Organic & Biomolecular Chemistry

Accepted Manuscript

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Synthesis and antitumor activity of novel 2-substituted indoline imidazolium salt derivatives

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Received Xth XXXXXXXXX 2014, Accepted Xth XXXXXXXXX 2014

First published on the web Xth XXXXXXXXX 2014

DOI: 10.1039/c1ob00000x

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† Electronic supplementary information (ESI) available: Details of experimental procedure, spectral data and copies of all novel compounds. CCDC 1012979. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob00000x.

A series of novel 2-substituted indoline imidazolium salt derivatives has been prepared and evaluated *in vitro* against a panel of human tumor cell lines. The results suggest that the existence of substituted benzimidazole ring and substitution of the imidazolyl-3-position with a naphthylacyl or 2-naphthylmethyl group were vital for modulating cytotoxic activity. Compound **25** was found to be the most potent derivatives with IC_{50} values of 0.24–1.18 μM and exhibited cytotoxic activity selectively against MCF-7, SW480, SMMC-7721 and HL-60 cell lines, while compound **26** showed powerful inhibitory activities selectively against SMMC-7721 and A549 cell lines. Compound **25** can induce the G2/M phase cell cycle arrest and apoptosis in SMMC-7721 cells.

Introduction

Indolines are an important class of biologically active nitrogen-containing heterocycles. Biologically active agents and natural products with the 2-substituted indoline framework display a broad range of biological and pharmacological activities.[1] In particular, 2-substituted indoline derivatives show significant antitumor activity. As illuminated in Scheme 1, AC-93253 significantly enhanced acetylation of tubulin and exhibited submicromolar selective cytotoxicity towards tumor cell lines (DU145, MiaPaCa2, A549 and NCI-H460),[2] while indoline-2-carboxylic acid N-(substituted)phenylamide (ICNP) showed potent cytotoxic activities against human lung and prostate carcinoma cells (NCI-H23 and PC-3).[3]

On the other hand, imidazolium salts have gained considerable interests thanks to their biological and pharmacological activity,[4] especially antitumor activity.[5] For example, two new imidazolium chlorides (Fig. 1), Lepidiline A and B, displayed potent cytotoxic activity against human cancer cell lines (UMUC3, PACA2, MDA231, and FDIGROV).[6] In this respect, we have previously reported the synthesis of a series of novel imidazolium salt derivatives, such as NMIB (Fig. 1), and their potential antitumor activity.[7] Studies on molecular mechanisms demonstrated that the imidazolium salt hybrids can induce the cell cycle arrest and apoptosis in tumor cells.[7e]

