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Design, synthesis and antitumor activity of novel 8-substituted 2,3,5,6-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran imidazolium salt derivatives†

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A series of novel 8-substituted 2,3,5,6-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran 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 the 5,6-dimethyl-benzimidazole ring and substitution of the imidazolyl-3-position with a 2-naphthylmethyl or 4-methylbenzyl group were vital for modulating cytotoxic activity. Compound **43** was found to be the most potent derivative and exhibited cytotoxic activities selectively against breast carcinoma (MCF-7), colon carcinoma (SW480), myeloid leukaemia (HL-60) and lung carcinoma (A549) with an IC_{50} value 65.0-fold, 48.5-fold, 21.2-fold and 19.9-fold more sensitive to DDP, respectively.

Introduction

Cancer is one of the leading causes of human mortality and remains one of the most difficult diseases worldwide to treat.[1] Developing new anticancer drugs and more effective treatment strategies for cancer is of great importance.[2] Natural products represent a significant source of inspiration for the design of structural analogues with an improved pharmacological profile in medicinal chemistry.[3] Naturally occurring benzofurans are an important class of biologically active oxygen-containing heterocycles. Natural products possessing the dihydrobenzofuran and tetrahydrobenzodifuran moieties exhibit a broad range of biological and pharmacological activities.[4] Recently, naturally occurring dihydrobenzofurans and tetrahydrobenzodifurans have been identified to possess antitumor activity. As exemplified in Scheme 1, mesocyperusphenol A is an anti-leukaemic agents, which is tetrahydrobenzodifuran derived compounds exhibiting potent cytotoxic activity against human T-cell leukemia cells.[5]

Imidazolium salts have attracted considerable interests for their broad range of biological and pharmacological activity,[6] especially antitumor activity.[7] For example, two new imidazolium halides (Fig. 1), lepidiline A and lepidiline B, isolated from the roots of *Lepidium meyenii*, showed potent cytotoxic activity against human cancer cell lines (UMUC3, PACA2, MDA231, and FDIGROV).[8] More recently, we have previously reported the synthesis of a series of novel imidazolium salt derivatives, such as MNIB (Fig. 1), and their potential antitumor activity.[9] Studies on molecular mechanisms demonstrated that the imidazolium salt hybrids can induce the G1 phase cell cycle arrest and apoptosis in tumor cells.[9c]

Molecular hybridization as a drug discovery strategy, involves the rational design of new chemical entities by the fusion of two drugs. The active compounds and/or pharmacophoric units are identified and derived from known bioactive molecules, as shown in the development of new anticancer, anti-Alzheimer, and antimalarial agents.[10] Considering the anticancer activities of naturally occurring substituted tetrahydrobenzodifuran as well as the potent cytotoxic activities of natural and synthetic imidazolium derivatives, we were interested in synthesizing a number of new hybrid compounds bearing tetrahydrobenzodifuran and imidazolium moieties.

Although dihydrobenzofuran–triazole hybrid compounds were synthesized and found to possess antitubercular activity by Tripathi,[11] and some benzofuran-based hybrid compounds were synthesized and found to exhibit cholinesterase inhibitory activity by Rampa,[12] to the best of our knowledge, no reports concerning antitumor activity for hybrid compounds between tetrahydrobenzodifuran and imidazole have been reported.

In the present research, we have designed and synthesized a series of novel 8-substituted 2,3,5,6-tetrahydrobenzo[1,2-*b*:4,5-*b*′]-difuran imidazolium salt derivatives. The purpose of this study was to investigate the antitumor activity of tetrahydrobenzodifuran-

