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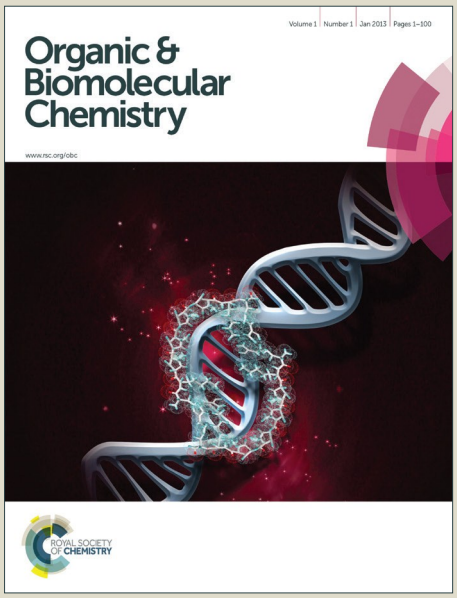

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Synthesis and antitumor activity of novel *N*-substituted tetrahydro-β-carboline–imidazolium salt derivatives

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The synthesis of a series of novel *N*-substituted tetrahydro-β-carboline–imidazolium salt derivatives is presented. Biological property of such compounds was further evaluated *in vitro* against a panel of human tumor cell lines. The results suggest that benzimidazole ring and 1-(naphthalen-2-yl)ethan-1-one or 2-naphthylmethyl substituent at imidazolyl-3-position were vital for modulating cytotoxic activity. Compound **41** was observed as potent derivative with IC_{50} values of 3.24-8.78 μM and exhibited cytotoxic activity selectively against HL-60, A-549 and MCF-7 cell lines, additionally. Meanwhile, high inhibitory activities selectively against HL-60 and MCF-7 cell lines were observed in compound **51**. Moreover, compound **51** was able to induce the G1 phase cell cycle arrest and apoptosis in MCF-7 cells. The cytotoxicity of compound **51** against human normal lung epithelial cell line (BEAS-2B) was further evaluated.

Introduction

β-carboline alkaloids are widely present as anticancer natural products and are widely distributed in plants, animals and humans.[1-3] They have attracted significant attention due to their biological effects such as antitumor, anti-leishmanial, anti-trypanosomal, anti-HIV and anti-inflammatory, etc.[4] Specifically, tetrahydro-β-carbolines[5], such as eleagnine, harmicine and *N*-substituted 2-benzoyl-1,3,4,9-tetrahydro-β-carboline, are one of the important analogs in carboline alkaloid (Fig. 1), which are reported possessing antinociceptive, anti-inflammatory and antioxidant, antileishmanial and antinociceptive activities.[6]

Imidazolium salts are important building block in drug discovery with pharmacological activities[7] and antitumor activity.[8] For example, two new imidazolium chlorides, Lepidiline A and B, illustrated in Figure 1, displayed potent cytotoxic activity against human cancer cell lines (UMUC3, PACA2, MDA231, and FDIGROV).[9] Considering the value of imidazolium salts, we recently introduced the synthesis of a novel imidazolium salt NMIB (Fig. 1) and studied their potential antitumor activity.[10] Further study of molecular mechanisms showed that the imidazolium salt hybrids can induce the cell cycle arrest and apoptosis in tumor cells.[10i]

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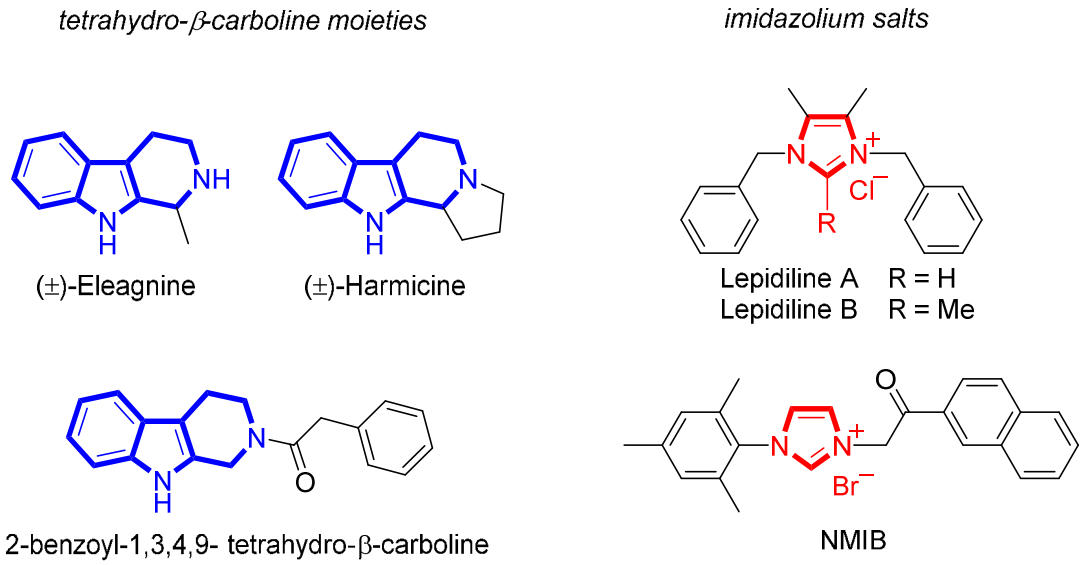

Fig. 1 Representative structures of tetrahydro-β-carboline and its N-substituted derivative and imidazolium salts.