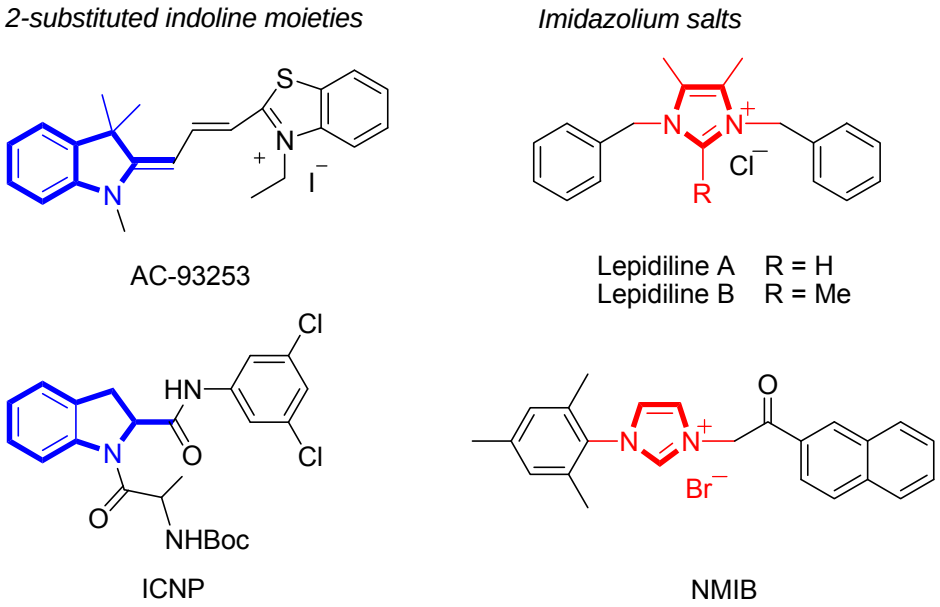


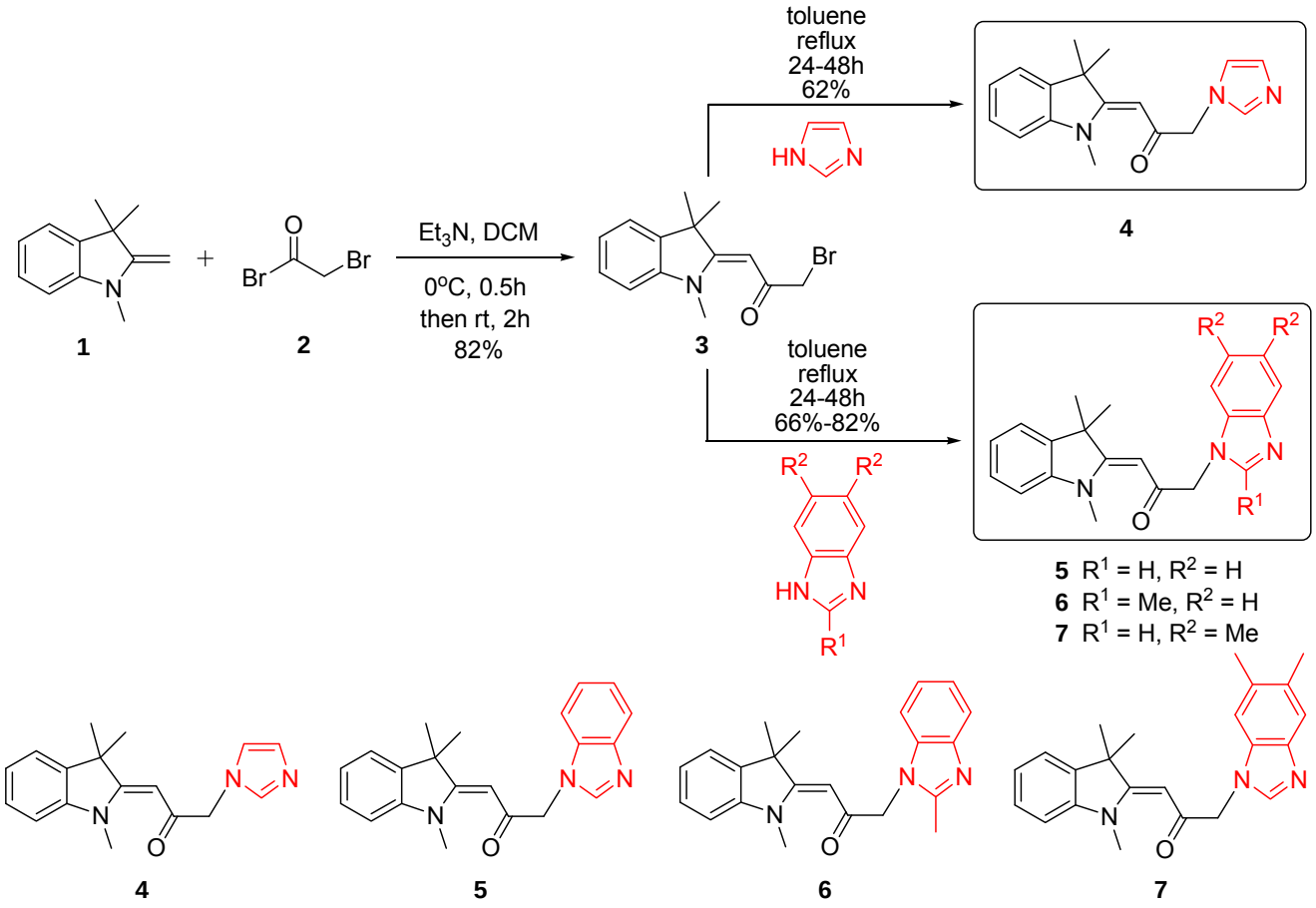
Fig. 1 Representative structures of 2-substituted indoline derivatives and imidazolium salts.

Constructing novel pharmacologically interesting hybrid compounds for drug discovery have attracted much attention during the past two decades.[8] To validate synergic integration of the anticancer activities of 2-substituted indolines derivatives and the potent cytotoxic activities of imidazolium salts, we were interested in synthesizing the hybridizing compounds of 2-substituted indoline with imidazole moieties. To the best of our knowledge, no reports concerning antitumor activity of 2-substituted indoline–imidazole hybrids have been found in the literature.

In this paper, a series of novel 2-substituted indoline imidazolium salt derivatives were synthesized to investigate the antitumor activity of indoline imidazolium salts with the ultimate aim of developing potent antitumor agents.

Results and discussion

Chemistry

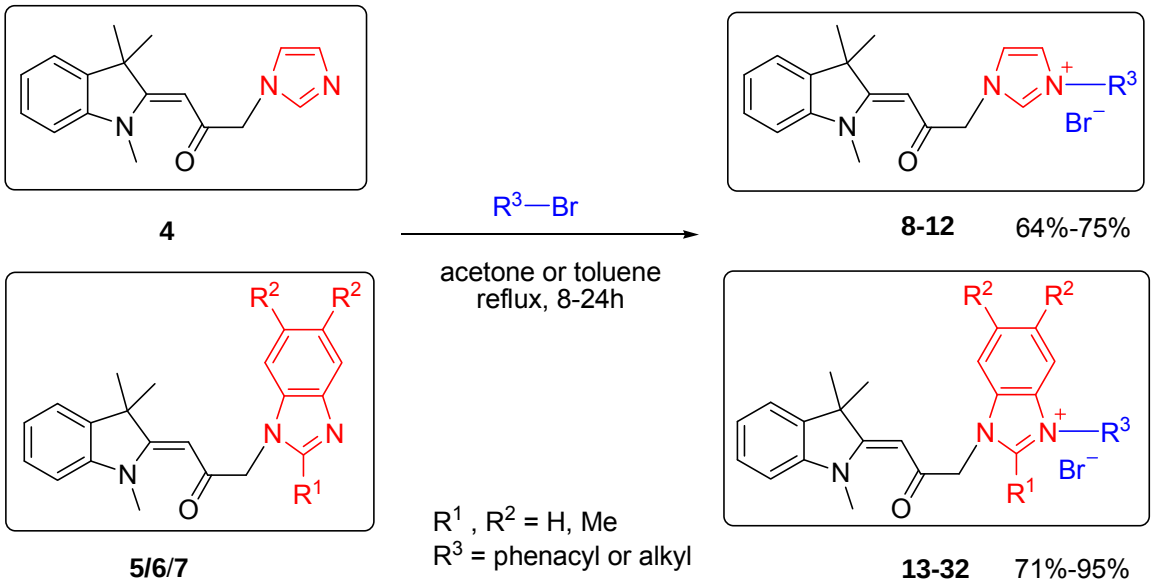


Scheme 1 Synthesis of hybrid compounds **4**–**7**.

To synthesize the indoline-imidazole derivatives, we used commercially available imidazole derivatives that were alkylated with 1-bromo-3-(1,3,3-trimethylindolin-2-ylidene)propan-2-one, which was synthesized from readily available starting materials as shown in scheme 1. Commercial 1,3,3-trimethyl-2-methyleneindoline **1** was chosen as the starting material for the preparation of a series of 2-substituted indoline-imidazole hybrids (**4**–**7**). Treatment of 1,3,3-trimethyl-2-methyleneindoline **1** with bromoacetyl bromide **2** in the presence of triethylamine gave the corresponding 1-bromo-3-(1,3,3-trimethylindolin-2-ylidene)propan-2-one **3** in 82% yield. Subsequently, Bromide **3** was transformed to the respective four 2-substituted indoline–imidazole hybrids **4**–**7** with imidazole or various substituted benzimidazole (benzimidazole, 2-methyl-benzimidazole or 5,6-dimethyl-benzimidazole) by refluxing under toluene with 62–82% yields (two steps).

