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Synthesis and antitumor activity of aza-brazilian derivatives containing imidazolium salt pharmacophores†

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The synthesis of a series of novel aza-brazilian derivatives containing imidazolium salt pharmacophores is presented. The biological activity of such imidazolium salts was further evaluated *in vitro* against a panel of human tumor cell lines. The results suggest that the electron-withdrawing group on the aza-brazilian moiety, substituted 5,6-dimethyl-benzimidazole ring and substitution of the imidazolyl-3-position with a 4-methylbenzyl group were essential for modulating the cytotoxic activity. Compounds **55** and **39**, bearing a 4-methylbenzyl substituent at position-3 of 5,6-dimethyl-benzimidazole, were found to be the most potent compounds with IC₅₀ values of 0.52–1.30 μM and 0.56–1.51 μM against four human tumor cell lines investigated. Particularly, compound **57** exhibited inhibitory activity against the MCF-7 cell line with an IC₅₀ value of 0.35 μM and was 56-fold more sensitive than DDP. Moreover, compound **55** inhibited cell proliferation through inducing G0/G1 cell cycle arrest and apoptosis in SMMC-7721 cells.

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Introduction

Caesalpinia sappan L. (Chinese name Su-Mu) has long been used as a traditional folk medicine in Southeast and East Asia.¹ Scientific studies have confirmed that the heartwood of *C. sappan* is rich in homoisoflavones. Among them, brazilin (Fig. 1) is considered as one of the most significant bioactive constituents and has attracted the attention of researchers owing to its many pharmacological activities.² Previous studies have reported that brazilin possesses a wide range of pharmacological activities such as anticancer, antioxidant, antibacterial, anti-inflammatory, hypoglycemic, vasorelaxant and hepatoprotective activities.^{3–9} We initiated the research towards the antitumor activity of aza-brazilin derivatives in 2011 and a number of cytotoxic aza-brazilin derivatives were designed and synthesized. We found that aza-brazilin derivatives (such as DMAB, Fig. 1) showed potent cytotoxic activity against a panel of human cancer cell lines.¹⁰

On the other hand, N-heterocycles are of great interest to medicinal chemists because of their widespread occurrence

in active natural products and drug molecules.^{11–15} Of them, imidazolium salts have received significant attention due to their significant and widespread biological and pharmacological activities,^{16–20} especially antitumor activity.^{21–24} For instance, two novel imidazolium salts, lepidiline A and B (Fig. 1), showed effective cytotoxic activity against human tumor cell lines (UMUC3, PACA2, MDA231, and FDIGROV).²⁵ In this respect, our group has long been devoted to the synthesis of novel imidazolium salt derivatives and their potential antitumor activity, aiming to discover new antitumor agents.^{26–31} One representative example is the imidazolium salt NMIB (Fig. 1) which exhibits potent *in vitro* and *in vivo* antitumor activities.^{32,33} Further mechanism research

Fig. 1 Representative structures of brazilin, aza-brazilin derivatives and imidazolium salts.

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demonstrated that these imidazolium salt derivatives could induce cell cycle arrest and apoptosis in tumor cells.^{34–36} Recently, we have reported that imidazolium salts, as novel specific pan-PI3K inhibitors with potent inhibitory activity against class I PI3K isoforms, showed effective inhibition of the PI3K/mTOR signaling pathway.³⁷

Molecular hybridization has played an important role in drug discovery during the past two decades. Thus, it is clear that new pharmacologically interesting hybrid compounds would directly benefit patients by providing access to novel drugs.^{38–41} In view of the potential anticancer activity of aza-brazilian derivatives and imidazolium salts, we conducted the synthesis of hybridized compounds bearing aza-brazilian derivatives and imidazolium salts. Although some hybrid compounds between aza-brazilian (desoxo-brazilin) and imidazole were synthesized and found to display moderate antitumor activity in our previous study,⁴² further design, synthesis and structural modification of new aza-brazilian-imidazolium salt derivatives guided by the structure-activity relationship (SAR) results are needed. With this in mind, we turned our attention to the synthesis and antitumor activity of a series of novel aza-brazilian derivatives containing imidazolium salt pharmacophores and reported our results herein.

Results and discussion

Chemistry

For the synthesis of hybrid molecules containing aza-brazilian derivatives and imidazolium salts, we utilized commercially available imidazole derivatives that were alkylated with *N*-chloropropyl substituted aza-brazilian derivatives, which were prepared from readily obtainable starting materials as illustrated in Scheme 1. Commercial substituted phenylpropanoic acids **1a–1c** were selected as the starting materials for the synthesis of a series of aza-brazilian-imidazole hybrids (compounds **23–28**). Phenylpropanoic acids **1a–1c** were treated with oxalyl chloride, and then under Friedel-Crafts alkylation conditions yielded the corresponding indanones **2–4** in 95–97% yields. The acylation of compounds **2–4** with dimethyl carbonate led to methyl carboxylates **5–7** in 90–92% yields. Compounds **5–7** reacted with *o*-methoxyaniline to give amides **8–10** in refluxing xylene (74–80% yields). Subsequently, the ketone groups of compounds **8–10** were reduced by NaBH₄ yielding hydroxy groups (**11–13**, 74–80% yields), and amides **11–13** were reduced by LiAlH₄ leading to amines (**14–16**, 50–75% yields). Next, the intramolecular Friedel-Crafts alkylation of compounds **14–16** resulted in aza-brazilian derivatives **17–19** in refluxing toluene and pyridinium *p*-toluenesulfonate (PPTS) in 50–63% yields. Key intermediates **17–19** were treated with chloropropanoyl chloride, and then reduced by borane giving *N*-chloropropyl substituted aza-brazilian derivatives (**20–22**, 84–86% yields for two steps). Compounds **20–22** were transformed into the respective aza-brazilian-imidazole

hybrids **23–28** with various substituted benzimidazoles (2-methylbenzimidazole and 5,6-dimethylbenzimidazole) by heat in DMF with 53–74% yields. At the same time, key intermediates **17–19** were also treated with chloropropanoyl chloride, and then coupled directly with various substituted benzimidazoles to give the respective aza-brazilian-imidazole hybrids **29–34** under the same conditions with 61–82% yields.