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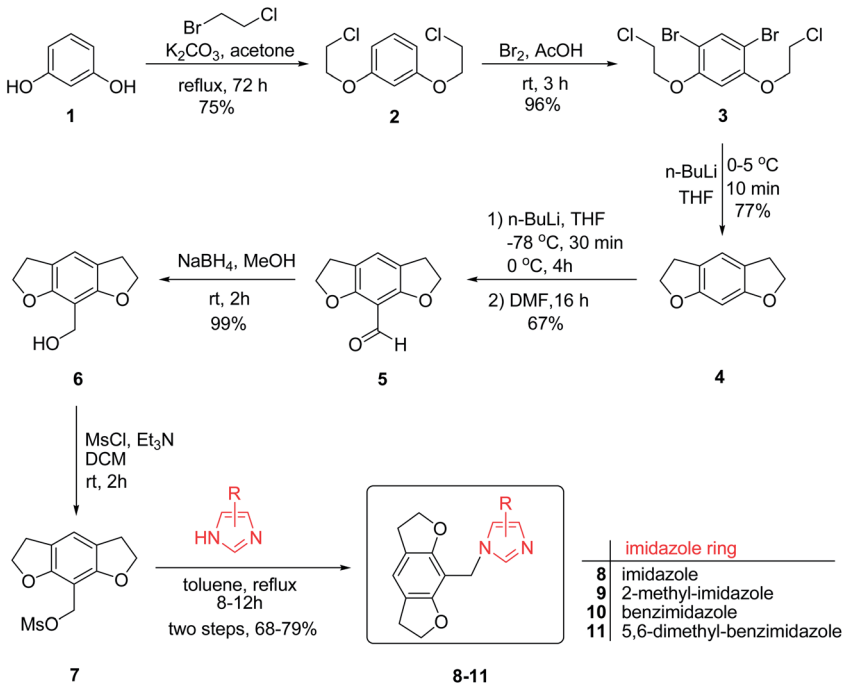
† Electronic supplementary information (ESI) available: Details of experimental procedure, spectral data and copies of all novel compounds. CCDC 941506. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ra43183e



Scheme 1 Synthesis of hybrid compounds **8–11**.

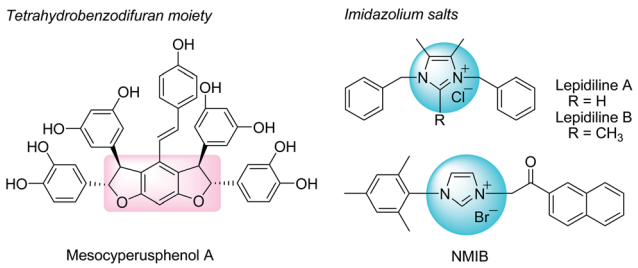


Fig. 1 Representative structures of tetrahydrobenzodifuran and imidazolium salts.

based imidazolium salt compounds, with the ultimate aim of developing novel potent antitumor agents.

Results and discussion

Chemistry

To synthesize the tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran–imidazole hybrids, we used commercially available imidazole derivatives that were alkylated with tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-8-methanol, which was synthesized over five steps from readily available starting materials as shown in Scheme 1. Resorcinol **1** was chosen as the starting material for the preparation of a series of tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran–imidazole hybrids (**8–11**). The dialkylation of resorcinol **1** was achieved by reacting with excess 1-bromo-2-chloroethane and potassium carbonate in acetone at reflux (75% yield). Aromatic dibromination of ether **2** was accomplished using bromine in acetic acid (96% yield), and cyclization of dibromo compound **3** with 2 equiv. of *n*-butyllithium in THF at 0 °C gave the key intermediate tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran **4** in 77% yield.[13] This tricyclic compound **4** was regioselectively lithiated at the position *ortho* to the aryl-oxygens and the resulting anion quenched with DMF to afford compound **5** in 67% yield.[14] Then, the tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-8-carboxaldehyde **5** were reduced with $NaBH_4$ to the respective tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-8-methanol (**6**, 99% yields). Subsequently, compound **6** was transformed *via* the mesylate to the respective four 8-substituted tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran–imidazole hybrids **8–11** with various substituted imidazole (imidazole, 2-methyl-imidazole, benzimidazole or 5,6-dimethyl-benzimidazole) by refluxing under toluene with 68–79% yields (two steps).

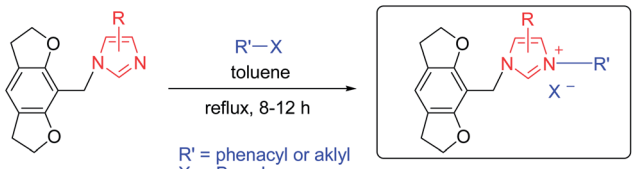
Finally, thirty-six 8-substituted tetrahydrobenzo[1,2-*b*:4,5-*b*′]-difuran imidazolium salts **12–47** were prepared with excellent yields by reaction of tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran–imidazole hybrids **8–11** with the corresponding alkyl and phenacyl halides in refluxing acetone (64–99% yields). The structures and yields of derivatives are shown in Table 1.