Molecular hybridization is a useful tool in new drug design and development during the past two decades.[11] We were curious if any pharmacological activities would be identified in the hybridizing compounds of *N*-substituted tetrahydro-β-carboline together with imidazole moieties. To the best of our knowledge, antitumor activity hasn't been observed in any *N*-substituted tetrahydro-β-carboline–imidazole hybrids in the literature. Herein, we have designed and synthesized a series of novel imidazole scaffold-based *N*-substituted tetrahydro-β-carboline and investigated their antitumor activity.

Results and discussion

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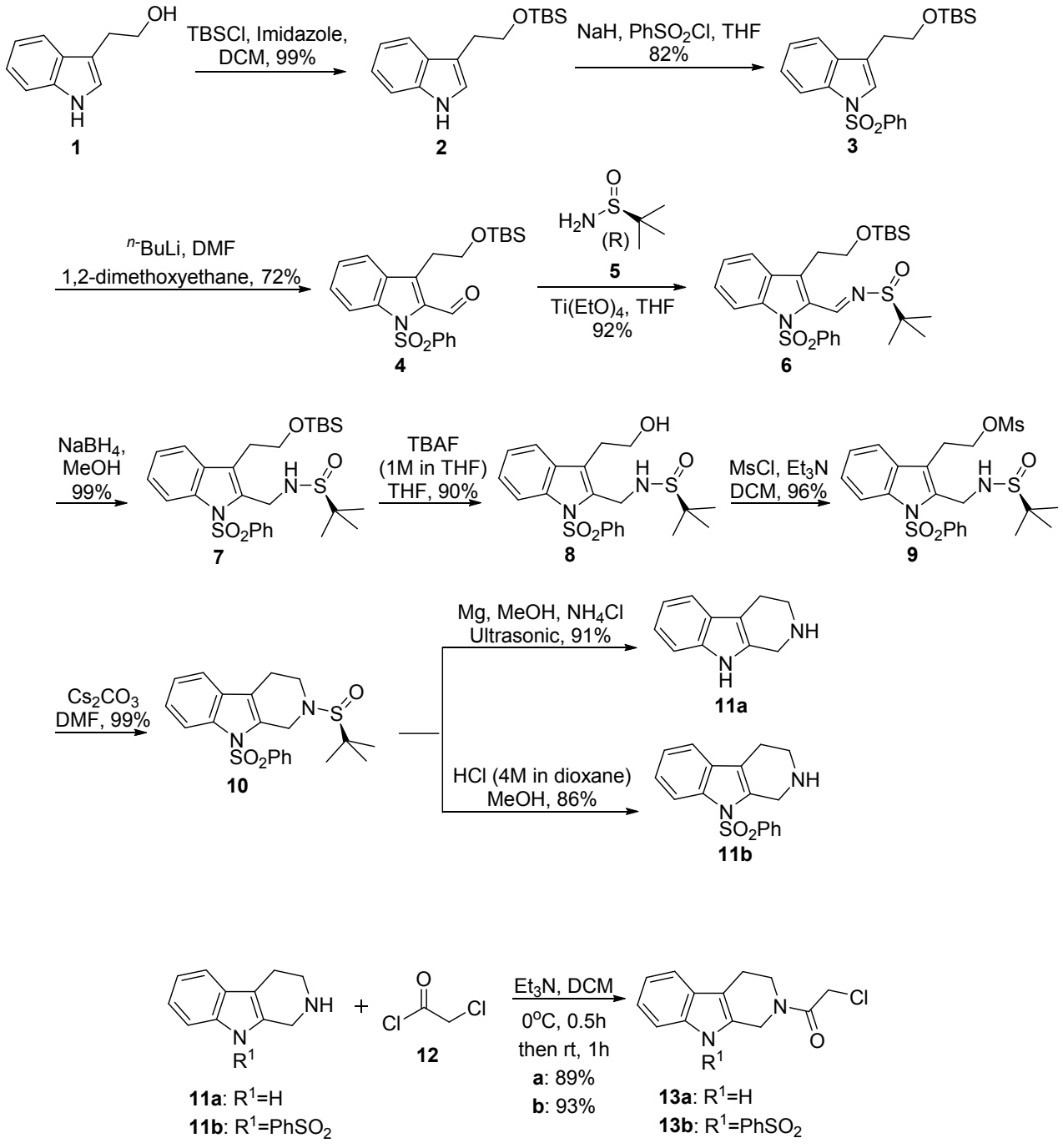