Finally, thirty-six aza-brazilian-imidazolium salt derivatives **35–58** and **59–70** were synthesized by the reaction of aza-brazilian-imidazole hybrids **23–28** and **29–34** with the corresponding alkyl and phenacyl bromides in the refluxing mixed solvents of toluene and acetone (49–87% and 13–45% yields). The structures and yields of the aza-brazilian-imidazolium salt derivatives are listed in Table 1.

Biological evaluation and structure-activity relationship analysis

The potential cytotoxicity of all the prepared aza-brazilian-imidazolium salts was evaluated *in vitro* against a panel of human tumor cell lines. The panel comprised liver carcinoma (SMMC-7721), lung carcinoma (A-549), breast carcinoma (MCF-7) and colon carcinoma (SW480). Cisplatin (DDP) served as the reference drug. The results are listed in Table 2.

As shown in Table 2, the structures of aza-brazilian derivatives have an essential effect on the cytotoxic potential. The corresponding imidazoles **23–34**, as controls, lacked activity against all tumor cell lines investigated at a concentration of 20 μ M (entry 1). For the substituents of the aza-brazilian moiety, aza-brazilian-imidazolium salts **35–42** and **59–65** with no substituents on the aza-brazilian moiety ($R^1 = R^2 = H$) showed moderate cytotoxic activities with IC₅₀ values of 0.56–10.61 μ M. When the substituents were replaced with electron-donating groups ($R^1 = R^2 = OMe$), the cytotoxic activities of aza-brazilian-imidazolium salts **43–50** and **66–67** were decreased remarkably (IC₅₀ values of 1.07 $\geq$ 20 μ M). In contrast, when the substituents were changed with electron-withdrawing groups ($R^2 = Br$), aza-brazilian-imidazolium salts **51–58** and **68–70** tend to have higher inhibitory activities with IC₅₀ values of 0.35–5.62 μ M than the others.

For the chain between aza-brazilian and the imidazole ring, aza-brazilian-imidazolium salt derivatives (**35–58**) with alkyl chains displayed higher cytotoxic activities against the four tumor cell lines. When the chain was replaced with an acyl chain (**59–70**), the cytotoxic activities of aza-brazilian-imidazolium salts dropped slightly, and some compounds kept the same activity.

In the case of the imidazole ring, aza-brazilian-imidazolium salt derivatives (**35–38/43–46/51–54/59–62/66–68**) with a 2-methyl-benzimidazole ring exhibited medium cytotoxic activities. However, aza-brazilian-imidazolium salt derivatives (**39–42/47–50/55–58/63–65/69–70**) with a 5,6-

Scheme 1 Synthesis of aza-brazilan-imidazole hybrid compounds 23-34.

dimethyl-benzimidazole ring displayed significant inhibitory activities. Interestingly, aza-brazilan-imidazolium salt derivatives 39-42/55/57/69/70, with the 5,6-dimethyl-benzimidazole ring, showed powerful inhibitory activity with IC_{50} values below 2.00 μM (0.35-1.83 μM) against the four tumor cell lines.

For the substituent at position-3 of the imidazole ring, aza-brazilan-imidazolium salt derivatives with a 4-methoxyphenacyl substituent such as 37, 41, 45, 49, 53, 57, 61, 64 and 69 showed relatively weak inhibitory activities against the four tumor cell lines with IC_{50} values of 0.35-14.42 μM . Compound 66 lacked activities against three tumor cell lines tested at a concentration of 20 μM . Imidazolium salts with a 2-naphthylmethyl substituent

such as 36, 40, 44, 48, 52, 56, 60, 63 and 68, as well as imidazolium salts with a 2-naphthylacyl substituent such as 38, 42, 46, 50, 54, 58, 62, 65, 67 and 70 had moderate cytotoxic activities (with IC_{50} values of 0.85-5.83 μM and 0.91-8.26 μM). Notably, imidazolium salts with a 4-methylbenzyl substituent such as 35, 39, 47, 51, 55 and 59 exhibited higher inhibitory activity ($IC_{50} = 0.52-6.27 \mu M$). Among them, compounds 55 and 39, bearing a 4-methylbenzyl substituent at position-3 of 5,6-dimethyl-benzimidazole, were found to be the most potent compounds with IC_{50} values of 0.52-1.30 μM and 0.56-1.51 μM against the four human tumor cell lines investigated. Particularly, compound 57, with a 4-methoxyphenacyl substituent at position-3 of 5,6-dimethyl-benzimidazole, exhibited

Table 1 Synthesis of aza-brazilan-imidazolium salt derivatives **35–70** from hybrids **23–34**