To verify the structures of the 8-substituted tetrahydrobenzo [1,2-*b*:4,5-*b*′]difuran imidazolium salt derivatives, imidazolium salt **20** was selected as a representative compound and characterized by X-ray crystallography (ESI†),[15] as shown in Fig. 2.

Biological evaluation and structure–activity relationship analysis

The cytotoxic potential of all newly synthesized imidazole and imidazolium salt derivatives was evaluated *in vitro* against a panel of human tumor cell lines according to procedures described in the literature.[16] The panel consisted of myeloid leukaemia (HL-60), breast carcinoma (MCF-7), colon carcinoma

Table 1 Synthesis of imidazolium salt derivatives **12–47** from **8–11**



8-11 **12-47** 64-99%

Entry	Compound no.	Imidazole ring	R′	X	Yields (%)
1	**8**	Imidazole	—	—	73
2	**9**	2-Methyl-imidazole	—	—	68
3	**10**	Benzimidazole	—	—	79
4	**11**	5,6-Dimethyl-benzimidazole	—	—	68
5	**12**	Imidazole	Benzyl	Br	92
6	**13**	Imidazole	4-Methylbenzyl	Br	83
7	**14**	Imidazole	4-Bromobenzyl	Br	88
8	**15**	Imidazole	4-Nitrobenzyl	Br	95
9	**16**	Imidazole	2-Naphthylmethyl	Br	90
10	**17**	Imidazole	Phenacyl	Br	91
11	**18**	Imidazole	4-Bromophenacyl	Br	95
12	**19**	Imidazole	4-Methoxyphenacyl	Br	95
13	**20**	Imidazole	Naphthylacyl	Br	90
14	**21**	2-Methyl-imidazole	Benzyl	Br	84
15	**22**	2-Methyl-imidazole	4-Bethylbenzyl	Br	97
16	**23**	2-Methyl-Imidazole	4-Bromobenzyl	Br	79
17	**24**	2-Methyl-imidazole	4-Nitrobenzyl	Br	97
18	**25**	2-Methyl-imidazole	2-Naphthylmethyl	Br	91
19	**26**	2-Methyl-imidazole	Phenacyl	Br	98
20	**27**	2-Methyl-imidazole	4-Bromophenacyl	Br	79
21	**28**	2-Methyl-imidazole	4-Methoxyphenacyl	Br	86
22	**29**	2-Methyl-imidazole	Naphthylacyl	Br	93
23	**30**	Benzimidazole	Butyl	I	77
24	**31**	Benzimidazole	Benzyl	Br	85
25	**32**	Benzimidazole	4-Methylbenzyl	Br	77
26	**33**	Benzimidazole	4-Bromobenzyl	Br	70
27	**34**	Benzimidazole	2-Naphthylmethyl	Br	64
28	**35**	Benzimidazole	Phenacyl	Br	95
29	**36**	Benzimidazole	4-Bromophenacyl	Br	88
30	**37**	Benzimidazole	4-Methoxyphenacyl	Br	96
31	**38**	Benzimidazole	Naphthylacyl	Br	86
32	**39**	5,6-Dimethyl-benzimidazole	Butyl	I	75
33	**40**	5,6-Dimethyl-benzimidazole	Benzyl	Br	96
34	**41**	5,6-Dimethyl-benzimidazole	4-Methylbenzyl	Br	83
35	**42**	5,6-Dimethyl-benzimidazole	4-Bromobenzyl	Br	93
36	**43**	5,6-Dimethyl-benzimidazole	2-Naphthylmethyl	Br	70
37	**44**	5,6-Dimethyl-benzimidazole	Phenacyl	Br	99
38	**45**	5,6-Dimethyl-benzimidazole	4-Bromophenacyl	Br	93
39	**46**	5,6-Dimethyl-benzimidazole	4-Methoxyphenacyl	Br	98
40	**47**	5,6-Dimethyl-benzimidazole	Naphthylacyl	Br	89

(SW480), lung carcinoma (A549), and liver carcinoma (SMMC-7721). Cisplatin (DDP) was used as the reference drug. The results are summarized in Table 2 (IC_{50} value, defined as the concentrations corresponding to 50% growth inhibition).

