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Novel 3-substituted fluorine imidazolium/triazolium salt derivatives: Synthesis and antitumor activity†

Jin-Mei Liu,‡[a] Min Wang,‡[b] Yun-Jing Zhou,[a] Ju-Ming Yan,[b] Li-Juan Yang,*[c] Yan Li,*[b] Hong-Bin Zhang[a] and Xiao-Dong Yang*[a]

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[a]*Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming, 650091, P. R. China. Tel.: +86-871-65031119; Fax.: +86-871-65035538. E-mail: xdyang@ynu.edu.cn*

[b]*State Key Laboratory for Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science, Kunming, 650204, P. R. China. E-mail: liyanb@mail.kib.ac.cn*

[c]*Key Laboratory of Ethnic Medicine Resource Chemistry, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University, Kunming, 650500, P. R. China. E-mail: yangljyang@sina.com*

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‡ These authors contributed equally to this paper.

A series of novel (±)-3-substituted fluorene–imidazolium/triazolium salt derivatives has been prepared and evaluated *in vitro* against a panel of human tumor cell lines. The results suggest that the existence of 2-methyl-benzimidazole or 5,6-dimethyl-benzimidazole ring and substitution of the imidazolyl/triazolyl-3/4-position with a naphthylacyl or 4-methoxyphenacyl group were important for modulating cytotoxic activity. Compounds **37** and **42** were found to be the most potent derivatives with IC_{50} values of 0.51–2.51 μM and exhibited cytotoxic activities selectively against myeloid leukaemia (HL-60), liver carcinoma (SMMC-7721) and lung carcinoma (A549). Compound **37** can remarkablely induce the G2/M phase cell cycle arrest and apoptosis in SMMC-7721 cells. Additionally, compound **30** exhibited selective cytotoxicity to some extent between cancer cells (A549) and normal cells (BEAS-2B).

Introduction

Constructing novel pharmacologically interesting hybrid compounds for drug discovery has attracted much attention during the past two decades.[1] Fluorenes are an important class of biologically active compounds. Natural products and biologically active agents possessing the fluorene framework display a broad range of biological and pharmacological activities.[2] In particular, fluorene derivatives have been identified to possess antitumor activity. As illuminated in Scheme 1, Ixorapeptide I exhibited selective potency against Hep3B liver cancer cell line,[3] while N-(2-(1H-pyrazol-1-yl)phenyl)-7-amino-9-oxo-9H-fluorene-1-carboxamide (PAFC) significantly showed selective cytotoxicity towards breast, colon and hepatocellular carcinoma cells (T47D, HCT116 and SNU398).[4]

On the other hand, imidazolium and triazolium salts have gained considerable interests because of their broad range of biological and pharmacological activity,[5] especially antitumor activity.[6] For example, two new imidazolium chlorides (Fig. 1), Lepidiline A and B, isolated from the roots of *Lepidium meyenii*, showed potent cytotoxic activity against human cancer cell lines.[7] We have previously reported the synthesis of a series of novel imidazolium and triazolium salt derivatives, such as NPTB (Fig. 1), and their potential antitumor

activity.[8] Studies on molecular mechanisms demonstrated that the imidazolium salt hybrids can induce the G1 phase cell cycle arrest and apoptosis in tumor cells.[8c]

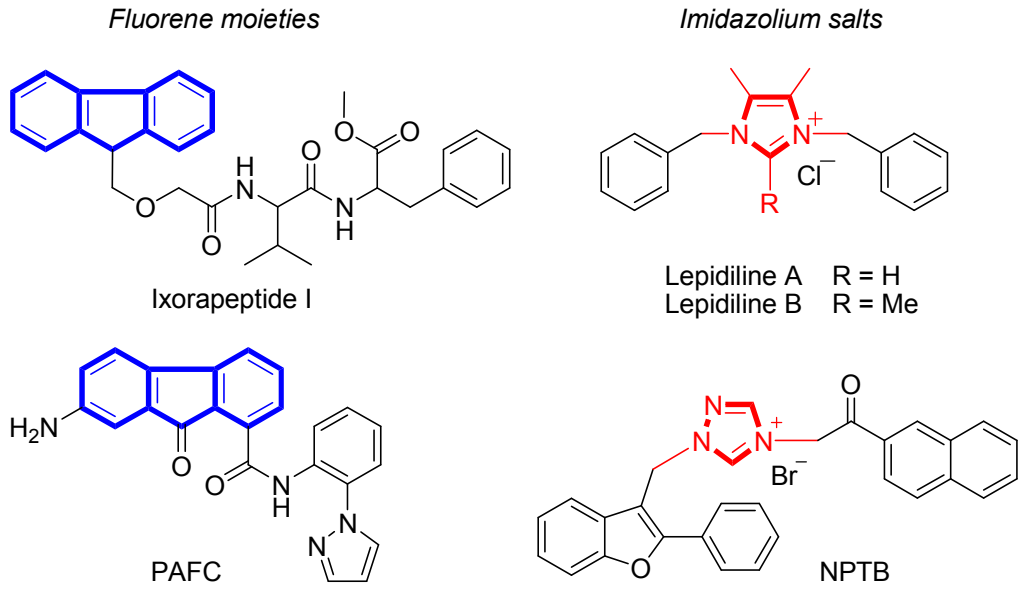


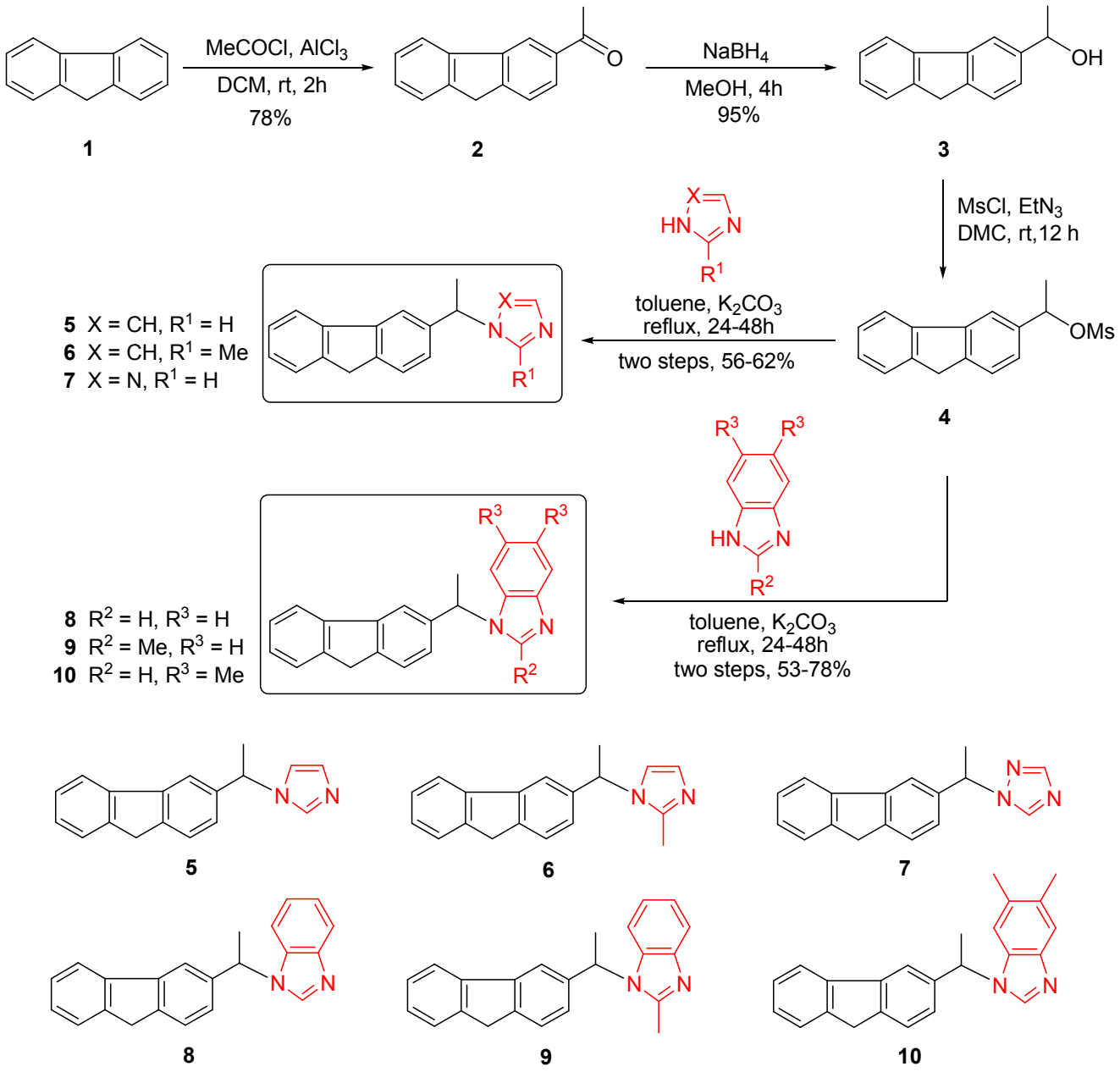
Fig. 1 Representative structures of fluorene derivatives and imidazolium/triazolium salts.