Entry	Compound no.	R ¹	R ²	Imidazole ring	R ³	Molecular formula	Yields (%)
1	23	H	H	2-Methylbenzimidazole	—	C ₂₈ H ₂₉ N ₃ O	74
2	24	H	H	5,6-Dimethylbenzimidazole	—	C ₂₉ H ₃₁ N ₃ O	66
3	25	OMe	OMe	2-Methylbenzimidazole	—	C ₃₀ H ₃₃ N ₃ O ₃	74
4	26	OMe	OMe	5,6-Dimethylbenzimidazole	—	C ₃₁ H ₃₅ N ₃ O ₃	53
5	27	H	Br	2-Methylbenzimidazole	—	C ₂₈ H ₂₈ BrN ₃ O	69
6	28	H	Br	5,6-Dimethylbenzimidazole	—	C ₂₉ H ₃₀ BrN ₃ O	71
7	29	H	H	2-Methylbenzimidazole	—	C ₂₈ H ₂₇ N ₃ O ₂	64
8	30	H	H	5,6-Dimethylbenzimidazole	—	C ₂₉ H ₂₉ N ₃ O ₂	61
9	31	OMe	OMe	2-Methylbenzimidazole	—	C ₃₀ H ₃₁ N ₃ O ₄	82
10	32	OMe	OMe	5,6-Dimethylbenzimidazole	—	C ₃₁ H ₃₃ N ₃ O ₄	81
11	33	H	Br	2-Methylbenzimidazole	—	C ₂₈ H ₂₆ BrN ₃ O ₂	69
12	34	H	Br	5,6-Dimethylbenzimidazole	—	C ₂₉ H ₂₈ BrN ₃ O ₂	71
13	35	H	H	2-Methylbenzimidazole	4-Methylbenzyl	C ₃₆ H ₃₈ BrN ₃ O	66
14	36	H	H	2-Methylbenzimidazole	2-Naphthylmethyl	C ₃₉ H ₃₈ BrN ₃ O	81
15	37	H	H	2-Methylbenzimidazole	4-Methoxyphenacyl	C ₃₇ H ₃₈ BrN ₃ O ₃	84
16	38	H	H	2-Methylbenzimidazole	2-Naphthylacyl	C ₄₀ H ₃₈ BrN ₃ O ₂	58
17	39	H	H	5,6-Dimethylbenzimidazole	4-Methylbenzyl	C ₃₆ H ₄₀ BrN ₃ O	63
18	40	H	H	5,6-Dimethylbenzimidazole	2-Naphthylmethyl	C ₄₀ H ₄₀ BrN ₃ O	87
19	41	H	H	5,6-Dimethylbenzimidazole	4-Methoxyphenacyl	C ₃₈ H ₄₀ BrN ₃ O ₃	80
20	42	H	H	5,6-Dimethylbenzimidazole	2-Naphthylacyl	C ₄₁ H ₄₀ BrN ₃ O ₂	71
21	43	OMe	OMe	2-Methylbenzimidazole	4-Methylbenzyl	C ₃₈ H ₄₂ BrN ₃ O ₄	65
22	44	OMe	OMe	2-Methylbenzimidazole	2-Naphthylmethyl	C ₄₂ H ₄₂ BrN ₃ O ₃	76
23	45	OMe	OMe	2-Methylbenzimidazole	4-Methoxyphenacyl	C ₃₉ H ₄₂ BrN ₃ O ₅	68
24	46	OMe	OMe	2-Methylbenzimidazole	2-Naphthylacyl	C ₄₂ H ₄₂ BrN ₃ O ₄	86
25	47	OMe	OMe	5,6-Dimethylbenzimidazole	4-Methylbenzyl	C ₃₉ H ₄₄ BrN ₃ O ₃	74
26	48	OMe	OMe	5,6-Dimethylbenzimidazole	2-Naphthylmethyl	C ₄₂ H ₄₄ BrN ₃ O ₃	52
27	49	OMe	OMe	5,6-Dimethylbenzimidazole	4-Methoxyphenacyl	C ₄₀ H ₄₄ BrN ₃ O ₅	63
28	50	OMe	OMe	5,6-Dimethylbenzimidazole	2-Naphthylacyl	C ₄₃ H ₄₄ BrN ₃ O ₄	71
29	51	H	Br	2-Methylbenzimidazole	4-Methylbenzyl	C ₃₆ H ₃₇ Br ₂ N ₃ O	53
30	52	H	Br	2-Methylbenzimidazole	2-Naphthylmethyl	C ₃₉ H ₃₇ Br ₂ N ₃ O	49
31	53	H	Br	2-Methylbenzimidazole	4-Methoxyphenacyl	C ₃₇ H ₃₇ Br ₂ N ₃ O ₃	67
32	54	H	Br	2-Methylbenzimidazole	2-Naphthylacyl	C ₄₀ H ₃₇ Br ₂ N ₃ O ₂	80
33	55	H	Br	5,6-Dimethylbenzimidazole	4-Methylbenzyl	C ₃₇ H ₃₉ Br ₂ N ₃ O	69
34	56	H	Br	5,6-Dimethylbenzimidazole	2-Naphthylmethyl	C ₄₀ H ₃₉ Br ₂ N ₃ O	56
35	57	H	Br	5,6-Dimethylbenzimidazole	4-Methoxyphenacyl	C ₃₈ H ₃₉ Br ₂ N ₃ O ₃	69
36	58	H	Br	5,6-Dimethylbenzimidazole	2-Naphthylacyl	C ₄₁ H ₃₉ Br ₂ N ₃ O ₂	68
37	59	H	H	2-Methylbenzimidazole	4-Methylbenzyl	C ₃₆ H ₃₆ BrN ₃ O ₂	28
38	60	H	H	2-Methylbenzimidazole	2-Naphthylmethyl	C ₃₉ H ₃₆ BrN ₃ O ₂	27
39	61	H	H	2-Methylbenzimidazole	4-Methoxyphenacyl	C ₃₇ H ₃₆ BrN ₃ O ₄	33
40	62	H	H	2-Methylbenzimidazole	2-Naphthylacyl	C ₄₀ H ₃₆ BrN ₃ O ₃	34
41	63	H	H	5,6-Dimethylbenzimidazole	2-Naphthylmethyl	C ₄₀ H ₃₈ BrN ₃ O ₂	16
42	64	H	H	5,6-Dimethylbenzimidazole	4-Methoxyphenacyl	C ₃₈ H ₃₈ BrN ₃ O ₄	17
43	65	H	H	5,6-Dimethylbenzimidazole	2-Naphthylacyl	C ₄₁ H ₃₈ BrN ₃ O ₃	13
44	66	OMe	OMe	2-Methylbenzimidazole	4-Methoxyphenacyl	C ₃₉ H ₄₀ BrN ₃ O ₆	21
45	67	OMe	OMe	2-Methylbenzimidazole	2-Naphthylacyl	C ₄₂ H ₄₀ BrN ₃ O ₅	20
46	68	H	Br	2-Methylbenzimidazole	2-Naphthylacyl	C ₄₀ H ₃₅ Br ₂ N ₃ O ₃	45
47	69	H	Br	5,6-Dimethylbenzimidazole	2-Naphthylmethyl	C ₄₀ H ₃₇ Br ₂ N ₃ O ₂	27
48	70	H	Br	5,6-Dimethylbenzimidazole	2-Naphthylacyl	C ₄₁ H ₃₇ Br ₂ N ₃ O ₃	24