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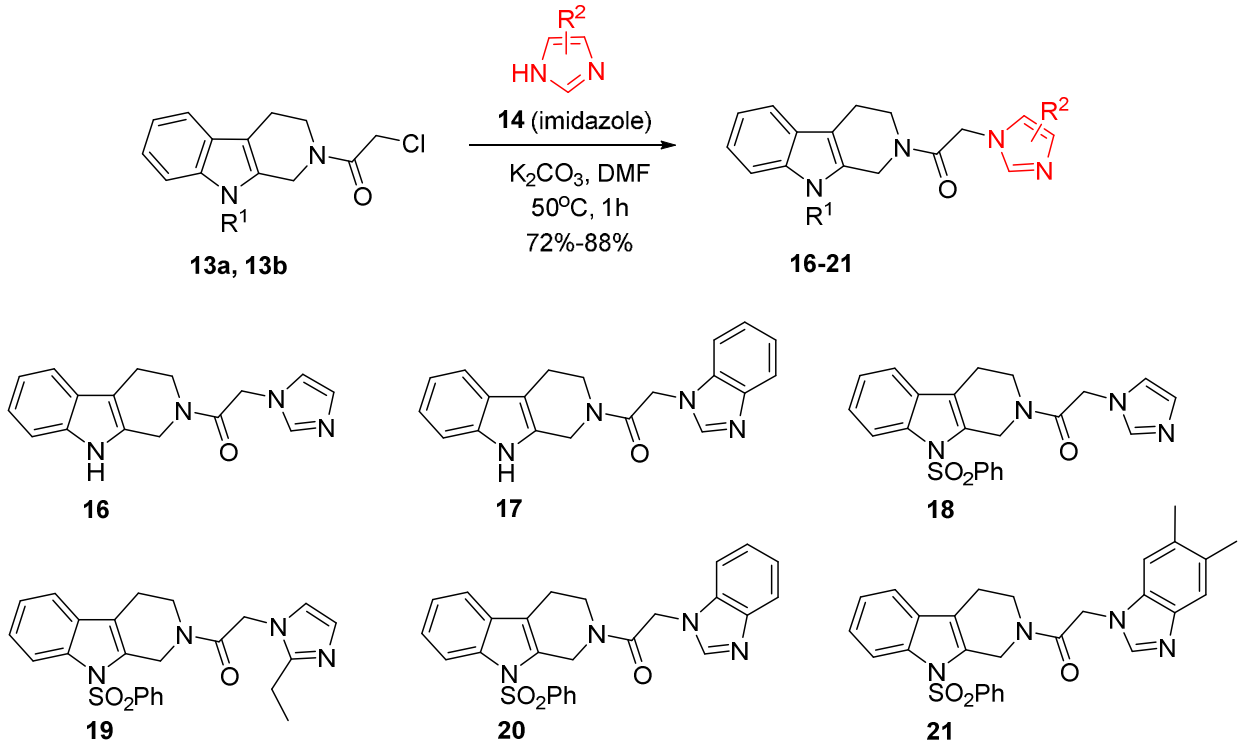

Scheme 1 Synthesis of hybrid compounds **16-21**.

For the synthesis of *N*-substituted tetrahydro-β-carboline–imidazole derivatives (Compounds **16-21**), we started with commercial tryptophol **1**, after OH and NH groups were protected with tert-butyl(dimethyl)silyl with phenylsulfonyl groups, and an aldehyde group was installed at the 3-position of indole motif to afford compound **4**. The condensation of **4** with (R)-(+)-2-methyl-2-propanesulfinamide **5** led to imine **6.** Started form compound **6**, tetrahydro-β-carboline **10** was formed through sequential three steps including reduction of imine, deprotection of OH group and ring closure. Next, key intermediate **10** was converted to **11a** and **11b** under two deportation conditions. The acylation of **11a** and **11b** led to amides **13a** and **13b**. Subsequently, *N*-substituted tetrahydro-β-carboline–imidazole hybrids **16**–**21** were formed from **13a** and **13b** with various substituted imidazole (imidazole, 2-ethyl-imidazole, benzimidazole or 5,6-dimethyl-benzimidazole) at 72–88% yields (Scheme 1).

Finally, imidazolium salts **22**–**51** were prepared from coupling of imidazole hybrids **16**–**21** with various alkyl and phenacyl bromides at 72–92% yields. The structures and yields of derivatives are shown in Table 1.

Table 1 Synthesis of *N*-substituted tetrahydro-β-carboline imidazolium salt derivatives **22-54** from **16-21**

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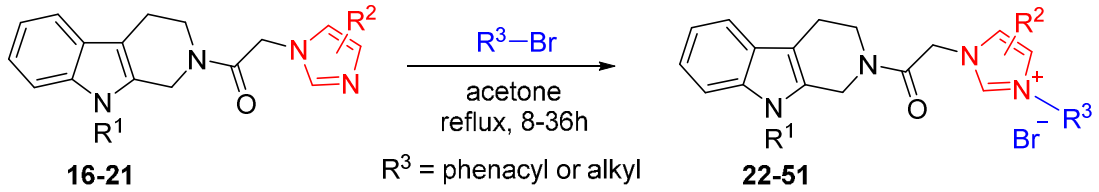

