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Synthesis and biological evaluation of novel 3-benzylcoumarin-imidazolium salts

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ABSTRACT

A series of novel 3-benzylcoumarin-imidazolium salts were prepared and evaluated in vitro against a panel of human tumor cell lines. The results showed that the existence of 5,6-dimethyl-benzimidazole ring and substitution of the imidazolyl-3-position with a naphthylacyl group were vital for modulating cytotoxic activity. Notably, compound **38** was found to be the most potent derivative with IC_{50} values of 2.04–4.51 μM against five human tumor cell lines, while compound **34** were more selective to SW-480 cell lines with IC_{50} value 40.0-fold lower than DDP. Mechanism of action studies indicated that compound **38** can cause the G0/G1 phase cell cycle arrest and apoptosis in SMMC-7721 cell lines.

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Coumarins are an important class of biologically active heterocycles consisting of benzene and 2-pyrone rings. Natural and synthetic products with 3-benzylcoumarin moiety exhibit a broad range of biological and pharmacological activities.[1] In particular, 3-benzylcoumarin derivatives have been identified to possess antitumor activity.[2] As illuminated in Fig. 1, compound **1** exhibited potent cytotoxic activity against colon carcinoma cells (HCT116) with IC_{50} values of 8 nM,[3] while compound **2** showed effective cytotoxicity to urinary bladder carcinoma cells (EJ-60) and leukemia cells (HL-60).[4]

Heterocycles are of great interest to medicinal chemists owing to their widespread biological and pharmacological activities.[5] Particularly, imidazole and imidazolium salts are key building block of many bioactive compounds. Natural products and biologically active agents possessing the imidazole or imidazolium salts moieties display a variety of biological activities, such as antitumor, antiprotozoal, antidengue virus and antifungal activities.[6] For example, two natural imidazolium salts, Lepidiline A and B (Fig. 1) isolated from *Lepidium meyenii* were found to be potently active against four human cancer cell lines.[7] Recently, we have reported the synthesis of a series of novel imidazolium derivatives, such as NMIB (Fig. 1) and their potential antitumor activities.[8] Further mechanism study demonstrated that the imidazolium salt derivatives can induce the cell cycle arrest and apoptosis in tumor cells.[8h] In previous structure-activity relationships (SARs) studies, we found that the existence of substituted benzimidazole (such as 5,6-dimethyl-benzimidazole) ring and substitution of the imidazolyl-3-position with a substituted phenacyl group (such as 2-naphthylacyl) could be crucial for antitumor activity, which provides the valuable information for further rational design and synthesis of imidazolium salts.

Hence, considering the potent cytotoxic activities of natural and synthetic 3-benzylcoumarin derivatives, as well as the anticancer activities of imidazole derivatives, we report design and synthesis of novel 3-benzylcoumarin-imidazolium derivatives as potent antitumor agents. To the best of our knowledge, no reports concerning antitumor activity for 3-benzylcoumarin-imidazolium salt hybrids have been reported.

In the present research, twenty-four new 3-benzylcoumarin-imidazolium hybrids were synthesized to explore the antitumor activity of these derivatives. The synthetic route for 3-benzylcoumarin-imidazolium salt was shown in Scheme 1. Salicylaldehyde **6** and ethyl 3-oxo-3-phenylpropanoate **7** were selected as the starting materials. Initially, Knoevenagel condensation of salicylaldehyde **6** with ethyl benzoylacetate **7** afforded 3-benzoyl-coumarin **8** in 95% yield. Subsequently, reduction of **8** using $NaBH_4$-$CeCl_3$ in methanol and dichloromethane gave the 3-(hydroxy(phenyl)methyl)-coumarin (**9**, 87% yield). The 3-(hydroxy(phenyl)methyl)-coumarin **9** was transformed via the mesylate to the respective four 3-benzylcoumarin-imidazole hybrids **11**–**14** with various substituted imidazole (imidazole, benzimidazole, 2-methyl-benzimidazole or 5,6-dimethyl-benzimidazole) by refluxing under acetonitrile with 49–72% yields for two steps. Finally, twenty-four novel 3-benzylcoumarin-imidazolium salts **15**–**38** were prepared with excellent yields by reaction of 3-benzylcoumarin-imidazole hybrids **11**–**14** with the corresponding phenacyl bromides in refluxing acetone (65–98% yields). The structures and yields of 3-benzylcoumarin-imidazole derivatives are shown in Table 1.