Finally, twenty-five 2-substituted indoline imidazolium salts **8–32** were prepared with excellent yields by reaction of 2-substituted indoline–imidazole hybrids **4–7** with the corresponding alkyl and phenacyl bromides in refluxing acetone or toluene (64–95% yields). The structures and yields of derivatives are shown in Tables 1.

Table 1 Synthesis of indoline imidazolium salt derivatives **8–32** from **4–7**



Entry	Compound no.	imidazole ring	R^3	molecular formula	mp (°C)	Yields (%)
1	**4**	imidazole	–	$C_{17}H_{19}N_3O$	134-136	62
2	**5**	benzimidazole	–	$C_{21}H_{21}N_3O$	192-193	82
3	**6**	2-methyl-benzimidazole	–	$C_{22}H_{23}N_3O$	142-144	80
4	**7**	5,6-dimethyl-benzimidazole	–	$C_{23}H_{25}N_3O$	151-153	66
5	**8**	imidazole	2-bromobenzyl	$C_{24}H_{25}Br_2N_3O$	193-194	65
6	**9**	imidazole	phenacyl	$C_{25}H_{26}BrN_3O_2$	282-284	77
7	**10**	imidazole	4-bromophenacyl	$C_{25}H_{25}Br_2N_3O_2$	163-164	67
8	**11**	imidazole	4-methoxyphenacyl	$C_{26}H_{28}BrN_3O_3$	241-243	75
9	**12**	imidazole	naphthylacyl	$C_{29}H_{28}BrN_3O_2$	155-156	73
10	**13**	benzimidazole	4-methylbenzyl	$C_{29}H_{30}BrN_3O$	264-265	91
11	**14**	benzimidazole	2-bromobenzyl	$C_{28}H_{27}Br_2N_3O$	275-277	84
12	**15**	benzimidazole	2-naphthylmethyl	$C_{32}H_{30}BrN_3O$	247-248	85
13	**16**	benzimidazole	phenacyl	$C_{29}H_{28}BrN_3O_2$	282-284	77
14	**17**	benzimidazole	4-bromophenacyl	$C_{29}H_{27}Br_2N_3O_2$	299-301	88
15	**18**	benzimidazole	naphthylacyl	$C_{33}H_{30}BrN_3O_2$	177-180	80
16	**19**	2-methyl-benzimidazole	4-methylbenzyl	$C_{30}H_{32}BrN_3O$	304-305	94
17	**20**	2-methyl-benzimidazole	2-bromobenzyl	$C_{29}H_{29}Br_2N_3O$	260-263	81
18	**21**	2-methyl-benzimidazole	2-naphthylmethyl	$C_{33}H_{32}BrN_3O$	279-281	91

19	**22**	2-methyl-benzimidazole	phenacyl	$C_{30}H_{30}BrN_3O_2$	257-260	85
20	**23**	2-methyl-benzimidazole	4-bromophenacyl	$C_{30}H_{29}Br_2N_3O$	242-245	89
21	**24**	2-methyl-benzimidazole	4-methoxyphenacyl	$C_{31}H_{32}BrN_3O_3$	264-266	90
22	**25**	2-methyl-benzimidazole	naphthylacyl	$C_{34}H_{32}BrN_3O_2$	255-257	92
23	**26**	5,6-dimethyl-benzimidazole	4-methylbenzyl	$C_{31}H_{34}BrN_3O$	211-214	91
24	**27**	5,6-dimethyl-benzimidazole	2-bromobenzyl	$C_{30}H_{31}Br_2N_3O$	281-284	88
25	**28**	5,6-dimethyl-benzimidazole	2-naphthylmethyl	$C_{34}H_{34}BrN_3O$	228-230	92
26	**29**	5,6-dimethyl-benzimidazole	phenacyl	$C_{31}H_{32}BrN_3O_2$	239-242	80
27	**30**	5,6-dimethyl-benzimidazole	4-bromophenacyl	$C_{31}H_{31}Br_2N_3O_2$	201-202	95
28	**31**	5,6-dimethyl-benzimidazole	4-methoxyphenacyl	$C_{32}H_{34}BrN_3O_3$	183-185	71
29	**32**	5,6-dimethyl-benzimidazole	naphthylacyl	$C_{35}H_{34}BrN_3O_2$	193-195	82

To verify the structures of the 2-substituted indoline imidazolium salt derivatives, imidazolium salt **24** was selected as a representative compound and characterized by X-ray crystallography (the Cambridge crystallographic data centre (CCDC) 1012979)[9], as shown in Figure 2.

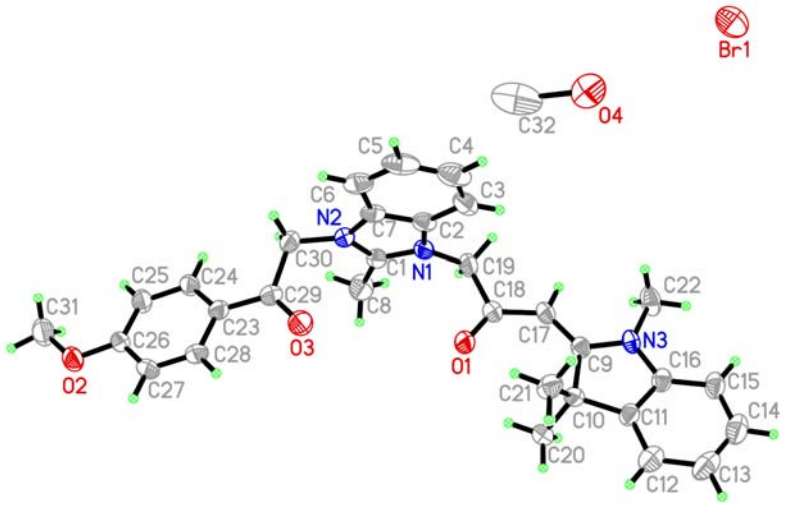


Fig. 2 X-ray crystal structure of compound **24**.