Entry	Compound No.	R^1	Imidazole ring	R^3	Molecular formula	mp (°C)	Yields (%)
1	**16**	H	imidazole	–	$C_{16}H_{16}N_4O$	85-86	85
2	**17**	H	benzimidazole	–	$C_{20}H_{18}N_4O$	275-276	88
3	**18**	$PhSO_2$	imidazole	–	$C_{22}H_{20}N_4O_3S$	77-79	82
4	**19**	$PhSO_2$	2-ethyl-imidazole	–	$C_{24}H_{24}N_4O_3S$	147-148	88
5	**20**	$PhSO_2$	benzimidazole	–	$C_{26}H_{22}N_4O_3S$	139-140	72
6	**21**	$PhSO_2$	5,6-dimethyl-benzimidazole	–	$C_{28}H_{26}N_4O_3S$	278-279	76
7	**22**	H	imidazole	phenacyl	$C_{28}H_{25}BrN_4O_2$	201-202	78
8	**23**	H	imidazole	1-(naphthalen-2-yl)ethan-1-one	$C_{28}H_{25}BrN_4O_2$	249-251	92
9	**24**	H	benzimidazole	phenacyl	$C_{28}H_{25}BrN_4O_2$	236-238	92
10	**25**	H	benzimidazole	4-methoxyphenacyl	$C_{29}H_{27}BrN_4O_3$	258-260	88
11	**26**	H	benzimidazole	4-bromophenacyl	$C_{28}H_{24}Br_2N_4O_2$	240-242	78
12	**27**	H	benzimidazole	1-(naphthalen-2-yl)ethan-1-one	$C_{32}H_{27}BrN_4O_2$	270-271	83
13	**28**	H	benzimidazole	4-bromobenzyl	$C_{27}H_{24}Br_2N_4O$	272-274	88
14	**29**	H	benzimidazole	4-nitrobenzyl	$C_{27}H_{24}BrN_5O_3$	279-280	82
15	**30**	H	benzimidazole	3-naphthylmethyl	$C_{31}H_{27}BrN_4O$	272-273	76
17	**31**	$PhSO_2$	imidazole	phenacyl	$C_{30}H_{27}BrN_4O_4S$	222-223	89
18	**32**	$PhSO_2$	imidazole	1-(naphthalen-2-yl)ethan-1-one	$C_{34}H_{29}BrN_4O_4S$	195-196	86
29	**33**	$PhSO_2$	2-ethyl-imidazole	phenacyl	$C_{32}H_{31}BrN_4O_4S$	272-273	86
20	**34**	$PhSO_2$	2-ethyl-imidazole	4-bromophenacyl	$C_{32}H_{30}Br_2N_4O_4S$	279-280	89
21	**35**	$PhSO_2$	2-ethyl-imidazole	1-(naphthalen-2-yl)ethan-1-one	$C_{36}H_{33}BrN_4O_4S$	273-274	78
22	**36**	$PhSO_2$	2-ethyl-imidazole	4-methylbenzyl	$C_{32}H_{33}BrN_4O_3S$	244-245	81
23	**37**	$PhSO_2$	2-ethyl-imidazole	3-naphthylmethyl	$C_{35}H_{33}BrN_4O_3S$	253-254	87
24	**38**	$PhSO_2$	benzimidazole	phenacyl	$C_{34}H_{29}BrN_4O_4S$	200-201	86
25	**39**	$PhSO_2$	benzimidazole	4-bromophenacyl	$C_{34}H_{28}Br_2N_4O_4S$	197-198	75
26	**40**	$PhSO_2$	benzimidazole	4-methoxyphenacyl	$C_{35}H_{31}BrN_4O_5S$	209-210	89
27	**41**	$PhSO_2$	benzimidazole	1-(naphthalen-2-yl)ethan-1-one	$C_{38}H_{31}BrN_4O_4S$	214-215	77
28	**42**	$PhSO_2$	benzimidazole	4-methylbenzyl	$C_{34}H_{31}BrN_4O_3S$	290-291	84
29	**43**	$PhSO_2$	benzimidazole	4-nitrobenzyl	$C_{33}H_{28}BrN_5O_5S$	272-273	86
30	**44**	$PhSO_2$	benzimidazole	3-naphthylmethyl	$C_{37}H_{31}BrN_4O_3S$	136-137	72
31	**45**	$PhSO_2$	5,6-dimethyl-benzimidazole	phenacyl	$C_{36}H_{33}BrN_4O_4S$	239-240	91
32	**46**	$PhSO_2$	5,6-dimethyl-benzimidazole	4-bromophenacyl	$C_{36}H_{32}Br_2N_4O_4S$	250-252	88
33	**47**	$PhSO_2$	5,6-dimethyl-benzimidazole	4-methoxyphenacyl	$C_{37}H_{35}BrN_4O_5S$	244-245	90
34	**48**	$PhSO_2$	5,6-dimethyl-benzimidazole	1-(naphthalen-2-yl)ethan-1-one	$C_{40}H_{35}BrN_4O_4S$	234-235	91