inhibitory activity against the MCF-7 cell line with an IC_{50} value of 0.35 μ M and was 56-fold more sensitive than DDP.

The results implied that the existence of an electron-withdrawing group on the aza-brazilian moiety, the substituted 5,6-dimethyl-benzimidazole ring and substitution of the imidazolyl-3-position with a 4-methylbenzyl group could be important for improving the cytotoxic activity. The SAR results are summarized in Scheme 2.

Imidazolium salt 55 induced G0/G1 phase arrest and apoptosis in cancer cells

To determine the proliferation selectivity of compound 55, the IC_{50} values of compound 55 on three normal cell lines (normal human liver cell line L02, normal human lung cell line Beas-2B and normal human colon cell line NCM-460) were detected. As shown in Fig. 2, compound 55 inhibited the growth of human cancer cells with mod-

erate selectivity, compared with the corresponding normal human cell lines. All the compounds used as chemotherapeutic agents do have an effect on normal cells as do the present compounds. However, their effect is higher on cancer cells. This is the reason why the present drugs are potential chemotherapeutic agents. To determine the proliferation inhibitory effect of aza-brazilian-imidazolium salt 55 caused by cell cycle arrest, propidium iodide (PI) staining and flow cytometry analysis of cells were performed in SMMC-7721 cells treated with indicated concentrations of imidazolium salt 55 (1, 2, and 4 μ M). As shown in Fig. 3, the results suggested that imidazolium salt 55 may induce G0/G1 phase arrest in the cell cycle, and a sub-G1 peak (apoptotic peak) appeared when the concentration was at 4 μ M.

Aza-brazilian-imidazolium salt 55 induced cell apoptosis was determined with Annexin V-FITC/PI double-labeled cell cytometry. As shown in Fig. 4, after treatment of cells with imidazolium salt 55 at 0.5, 1, 2, 4 and 8 μ M for 48 h, cell

Table 2 Cytotoxic activities of aza-brazilian-imidazole hybrids **23–34** and aza-brazilian-imidazolium salts **35–70** *in vitro*^b (IC_{50} , μ M^a)