Biological evaluation and structure-activity relationship analysis

The cytotoxic potential of all newly synthesized imidazole and imidazolium salt derivatives were assessed *in vitro* against a panel of human tumor cell lines, on the basis of the procedures in the literature[10]. The panel comprising myeloid leukaemia (HL-60), liver carcinoma (SMMC-7721), lung carcinoma (A549), breast carcinoma (MCF-7) and colon carcinoma (SW480). Cisplatin (DDP) was used as the reference drug. The results are summarized in Table 2.

Table 2 Cytotoxic activities of imidazole and imidazolium salt derivatives in vitro[b] (IC_{50}, μM[a])

Entry	Compound no.	HL-60	SMMC-7721	A549	MCF-7	SW480
1	**4**	>40	>40	>40	>40	>40
2	**5**	>40	>40	>40	>40	>40
3	**6**	>40	>40	>40	>40	>40
4	**7**	>40	>40	>40	>40	>40
5	**8**	0.69	5.45	3.00	6.33	11.71
6	**9**	3.54	22.13	>40	22.39	>40
7	**10**	2.20	8.42	15.48	10.45	18.48
8	**11**	1.84	5.70	10.77	23.90	15.59
9	**12**	0.73	2.45	8.37	3.37	9.29
10	**13**	0.70	1.98	2.93	2.96	4.54
11	**14**	0.39	1.10	1.21	3.96	2.54
12	**15**	0.47	1.10	1.40	1.91	2.23
13	**16**	2.03	10.26	16.95	23.76	15.64
14	**17**	1.48	5.80	8.41	3.76	4.47
15	**18**	0.67	2.13	2.85	4.47	2.85
16	**19**	0.24	1.09	0.77	2.03	4.95
17	**20**	0.29	1.29	0.96	1.68	1.86
18	**21**	0.40	0.87	1.02	1.92	2.04
19	**22**	1.86	4.46	10.64	8.04	5.49
20	**23**	0.69	3.01	8.25	1.95	4.19
21	**24**	0.43	1.03	2.78	2.17	1.71
22	**25**	0.24	1.09	0.98	1.13	1.18
23	**26**	0.41	0.75	0.64	2.06	2.02
24	**27**	0.60	1.27	0.89	1.38	2.04
25	**28**	0.69	1.21	1.21	1.20	2.34
26	**29**	0.64	2.45	3.74	2.36	3.84
27	**30**	0.94	2.44	4.28	2.42	2.74
28	**31**	0.38	1.88	1.67	2.50	3.46
29	**32**	0.47	1.11	1.82	1.83	2.33
30	DDP	1.00	6.33	7.25	15.93	13.57

[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 expected, for five tumor cell lines, all imidazolium salts **8**–**32** gave more selectivity towards HL-60, with IC_{50} values of 0.24–2.20 μM (except **9**). Among them, nineteen imidazolium salts showed higher inhibitory activity against HL-60 cell line than DDP (IC_{50} values below 1.00 μM). Meanwhile, twenty-two and twenty-one

imidazolium salts exhibited higher inhibitory activities against MCF-7 and SW480 cell lines than DDP. Compound **25** showed powerful inhibitory activities selectively against MCF-7 and SW480 cell lines, with IC_{50} values 14.1-fold and 11.5-fold more sensitive to DDP. Additionally, twenty-two and seventeen imidazolium salts displayed higher inhibitory activities against SMMC-7721 and A549 cell lines than DDP. Compound **26** exhibited powerful inhibitory activities selectively against SMMC-7721 and A549 cell lines, with IC_{50} values of 0.75 μM and 0.64 μM, respectively.