Considering the potent anticancer activities of fluorene derivatives and imidazolium or triazolium salts, we were interested in synthesizing the hybridizing compounds bearing 3-substituted fluorene and imidazolium or triazolium moieties. To the best of our knowledge, no reports concerning antitumor activity of 3-substituted fluorene–imidazole/triazole hybrid compounds have been found in the literature.

In the present research, a series of novel 3-substituted fluorene–imidazolium/triazolium salt derivatives were synthesized. The purpose of this study was to investigate the antitumor activity of fluorene-based imidazolium/triazolium salt compounds, with the ultimate aim of developing novel potent antitumor agents.

Results and discussion

Chemistry

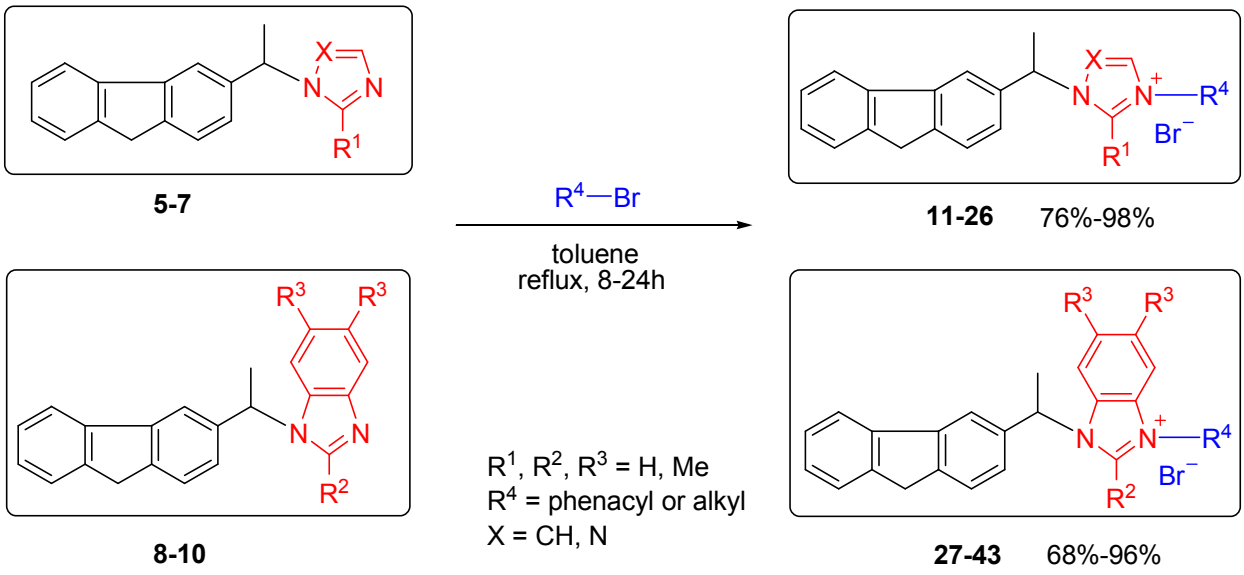


Scheme 1 Synthesis of hybrid compounds **5**–**10**.

To synthesize 3-substituted fluorene–imidazolium/triazolium salt derivatives, we used commercially available imidazole or triazole derivatives that were alkylated with 1-(9*H*-fluoren-3-yl)ethanol, which was synthesized from readily available starting materials as shown in Scheme 1. Commercial fluorene **1** was chosen as the starting material for the preparation of a series of 3-substituted fluorene–imidazole/triazole hybrids (**5**–**10**). The

acetylation of fluorene **1** under Friedel–Craft acylation conditions gave the corresponding 1-(9*H*-fluoren-3-yl)ethanone **2** in 78% yield. The ketone compounds **2** were reduced via $NaBH_4$ leading to the formation of (±)-1-(9*H*-fluoren-3-yl)ethanol (**3**, 95% yield). Subsequently, ethanol **3** was transformed to the respective five (±)-3-substituted fluorene–imidazole hybrids **5**, **6**, **8**–**10** with various substituted imidazole or benzimidazole (imidazole, 2-methyl- imidazole, benzimidazole, 2-methyl-benzimidazole or 5,6-dimethyl-benzimidazole) and a (±)-3-substituted fluorene–triazole hybrid **7** with 1,2,4-triazole by refluxing under toluene with 53–78% yields (two steps).

Finally, thirty-three (±)-3-substituted fluorene–imidazolium/triazolium salts **11**–**43** were prepared with excellent yields by reaction of (±)-3-substituted fluorene–imidazole hybrids **5**–**10** with the corresponding alkyl and phenacyl bromides in refluxing toluene (68–98% yields). The structures and yields of 3-substituted fluorene–imidazole/triazole derivatives are shown in Scheme 2.



Scheme 2 Synthesis of (±)-3-substituted fluorene–imidazolium/triazolium salt derivatives **11**–**43** from **5**–**10**.

Biological evaluation and structure-activity relationship analysis

The potential cytotoxicity of all newly synthesized (±)-3-substituted fluorene imidazolium/triazolium salt derivatives was evaluated *in vitro* against a panel of human tumor cell lines according to procedures described in the literature[9]. The panel consisted of myeloid leukaemia (HL-60), liver carcinoma (SMMC-7721), lung carcinoma (A549), breast carcinoma (MCF-7) and colon carcinoma (SW480). Cisplatin (DDP) and taxol were used as the reference drugs. The results are summarized in Table 1.