Entry	Compound no.	SMMC-7721	A-549	MCF-7	SW480
1	23–34	>20	>20	>20	>20
2	35	1.80 $\pm$ 0.24	4.25 $\pm$ 0.32	1.47 $\pm$ 0.17	1.66 $\pm$ 0.18
3	36	2.06 $\pm$ 0.37	5.74 $\pm$ 0.39	1.02 $\pm$ 0.03	2.16 $\pm$ 0.51
4	37	1.32 $\pm$ 0.14	3.65 $\pm$ 1.56	0.40 $\pm$ 0.25	1.27 $\pm$ 0.17
5	38	2.01 $\pm$ 0.19	5.32 $\pm$ 0.15	1.36 $\pm$ 0.17	1.80 $\pm$ 0.16
6	39	0.66 $\pm$ 0.11	1.51 $\pm$ 0.07	0.67 $\pm$ 0.10	0.56 $\pm$ 0.08
7	40	0.85 $\pm$ 0.17	1.51 $\pm$ 0.18	0.91 $\pm$ 0.27	0.93 $\pm$ 0.32
8	41	1.42 $\pm$ 0.14	1.40 $\pm$ 0.10	1.26 $\pm$ 0.13	1.73 $\pm$ 0.06
9	42	1.09 $\pm$ 0.11	1.47 $\pm$ 0.06	0.91 $\pm$ 0.15	1.46 $\pm$ 0.19
10	43	6.49 $\pm$ 0.76	14.40 $\pm$ 2.16	5.54 $\pm$ 1.06	3.51 $\pm$ 0.59
11	44	1.13 $\pm$ 0.08	1.53 $\pm$ 0.15	1.07 $\pm$ 0.06	1.50 $\pm$ 0.03
12	45	5.36 $\pm$ 1.12	14.42 $\pm$ 0.37	3.36 $\pm$ 0.45	2.34 $\pm$ 0.61
13	46	5.46 $\pm$ 0.50	8.26 $\pm$ 0.84	2.16 $\pm$ 0.21	2.06 $\pm$ 0.25
14	47	1.71 $\pm$ 0.35	4.83 $\pm$ 0.35	2.00 $\pm$ 0.01	2.00 $\pm$ 0.09
15	48	1.82 $\pm$ 0.41	5.83 $\pm$ 0.78	1.49 $\pm$ 0.07	1.62 $\pm$ 0.16
16	49	4.50 $\pm$ 0.50	8.27 $\pm$ 0.17	2.14 $\pm$ 0.19	1.80 $\pm$ 0.29
17	50	1.71 $\pm$ 0.04	4.91 $\pm$ 0.51	1.12 $\pm$ 0.14	1.38 $\pm$ 0.12
18	51	0.98 $\pm$ 0.29	1.42 $\pm$ 0.45	1.08 $\pm$ 0.28	1.62 $\pm$ 0.43
19	52	1.08 $\pm$ 0.26	1.89 $\pm$ 0.47	0.92 $\pm$ 0.01	1.28 $\pm$ 0.05
20	53	0.93 $\pm$ 0.05	1.22 $\pm$ 0.08	1.01 $\pm$ 0.12	1.24 $\pm$ 0.06
21	54	1.12 $\pm$ 0.09	1.61 $\pm$ 0.23	0.95 $\pm$ 0.10	1.26 $\pm$ 0.06
22	55	0.52 $\pm$ 0.06	1.30 $\pm$ 0.01	0.68 $\pm$ 0.14	0.75 $\pm$ 0.01
23	56	2.16 $\pm$ 0.74	5.62 $\pm$ 0.76	0.84 $\pm$ 0.78	3.92 $\pm$ 0.64
24	57	0.89 $\pm$ 0.02	1.53 $\pm$ 0.11	0.35 $\pm$ 0.13	1.40 $\pm$ 0.18
25	58	1.35 $\pm$ 0.16	3.48 $\pm$ 1.00	1.58 $\pm$ 0.15	4.26 $\pm$ 0.67
26	59	2.04 $\pm$ 0.57	6.27 $\pm$ 0.42	1.57 $\pm$ 0.38	1.57 $\pm$ 0.24
27	60	1.45 $\pm$ 0.32	3.68 $\pm$ 0.62	0.96 $\pm$ 0.20	0.77 $\pm$ 0.19
28	61	4.62 $\pm$ 0.73	10.61 $\pm$ 0.91	3.12 $\pm$ 0.71	2.65 $\pm$ 0.38
29	62	1.51 $\pm$ 0.11	4.71 $\pm$ 0.26	1.46 $\pm$ 0.06	1.44 $\pm$ 0.03
30	63	1.03 $\pm$ 0.15	2.58 $\pm$ 0.24	0.93 $\pm$ 0.14	0.97 $\pm$ 0.21
31	64	3.16 $\pm$ 1.72	7.53 $\pm$ 0.98	2.54 $\pm$ 0.81	0.80 $\pm$ 0.32
32	65	1.12 $\pm$ 0.23	1.99 $\pm$ 0.14	1.01 $\pm$ 0.25	1.35 $\pm$ 0.28
33	66	>20	>20	>20	15.40 $\pm$ 2.36
34	67	6.53 $\pm$ 0.72	9.29 $\pm$ 1.12	4.67 $\pm$ 0.20	3.79 $\pm$ 1.38
35	68	1.58 $\pm$ 0.20	2.63 $\pm$ 0.49	1.43 $\pm$ 0.20	1.75 $\pm$ 0.19
36	69	1.09 $\pm$ 0.01	1.45 $\pm$ 0.07	1.05 $\pm$ 0.10	1.05 $\pm$ 0.17
37	70	1.00 $\pm$ 0.08	1.11 $\pm$ 0.05	1.22 $\pm$ 0.16	1.83 $\pm$ 0.10
38	DDP	9.25 $\pm$ 0.39	5.55 $\pm$ 0.30	19.56 $\pm$ 0.49	6.84 $\pm$ 0.10

^a Cytotoxicity as IC_{50} for each cell line, which is the concentration of a compound which reduced the optical density of treated cells by 50% with respect to untreated cells using the MTS assay. ^b Data represent the mean values of three independent determinations.

Scheme 2 Structure-activity relationship of aza-brazilin-imidazolium salt derivatives.

Fig. 2 The proliferation selectivity of imidazolium salt 55 against human cancer cells and normal human cell lines.

apoptosis in SMMC-7721 cells remarkably elevated to 8.97%, 15.13%, 16.22%, 77.72% and 81.56%, respectively. The data suggested that steroidal imidazolium salt 55 inhibited cell proliferation through induction of G0/G1 cell cycle arrest and apoptosis of the SMMC-7721 cells.