The potential cytotoxicity of 3-benzylcoumarin-imidazole derivatives **11**–**38** were then evaluated *in vitro* against five human cancer cell lines by MTS assay. [9] The cell lines include

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leuk ined (SW480), breast carcinoma (MCF-7) and liver carcinoma as the concentrations corresponding to 50% growth inhibition). (SMMC-7721). Cisplatin (DDP) was chosen as the reference

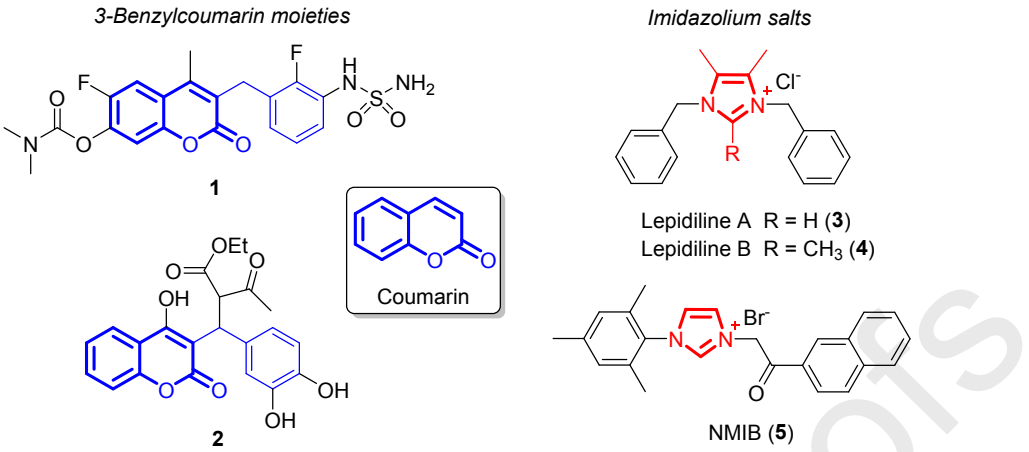
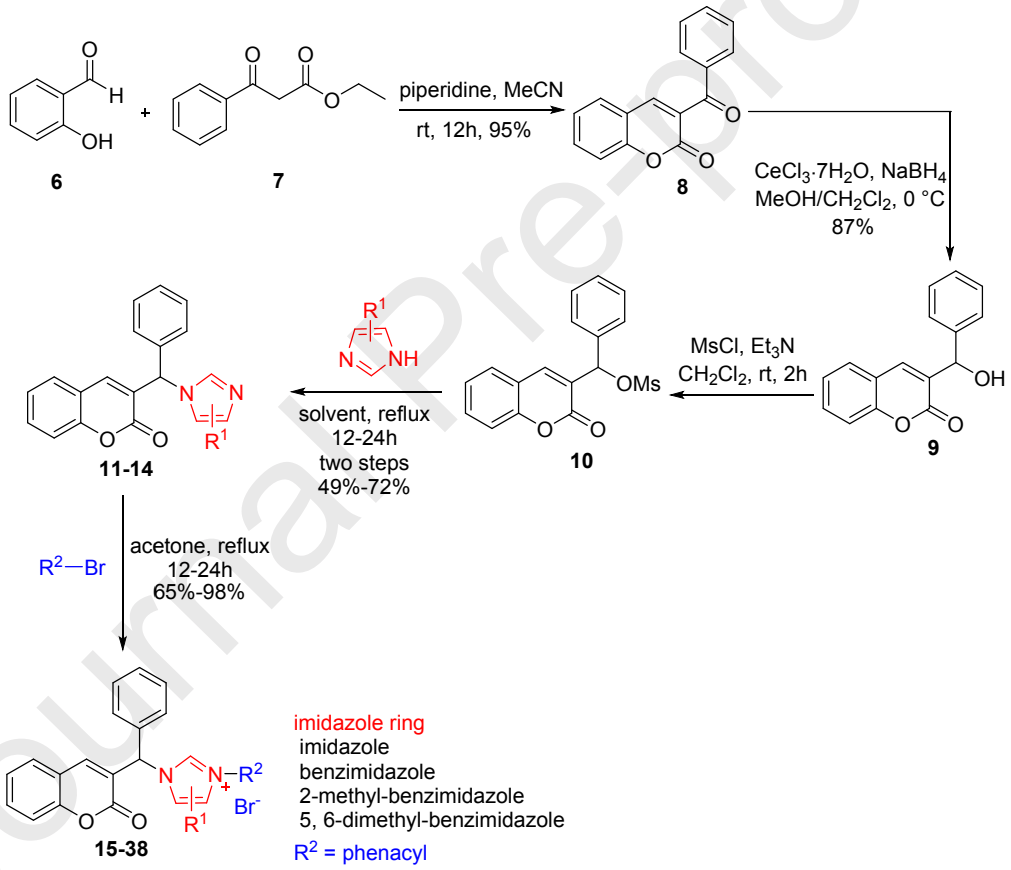


Fig. 1. Representative structures of coumarin, 3-benzylcoumarins and imidazolium salts.