Table 1 Cytotoxic activities of (±)-3-substituted fluorene–imidazole/triazole derivatives **5**–**43** in vitro[b] (IC_{50}, mean ± SD, μM[a])

Entry	Compd.	Imidazole/triazole ring	R^4	HL-60	SMMC-7721	A549	MCF-7	SW480
1	**5**	imidazole	–	>40	>40	>40	>40	>40
2	**6**	2-methyl-imidazole	–	>40	>40	>40	>40	>40
3	**7**	triazole	–	>40	>40	>40	>40	>40
4	**8**	benzimidazole	–	>40	>40	>40	>40	>40
5	**9**	2-methyl-benzimidazole	–	10.75 ± 0.85	23.36 ± 3.74	18.21 ± 0.19	36.89 ± 0.91	25.92 ± 3.57
6	**10**	5,6-dimethyl-benzimidazole	–	31.50 ± 6.37	>40	35.91 ± 1.08	>40	>40
7	**11**	imidazole	4-bromobenzyl	2.17 ± 0.22	6.76 ± 1.88	10.45 ± 0.80	4.42 ± 0.15	11.94 ± 0.59
8	**12**	imidazole	phenacyl	3.49 ± 0.03	18.08 ± 0.16	23.41 ± 2.25	17.68 ± 0.94	13.74 ± 0.52
9	**13**	imidazole	4-bromophenacyl	1.75 ± 0.04	5.34 ± 0.29	4.02 ± 0.35	3.03 ± 0.23	3.85 ± 0.22
10	**14**	imidazole	4-fluorophenacyl	2.92 ± 0.39	16.49 ± 0.44	15.29 ± 0.91	15.70 ± 1.03	12.56 ± 0.89
11	**15**	imidazole	4-methoxyphenacyl	1.10 ± 0.04	7.56 ± 0.29	9.38 ± 0.82	4.52 ± 0.11	9.00 ± 0.71
12	**16**	imidazole	naphthylacyl	1.01 ± 0.04	4.13 ± 0.22	3.40 ± 0.49	3.17 ± 0.13	3.44 ± 0.14
13	**17**	2-methyl-imidazole	4-bromobenzyl	1.09 ± 0.09	4.47 ± 0.46	6.75 ± 0.88	6.64 ± 1.17	11.03 ± 0.48
14	**18**	2-methyl-imidazole	phenacyl	1.47 ± 0.24	8.25 ± 0.20	11.01 ± 0.33	12.35 ± 0.98	13.11 ± 0.28
15	**19**	2-methyl-imidazole	4-bromophenacyl	1.90 ± 0.10	8.80 ± 0.35	9.06 ± 0.48	7.92 ± 0.57	12.68 ± 0.92
16	**20**	2-methyl-imidazole	4-methoxyphenacyl	0.52 ± 0.09	2.70 ± 0.81	2.86 ± 0.34	3.01 ± 0.23	10.84 ± 0.44
17	**21**	2-methyl-imidazole	naphthylacyl	0.79 ± 0.01	2.65 ± 0.22	2.15 ± 0.20	2.92 ± 0.06	8.89 ± 0.78
18	**22**	triazole	4-bromobenzyl	2.05 ± 0.10	8.72 ± 0.07	10.00 ± 0.52	4.07 ± 0.70	11.16 ± 1.19
19	**23**	triazole	phenacyl	8.29 ± 1.50	17.03 ± 0.65	15.34 ± 0.63	17.80 ± 0.38	16.15 ± 0.30
20	**24**	triazole	4-bromophenacyl	2.07 ± 0.18	3.15 ± 0.11	2.97 ± 0.16	3.41 ± 0.18	3.51 ± 0.04
21	**25**	triazole	4-methoxyphenacyl	2.55 ± 0.14	13.36 ± 0.50	12.32 ± 1.10	9.37 ± 2.48	11.94 ± 1.94
22	**26**	triazole	naphthylacyl	1.70 ± 0.10	3.30 ± 0.03	3.17 ± 0.04	3.41 ± 0.48	3.11 ± 0.33
23	**27**	benzimidazole	4-bromobenzyl	0.74 ± 0.05	3.42 ± 0.40	4.05 ± 0.23	2.61 ± 0.08	3.14 ± 0.11
24	**28**	benzimidazole	phenacyl	0.76 ± 0.05	4.54 ± 0.20	8.84 ± 1.07	3.17 ± 0.15	2.89 ± 0.10
25	**29**	benzimidazole	4-bromophenacyl	1.38 ± 0.13	3.40 ± 0.13	3.01 ± 0.12	2.30 ± 0.11	3.25 ± 0.10
26	**30**	benzimidazole	4-methoxyphenacyl	0.56 ± 0.02	2.22 ± 0.09	2.58 ± 0.17	1.80 ± 0.18	2.54 ± 0.22
27	**31**	benzimidazole	naphthylacyl	1.23 ± 0.01	3.23 ± 0.11	4.04 ± 0.43	2.44 ± 0.13	3.11 ± 0.14
28	**32**	2-methyl-benzimidazole	4-bromobenzyl	0.60 ± 0.07	2.38 ± 0.14	3.64 ± 0.10	2.78 ± 0.03	2.15 ± 0.13
29	**33**	2-methyl-benzimidazole	phenacyl	0.63 ± 0.15	1.97 ± 0.09	4.49 ± 0.81	2.58 ± 0.01	2.43 ± 0.13

30	**34**	2-methyl-benzimidazole	4-bromophenacyl	0.81 ± 0.04	2.43 ± 0.42	4.63 ± 0.85	3.43 ± 0.02	2.82 ± 0.08
31	**35**	2-methyl-benzimidazole	4-fluorophenacyl	0.68 ± 0.06	5.47 ± 0.15	9.08 ± 0.65	3.12 ± 0.19	2.72 ± 0.10
32	**36**	2-methyl-benzimidazole	4-methoxyphenacyl	0.59 ± 0.03	2.04 ± 0.03	2.47 ± 0.27	2.79 ± 0.09	2.28 ± 0.11
33	**37**	2-methyl-benzimidazole	naphthylacyl	0.57 ± 0.02	1.38 ± 0.04	1.82 ± 0.24	2.51 ± 0.13	2.36 ± 0.04
34	**38**	5,6-dimethyl-benzimidazole	4-bromobenzyl	0.45 ± 0.02	2.17 ± 0.11	2.62 ± 0.06	2.99 ± 0.10	3.04 ± 0.20
35	**39**	5,6-dimethyl-benzimidazole	phenacyl	0.68 ± 0.07	2.44 ± 0.25	3.43 ± 0.14	3.14 ± 0.08	3.28 ± 0.08
36	**40**	5,6-dimethyl-benzimidazole	4-bromophenacyl	0.58 ± 0.02	2.30 ± 0.08	3.25 ± 0.45	2.79 ± 0.07	2.70 ± 0.11
37	**41**	5,6-dimethyl-benzimidazole	4-fluorophenacyl	1.78 ± 0.13	2.82 ± 0.38	6.74 ± 0.16	3.71 ± 0.31	4.43 ± 0.06
38	**42**	5,6-dimethyl-benzimidazole	4-methoxyphenacyl	0.50 ± 0.03	1.69 ± 0.15	1.61 ± 0.17	2.41 ± 0.18	2.41 ± 0.02
39	**43**	5,6-dimethyl-benzimidazole	naphthylacyl	0.87 ± 0.17	2.31 ± 0.01	2.59 ± 0.13	3.02 ± 0.15	3.04 ± 0.14
40				1.16 ± 0.10	6.72 ± 0.28	7.25 ± 0.46	15.06 ± 0.81	15.11 ± 0.92
41				<0.008	<0.008	<0.008	<0.008	<0.008

[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 MTT assay.