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35	**49**	$PhSO_2$	5,6-dimethyl-benzimidazole	4-methylbenzyl	$C_{36}H_{35}BrN_4O_3S$	270-271	79
36	**50**	$PhSO_2$	5,6-dimethyl-benzimidazole	4-nitrobenzyl	$C_{35}H_{32}BrN_5O_5S$	227-228	89
37	**51**	$PhSO_2$	5,6-dimethyl-benzimidazole	3-naphthylmethyl	$C_{39}H_{35}BrN_4O_3S$	252-254	82

Biological evaluation and structure-activity relationship analysis

The potential cytotoxicity of all newly synthesized hybrid compounds was assessed *in vitro* against a panel of human tumor cell lines, on the basis of the procedures in the literature[12]. The panel comprised with myeloid leukaemia (HL-60), liver carcinoma (SMMC-7721), lung carcinoma (A-549), breast carcinoma (MCF-7) and colon carcinoma (SW480). Cisplatin (DDP) were used as reference drug (See Table 2 to for complete results).

Table 2 Cytotoxic activities of hybrid compounds **16-51** in vitro[b] (IC_{50}, μM[a])

Entry	Compound No.	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	**17**	>40	>40	>40	>40	>40
2	**18**	>40	>40	>40	>40	>40
3	**19**	17.81	>40	28.16	21.00	29.80
4	**20**	36.73	>40	>40	>40	>40
5	**21**	>40	>40	>40	>40	>40
6	**22**	>40	>40	>40	>40	>40
7	**23**	>40	>40	>40	>40	>40
8	**24**	>40	>40	>40	>40	>40
9	**25**	>40	>40	>40	>40	>40
10	**26**	21.81	27.59	>40	20.47	32.35
11	**27**	11.09	19.80	>40	17.56	16.69
12	**28**	10.68	30.39	>40	19.51	>40
13	**29**	>40	>40	>40	>40	>40
14	**30**	3.54	13.23	18.02	12.24	17.46
15	**31**	>40	>40	>40	>40	>40
16	**32**	3.32	12.11	14.21	3.74	11.80
17	**33**	>40	>40	>40	>40	>40
18	**34**	11.87	16.77	>40	8.28	35.62
19	**35**	2.47	10.67	13.39	10.44	10.14
20	**36**	2.56	12.48	22.13	3.37	11.84
21	**37**	2.77	12.81	14.46	2.61	12.81
22	**38**	14.39	24.60	21.41	16.44	13.60
23	**39**	10.61	17.28	31.23	16.59	11.81
24	**40**	3.97	14.95	18.27	11.34	13.58
25	**41**	3.24	15.03	8.78	8.05	11.01
26	**42**	3.04	14.78	17.01	7.68	11.70

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27	**43**	13.58	23.35	35.36	17.42	12.44
28	**44**	4.34	14.74	17.28	10.33	11.76
29	**45**	10.18	14.50	22.75	11.26	13.19
30	**46**	3.75	15.30	>40	4.97	10.47
31	**47**	3.39	13.18	23.70	8.23	16.37
32	**48**	3.08	14.77	>40	3.90	16.17
33	**49**	4.30	15.13	29.52	10.17	14.20
34	**50**	12.81	33.19	>40	10.81	>40
35	**51**	2.61	14.15	17.13	2.79	9.46
36	DDP (MW 300)	2.27	9.98	8.25	14.69	15.11

[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 2, at the concentration of 40 μM, imidazole derivatives **17, 18** and **21** were inactive at concentrations lower than 40 μM, and **19** and **20** were observed processing weak activities against all tumor cell lines, while their imidazolium salts anallogs **26, 27, 28, 30, 32, 34-51** exhibited cytotoxic activities. Such difference in cytotoxicity between neutral compounds and imidazolium salts may be attributed to the differences in molecular structure, charge distribution and water solubility.[13]

In the case of the *N*-substituents of indole moiety, *N*-benzenesulfonylated imidazolium salts **32, 34-51** showed higher cytotoxic activities than *N*-H imidazolium salts **22-29**. All of *N*-benzenesulfonylated salts (except **31** and **33**) exhibited moderated or high inhibitory activities. Thus, we will mainly focus on the *N*-benzenesulfonylated indole moiety in the following discussion.

Substituted imidazole part of imidazolium salts plays an important role in bioactivity, for example, only compounds **32** and **37** which have 1-(naphthalen-2-yl)ethan-1-one and 3-naphthylmethyl at position-3 of the imidazole and 2-ethyl-imidazole rings, showed higher cytotoxic activity with IC_{50} values of 2.61–14.46 μM. Meanwhile, compounds **38-44** with benzimidazole ring exhibited medium inhibitory activities. Different substituents at position-3 of the benzimidazole ring such as 4-methoxyphenacyl (**40**), 1-(naphthalen-2-yl)ethan-1-one (**41**) and 4-methylbenzyl (**42**) substituent displayed better inhibitory activity toward MCF-7 and SW480 compared with DDP. Notably, compounds **45-51** with 5,6-dimethyl-benzimidazole rings showed significant inhibitory activities. Among them, compounds **46, 49** and **51**, with a 4-bromophenacyl, 4-methylbenzyl or 3-

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naphthylmethyl, displayed potent higher inhibitory activity *in vitro* than DDP with IC_{50} values of 2.79-14.20 μM against MCF-7 and SW480 tumor cell lines.