Scheme 1. Synthesis of 3-benzylcoumarin–imidazolium salts **15**–**38**.

Table 1

Structures and yields of compounds **11**–**38**.

Entry	Compound	Imidazole ring	R^2	Molecular formula	Yields (%)
1	**11**	imidazole	-	$C_{19}H_{14}N_2O_2$	72
2	**12**	benzimidazole	-	$C_{23}H_{16}N_2O_2$	49
3	**13**	2-methyl-benzimidazole	-	$C_{24}H_{18}N_2O_2$	52
4	**14**	5,6-dimethyl-benzimidazole	-	$C_{25}H_{20}N_2O_2$	58
5	**15**	imidazole	phenacyl	$C_{27}H_{21}BrN_2O_3$	88
6	**16**	imidazole	4-bromophenacyl	$C_{27}H_{20}Br_2N_2O_3$	98

Journal Pre-proofs

18	imidazole	4-methoxyphenacyl	$C_{28}H_{23}BrN_2O_4$	98
19	imidazole	4-phenylphenacyl	$C_{33}H_{25}BrN_2O_3$	98
20	imidazole	naphthylacyl	$C_{31}H_{23}BrN_2O_3$	94
21	benzimidazole	phenacyl	$C_{31}H_{23}BrN_2O_3$	65
22	benzimidazole	4-bromophenacyl	$C_{31}H_{22}Br_2N_2O_3$	65
23	benzimidazole	4-trifluoromethylphenacyl	$C_{32}H_{22}BrF_3N_2O_3$	76
24	benzimidazole	4-methoxyphenacyl	$C_{32}H_{25}BrN_2O_4$	74
25	benzimidazole	4-phenylphenacyl	$C_{37}H_{27}BrN_2O_3$	80
26	benzimidazole	naphthylacyl	$C_{35}H_{25}BrN_2O_3$	87
27	2-methyl-benzimidazole	phenacyl	$C_{32}H_{25}BrN_2O_3$	82
28	2-methyl-benzimidazole	4-bromophenacyl	$C_{32}H_{24}Br_2N_2O_3$	92
29	2-methyl-benzimidazole	4-trifluoromethylphenacyl	$C_{33}H_{24}BrF_3N_2O_3$	68
30	2-methyl-benzimidazole	4-methoxyphenacyl	$C_{33}H_{27}BrN_2O_4$	78
31	2-methyl-benzimidazole	4-phenylphenacyl	$C_{38}H_{29}BrN_2O_3$	86
32	2-methyl-benzimidazole	naphthylacyl	$C_{36}H_{27}BrN_2O_3$	88
33	5,6-dimethyl-benzimidazole	phenacyl	$C_{33}H_{27}BrN_2O_3$	82
34	5,6-dimethyl-benzimidazole	4-bromophenacyl	$C_{33}H_{26}Br_2N_2O_3$	85
35	5,6-dimethyl-benzimidazole	4-trifluoromethylphenacyl	$C_{34}H_{26}BrF_3N_2O_3$	79
36	5,6-dimethyl-benzimidazole	4-methoxyphenacyl	$C_{34}H_{29}BrN_2O_4$	81
37	5,6-dimethyl-benzimidazole	4-phenylphenacyl	$C_{39}H_{31}BrN_2O_3$	87
38	5,6-dimethyl-benzimidazole	naphthylacyl	$C_{37}H_{29}BrN_2O_3$	87

As show in Table 2, the 3-benzylcoumarin-imidazole derivatives gave more selectivity towards colon carcinoma (SW-480) and breast carcinoma (MCF-7) cell lines. Meanwhile, the structures of hybrid compounds have an obvious influence on the inhibitory activities. 3-benzylcoumarin-imidazoles **11**–**14** and 3-benzylcoumarin-imidazolium salts **15**–**20** lacked activity against five tumor cell lines investigated at the concentration of 20 μM. Nevertheless, their benzimidazolium salts **21**–**38** displayed some extent of activity or higher levels activity. This difference in cytotoxicity between neutral compounds and imidazolium salts may be dependent on variations of molecular structure, charge distribution and water solubility.[10]