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

As shown in Table 1, the structures of the hybrid compounds have an obvious influence on the inhibitory activities. (±)-3-Substituted fluorene–imidazole/triazole hybrids **5**–**10** almost lacked activities against all tumor cell lines investigated at the concentration of 40 μM. However, their imidazolium/triazolium salts **11**–**43** exhibited higher cytotoxic activities. This could be understandable because of the changes of molecular structure, charge distribution and water solubility.[10]

All imidazolium/triazolium salts **11**–**43** gave more selectivity towards HL-60, with IC_{50} values of 0.45–3.49 μM. Among them, nineteen imidazolium salts (19/28) showed higher inhibitory activity against HL-60 cell line than DDP (IC_{50} values below 1.16 μM). Meanwhile, twenty-four, twenty-two, thirty and thirty-two imidazolium /triazolium salts displayed higher inhibitory activities against SMMC-7721, A549, MCF-7 and SW480 cell lines than DDP. Compounds **38**, **37**, **42**, **30** and **32** showed powerful inhibitory activities selectively against HL-60 (IC_{50}, 0.45 ± 0.02 μM), SMMC-7721 (IC_{50}, 1.38 ± 0.04 μM), A549 (IC_{50}, 1.61 ± 0.17 μM), MCF-7 (IC_{50}, 1.80 ± 0.18 μM) and SW480 (IC_{50}, 2.15 ± 0.13 μM) cell lines, respectively.

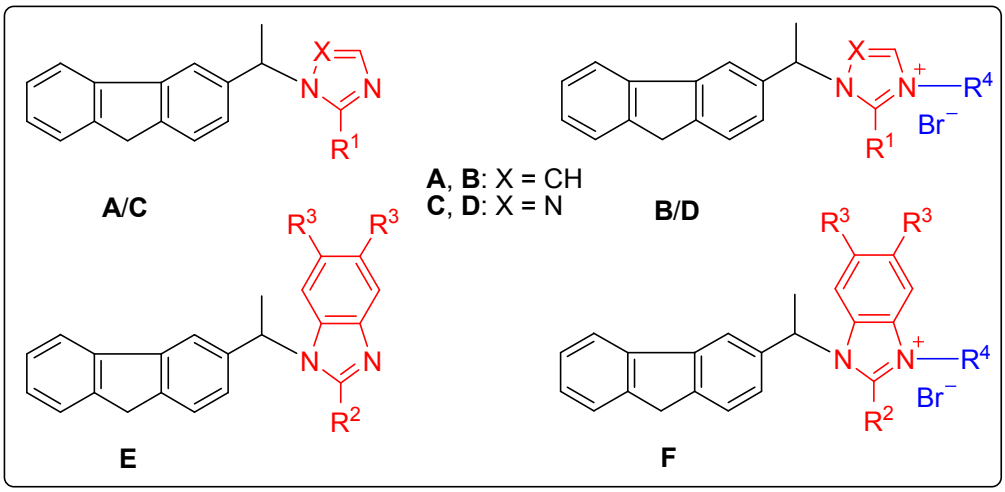
In terms of the imidazole ring (imidazole, 2-methyl-imidazole, benzimidazole, 2-methyl-benzimidazole, or 5,6-dimethyl-benzimidazole) and triazole ring, imidazolium salt derivatives **11**–**16** with imidazole ring, **17**–**21** with 2-methyl-imidazole and triazolium salts **22**–**26** exhibited some inhibitory activities. Only compounds **16**, **21** and **26**, bearing a naphthylacyl substituent at position-3/4 of the imidazole/triazole, showed higher cytotoxic

activity compared with DDP with IC_{50} values of 0.79–8.89 μM. Meanwhile, imidazolium salt derivatives **27**–**31** with benzimidazole ring displayed medium or high cytotoxic activities. Among them, compounds **30** and **31**, bearing a 4-methoxyphenacyl or naphthylacyl substituent at position-3 of the benzimidazole, showed higher cytotoxic activities compared with DDP with IC_{50} values of 0.56–4.04 μM. However, imidazolium salt derivatives **32**–**37** with 2-methyl-benzimidazole ring and **38**–**43** with 5,6-dimethyl-benzimidazole ring displayed powerful cytotoxic activities. All of these kinds of derivatives (12 compounds) were found to be much more active than DDP. Among them, compounds **36**, **37**, **42** and **43**, also bearing a 4-methoxyphenacyl or naphthylacyl substituent at position-3 of the 2-methyl-benzimidazole or 5,6-dimethyl-benzimidazole, exhibited potent cytotoxic activities with IC_{50} values of 0.50–3.04 μM against five human tumor cell lines investigated.

In terms of the substituent at position-3 of imidazole or position-4 of triazole ring, salts **11**, **12**, **14**, **17**, **18**, **22**, **23**, **27**, **28**, **32**, **33**, **35**, **38**, **39** and **41** with a 4-bromobenzyl, phenacyl or 4-fluorophenacyl substituent at position-3/4 of imidazole/triazole ring showed weak activities against five tumor cell lines. Meanwhile, compounds **13**, **19**, **24**, **29**, **34** and **40** with a 4-bromobphenacyl substituent at position-3/4 of imidazole/triazole ring exhibited medium cytotoxic activities (IC_{50}, 0.58–12.68 μM). However, compared with above benzyl or phenacyl substituent derivatives, imidazolium/triazolium salts with 4-methoxyphenacyl or naphthylacyl group at position-3/4 of imidazole/triazole ring exhibited higher cytotoxic activity. Most of these kinds of derivatives showed moderate or potent activity. Especially, compounds **16**, **26**, **31**, **37** and **43** with a naphthylacyl substituent, as well as compounds **30**, **36** and **42** with a 4-methoxyphenacyl substituent at position-3 of the imidazole ring much exhibited higher cytotoxic activity in vitro compared with DDP. Interestingly, compound **37**, bearing a naphthylacyl substituent at position-3 of 2-methyl-benzimidazole, and compound **42**, bearing a 4-methoxyphenacyl substituent at position-3 of 5,6-dimethyl-benzimidazole, were found to be the most potent derivatives with IC_{50} values of 0.51–2.51 μM against all of human tumor cell lines investigated and more active than DDP. Notably, compound **37** and **42** displayed cytotoxic activity selectively against HL-60, SMMC-7721 and A549 cell lines with IC_{50} values below 1.82 μM. This finding shows that steric and electronic effects have an important role in the cytotoxic activity of imidazolium/triazolium salts.

The results suggest that the existence of substituted 2-methyl-benzimidazole and 5,6-dimethyl-benzimidazole ring and substitution of the imidazolyl/triazolyl-3/4-position with a naphthylacyl or 4-methoxyphenacyl group

were important for prommoting cytotoxic activity. The structure-activity relationship (SAR) results were illustrated in Scheme 3.