In the case of the substituent at position-3 of imidazole ring, imidazolium salt **31, 33, 38** and **45** with phenacyl substituent, as well as derivatives **43** and **50** with 4-nitrobenzyl substituent showed either negative or weak activities against five tumor cell lines. Compounds **34, 36, 39, 40, 42, 46, 47** and **49** with, 4-bromophenacyl, 4-methylbenzyl or 4-methoxyphenacyl substituent at position-3 of benzimidazole ring exhibited medium cytotoxic activities (IC_{50} = 2.56–35.62 μM). However, compared with above phenacyl or substituted phenacyl substituent derivatives, compounds **30, 37, 44** and **51** with 3-naphthylmethyl substituent, as well as compounds **32, 35, 41** and **48** with 1-(naphthalen-2-yl)ethan-1-one substituent at position-3 of the imidazole ring displayed much higher cytotoxic activity *in vitro*. Interestingly, compound **51**, bearing a 3-naphthylmethyl substituent at position-3 of 5,6-dimethyl-benzimidazole, was found to be one of the most potent derivatives with IC_{50} values of 2.61–17.13 μM against the five human tumor cell lines investigated. Notably, compound **51** exhibited cytotoxic activity selectively against MCF-7 and SW480 cell lines with IC_{50} values 5.3-fold and 1.6-fold more sensitive to DDP. 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 5,6-dimethyl-benzimidazoless ring and substitution of the imidazolyl-3-position with a 1-(naphthalen-2-yl)ethan-1-one or 2-naphthylmethyl group could enhance the cytotoxic activity of imidazolium salts. In addition, the structure-activity relationship (SAR) results were illustrated in Scheme 2.

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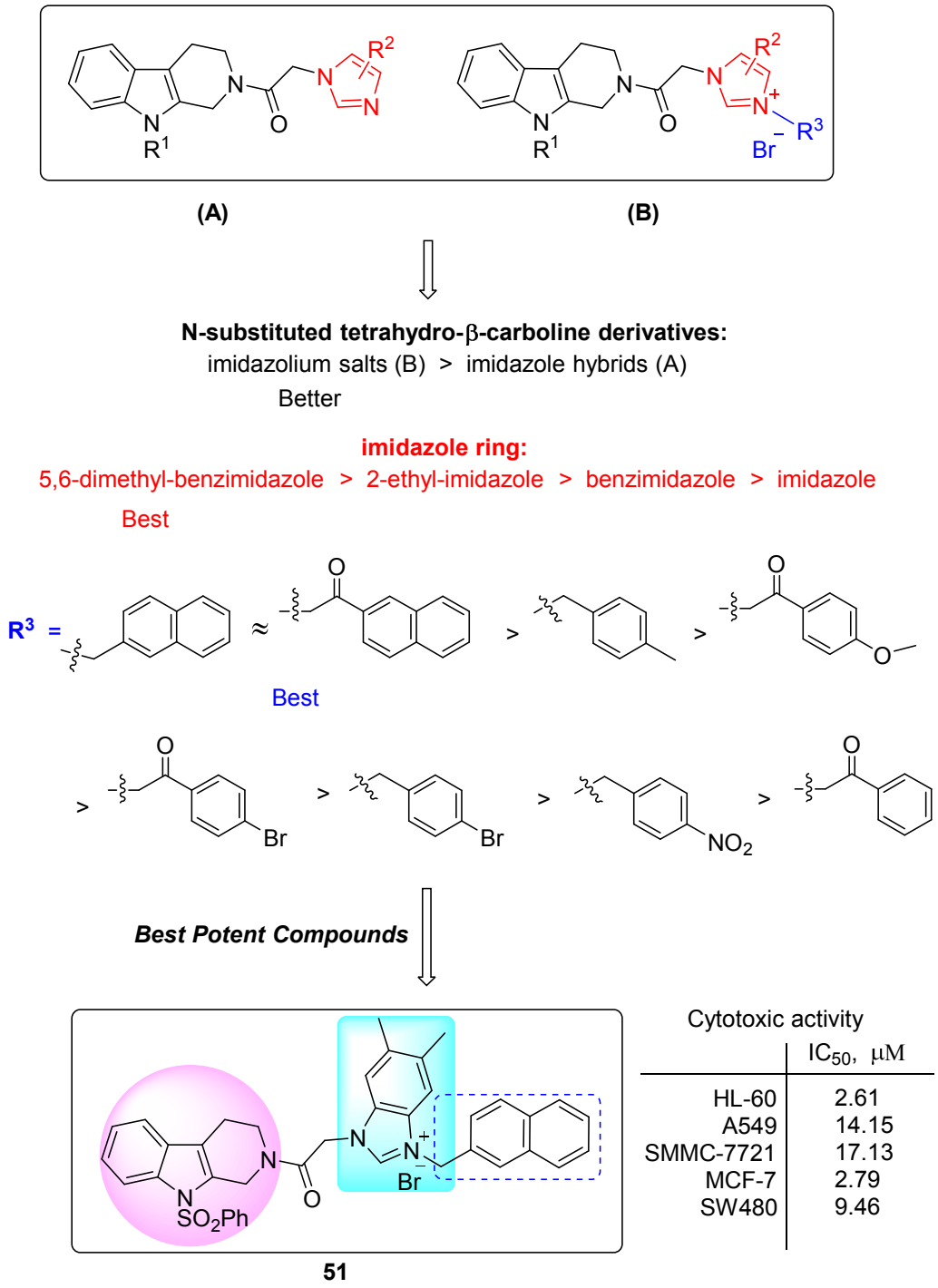