As shown in Table 2, the structures of imidazole and imidazolium salt derivatives have an obvious influence on the cytotoxic activities. Tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran–imidazole hybrids **8–11** lacked activities against all tumor cell lines investigated at the concentration of 40 μM. However, their imidazolium salts **12–47** exhibited some degree of cytotoxic activities or higher cytotoxic activities. This difference in cytotoxicity between neutral compounds and imidazolium salts may be due to the changes of molecular structure, charge distribution and water solubility.[17]

In terms of the imidazole ring (imidazole, 2-methyl-imidazole, benzimidazole or 5,6-dimethyl-benzimidazole), imidazolium salt derivatives **12–20** with imidazole ring displayed weak cytotoxic activities. Only compounds **13**, **16** and **20**, bearing a 4-methylbenzyl, 2-naphthylmethyl or naphthylacyl substituent at position-3 of the imidazole, showed higher cytotoxic activity

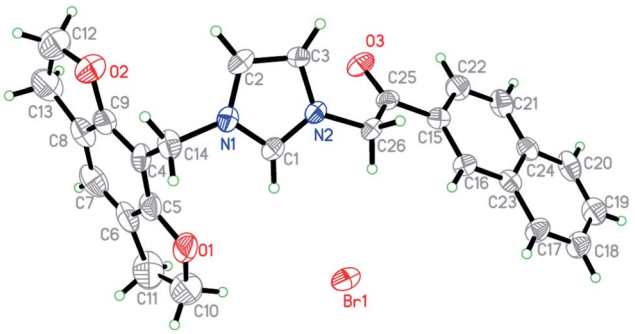


Fig. 2 X-ray crystal structure of compound **20**.

compared with DDP with IC_{50} values of 1.09–10.49 μM. Meanwhile, imidazolium salt derivatives **21–29** with 2-methyl-imidazole ring exhibited medium cytotoxic activities. Among them, compounds **22**, **25** and **29**, bearing above same substituents at position-3 of the 2-methyl-imidazole, displayed higher cytotoxic activities compared with DDP with IC_{50} values of 0.51–7.64 μM. However, imidazolium salt derivatives **30–38** with benzimidazole ring and **39–47** with 5,6-dimethyl-benzimidazole ring exhibited powerful cytotoxic activities. All of these kinds of derivatives (18 compounds) were found to be much more active than DDP. Among them, compounds **32**, **34**, **41** and **43**, also bearing a 4-methylbenzyl and 2-naphthylmethyl substituent at position-3 of the benzimidazole or 5,6-dimethyl-benzimidazole, showed potent cytotoxic activities with IC_{50} values of 0.20–2.21 μM against five human tumor cell lines investigated. As for the anion (Br^- and I^-) of imidazolium salts, iodide derivatives (compounds **30** and **39**) displayed similar cytotoxic activities compared with bromide derivatives.

In terms of the substituent at position-3 of imidazole ring, imidazolium salt derivatives **15** and **24** with 4-nitrobenzyl substituent, as well as derivative **17** with a phenacyl substituent at position-3 of imidazole ring showed lacked activities against five tumor cell lines. However, compared with above 4-nitrobenzyl or phenacyl substituent derivatives, imidazolium salts with 2-naphthylmethyl, 4-methylbenzyl or substituted phenacyl groups at position-3 of imidazole ring exhibited higher cytotoxic activity. Most of these kinds of derivatives showed moderate or potent activity. Especially, compounds **16**, **25**, **34** and **43** with a 2-naphthylmethyl substituent, as well as compounds **13**, **22**, **32** and **41** with a 4-methylbenzyl substituent at position-3 of the imidazole ring displayed much higher cytotoxic activity *in vitro* compared with DDP. Interestingly, compound **43**, bearing a 2-naphthylmethyl substituent at position-3 of 5,6-dimethyl-benzimidazole, was found to be the most potent derivatives with IC_{50} values of 0.20–1.81 μM against all of human tumor cell lines investigated and more active than DDP. Notably, this compound exhibited cytotoxic activity selectively against breast carcinoma (MCF-7), colon carcinoma (SW480), myeloid leukaemia (HL-60) and lung carcinoma (A549) with IC_{50} value 65.0-fold, 48.5-fold, 21.2-fold and 19.9-fold more sensitive to DDP, respectively. This finding shows that steric and electronic effects have an important role in the cytotoxic activity of imidazolium salt hybrids. Generally, a bulkier 2-naphthylmethyl substituent, as well as an electron-donating 4-methylbenzyl substituent at position-3 of imidazole ring exhibit higher cytotoxic activity against tumor cells.[9]