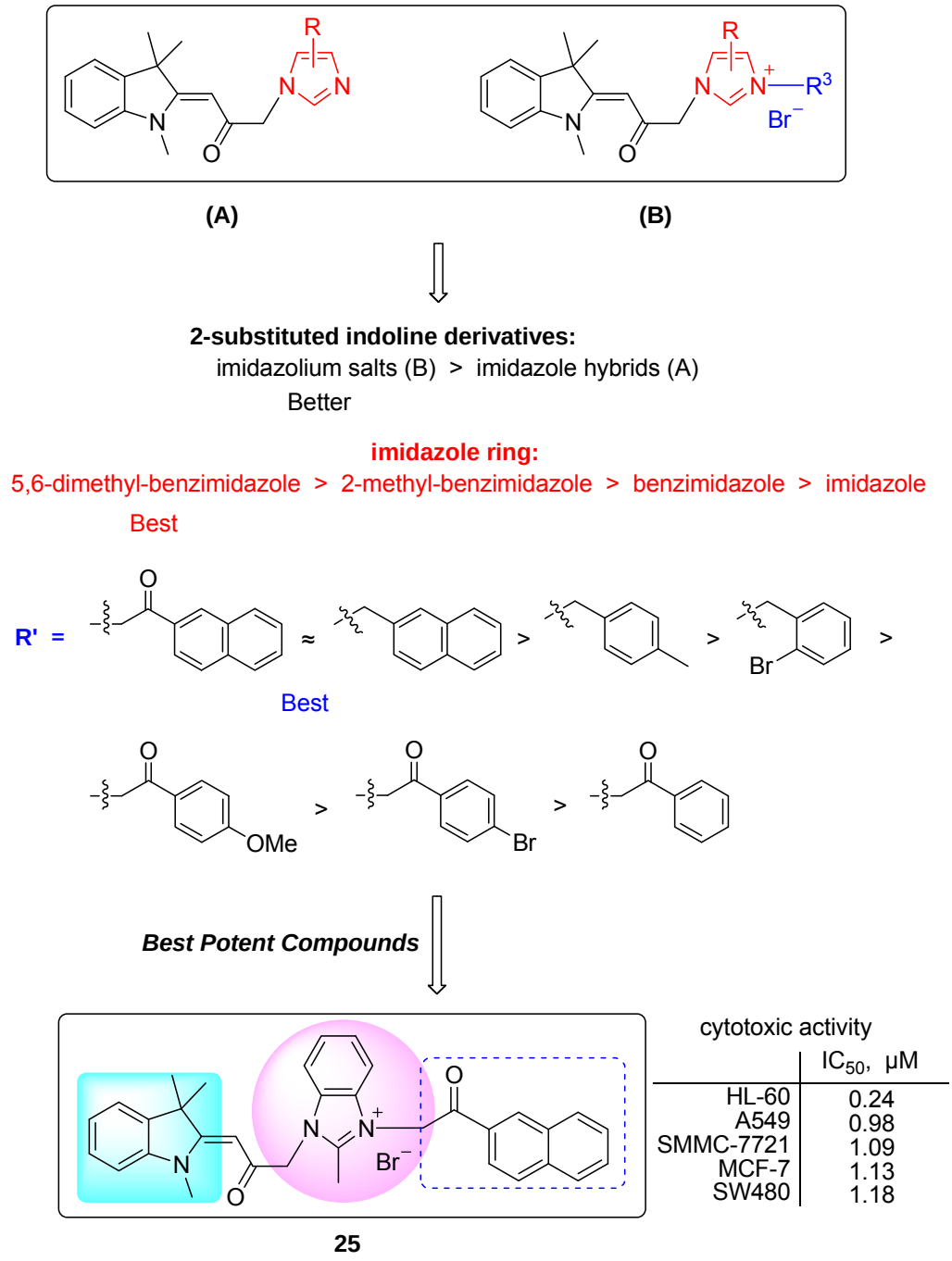
Particularly, the structures of imidazole and imidazolium salt derivatives have a remarkable impact on the cytotoxic activities. 2-substituted indoline–imidazole hybrids **4**–**7** lacked activities against all tumor cell lines investigated at the concentration of 40 μM. However, their imidazolium salts **8**–**32** exhibited some degree of cytotoxic activities or higher cytotoxic activities. This could be understandable because of the changes of molecular structure, charge distribution and water solubility.[11]

In the case of the imidazole ring (imidazole, benzimidazole, 2-methyl-benzimidazole, or 5,6-dimethyl-benzimidazole), imidazolium salt derivatives **8**–**12** with imidazole ring displayed weak cytotoxic activities. Only compounds **8** and **12**, bearing a 4-methylbenzyl or naphthylacyl substituent at position-3 of the imidazole, showed higher cytotoxic activity compared with DDP with IC_{50} values of 0.69–11.71 μM. Meanwhile, imidazolium salt derivatives **13**–**18** with benzimidazole ring exhibited medium or high cytotoxic activities. Among them, compounds **13**, **14**, **15** and **18**, bearing 4-methylbenzyl, 2-bromobenzyl, 2-naphthylmethyl or naphthylacyl substituent at position-3 of the benzimidazole, displayed higher cytotoxic activities compared with DDP with IC_{50} values of 0.39–4,54 μM. However, imidazolium salt derivatives **19**–**25** with 2-methyl-benzimidazole ring and **26**–**32** with 5,6-dimethyl-benzimidazole ring exhibited powerful cytotoxic activities. All of these kinds of derivatives (14 compounds) were found to be much more active than DDP. Among them, compounds **21**, **25**, **26**, **28** and **32**, also bearing a 4-methylbenzyl, 2-naphthylmethyl or naphthylacyl substituent at position-3 of the 2-methyl-benzimidazole or 5,6-dimethyl-benzimidazole, showed potent cytotoxic activities with IC_{50} values of 0.24–2.34 μM against five human tumor cell lines investigated.

In the case of the substituent at position-3 of imidazole ring, imidazolium salt derivatives **9**, **16**, **22** and **29** with a phenacyl substituent at position-3 of imidazole ring showed lacked or weak activities against five tumor cell lines. Meanwhile, compounds **10**, **11**, **17**, **23** and **30** with a 4-bromophenacyl or 4-methoxyphenacyl

substituent at position-3 of imidazole ring exhibited medium cytotoxic activities (IC_{50} = 0.69–23.90 μM). However, compared with above phenacyl or substituted phenacyl substituent derivatives, imidazolium salts with 2-naphthylmethyl, 4-methylbenzyl or naphthylacyl groups at position-3 of imidazole ring exhibited higher cytotoxic activity. Most of these kinds of derivatives showed moderate or potent activity. Especially, compounds **15**, **21** and **28** with a 2-naphthylmethyl substituent, as well as compounds **18**, **25** and **32** with a naphthylacyl substituent at position-3 of the imidazole ring displayed much higher cytotoxic activity in vitro compared with DDP. Interestingly, compound **25**, bearing a naphthylacyl substituent at position-3 of 2-methyl-benzimidazole, was found to be the most potent derivatives with IC_{50} values of 0.24–1.18 μM against all of human tumor cell lines investigated and more active than DDP. Notably, compound **25** exhibited cytotoxic activity selectively against MCF-7, SW480, SMMC-7721 and HL-60 cell lines with IC_{50} values 14.1-fold, 11.5-fold, 6.1-fold and 5.0-fold more sensitive to DDP, while compound **26** showed powerful inhibitory activities selectively against SMMC-7721 and A549 cell lines with IC_{50} values of 0.75 μM and 0.64 μM. This finding shows that steric and electronic effects have an important role in the cytotoxic activity of imidazolium salts.