Derivatives:

fluorene-imidazolium salt (B/F) > fluorene-imidazole hybrids (A/E)
fluorene-triazolium salt (D) > fluorene-triazole hybrids (C)
fluorene-imidazolium salt (B/D/F) > fluorene-triazolium salt (A/C/E)

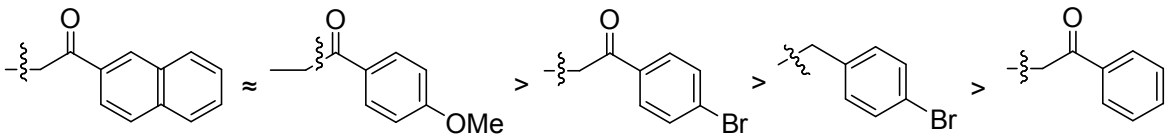
Better

Imidazole / Triazole ring:

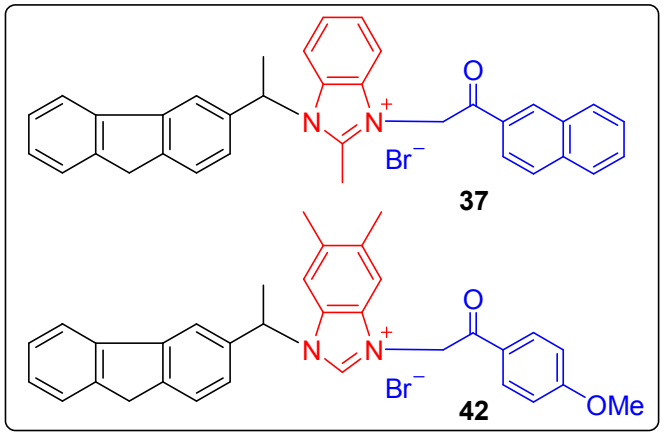
5,6-dimethyl-benzimidazole ≈ 2-methyl-benzimidazole > benzimidazole

Best > 2-methyl-imidazole > imidazole

R^4 substituent:



Best Potent Compounds



Scheme 3 Structure-activity relationship of (±)-3-substituted fluorene–imidazole/triazole derivatives.

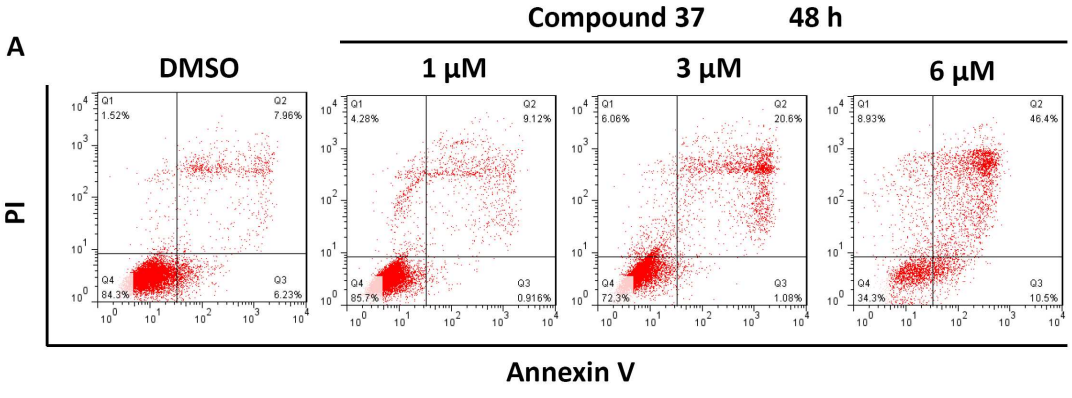
Furthermore, we also evaluated the cytotoxicity of the representative compounds **30**, **37** and **42** against human normal lung epithelial cell line (BEAS-2B). The results were showed in Table 2. By comparing the IC_{50} values of the tested compounds towards cancer cell lines with those towards the normal lung epithelial cells BEAS-2B, compound **30** exhibited selective cytotoxicity between cancer and normal cells, with an IC_{50} value of 16.26 μM against normal BEAS-2B cells, 6.3-fold less toxic than that against lung carcinoma A549 cancer cells. Contrarily, compounds **37** and **42** had not obvious selectivity between cancer and normal cells.

Table 2 Cytotoxicity of compounds **30**, **37** and **42** against A549 and BEAS-2B cells in vitro (IC_{50}, μM)

Entry	Compound no.	BEAS-2B	A549
1	**30**	16.26 ± 0.33	2.58 ± 0.17
2	**37**	3.32 ± 0.05	1.82 ± 0.24
3	**42**	2.25 ± 0.04	1.61 ± 0.17
4	DDP	9.16 ± 0.23	7.25 ± 0.46

Compound 37 induces G2/M phase arrest and apoptosis in cancer cells

SMMC-7721 cells were exposed to increasing concentrations of compound **37** 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 **37** at 1, 3, 6 μM for 48 h, the apoptotic cell rate was 10.04 ± 0.51 %, 21.68 ± 0.69 % and 56.90 ± 0.99 %, respectively, which were statistically different from the control (14.19 ± 0.29 %) (Fig. 2). These results showed that 3-substituted fluorene–triazolium salt **37** can remarkably induce apoptosis of the SMMC-7721 cells.



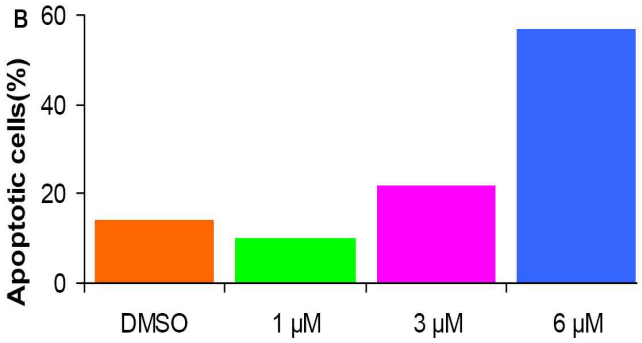
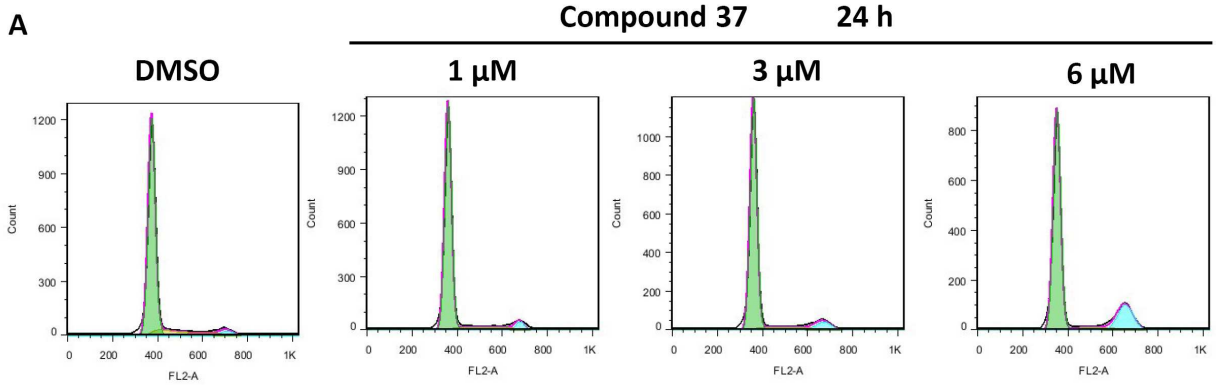


Fig. 2 Compound **37** caused significant apoptosis of SMMC-7721 cells. (A) Cells were treated with 1, 3 and 6 μM compound **37** for 48 h. Cell apoptosis was determined by Annexin V-FITC/PI double-staining assay. (B) The quantification of cell apoptosis. Data represents the mean ± S.D. of three independent experiments.