Scheme 2 Structure-activity relationship of N-substituted tetrahydro-β-carboline imidazolium salts.

Furthermore, the cytotoxicity of the representative compound **51** against human normal lung epithelial cell line (BEAS-2B) was also evaluated. 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,

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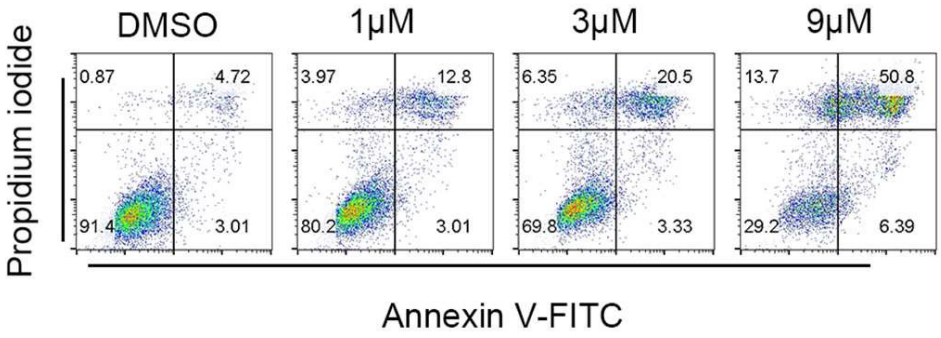
compound **51** exhibited selective cytotoxicity between cancer and normal cells, with an IC_{50} value above 40 μM against normal BEAS-2B cells, and less toxic than that against lung carcinoma A549 cancer cells. Contrarily, DDP had not obvious selectivity between cancer and normal cells.

Table 2 Cytotoxicity of compound **15** against A549 and BEAS-2B cells in vitro (IC_{50}, μM)

Entry	Compound no.	BEAS-2B	A549
1	**51**	>40	17.13
2	DDP	9.12	8.25

Compound 51 induces G1 phase arrest and apoptosis in cancer cells

MCF-7 cells were exposed to increasing concentrations of compound **51** 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 **51** at 1, 3, 9 μM for 48 h, the apoptotic cell rate was 15.1±0.9 %, 25.5±1.7 % and 59.8±2.6 %, respectively, which were statistically different from the control (6.5±0.9%).



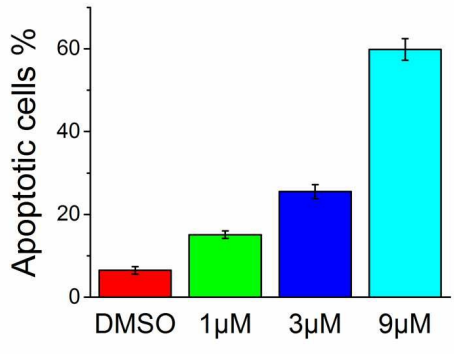


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Fig. 2 Compound **51** caused significant apoptosis of MCF-7 cells. (A) Cells were treated with 1, 3 and 9 μM compound **51** for 48 h. Cell apoptosis was determined by Annexin V-FITC/PI double-staining assay. (B) The quantification of cell apoptosis. Data represents the mean ± S.D. of three independent experiments.

The results of cell cycle analysis on MCF-7 cells treated with compound **51** were summarized in Fig. 3. Compared with the control cells, the percentage of cells of G1 phase increased during the cells incubated with compound **51** with a dose dependent manner. In the meanwhile, the fraction of cells in S phase decreased slightly accordingly, while the proportion of G2 phase cells showed no obvious change. Our data suggest that compound **51** may induce G1 phase arrest in the cell cycle.