The results suggest that the existence of substituted benzimidazole ring and substitution of the imidazolyl-3-position with a naphthylacyl or 2-naphthylmethyl group could be crucial for prommoting cytotoxic activity. In addition, the structure-activity relationship (SAR) results were illustrated in Scheme 3.



Scheme 3 Structure-activity relationship of 2-substituted indoline imidazolium salts.

Compound 25 induces G1 phase arrest and apoptosis in cancer cells

SMMC-7721 cells were exposed to increasing concentrations of compound **25** and cell apoptosis was determined with Annexin V-FITC/PI double-labeled cell cytometry. As shown in Fig. 3, after treatment of cells

with compound **25** at 1, 2, 4 μM for 48 h, the apoptotic cell rate was 7.13±1.25 %, 9.14±1.82 % and 25.67± 2.98 %, respectively, which were statistically different from the control (2.23±0.42 %).

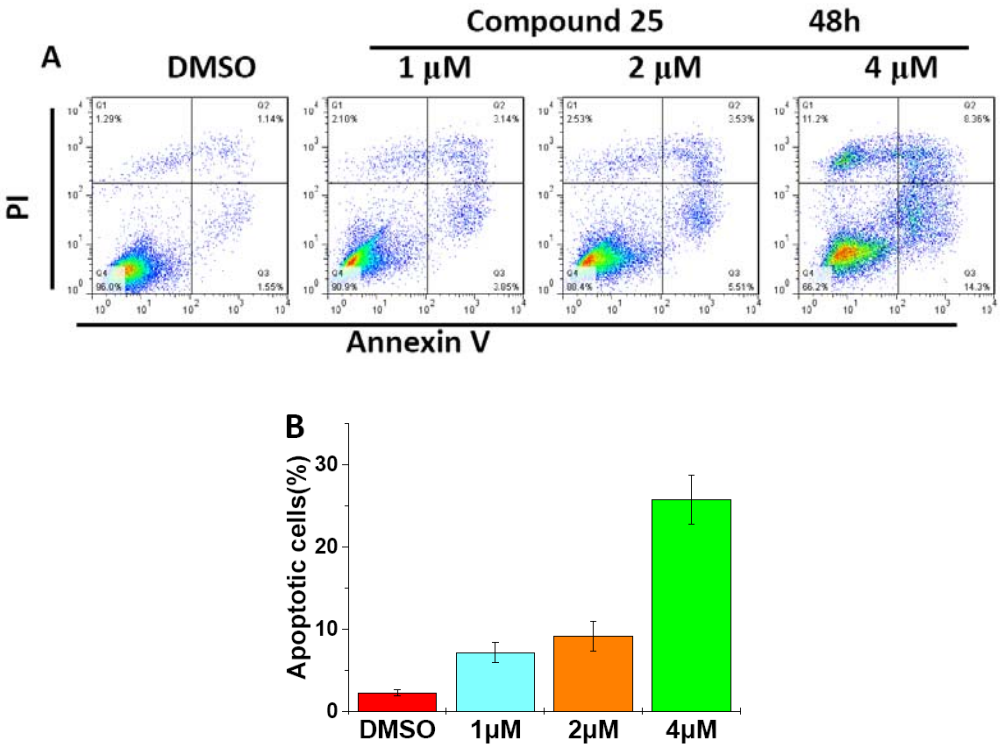
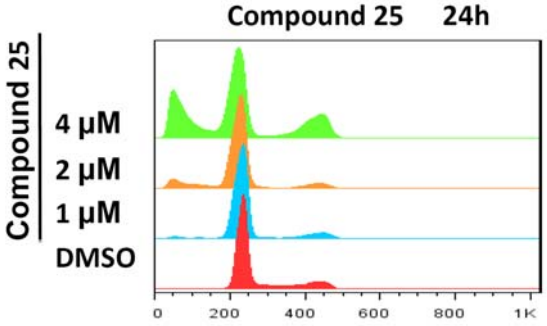


Fig. 3 Compound **25** caused significant apoptosis of SMMC-7721 cells. (A) Cells were treated with 4, 8 and 16 μM compound **25** for 48 h. Cell apoptosis was determined by Annexin V-FITC/PI double-staining assay. (B) The quantification of cell apoptosis. Data represents the mean ± S.D. of three independent experiments.

The results of cell cycle analysis on SMMC-7721 cells treated with compound **25** were summarized in Fig. 4. Compared with the control cells, the percentage of cells of G2/M phase was increased in the cells incubated with compound **25** with a dose dependent manner. In the meanwhile, the fraction of cells in S phase decreased slightly accordingly, while the proportion of G0/G1 phase cells showed no obvious change. Our dada suggest that compound **25** may induce G2/M phase arrest in the cell cycle.



Treatment	Cells (%)		
	G0/G1	S	G2/M
DMSO	72.56±3.29	14.32±3.68	15.21±2.57
Compound **25** (1 μM)	76.21±1.35	2.32±0.24	8.43±0.85
Compound **25** (2 μM)	82.59±3.75	1.64±0.19	7.98±1.24
Compound **25** (4 μM)	60.15±4.29	1.18±0.31	11.08±1.23

Fig. 4 Compound **25** induces S phase arrest in SMMC-7721 cells. (A) Cells were treated with 1, 2 and 4 μM of compound **25** 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.