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

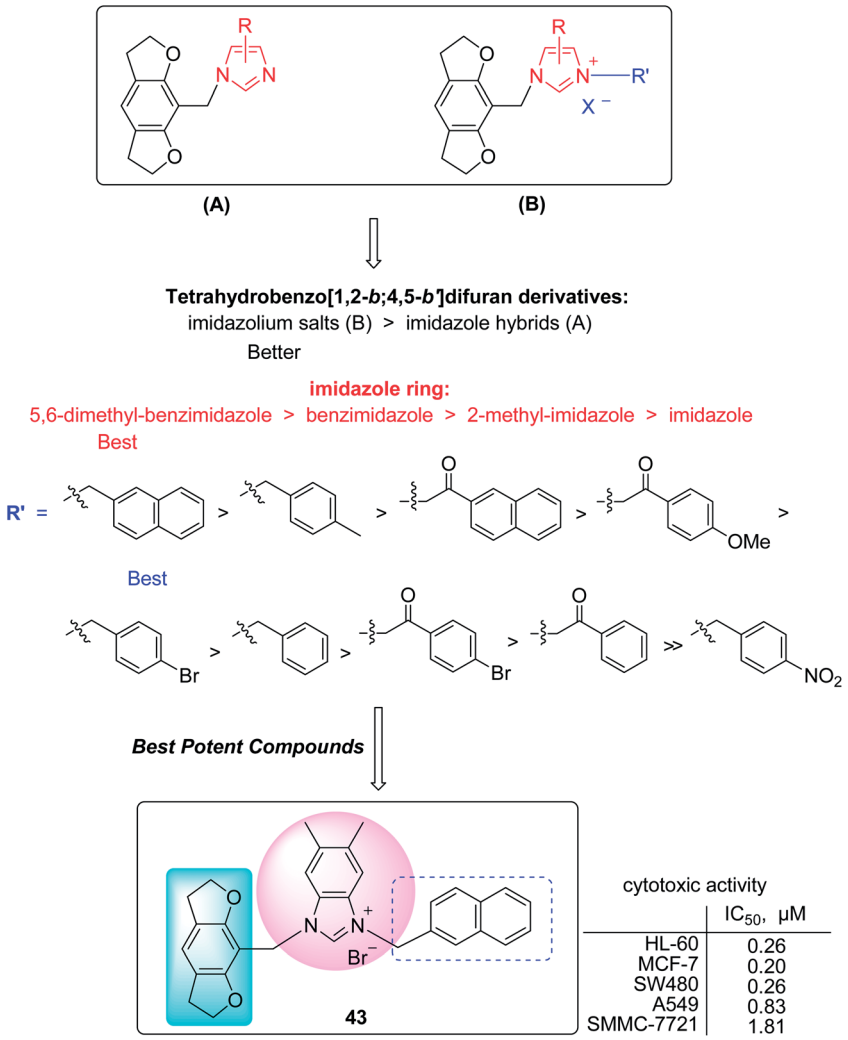
Entry	Compound no.	HL-60	MCF-7	SW480	A549	SMMC-7721
1	**8**	>40	>40	>40	>40	>40
2	**9**	>40	>40	>40	>40	>40
3	**10**	>40	>40	>40	>40	>40
4	**11**	>40	>40	>40	>40	>40
5	**12**	12.27	16.70	25.07	32.92	>40
6	**13**	1.84	3.49	4.72	6.64	10.28
7	**14**	2.22	12.16	15.67	34.29	28.60
8	**15**	>40	>40	>40	>40	>40
9	**16**	1.13	2.90	3.62	7.22	10.49
10	**17**	>40	>40	>40	>40	>40
11	**18**	3.66	14.65	17.46	39.93	>40
12	**19**	>40	>40	>40	>40	>40
13	**20**	1.09	3.43	4.63	9.08	9.02
14	**21**	3.96	15.79	9.65	11.48	17.17
15	**22**	0.63	6.98	3.50	3.39	2.59
16	**23**	0.77	3.46	10.08	7.81	13.08
17	**24**	>40	>40	>40	>40	>40
18	**25**	0.51	0.65	3.89	1.86	3.36
19	**26**	3.61	18.80	32.26	32.80	>40
20	**27**	1.99	4.26	13.85	10.22	15.03
21	**28**	0.82	4.66	15.11	5.82	6.45
22	**29**	1.04	1.21	4.61	3.61	7.64
23	**30**	1.65	1.58	4.06	9.60	8.56
24	**31**	1.21	0.80	2.45	3.83	5.48
25	**32**	0.42	0.27	0.92	0.96	2.13
26	**33**	0.58	2.36	1.84	3.58	7.45
27	**34**	0.31	1.13	0.57	0.55	1.35
28	**35**	2.03	5.16	3.77	3.16	8.22
29	**36**	1.17	1.60	3.20	5.44	6.41
30	**37**	0.87	2.96	2.75	5.63	5.13
31	**38**	0.83	1.19	2.93	3.30	5.17
32	**39**	0.57	0.94	0.89	1.48	1.25
33	**40**	0.50	0.69	1.01	1.62	0.73
34	**41**	0.40	0.65	0.64	1.06	2.21
35	**42**	0.79	0.97	0.96	1.45	1.81
36	**43**	0.26	0.20	0.26	0.83	1.81
37	**44**	1.23	1.04	1.21	4.39	3.97
38	**45**	1.18	1.02	1.63	4.13	3.07
39	**46**	0.95	0.61	1.41	2.55	4.89
40	**47**	0.98	0.83	1.36	3.28	3.92
41	DDP	5.52	12.99	12.61	16.51	18.77