Conclusion

In summary, a series of novel aza-brazilin derivatives containing imidazolium salt pharmacophores prepared in this research have been verified to be potential antitumor

Fig. 3 Imidazolium salt **55** induced G0/G1 phase arrest in SMMC-7721 cells. (A) Cells were treated with 1, 2 and 4 μM compound **55** for 24 h. The 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 and data of one representative experiment are shown.

Fig. 4 Imidazolium salt **55** caused apoptosis of SMMC-7721 cells. (A) Cells were treated with 0.5, 1, 2, 4 and 8 μM imidazolium salt **55** for 48 h. Cell apoptosis was determined by the Annexin V-FITC/PI double-staining assay. (B) The quantification of cell apoptosis. Four independent experiments were performed and data of one representative experiment are shown. The significance was determined by Student's test (** $p < 0.01$ and *** $p < 0.001$ vs. DMSO).

agents. The aza-brazilan-imidazolium salt derivatives **39**, **40**, **51**, **52**, **53**, **55** and **57**, with an electron-withdrawing group on the aza-brazilan moiety, or a 5,6-dimethyl-benzimidazole ring or a 4-methylbenzyl group at position-3 of the imidazole ring, were found to be the most potent compounds. Notably, compounds **55** and **39**, bearing a 4-methylbenzyl substituent at position-3 of 5,6-dimethyl-benzimidazole, were found to be the most potent compounds with IC_{50} values of 0.52–1.30 μ M and 0.56–1.51 μ M against the four human tumor cell lines investigated. Particularly, compound **57** exhibited inhibitory activity against the MCF-7 cell line with an IC_{50} value of 0.35 μ M and was 56-fold more sensitive than DDP. Compound **55** can induce G0/G1 phase cell cycle arrest and apoptosis in SMMC-7721 cells. This kind of aza-brazilan-imidazolium salt derivative could thus serve as a novel starting point to investigate better lead compounds for further structural modifications.

Experimental section

General procedures

Melting points were obtained on an XT-4 melting-point apparatus and were uncorrected. Proton nuclear magnetic resonance (1 H-NMR) spectra were recorded on a Bruker Avance 300 or Bruker DRX 400 spectrometer at 300 or 400 MHz. Carbon-13 nuclear magnetic resonance (13 C-NMR) spectra were recorded on a Bruker DRX 400 spectrometer at 100 MHz. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (TMS) for all the recorded NMR spectra. Low-resolution mass spectra were recorded on a VG Auto Spec-3000 magnetic sector MS spectrometer. High resolution mass spectra were taken on an AB QSTAR Pulsar mass spectrometer. Silica gel (200–300 mesh) for column chromatography and silica GF₂₅₄ for TLC were produced by Qingdao Marine Chemical Company (China). All air- or moisture-sensitive reactions were conducted under an argon atmosphere. Starting materials and reagents used in reactions were obtained commercially from Acros, Aldrich, and Fluka and were used without purification, unless otherwise indicated.

Synthesis of compounds 2–4, 5–7, 8–10, 11–13, 14–16, 17–19 and 20–22. See the ESI† for characterization data.

Synthesis of aza-brazilan-imidazole hybrid compounds 23–28. A mixture of compound **20–22** (0.44 mmol), 2-methylbenzimidazole or 5,6-dimethylbenzimidazole (0.53 mmol) and K_2CO_3 (365 mg, 2.64 mmol) was stirred in DMF (20 ml) at 120 °C under nitrogen for 12 h. The reaction progress was monitored by TLC. After cooling to room temperature, the solvent was evaporated under reduced pressure and the crude product was purified by flash column chromatography on silica gel (eluted with CH_2Cl_2 :MeOH:Et₃N = 500:1:5 $\rightarrow$ 300:1:3) to give **23–28** as a white powder or colorless oil in 53–74% yields. See the ESI† for characterization data.

Synthesis of aza-brazilan-imidazole hybrid compounds 29–34. 3-Chloropropanoyl chloride (367 mg, 2.89 mmol) was slowly added to a solution of compound **17–19** (0.96 mmol)

and K_2CO_3 aqueous solution (0.5 M, 6 ml, 3.00 mmol) in CH_2Cl_2 (10 mL) in an ice bath. The solution was warmed to room temperature and stirred for 30 min. After cooling at 0 °C, it was quenched with H_2O . The resulting mixture was extracted with CH_2Cl_2 , and the organic layer was washed with brine, dried over Na_2SO_4 , and concentrated. The obtained product was used without any further purification. A mixture of the previous product (0.44 mmol), 2-methylbenzimidazole or 5,6-dimethylbenzimidazole (0.53 mmol) and K_2CO_3 (365 mg, 2.64 mmol) in dry DMF (5 ml) was stirred in DMF (20 ml) at 120 °C under nitrogen for 12 h. The reaction progress was monitored by TLC. The solvent was evaporated under reduced pressure and the crude product was purified by flash column chromatography on silica gel (eluted with CH_2Cl_2 :MeOH:Et₃N = 500:1:5 $\rightarrow$ 300:1:3) to give **29–34** as a white powder or colorless oil in 61–82% yields. See the ESI† for characterization data.