The results of cell cycle analysis on SMMC-7721 cells treated with compound **37** were summarized in Fig. 3. Compared with the control cells, the percentage of cells of G2/M phase was increased in the cells incubated with compound **37** with a dose dependent manner. Compound **37** treatment caused 17.97% cells in G2/M phase as compared to control showing 3.18%. In the meanwhile, the fraction of cells in S phase decreased slightly accordingly from 84.15% to 74.45%, while the proportion of G0/G1 phase cells showed no obvious change. The data suggest that compound **37** may induce G2/M phase arrest in the cell cycle. Disruption or malfunction of cell cycle control within the G2/M phase has been recognized as one of the most important biochemical phenomenon for tumor progression and tumorigenesis. The ability of certain small molecules to control cell cycle machinery within the G2/M phase has provided exciting new opportunities with hopes of developing new types of drugs efficacious against refractory cancers.[11]



B

Treatment	Cells (%)		
	G0/G1	S	G2/M
DMSO	81.98 ± 2.18	12.28 ± 2.74	3.18 ± 2.23
Compound **37** (1 μM)	84.15 ± 1.76	8.72 ± 0.36	4.03 ± 0.67
Compound **37** (3 μM)	83.00 ± 2.85	7.97 ± 0.18	6.66 ± 1.56
Compound **37** (6 μM)	74.45 ± 3.27	5.51 ± 0.22	17.97 ± 1.54

Fig. 3 Compound **37** induces G2/M phase arrest in SMMC-7721 cells. (A) Cells were treated with 1, 3 and 6 μM of compound **37** 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.

Conclusion

In summary, a series of novel (±)-3-substituted fluorene–imidazolium/triazolium salt derivatives prepared proved to be potent antitumor agents. The imidazolium salt derivatives **36**, **37**, **42** and **43**, bearing 2-methyl-benzimidazole or 5,6-dimethyl-benzimidazole ring and a naphthylacyl or 2-naphthylmethyl at position-3 of the imidazole ring, were found to be the most potent compounds. compound **37**, bearing a naphthylacyl substituent at position-3 of 2-methyl-benzimidazole, and compound **42**, bearing a 4-methoxyphenacyl substituent at position-3 of 5,6-dimethyl-benzimidazole, were found to be the most potent derivatives with IC_{50} values of 0.51–2.51 μM against all of human tumor cell lines investigated. Notably, compound **37** and **42** displayed cytotoxic activity selectively against HL-60, SMMC-7721 and A549 cell lines with IC_{50} values below 1.82 μM. Compound **37** can remarkablely induce the G2/M phase cell cycle arrest and apoptosis in SMMC-7721 cells. Interestingly, compound **30** exhibited selective cytotoxicity between cancer and normal cells, which an IC_{50} value of 16.26 μM against normal BEAS-2B cells, 6.3-fold less toxic than that against lung carcinoma A549 cancer cells. The fluorene-based imidazolium salts **30**, **36**, **37**, **42** and **43** can be considered promising leads for further structural modifications guided by the valuable information derivable from our detailed SARs.

Experimental Section

General procedures

Melting points were obtained on a XT-4 melting-point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker Avance 300 spectrometer at 300 MHz. Carbon-13 nuclear magnetic resonance (^{13}C-NMR) was recorded on Bruker Avance 300 spectrometer at 75 MHz. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (TMS) for all 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 AB QSTAR Pulsar mass spectrometer. Elemental analysis were carried out on a Vario-EL analyzer. Silica gel (200–300 mesh) for column chromatography and silica GF_{254} 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, Fluka and were used without purification, unless otherwise indicated.

Synthesis of 1-(9*H*-fluoren-6-yl)ethanone (2). Anhydrous $AlCl_3$ (4.83 g, 36.20 mmol) in dichloromethane (50 mL) was added to acetyl chloride (1.71 g, 21.70 mmol) at 0 °C and then fluorene **1** (3.00 g, 18.10 mmol) in dichloromethane (100 mL) slowly, and then at ambient temperature for 2 h. After the reaction (TLC) was completed, the reaction mixture was quenched with 1 N HCl and extracted with dichloromethane (3 × 100 mL). The combined organic extracts were washed with H_2O, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was chromatographed on silica gel (petroleum ether 60-90 °C : EtOAc = 15:1) to afford the product **2** (2.94 g, 78%) as white powder. See ESI file for characterization data.†

Synthesis of (±)-1-(9*H*-fluoren-6-yl)ethanol (3). To a stirred solution of 1-(9*H*-fluoren-3-yl)ethanone **2** (2.30 g, 11.10 mmol) in methanol (25 mL) at 0 °C was added $NaBH_4$ (0.63 g, 16.65 mmol) in small portions over a period of 20 minutes, and then at ambient temperature for 4 h. Reaction progress was monitored by TLC. A small amount of water was added and the mixture was stirred for 15 min before rotary evaporation. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether 60-90 °C : EtOAc = 10:1) to afford the products **3** (2.21 g, 95%) as white powder. See ESI file for characterization data.†

Synthesis of (±)-3-substituted fluorene–imidazole hybrids (5-10). To a solution of (±)-1-(9*H*-fluoren-3-yl)ethanol **3** (210 mg, 1.00 mmol) in dichloromethane (50 mL) was added methanesulfonyl chloride (1.2 mmol) and triethylamine (2 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 12 h. After quenching the reaction with water (50 mL), the layers were separated. The organic phase was dried over anhydrous Na_2SO_4 and concentrated, and used for the next synthetic step. A mixture of the previous methanesulfonate **4** and various substituted imidazole, benzimidazole or triazole (6 mmol) and K_2CO_3 (3 mmol) was stirred in tuloene (20 ml) at reflux for 24–48 h (monitored by TLC). After cooling to room temperature, the solvent was concentrated, and the residue was diluted with EtOAc (20 mL). The organic layer was washed with water (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography (silica gel, petroleum ether 60–90 °C : EtOAc = 1:1) to afford **5-10** in 53-78% yield as white powder. See ESI file for characterization data.†

(±)-1-(1-(9H-Fluoren-3-yl)ethyl)-1H-imidazole (**5**). Yield 62%. White solid, Mp 188-190 °C. IR ν_{max} (cm^{-1}): 2971, 1605, 1498, 1397, 1225, 1085, 740. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.70-7.75 (2H, m), 7.62 (1H, s), 7.51 (1H, d, *J* = 7.2 Hz), 7.31-7.38 (3H, m), 7.16 (1H, d, *J* = 7.8 Hz), 7.09 (1H, s), 6.95 (1H, s), 5.34-5.41 (1H, m), 3.83 (2H, s), 1.87 (3H, d, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ : 143.97 (C), 143.34 (C), 141.75 (C), 140.94 (C), 140.03 (C), 136.08 (CH), 129.32 (CH), 127.02 (CH), 126.86 (CH), 125.08 (CH), 124.85 (CH), 122.68 (CH), 120.11 (CH), 120.00 (CH), 118.03 (CH), 56.78 (CH), 36.87 (CH_2), 22.21 (CH_3). Anal. Calcd for $C_{18}H_{16}N_2$: C, 83.04; H, 6.19; N, 10.76. Found: C, 82.74; H, 5.81; N 10.65. HRMS (ESI-TOF) *m/z* Calcd for $C_{18}H_{17}N_2$ $[M+H]^+$ 261.1392, found 261.1395.