[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.

The results suggest that the existence of 5,6-dimethyl-benzimidazole ring and substitution of the imidazolyl-3-position with a 2-naphthylmethyl or 4-methylbenzyl group were vital for modulating cytotoxic activity. The structure–activity relationship (SAR) results were summarized in Scheme 2.

Molecule docking

In addition, we found these 8-substituted tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran imidazolium salts could inhibit the mTOR



Scheme 2 Structure–activity relationship of tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran imidazolium salts.

(mammalian target of rapamycin) signal pathway during our research. In order to rationalize the observed SARs for this series of compound, we attempted to dock imidazolium salts **43** and **34** with some crystal structure of proteins in this signaling pathway, *e.g.* mTORC1, mTORC2, and PI3K using Autodock 4.0 (see ESI† for a detailed description of the docking experiments). Although these compounds could not dock with mTORC1 or mTORC2, it could dock well with PI3Kγ (PDB code 3PRZ). Fig. 3 shows the tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran ring of hybrid **43** can foster van der Waals interactions with the pocket bounded by LYS572, LYS579 and GLU573, and it also shows 5,6-dimethyl-benzimidazole ring can interact with the gap bounded by SER595, TRP576, LYS606 and ILE603, while 2-naphthylmethyl ring is placing in the pocket bounded by LEU574, GLU570, LEU551, PHE578, and HIS577. Similar to hybrid **43**, hybrid **34** can foster van der Waals interactions with the pocket bounded by GLU573, LEU574, PHE578 and HIS577 using tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran ring, and it also interact with the gap bounded by GLU570 and ALA569 using benzimidazole ring, while its 2-naphthylmethyl ring is placing in the pocket bounded by GLN550, GLN554 and LEU551. In addition, hybrid **34**

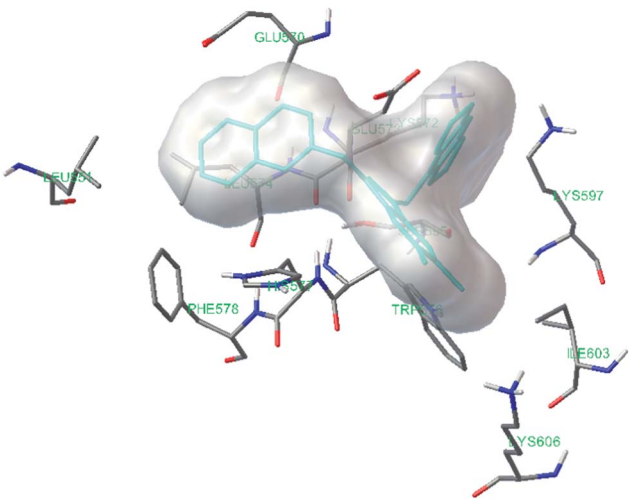


Fig. 3 Model of compound **43** docked into PI3Kγ.