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.[12]

Conclusion

In summary, a series of novel 2-substituted indoline imidazolium salt derivatives prepared proved to be potent antitumor agents. The imidazolium salt derivatives **15**, **21**, **25, 28** and **32**, 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 **25**, bearing a naphthylacyl substituent at position-3 of 2-methyl-benzimidazole, was found to be the most potent derivatives with IC_{50} values of 0.24–1.18 μM against

all of human tumor cell lines. Notably, compound **25** exhibited cytotoxic activity selectively against MCF-7, SW480, SMMC-7721 and HL-60 cell lines with IC_{50} values 14.1-fold, 11.5-fold, 6.1-fold and 5.0-fold more sensitive to DDP, while compound **26** showed powerful inhibitory activities selectively against SMMC-7721 and A549 cell lines with IC_{50} values of 0.75 μM and 0.64 μM. Compound **25** can induce the G2/M phase cell cycle arrest and apoptosis in SMMC-7721 cells. The indoline-based imidazolium salts **15**, **21**, **25, 26, 28** and **32** 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. 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 compound 3. To a stirred solution of 1,3,3-trimethyl-2-methyleneindoline **1** (8.66 g, 50 mmol) and triethylamine (6.57 g, 65 mmol) in dichloromethane (300 mL) at 0 °C was added bromoacetyl bromide **2** (12.11 g, 60 mmol) in small portions over a period of 30 min, and then at ambient temperature for 2 h. Reaction progress was monitored by TLC. A small amount of water was added and the mixture was stirred for 15 min. The aqueous phase was washed with CH_2Cl_2 (4×50 mL). The combined organic phases was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether 60-90 °C :

EtOAc : Et_3N = 30:1:0.1) to afford the product **3** (11.47g, 82%) as a red solid. See ESI file for characterization data.†

Synthesis of compounds 4-7. A mixture of compound **3** (2 mmol) and imidazole or various substituted benzimidazole (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 ^oC : EtOAc : Et_3N = 1:1:0.1) to afford **4-7** in 62-82% yield as yellow powder.

Compound 4: Yield 62%. Yellow solid, mp 134-136°C. IR ν_{max} (cm^{-1}): 3436, 2955, 1651, 1544, 1488, 1365, 1129, 1071, 941, 742. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.51 (1H, s), 7.21-7.16 (2H, m), 7.14 (1H, s), 7.02 (1H, d, J = 7.5 Hz), 6.96 (1H, s), 6.76 (1H, d, J =7.8 Hz), 4.91 (1H, s), 4.65 (2H, s), 3.06 (3H, s), 1.69 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 187.24, 174.02, 143.09, 140.16, 138.22, 129.64, 127.65, 123.03, 121.97, 120.26, 108.15, 88.21, 56.21, 48.78, 29.66, 22.91. HRMS (ESI-TOF) *m/z* Calcd for $C_{17}H_{20}N_3O$ $[M+1]^+$, 282.1606, found, 282.1600.

Compound 5: Yield 82%. Yellow solid, mp 192-193°C. IR ν_{max} (cm^{-1}): 3439, 2913, 2351, 1679, 1539, 1486, 1360, 1119, 934, 750. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.02 (1H, s), 7.89-7.87 (1H ,m), 7.44-7.41 (1H, m), 7.35-7.32 (2H, m), 7.26-7.20 (2H, m), 7.06 (1H, t, J = 7.2 Hz), 6.77 (1H, d, J = 7.5 Hz), 5.02 (1H, s), 4.93 (2H, s), 3.00 (3H, s), 1.75 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 186.79, 173.99, 143.91, 143.05, 140.14, 134.39, 127.65, 123.19, 123.05, 122.26, 121.98, 120.49, 109.94, 108.15, 88.32, 54.26, 48.82, 29.61, 27.0, 22.91. HRMS (ESI-TOF) *m/z* Calcd for $C_{21}H_{22}N_3O$ $[M+1]^+$ 332.1763, found 332.1753.

Compound 6: Yield 80%. Yellow solid, mp 151-153°C. IR ν_{max} (cm^{-1}): 3459, 2925, 1651, 1539, 1464, 1366, 1129, 937, 744. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.72 (1H, t, J = 3.6 Hz), 7.27-7.21 (m, 3H), 7.16 (2H, d, J = 8.1 Hz), 7.01 (1H, t, J = 7.2 Hz), 6.71 (1H, d, J = 7.8 Hz), 4.87 (1H, s), 4.77 (2H, s), 2.89 (3H, s), 2.58 (3H, s), 1.70 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 187.12, 173.94, 152.27, 143.04, 142.78, 140.10, 135.69, 127.63, 123.00, 122.28, 122.01, 121.95, 119.23, 109.21, 108.12, 88.08, 53.15, 48.76, 29.53, 22.95, 14.04. HRMS (ESI-TOF) *m/z* Calcd for $C_{22}H_{24}N_3O$ $[M+H]^+$ 346.1919. found 346.1911.

Compound 7: Yield 66%. Yellow solid, mp 142-144°C. IR ν_{max} (cm^{-1}): 3457, 2921, 1659, 1542, 1490, 1364, 1126, 940, 839, 745. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.85 (1H, s), 7.57 (1H, s), 7.14 (3H, t, *J* = 7.8 Hz), 7.00 (1H, t, *J* = 7.5 Hz), 6.72 (1H, d, *J* = 7.8 Hz), 4.94 (1H, s), 4.81 (2H, s), 2.94 (3H, s), 2.35 (6H, d, *J* = 1.5 Hz), 1.70 (6H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 187.39, 173.94, 143.07, 142.38, 140.19, 132.93, 132.42, 131.17, 127.64, 123.00, 121.98, 120.43, 110.10, 108.12, 88.33, 54.40, 48.80, 29.62, 22.96, 20.64, 20.32. HRMS (ESI-TOF) *m/z* Calcd for $C_{23}H_{26}N_3O$ $[M+1]^+$ 360.2076. found 360.2068.

Synthesis of compounds 8-32. A mixture of 2-substituted indoline–imidazole hybrids **4**–**7** (0.2 mmol) and phenacyl bromides or alkyl bromides (0.24 mmol) was stirred in acetone at reflux or toluene (5 ml) at 80 °C 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 **8-32** in 64–95% yields. See ESI file for characterization data of all novel compounds.†

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 °C 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.

Acknowledgments

This work was supported by grants from the Program for Changjiang Scholars and Innovative Research Team in University (IRT13095), Natural Science Foundation of China (21332007 and 21062026), Yunnan Province (2013FA028, 2012FB113 and 2010GA014) and Education Department of Yunnan Province (ZD2014010), Project of Recruited Top Talent of Sciences and Technology of Yunnan Province (2009C1120), and 100 Talents Program of CAS.