(±)-1-(1-(9H-Fluoren-3-yl)ethyl)-2-methyl-1H-imidazole (**6**). Yield 56%. White solid, Mp 134-136 °C. IR ν_{max} (cm^{-1}): 3161, 2974, 1660, 1490, 1268, 1105, 990, 737. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.70-7.76 (2H, m), 7.53 (1H, d, *J* = 7.2 Hz), 7.26-7.39 (2H, m), 7.18 (1H, s), 7.01-7.10 (3H, m), 5.33-5.40 (1H, m), 3.84 (2H, s), 2.31 (3H, s), 1.85 (3H, d, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ : 143.99 (C), 143.31 (C), 141.42 (C), 140.99 (C), 140.31(C), 126.95 (CH), 126.84 (CH), 125.07 (CH), 124.55 (CH), 122.32 (C), 120.09 (CH), 119.95 (CH), 116.78 (CH), 55.27 (CH), 36.89 (CH_2), 22.55 (CH_3), 13.52 (CH_3). Anal. Calcd for $C_{19}H_{18}N_2$: C, 83.18; H, 6.61; N, 10.21. Found: C, 83.13; H, 6.65; N 9.69. HRMS (ESI-TOF) m/z Calcd for $C_{19}H_{19}N_2$ $[M+H]^+$ 275.1548, found 275.1553.

(±)-1-(1-(9H-Fluoren-3-yl)ethyl)-1H-1,2,4-triazole (**7**). Yield 58%. White solid, Mp 108-110 °C. IR ν_{max} (cm^{-1}): 3118, 3085, 2968, 2933, 1678, 1614, 1499, 1269, 1140,1005, 946, 841, 734. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.07 (1H, s), 7.99 (1H, s), 7.70-7.74 (2H, m), 7.50 (1H, d, *J* = 7.2 Hz), 7.40 (1H, s), 7.37 (1H, s), 7.24-7.35 (2H, m), 5.55-5.61 (1H, m), 3.83 (2H, s), 1.95 (3H, d, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 151.93 (CH), 144.01 (CH), 143.39 (C), 142.11 (C), 140.91 (C), 138.38 (C), 127.10 (CH), 126.87 (CH), 125.37 (CH), 125.08 (CH), 123.23 (CH), 120.21 (CH), 120.07 (CH), 59.87 (CH), 36.88 (CH_2), 21.47 (CH_3). Anal. Calcd for $C_{17}H_{15}N_3$: C, 78.13; H, 5.79; N, 16.08. Found: C, 78.03; H, 5.72; N 15.80. HRMS (ESI-TOF) *m/z* Calcd for $C_{17}H_{16}N_3$ $[M+H]^+$ 262.1344, found 262.1349.

(±)-1-(1-(9H-Fluoren-3-yl)ethyl)-1H-benzo[d]imidazole (**8**). Yield 68%. White solid, Mp 179-181 °C. IR ν_{max} (cm^{-1}): 3051, 2970, 1607, 1484, 1223, 744. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.15 (1H, s), 7.83 (1H, d, *J* = 7.8 Hz), 7.71 (2H, dd, *J* = 15.0, 7.2 Hz), 7.48 (1H, d, *J* = 6.9 Hz), 7.27-7.36 (3H, m),7.14-7.23 (4H, m), 5.61-5.68 (1H, m), 3.78 (2H, s), 2.00 (3H, d, *J* = 6.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 144.11 (C), 143.71 (C), 143.35 (C), 141.85 (C), 141.03 (CH), 140.93 (CH), 139.11 (C), 133.64 (C), 127.05 (CH) , 126.87 (CH), 125.08 (CH), 124.84 (CH), 122.99 (CH), 122.63 (CH), 122.44 (CH), 120.22 (CH), 120.00 (CH), 110.82 (CH), 55.57 (CH), 36.87 (CH_2), 21.80 (CH_3). Anal. Calcd for $C_{22}H_{18}N_2$: C, 85.13; H, 5.85; N, 9.03. Found: C, 85.03; H, 5.89; N 8.86. HRMS (ESI-TOF) *m/z* Calcd for $C_{22}H_{19}N_2$ $[M+H]^+$ 311.1548, found 311.1551.

(±)-1-(1-(9H-Fluoren-3-yl)ethyl)-2-methyl-1H-benzo[d]imidazole (**9**). Yield 53%. White solid, Mp 166-168 °C. IR ν_{max} (cm^{-1}): 3048, 2991, 2880, 1609, 1520, 1391, 1283, 1145, 1007, 737. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.71-7.76 (3H, m), 7.52 (1H, d, *J* = 7.2 Hz), 7.25-7.39 (3H, m), 7.16-7.22 (2H, m), 7.07 (2H, d, *J* = 3.6 Hz), 5.80-5.87 (1H, m), 3.83 (2H, s), 2.64 (3H, s), 2.01 (3H, d, *J* = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 151.50 (C), 143.97 (C), 143.39 (C), 141.71 (C), 141.57 (C), 140.92 (C), 137.67 (C), 133.67 (C), 127.07 (CH), 126.89 (CH), 125.11 (CH), 124.95 (CH), 123.07 (CH), 122.24 (CH), 122.14 (CH), 120.07 (CH), 118.79 (CH), 111.25 (CH), 53.74 (CH), 36.92 (CH_2), 18.84 (CH_3), 14.45 (CH_3). Anal. Calcd for $C_{23}H_{20}N_2$: C, 85.15; H, 6.21; N, 8.63. Found: C, 84.97; H, 6.17; N 8.36. HRMS (ESI-TOF) m/z Calcd for $C_{23}H_{21}N_2$ $[M+H]^+$ 325.1705, found 325.1705.

(±)-1-(1-(9H-Fluoren-3-yl)ethyl)-5,6-dimethyl-1H-benzo[d]imidazole (**10**). Yield 60%. White solid, Mp 182-184 °C. IR ν_{max} (cm^{-1}): 3007, 2969, 2933, 2876, 1617, 1480, 1392, 1224, 1030, 839, 743. ^1H NMR (300 MHz,

$CDCl_3$) δ: 8.00 (1H, s), 7.73 (2H, t, *J* = 7.2 Hz), 7.58 (1H, s), 7.50 (1H, d, *J* = 7.2 Hz), 7.24-7.37 (2H, m), 7.21 (2H, d, *J* = 7.8 Hz), 6.98 (1H, s), 5.58-5.65 (1H, m), 3.80 (2H, s), 2.33 (3H, s), 2.27 (3H, s), 2.00 (3H, d, *J* = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ : 144.04 (C), 143.35 (C), 142.80 (C), 141.66 (C), 141.01 (C), 140.30 (CH), 139.57 (C), 132.31 (C), 131.96 (C), 131.13. (C), 126.97 (CH), 126.84 (CH), 125.06 (CH), 124.77 (CH), 122.55 (CH), 120.33 (CH), 120.16 (CH), 119.96 (CH), 110.77 (CH), 55.25 (CH), 36.87 (CH_2), 21.88 (CH_3), 20.59 (CH_3), 20.22 (CH_3). Anal. Calcd for $C_{24}H_{22}N_2$: C, 85.17; H, 6.55; N, 8.28. Found: C, 84.79; H, 6.39; N 7.98. HRMS (ESI-TOF) m/z Calcd for $C_{24}H_{23}N_2$ $[M+H]^+$ 339.1861, found 339.1863.