In terms of the various substituted imidazole ring (imidazole, benzimidazole, 2-methyl-benzimidazole or 5,6-dimethyl-benzimidazole), imidazolium salts **15**–**18** with imidazole ring lacked activity against all tumor cell lines, only **19** and **20** with a 4-phenylphenacyl or naphthylacyl substituent at position-3 of the imidazole ring displayed weak cytotoxic activities against HL-60 and MCF-7 cell lines. Meanwhile, benzimidazolium salt hybrids **21**–**26** with benzimidazole ring and **27**–**32** with 2-methyl-benzimidazole ring exhibited medium cytotoxic activities, but these hybrids displayed the better selective cytotoxic activity against SW-480 and MCF-7 cell lines with IC_{50} values of 0.96–9.13 μM. Among them, compound **31**, bearing a 4-phenylphenacyl substituent at position-3 of the benzimidazole, showed higher cytotoxic activities *in vitro* compared with DDP. However, benzimidazolium salt hybrids **33**–**38** with 5,6-dimethyl-benzimidazole ring displayed powerful cytotoxic activities. Most of this kind of derivatives was found to be much more active than DDP, such as compounds **34**, **36**, **37** and **38**. Among them, compounds **34**, **36** and **38**, with a 4-methoxyphenacyl, 4-bromophenacyl or naphthylacyl substituent at position-3 of the 5,6-dimethyl-benzimidazole, showed potent cytotoxic activities with IC_{50} values of 0.20–4.89 μM against five human tumor cell lines investigated.

In terms of the substituent at position-3 of imidazole ring, a phenacyl, 4-bromophenacyl or 4-trifluoromethylphenacyl substituent at position-3 of imidazole rings, such as **15**, **16**, **17**, **21**, **22**, **27**, **28**, **29** and **35**, lacked or decreased activities against all tumor cell lines. However, compound **34** with 4-bromophenacyl displayed cytotoxic activity selectively against colon carcinoma (SW-480) with IC_{50} value 40-fold more sensitive to DDP. A 4-methoxyphenacyl substituent at position-3 of imidazole rings, such as **24**, **30** and **36**, slightly improve the inhibitory activities. Especially, compound **36** was more selective to MCF-7 and SW-480 cell lines with IC_{50} value 6.2-fold and 22.2-fold lower than DDP. Interestingly, compared with above substituents, hybrid compounds **25**, **31** and **37** with substituted 4-phenylphenacyl or **26**, **32** and **38** with naphthylacyl substituent at position-3 of imidazole ring exhibited potent activities. Most of these kinds of derivatives showed higher activities than DDP. Furthermore, compounds **38**, bearing a naphthylacyl substituent at position-3 of the 5,6-dimethyl-benzimidazole was found to be the most potent derivatives with IC_{50} values of 2.04–4.51μM against all of human tumor cell lines investigated. Notably, hybrid **25** with a 4-phenylphenacyl substituent at position-3 of benzimidazole were more selective to MCF-7 and SW-480 cell lines with IC_{50} value 10.2-fold and 4.9-fold lower than DDP.

The results indicated that the existence of 5,6-dimethyl-benzimidazole ring and substitution of the imidazolyl-3-position with a 2-naphthylacyl or 4-phenylphenacyl group were important for the antitumor activity. The structure-activity relationship (SAR) results were summarized in Scheme 2.

SMMC-7721 cells were exposed to increasing concentrations of compound **38** and cell apoptosis was determined with Annexin V-FITC/PI double-labeled cell cytometry. As shown in Fig. 2, after treatment of cells with compound **38** at 2, 4, 8 μM for 48 h, the apoptotic cell rate was 1.89 ± 0.03%, 5.70 ± 0.14% and 37.30 ± 1.81%, respectively, which were statistically significantly

Journal Pre-proofs

diff… 1%. cycle analysis on SMMC-7721 cells treated with compound **38** were presented in Fig. 3. Compared with the control cells, the percentage of cells of G1 phase increased during the cells incubated with compound **38** with a dose dependent manner. Compound **38** treatment caused 70.88 ± 0.23% cells in G0/G1 Meanwhile, the percentage of S phase cells decreased slightly accordingly, while the proportion of cells in G2/M and Sub G1 phases showed no obvious change. The data suggest that compound **38** may induce G0/G1 phase arrest in the cell cycle.