Synthesis of aza-brazilan-imidazolium salt compounds 38–58 and 59–70. A mixture of compound **23–28** or **29–34** (0.19 mmol) and the corresponding alkyl and phenacyl bromides (0.38 mmol) was stirred in acetone/toluene (1:1, 6 ml) at reflux for 24–48 h. An insoluble substance was formed. After completion of the reaction as indicated by TLC, the precipitate was filtered through a small pad of Celite, washed with ethyl acetate (3 $\times$ 30 ml), and then dried to afford imidazolium salts **35–58** and **59–70** in 49–87% and 13–45% yields. See the ESI† for characterization data.

Cytotoxicity assay

The cytotoxicity of the compounds was determined by the MTS method. All cell lines used in this study were obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Briefly, 5×10^3 cells were plated in 96-well plates 12 h before treatment and continuously exposed to test compounds for 48 h. Then MTS (Promega, Madison, WI, USA) was added to each well. The samples were incubated at 37 °C for 1–4 h and the optical density (OD) was measured at 490 nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The IC_{50} values are calculated from appropriate dose-response curves.

Cell cycle analysis

To analyze the DNA content by flow cytometry, cells were collected and washed twice with PBS. Cells were fixed with 70% ethanol overnight. Fixed cells were washed with PBS, and then stained with a 50 μ g ml^{−1} propidium iodide (PI) solution (Sigma-Aldrich, Saint-Louis, MO, USA) containing 50 μ g ml^{−1} RNase A (Sigma-Aldrich) for 30 min at room temperature. The fluorescence intensity was analyzed using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). The percentages of the cells distributed in different phases of the cell cycle were determined using FlowJo V 7.6.1 software.

Cell apoptosis analysis

Cell apoptosis was analyzed using the Annexin V-FITC/PI Apoptosis kit (BD Biosciences, Franklin Lakes, NJ) according to the manufacturer's protocols. Cells were seeded in 6-well plates at a density of 3×10^5 cells per well. After 48 h of compound treatment at the indicated concentrations, cells were collected, washed twice with cold PBS, and then resuspended in a binding buffer containing Annexin V-FITC and propidium iodide (PI). After incubation for 15 min at room temperature in the dark, the fluorescence intensity was measured using a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ).

Conflicts of interest

There are no conflicts to declare.

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Notes and references

- N. P. Nirmal, M. S. Rajput, R. G. S. V. Prasad and M. Ahmad, *Asian Pac. J. Trop. Med.*, 2015, **8**, 421–430.
- I. K. Bae, H. Y. Min, A. R. Han, E. K. Seo and S. K. Lee, *Eur. J. Pharmacol.*, 2005, **513**, 237–242.
- B. Kim, S. H. Kim, S. J. Jeong, E. J. Sohn, J. H. Jung, M. H. Lee and S. H. Kim, *J. Agric. Food Chem.*, 2012, **60**, 9882–9889.
- C. C. Lee, C. N. Wang, J. J. Kang, J. W. Liao, B. L. Chiang, H. C. Chen, C. M. Hu, C. D. Lin, S. H. Huang and Y. T. Lai, *J. Agric. Food Chem.*, 2012, **60**, 9405–9414.
- Y. Sasaki, T. Hosokawa, M. Nagai and S. Nagumo, *Biol. Pharm. Bull.*, 2007, **30**, 193–196.
- H. X. Xu and S. F. Lee, *Phytother. Res.*, 2004, **18**, 647–651.
- C. K. Moon, S. H. Lee, M. O. Lee and S. G. Kim, *Life Sci.*, 1993, **53**, 1291–1297.
- C. M. Hu, J. J. Kang, C. C. Lee, C. H. Li, J. W. Liao and Y. W. Cheng, *Eur. J. Pharmacol.*, 2003, **468**, 37–45.
- C. K. Moon, K. S. Park, S. G. Kim, H. S. Won and J. H. Chung, *Drug Chem. Toxicol.*, 1992, **15**, 81–91.
- C. X. Pan, X. H. Zeng, Y. F. Guan, X. L. Jiang, L. A. Li and H. B. Zhang, *Synlett*, 2011, 425–429, DOI: 10.1055/s-0030-1259511.
- E. Vitaku, D. T. Smith and J. T. Njardarson, *J. Med. Chem.*, 2014, **57**, 10257–10274.
- Z. F. Liu, M. Y. Li, B. J. Wang, G. G. Deng, W. Chen, B. S. Kim, H. B. Zhang, X. D. Yang and P. J. Walsh, *Org. Chem. Front.*, 2018, **5**, 1870–1876.
- S. Duan, M. Li, X. Ma, W. Chen, L. Li, H. Zhang, X. Yang and P. J. Walsh, *Adv. Synth. Catal.*, 2018, **360**, 4837–4842.
- G. Deng, M. Li, K. Yu, C. Liu, Z. Liu, S. Duan, W. Chen, X. Yang, H. Zhang and P. J. Walsh, *Angew. Chem., Int. Ed.*, 2019, **58**, 2826–2830.
- G. Deng, B. Zhou, J. Wang, Z. Chen, L. Gong, Y. Gong, D. Wu, Y. Li, H. Zhang and X. Yang, *Eur. J. Med. Chem.*, 2019, **168**, 232–252.
- S. Manda, S. Sharma, A. Wani, P. Joshi, V. Kumar, S. K. Guru, S. S. Bharate, S. Bhushan, R. A. Vishwakarma, A. Kumar and S. B. Bharate, *Eur. J. Med. Chem.*, 2016, **107**, 1–11.
- C. Zheng, Y. Z. Fang, W. G. Tong, G. L. Li, H. G. Wu, W. B. Zhou, Q. X. Lin, F. F. Yang, Z. F. Yang, P. Wang, Y. R. Peng, X. F. Pang, Z. F. Yi, J. Luo, M. Y. Liu and Y. H. Chen, *J. Med. Chem.*, 2014, **57**, 600–612.
- S. Manda, S. I. Khan, S. K. Jain, S. Mohammed, B. L. Tekwani, I. A. Khan, R. A. Vishwakarma and S. B. Bharate, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 3247–3250.
- R. Otto, R. Penzsis, F. Gaube, T. Winckler, D. Appenroth, C. Fleck, C. Trankle, J. Lehmann and C. Enzensperger, *Eur. J. Med. Chem.*, 2014, **87**, 63–70.
- Y. Rook, K. U. Schmidtke, F. Gaube, D. Schepmann, B. Wunsch, J. Heilmann, J. Lehmann and T. Winckler, *J. Med. Chem.*, 2010, **53**, 3611–3617.
- M. R. Saberi, T. K. Vinh, S. W. Yee, B. J. N. Griffiths, P. J. Evans and C. Simons, *J. Med. Chem.*, 2006, **49**, 1016–1022.
- C. G. Fortuna, V. Barresi, G. Berellini and G. Musumarra, *Bioorg. Med. Chem.*, 2008, **16**, 4150–4159.
- W. Chen, X. D. Yang, Y. Li, L. J. Yang, X. Q. Wang, G. L. Zhang and H. B. Zhang, *Org. Biomol. Chem.*, 2011, **9**, 4250–4255.
- L. X. Liu, X. Q. Wang, B. Zhou, L. J. Yang, Y. Li, H. B. Zhang and X. D. Yang, *Sci. Rep.*, 2015, **5**, 13101.
- B. L. Cui, B. L. Zheng, K. He and Q. Y. Zheng, *J. Nat. Prod.*, 2003, **66**, 1101–1103.
- Y. Zhou, K. Duan, L. Zhu, Z. Liu, C. Zhang, L. Yang, M. Li, H. Zhang and X. Yang, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 460–465.
- B. Zhou, Z.-F. Liu, G.-G. Deng, W. Chen, M.-Y. Li, L.-J. Yang, Y. Li, X.-D. Yang and H.-B. Zhang, *Org. Biomol. Chem.*, 2016, **14**, 9423–9430.
- X.-L. Xu, C.-L. Yu, W. Chen, Y.-C. Li, L.-J. Yang, Y. Li, H.-B. Zhang and X.-D. Yang, *Org. Biomol. Chem.*, 2015, **13**, 1550–1557.
- X.-L. Xu, J. Wang, C.-L. Yu, W. Chen, Y.-C. Li, Y. Li, H.-B. Zhang and X.-D. Yang, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 4926–4930.
- X.-Q. Wang, L.-X. Liu, Y. Li, C.-J. Sun, W. Chen, L. Li, H.-B. Zhang and X.-D. Yang, *Eur. J. Med. Chem.*, 2013, **62**, 111–121.
- W. Chen, X.-Y. Deng, Y. Li, L.-J. Yang, W.-C. Wan, X.-Q. Wang, H.-B. Zhang and X.-D. Yang, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 4297–4302.