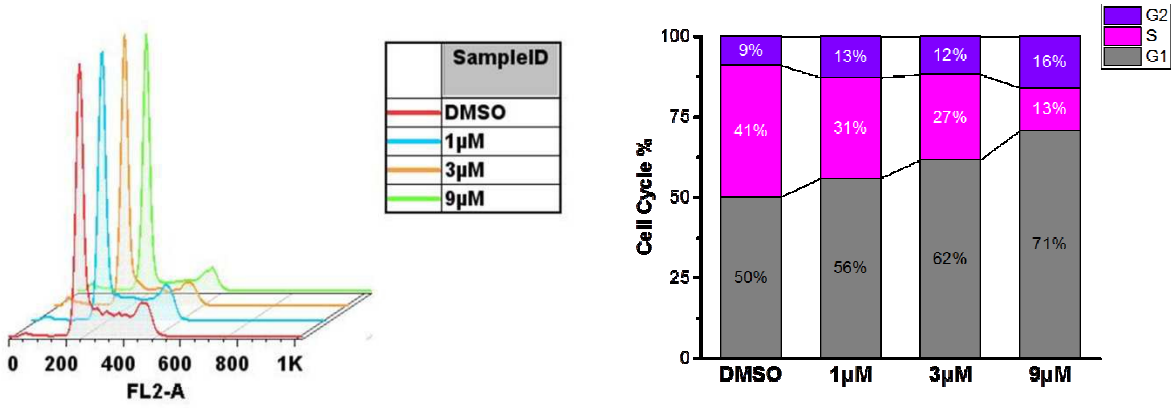

Fig. 3 Compound **51** induces G1 phase arrest in MCF-7 cells. (A) Cells were treated with 1, 3 and 9 μM of compound **51** for 24 h. Cell cycle was determined by PI staining and cell cytometry. (B) The percentages of cells in different phases were quantified. At least three independent experiments were performed and data of one representative experiment is shown.

Disruption or malfunction of cell cycle control within the G1 phase has been recognized as the most important biochemical phenomenon for tumor progression and tumorigenesis. The ability of certain small molecules to control cell cycle machinery within the G1 phase has provided exciting new opportunities with hopes of developing new types of drugs efficacious against refractory cancers.[14]

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Conclusion

In summary, a series of novel *N*-substituted tetrahydro-β-carboline imidazolium salt derivatives prepared in this research proved to be potent antitumor agents. The imidazolium salt derivatives **37**, **41**, **44** and **51**, bearing 2-ethyl-imidazole, benzimidazole or 5,6-dimethyl-benzimidazole ring and a 3-naphthylmethyl or 1-(naphthalen-2-yl)ethan-1-one at position-3 of the imidazole ring, were found to be the most potent compounds. Compound **41**, bearing a 1-(naphthalen-2-yl)ethan-1-one substituent at position-3 of benzimidazole, was found to be the most potent derivatives with IC_{50} values of 3.24–15.03 μM against five human tumor cell lines. Notably, compound **51** exhibited cytotoxic activity selectively against MCF-7 and SW480 cell lines with IC_{50} values 5.3-fold and 1.6-fold more sensitive to DDP. Compound **51** can induce the G1 phase cell cycle arrest and apoptosis in MCF-7 cells. The tetrahydro-β-carboline-based imidazolium salts **32, 35, 37, 41, 44** and **51** 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 400 (600) spectrometer at 400 (400) MHz. Carbon-13 nuclear magnetic resonance (^{13}C-NMR) was recorded on Bruker Avance 400 (600) spectrometer at 100 (120) 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 (%CHN) was conducted on a Vario EL Ⅲ 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

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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 compounds **2-4**, **6-11** and **13**. See ESI file for characterization data.†

Synthesis of hybrid compounds 16-21. A mixture of compound **13** (2 mmol) and imidazole or substituted imidazole (6mmol) and K_2CO_3 (3 mmol) was stirred in DMF (20 ml) at 50 ℃ for 1 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 : EtOAc : Et_3N = 1:1:0.1) to afford **16-21** in 62-82% yield as yellow powder. See ESI file for characterization data.†

Synthesis of compounds 22-51. A mixture of substituted imidazole **16-21** (0.25 mmol) and phenacylbromides or phenacyl or benzyl or 1-(naphthalen-2-yl)ethan-1-one or naphthylmethyl (0.75 mmol) was stirred in acetone (10 ml) at reflux 8-36 h. An insoluble substance was formed. After completion of the reaction as indicated by TLC, the precipitate was filteredand washed with acetone (3 × 10 ml), then dried to afford imidazolium salts **22-51** in 64–95% yields. See ESI file for characterization data.†

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).

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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 tetrahydro-β-carboline and its N-substituted derivative and imidazolium salts.

Fig. 2 Compound **51** caused significant apoptosis of MCF-7 cells. (A) Cells were treated with 1, 3 and 9 μM compound **51** 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 **51** induces G1 phase arrest in MCF-7 cells. (A) Cells were treated with 1, 3 and 9 μM of compound **51** 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 **16-21**.

Scheme 2 Structure-activity relationship of *N*-substituted tetrahydro-β-carboline imidazolium salts.

TABLE TITLES

Table 1 Synthesis of N-substituted tetrahydro-β-carboline imidazolium salt derivatives **22-51** from **16-21**

Table 2 Cytotoxic activities of hybrid compounds **17-51** in vitro[b] (IC_{50}, μM[a])