Notes and references

1 (a) T. Aboul-Fadl, F. A.S. Bin-Jubair, O. Aboul-Wafa, *Eur. J. Med. Chem.*, 2010, **45**, 4578. (b) F. Piu, N. K. Gauthier, R. Olsson, E. A. Currier, B. W. Lund, G. E. Croston, U. Hacksell, M. R. Brann, *Biochem. Pharmacol.*, 2005, **71**, 156. (c) M. P. Hay, G. J. Atwell, W. R. Wilson, S. M. Pullen, W. A. Denny, *J. Med. Chem.*, 2003, **46**, 2456. (d) E. Fattorusso, O. Taglialatela-Scafati, *Modern Alkaloids*, Wiley-VCH: Weinheim, 2008.

2 Y. Zhang, Q.Au, M. Zhang, J. R. Barber, S. C. Ng, B. Zhang, *Biochem. Biophys. Res. Commu.*, 2009, **36**, 729.

3 J. Kwak, Y. Kim, H. Park, J. Jang, K. K. Lee, W. Yi, J. Kwak, S. Park, H. Kim, K. Lee, J. S. Kang, S. Han, B. Y. Hwang, J. T. Hong, J. Jung, Y. Kim, J. Cho, H. Lee, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 4620.

4 (a) A. Vik, E. Hedner, C. Charnock, L. W. Tangen, Ø. Samuelsen, R. Larsson, L. Bohlinb, L. L. Gundersen, *Bioorg. Med. Chem.*, 2007, **15**, 4016. (b) H. Miyachi, H. Kiyota, M. Segawa, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 3003. (c) S. J. Dominianni, T. T. Yen, *J. Med. Chem.*, 1989, **32**, 2301. (d) E. E. Alberto, L. L. Rossato, S. H. Alves, D. Alves, A. L. Braga, *Org. Biomol. Chem.*, 2011, **9**, 1001.

5 (a) C. G. Fortuna, V. Barresi, G. Berellini, G. Musumarra, *Bioorg. Med. Chem.*, 2008, **16**, 4150. (b) F. P. Ballistreri, V. Barresi, P. Benedetti, G. Caltabiano, C. G. Fortuna, M. L. Longo, G. Musumarraa, *Bioorg. Med. Chem.*, 2004, **12**, 1689.

6 B. Cui, B. L. Zheng, K. He, Q. Y. Zheng, *J. Nat. Prod.*, 2003, **66**, 1101.

7 (a) C. J. Sun, W. Chen, Y. Li, L. X. Liu, X. Q. Wang, L. J. Li, H. B. Zhang, X. D. Yang, *RSC Adv.*, 2014, **4**, 16312. (b) W. Chen, X. H. Zeng, X. Y. Deng, Y. Li, L. J. Yang, W. C. Wan, X. Q. Wang, H. B. Zhang, X. D. Yang, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 4297. (c) W. J. Song, X. D. Yang, X. H. Zeng, X. L. Xu, G. L. Zhang, H. B. Zhang, *RSC Adv.*, 2012, **2**, 4612. (d) W. Chen, X. D. Yang, Y. Li, L. J. Yang, X. Q. Wang, G. L. Zhang, H. B. Zhang, *Org. Biomol. Chem.*, 2011, **9**, 4250. (e) X. H. Zeng, X. D. Yang, Y. L. Zhang, C. Qing, H. B. Zhang, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1844.

8 (a) C. Viegas Jnr, A. Danuello, V. S. Bolzani, E. J. Barreiro, C. A. M. Fraga, *Curr. Med. Chem.*, 2007, **14**, 1829. (b) J. J. Walsh, A. Bell, *Curr. Pharm. Des.*, 2009, **15**, 2970. (c) M. D'hooghe, K. Mollet, R. De Vreese, T. H. M. Jonckers, G. Dams, N. De Kimpe, *J. Med. Chem.*, 2012, **55**, 5637. (d) K. P. Kaliappan, V, Ravikumar, *Org. Biomol. Chem.*, 2005, **3**, 848.

9 CCDC 1012979 contains the supplementary crystallographic data for compound **24**. These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/ data_request/cif.

10 (a) D.-K. Kim, D. H. Ryu, J. Y. Lee, N. Lee, Y.-W. Kim, J.-S. Kim, K. Chang, G.-J. Im, T.-K. Kim, W.-S. Choi, *J. Med. Chem.*, 2001, **44**, 1594. (b) R. Cao, Q. Chen, X. Hou, H. Chen, H. Guan, Y. Ma, W. Peng, A. Xu, *Bioorg. Med. Chem.*, 2004, **12**, 4613.

11 J. Ranke, S. Stolte, R. Störmann, J. Arning, B. Jastorff, *Chem. Rev.*, 2007, **107**, 2183.

12 (a) Y. Wang, P. Ji, J. Liu, R. R. Broaddus, F. Xue, W. Zhang, *Mol. Cancer*, 2009, **8**, 8. (b) M. Löbrich, P. A. Jeggo, *Nat. Rev. Cancer*, 2007, **7**, 861. (c) X. Ling, R. J. Bernacki, M. G. F. Brattain, Li, *J. Biol. Chem.*, 2004, **279**, 15196.

FIGURE TITLES

Fig. 1 Representative structures of 2-substituted indoline derivatives and imidazolium salts.

Fig. 2 X-ray crystal structure of compound **24**.

Fig. 3 Model of compound **25** docked into PI3Kγ.

Fig. 4 Model of compound **26** docked into PI3Kγ.

SCHEME TITLES

Scheme 1 Synthesis of hybrid compounds **4**–**7**.

Scheme 2 Structure-activity relationship of 2-substituted indoline imidazolium salts.

TABLE TITLES

Table 1 Synthesis of indoline imidazolium salt derivatives **8**–**32** from **4**–**7**

Table 2 Cytotoxic activities of imidazole and imidazolium salt derivatives in vitro[b] (IC_{50}, μM[a])