- 32 X.-D. Yang, X.-H. Zeng, Y.-L. Zhang, C. Qing, W.-J. Song, L. Li and H.-B. Zhang, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1892–1895.
- 33 X. Zeng, X. Yang, Y. Zhang, C. Qing and H. Zhang, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1844–1847.
- 34 C.-B. Zhang, Y. Liu, Z.-F. Liu, S.-Z. Duan, M.-Y. Li, W. Chen, Y. Li, H.-B. Zhang and X.-D. Yang, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 1808–1814.
- 35 J.-M. Liu, M. Wang, Y.-J. Zhou, J.-M. Yan, L.-J. Yang, Y. Li, H.-B. Zhang and X.-D. Yang, *RSC Adv.*, 2015, **5**, 63936–63944.
- 36 L.-X. Liu, X.-Q. Wang, J.-M. Yan, Y. Li, C.-J. Sun, W. Chen, B. Zhou, H.-B. Zhang and X.-D. Yang, *Eur. J. Med. Chem.*, 2013, **66**, 423–437.
- 37 H. Zhou, C. Yu, L. Kong, X. Xu, J. Yan, Y. Li, T. An, L. Gong, Y. Gong, H. Zhu, H. Zhang, X. Yang and Y. Li, *Oncogene*, 2019, **38**, 3371–3386.
- 38 C. Viegas-Junior, A. Danuello, V. D. Bolzani, E. J. Barreir and C. A. M. Fraga, *Curr. Med. Chem.*, 2007, **14**, 1829–1852.
- 39 J. J. Walsh and A. Bell, *Curr. Pharm. Des.*, 2009, **15**, 2970–2985.
- 40 M. D'hooghe, K. Mollet, R. De Vreese, T. H. M. Jonckers, G. Dams and N. De Kimpe, *J. Med. Chem.*, 2012, **55**, 5637–5641.
- 41 H. Zhang, Y. Tian, D. W. Kang, Z. P. Huo, Z. X. Zhou, H. Q. Liu, E. De Clercq, C. Pannecouque, P. Zhan and X. Y. Liu, *Eur. J. Med. Chem.*, 2017, **130**, 209–222.
- 42 X. Wang, Y. Li, Y. X. Yang and H. Zhang, *Youji Huaxue*, 2015, **35**, 1276–1285.