393.

Compound **7c**: Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$)δ: 7.99(t, *J*=7.2Hz, 4H), 7.79(d, *J*=15.3Hz, 1H), 7.54(d, *J*=8.4Hz, 2H), 7.44(d, *J*=8.4Hz, 2H), 7.39(d, *J*=15.6Hz, 1H), 6.91(d, *J*=9.0Hz, 2H),, 6.69(d, *J*=8.7Hz, 2H), 3.82(s, 2H), 3.42(t, *J*=4.5Hz, 4H), 3.01(s, 6H), 2.75(t, *J*=4.8Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$)δ: 195.2, 188.4, 153.8, 151.9, 144.3, 139.9, 134.2, 130.4, 130.2, 129.8, 129.4, 129.0, 123.1, 116.9, 113.8, 111.9, 64.5, 53.2, 47.4, 40.2; HRMS: m/z calcd for $C_{29}H_{31}ClN_3O_2$ $(M+H)^+$488.2099, found 488.2100.

Compound **7d**: Yellow solid; ^{1}H NMR (300MHz, $CDCl_3$)δ: 7.99(d, *J*=8.7Hz, 2H), 7.88(d, *J*=8.7Hz, 2H), 7.79(d, *J*=15.3Hz, 1H), 7.59(d, *J*=8.4Hz, 2H), 7.54(d, *J*=8.7Hz, 2H), 7.39(d, *J*=15.6Hz, 1H), 6.90(d, *J*=9.0Hz, 2H), 6.68(d, *J*=8.7Hz, 2H), 3.80(s, 2H), 3.41(t, *J*=4.8Hz, 4H), 3.00(s, 6H), 2.73(t, *J*=4.8Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$)δ: 195.3, 188.3, 153.7, 151.8, 144.2, 134.6, 132.0, 130.4, 130.2, 129.8, 129.3, 128.6, 123.0, 116.8, 113.8, 111.9, 64.4, 53.1, 47.4, 40.2; HRMS: m/z calcd for $C_{29}H_{31}BrN_3O_2$ $(M+H)^+$532.1594, found 532.1592.

Antitumor activity: About 1×10^4 cell/well were seeded into 96-well microtiter plates. After twenty-four hours post-seeding, cells were treated with vehicle control or various concentrations of samples for 48h.20μL of MTT solution (5mg/mL) was added to each well and the tumor cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 air for 4 h. Upon removal of MTT/medium, 150μL of DMSO was added to each well and the plate was agitated at oscillator for 5 min to dissolve the MTT-formazan. The assay plate was read at a wavelength of 570 nm using a microplate reader.

Anti-tumoral effect of 7c *in vivo*: Female BALB/c mice was injected subcutaneously into the right forelimb armpit with 1×10^6 S180 tumor cells that resuspended in 0.2 ml of PBS, 24h after inoculated, the mouse were then randomly divided into two groups: a vehicle control group, and 7c group. Dosage of 7c group was orally administered 25mg/kg 7c.21 days after the last drug administration, the mice were sacrificed, tumors were harvest and weighed. Data are expressed as mean± s.e.m. of indicated experiments. Student's t test was used to determine significance between two groups where appropriate. $P<0.05$ was considered to be statistically significant.

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Synthesis and biological evaluation of novel hybrid compounds between chalcone and piperazine as potential antitumor agents

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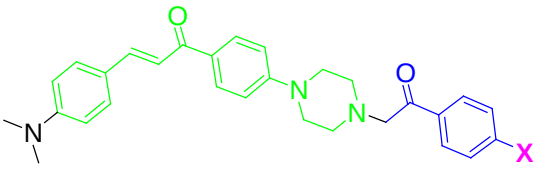

	X	Cell lines (IC_{50}, μM) A549	Hela	SGC7901
7a	H	11.12	2.08	8.84
7b	F	7.17	1.02	0.16
7c	Cl	5.24	0.19	0.41
7d	Br	9.43	0.26	4.07
DDP		11.54	20.52	12.44

A series of novel hybrid compounds between chalcone and piperazine have been synthesized, and their *in vitro* antitumor activity was evaluated against a panel of human tumor cell lines by MTT assay. The results demonstrated that hybrid compounds bearing acetophenone showed excellent antitumor activity, and **7c** was found to be the most potent compound.