Synthesis of (±)-3-substituted fluorene–imidazolium/triazolium salts (11-43). A mixture of (±)-3-substituted fluorene–imidazole hybrids **5**–**10** (0.2 mmol) and phenacyl bromides or alkyl bromides (0.24 mmol) was stirred in toluene (5 ml) at refluxat for 8-12 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, and washed with toluene (3 × 10 ml), then dried to afford imidazolium salts **11-43** in 68–98% yields. See ESI file for characterization data of all novel compounds.†

(±)-3-(Naphthalen-2-ylmethyl)-1-(1-(9H-fluoren-3-yl)ethyl)-2-methyl-1H-benzo[d]imidazol-3-ium bromide (**37**): Yield 80%. White solid, Mp 165-167 °C. IR ν_{max} (cm^{-1}): 3021, 1685, 1623,1520, 1470, 1075, 926, 823, 739. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.24 (1H, s), 8.21 (1H, d, J = 8.1 Hz), 8.12 (1H, d, J = 7.2 Hz), 7.92 (1H, d, J = 8.4 Hz), 7.74-7.86 (4H, m), 7.53-7.65 (4H, m), 7.27-7.46 (5H, m), 7.25 (1H, s), 6.95 (2H, s), 6.25-6.31 (1H, m), 3.91 (2H, s), 3.21 (3H, s), 2.21 (3H, d, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ : 190.66 (C), 152.64 (C), 144.58 (C), 143.55 (C), 142.72 (C), 140.49 (C), 136.37 (C), 134.14 (C), 132.60 (C), 132.48 (CH), 130.59 (CH), 130.49 (CH), 129.84 (C), 129.54 (CH), 129.04 (CH), 127.66 (CH), 127.48 (CH), 127.19 (CH), 126.97 (CH), 126.64 (CH), 126.35 (CH), 125.19 (CH), 123.40 (CH), 123.25 (CH), 120.60 (CH), 120.26 (CH), 114.03 (CH), 113.06 (CH), 57.03 (CH), 54.07 (CH_2), 36.99 (CH_2), 18.60 (CH_3), 13.52 (CH_3). Anal. Calcd for $C_{35}H_{29}BrN_2O$: C, 73.30; H, 5.10; N, 4.88. Found: C, 72.97; H, 5.29; N 4.47. HRMS (ESI-TOF) m/z Calcd for $C_{35}H_{29}N_2O$ $[M-Br]^+$ 493.2274, found 493.2277.

(±)-3-(2-(4-Methoxyphenyl)-2-oxoethyl)-1-(1-(9H-fluoren-3-yl)ethyl)-5,6-dimethyl-1H-benzo[d]imidazol-3-ium bromide (**42**). Yield 96%. White solid, Mp 239-241 °C. IR ν_{max} (cm^{-1}): 3191, 2986, 1682, 1597, 1450, 1018, 838, 740. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.13 (1H, s), 8.17 (2H, d, J = 8.1 Hz), 7.77 (2H, dd, J = 16.5,

8.4 Hz), 7.61 (1H, s), 7.52 (1H, d, J = 7.2 Hz), 7.30-7.43 (3H, m), 7.28 (1H, s), 7.20 (1H, s), 6.99 (2H, d, J = 8.1 Hz), 6.60 (2H, s), 5.85-5.87 (1H, m), 3.89 (2H, s), 3.88 (3H, s), 2.32 (3H, s), 2.28 (3H, s), 2.25 (3H, d, J = 6.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ : 188.76 (C), 164.85 (C), 144.69 (C), 143.57 (C), 142.79 (C), 141.24 (CH), 140.60 (C), 137.48 (C), 137.04 (C), 136.20 (C), 131.22 (CH), 129.17 (C), 127.31 (C), 126.86 (CH), 126.62 (CH), 125.14 (CH), 122.91 (CH), 120.61 (CH), 120.14 (CH), 114.44 (CH), 113.56 (CH), 113.11 (CH), 59.25 (CH), 55.66 (CH_3), 53.21 (CH_2), 36.95 (CH_2), 22.30 (CH_3), 20.68 (CH_3), 20.55 (CH_3). Anal. Calcd for $C_{33}H_{31}BrN_2O_2$: C, 69.84; H, 5.51; N, 4.94. Found: C, 69.77; H, 5.52; N 4.46. HRMS (ESI-TOF) m/z Calcd for $C_{33}H_{31}BrN_2O_2$ $[M-Br]^+$ 487.2380, found 487.2389.

Cytotoxicity assay. The assay was in five kinds of cell lines (HL-60, SMMC-7721, A549, MCF-7 and SW480). Cells were cultured at 37 ^oC under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal serum and dispersed in replicate 96-well plates. Compounds were then added. After 48 h exposure to the compounds, cells viability were determined by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) cytotoxicity assay by measuring the absorbance at 570 nm with a microplate spectrophotometer. Each test was performed in triplicate.

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 1.2×10^6 cells/well. After 48 h of compound treatment at the indicated concentrations, cells were collected and then washed twice with cold PBS, and then resuspended in a binding buffer containing Annexin V-FITC and propidium iodine (PI). After incubation for 15 min at room temperature in the dark, the fluorescent intensity was measured using a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ).

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 μg/ml propidium iodide (PI) solution containing 50 μg/ml RNase A for 30 min at room temperature. Fluorescence intensity was analyzed by 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 ModFIT LT 2.0.

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FIGURE TITLES

Fig. 1 Representative structures of fluorene derivatives and imidazolium/triazolium salts.

Fig. 2 Compound **37** caused significant apoptosis of SMMC-7721 cells. (A) Cells were treated with 2, 4 and 6 μM compound **37** 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.

Fig. 3 Compound **37** induces G2/M phase arrest in SMMC-7721 cells. (A) Cells were treated with 1, 3 and 6 μM of compound **37** 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 and data of one representative experiment is shown.

SCHEME TITLES

Scheme 1 Synthesis of hybrid compounds **5**–**10**.

Scheme 2 Synthesis of (±)-3-substituted fluorene–imidazolium/triazolium salt derivatives **11**–**43** from **5**–**10**

Scheme 3 Structure-activity relationship of (±)-3-substituted fluorene–imidazole/triazole derivatives.

TABLE TITLES

Table 1 Cytotoxic activities of (±)3-substituted fluorene–imidazole/triazole derivatives **5**–**43** in vitro[b] (IC_{50}, mean ± SD, μM[a])

Table 2 Cytotoxicity of compounds **30**, **37** and **42** against A549 and BEAS-2B cells in vitro (IC_{50}, μM)