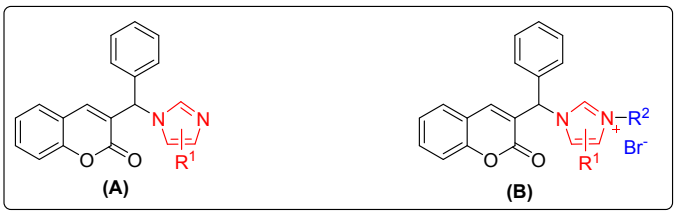
Table 2

Cytotoxic activities of compounds **11**–**38** *in vitro*[b] (IC_{50}, μM[a]).

Entry	Compound	HL-60	SMMC-7721	A-549	MCF-7	SW-480
1	**11**	>20	>20	>20	>20	>20
2	**12**	>20	>20	>20	>20	>20
3	**13**	>20	>20	>20	>20	>20
4	**14**	>20	>20	>20	>20	>20
5	**15**	>20	>20	>20	>20	>20
6	**16**	>20	>20	>20	>20	>20
7	**17**	>20	>20	>20	>20	>20
8	**18**	>20	>20	>20	>20	>20
9	**19**	5.28	>20	>20	12.65	>20
10	**20**	4.38	>20	>20	7.27	8.03
11	**21**	18.61	>20	>20	5.10	6.36
12	**22**	5.95	8.20	11.90	1.24	1.65
13	**23**	4.49	6.15	7.07	1.29	1.55
14	**24**	7.13	8.84	>20	1.74	4.82
15	**25**	2.91	4.70	7.47	0.96	1.63
16	**26**	4.13	5.72	9.11	1.28	1.48
17	**27**	>20	>20	>20	>20	>20
18	**28**	>20	>20	>20	>20	>20
19	**29**	7.72	>20	>20	8.15	9.13
20	**30**	3.69	6.92	8.56	1.21	3.43
21	**31**	2.77	5.05	5.10	2.86	2.88
22	**32**	8.34	11.36	15.83	2.81	6.02
23	**33**	4.81	7.17	12.42	4.17	1.24
24	**34**	2.45	4.19	4.89	2.29	0.20
25	**35**	8.81	9.50	11.68	6.61	8.19
26	**36**	1.55	4.72	5.65	1.58	0.36
27	**37**	2.07	1.91	2.14	1.66	8.03
28	**38**	2.07	3.77	4.51	2.04	2.32
29	DDP	3.02	5.70	7.21	9.80	7.99

[a]Cytotoxicity as IC_{50} for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTS assay.

[b]Data represent the mean values of three independent determinations.



hybrids:

3-benzylcoumarin-imidazolium salts (B) > 3-benzylcoumarin-imidazole hybrids (A)

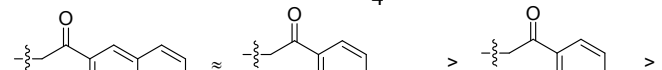
better

imidazole ring:

5,6-dimethyl-benzimidazole > benzimidazole > 2-methyl-benzimidazole > imidazole

Best

R^2 = phenacyl:



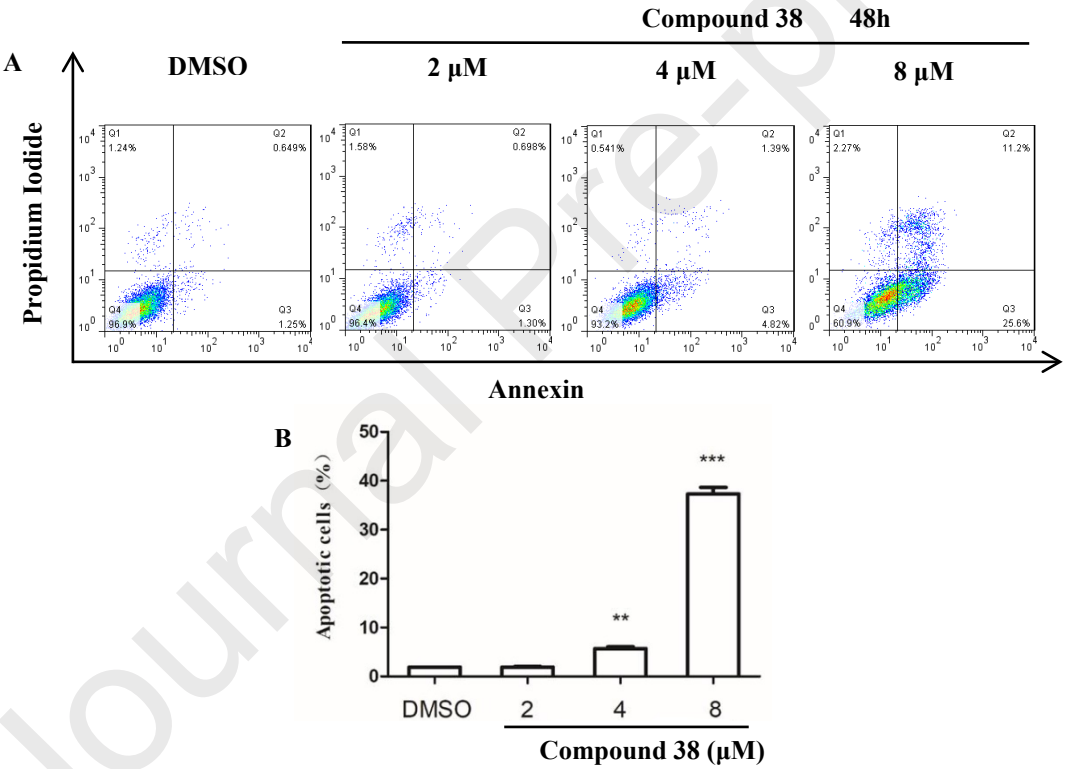
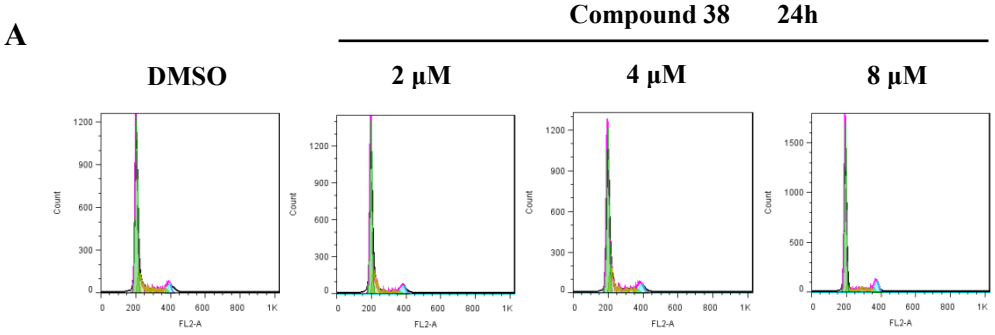


Fig. 2. Compound **38** caused significant apoptosis of SMMC-7721 cells. (A) Cells were treated with 2, 4 and 8 μM compound **38** for 48 h. Cell apoptosis was determined by Annexin V-FITC/PI double-staining assay. (B) The quantification of cell apoptosis. Data represents the mean ± S.D. of three independent experiments.



B

Treatment	SMMC-7721(%)			
	G0/G1	S	G2/M	Sub G1
DMSO	63.71±0.31	23.26±0.57	6.70±0.63	3.173±0.26

Fig. 3. Compound **38** induces G1 phase arrest in SMMC-7721 cells. (A) Cells were treated with 2, 4 and 8 μM of compound **38** for 24 h. Cell cycle was determined by PI staining and cell cytometry. (B) The percentages of cells in different phases were quantified. At least three independent experiments were performed.

In summary, a series of novel 3-benzylcoumarin-imidazolium salts were designed and prepared. Their antitumor activities of these agents were evaluated *in vitro* against five human tumor cell lines. The results showed that the hybrids gave more selectivity towards MCF-7 and SW-480. Compounds **31**, **34**, **36**, **37** and **38** with a 5,6-dimethyl-benzimidazole or 2-methyl-benzimidazole ring, and 2-naphthylacyl, 4-phenylphenacyl, 4-bromophenacyl or 4-methoxyphenacyl substituent at position-3 of imidazole ring were found to be the most potent compounds. Notably, compound **38** was found to be the most potent derivative with IC_{50} values of 2.04–4.51 μM against five human tumor cell lines. Compounds **25**, **34** and **36** were more selective to MCF-7 and SW-480 cell lines with IC_{50} values 10.2-fold, 40.0-fold and 22.2-fold lower than DDP. Study on the antitumor mechanism of action indicated that compound **38** can cause the G0/G1 phase cell cycle arrest and apoptosis in SMMC-7721 cell lines. The SARs was also established in this study, and the information showed that the 3-benzylcoumarin-based imidazolium salts **31**, **34**, **36**, **37** and **38** can be considered promising leads for design of new stronger antitumor compounds.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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