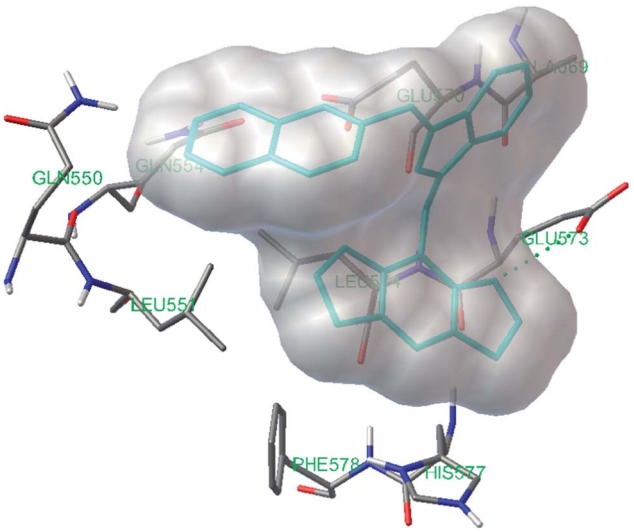


Fig. 4 Model of compound **34** docked into PI3Kγ.

establishes a hydrogen bond with GLU573 using a oxygen of tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran (Fig. 4). All these favorable interactions contribute to achieve a good docking score (AutoDock binding energy of **43** is −7.33 kcal mol^{-1}, and AutoDock binding energy of **34** is −7.13 kcal mol^{-1}) and an excellent inhibitory activity as it results from the experimental data. These interesting findings would be useful for our further research.

Conclusion

A number of novel 8-substituted benzo[1,2-*b*:4,5-*b*′]difuran imidazolium salt derivatives prepared in this research proved to be potent antitumor agents. The imidazolium salt derivatives **43**, **41**, **32** and **34**, bearing 5,6-dimethyl-benzimidazole or benzimidazole ring and a 2-naphthylmethyl or 4-methylbenzyl at position-3 of the imidazole ring, were found to be the most potent compounds. Compound **43**, bearing a 2-naphthylmethyl substituent at position-3 of 5,6-dimethyl-benzimidazole, was found to be the most potent derivatives with IC_{50} values of 0.20–1.81 μM against all of human tumor cell lines investigated and exhibited cytotoxic activities selectively against breast carcinoma (MCF-7), colon carcinoma (SW480), myeloid leukaemia (HL-60) and lung carcinoma (A549) with IC_{50} value 65.0-fold, 48.5-fold, 21.2-fold and 19.9-fold more sensitive to DDP, respectively. The 2-benzylbenzofuran-based imidazolium salts **43**, **41**, **32** and **34** 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 2. A mixture of resorcinol **1** (5.0 g, 45.4 mmol), 1-bromo-2-chloroethane (30 mL, 363 mmol), finely powdered K_2CO_3 (19.0 g, 137 mmol) and acetone (30 mL) was stirred and heated at reflux under argon for 72 h. The reaction was cooled to room temperature and filtered through a short pad of Celite. The Celite was washed with CH_2Cl_2, and the filtrate and washes were combined and evaporated to dryness by rotatory evaporation. The residue was partitioned between AcOEt (20 mL) and H_2O (20 mL). The organic phase was washed with 2 M NaOH (2 × 30 mL), then H_2O (2 × 30 mL) and brine (30 mL), dried over Na_2SO_4 and evaporated under reduced pressure to yield the products **2** (8.0 g, 75%) as white powder. See ESI file for characterization data.†

Synthesis of compound 3. The ether **2** (8.0 g, 34.0 mmol) was suspended in glacial acetic acid (25 mL) and a solution of Br_2 (4.4 mL) in glacial acetic acid (10 mL) was added dropwise at 0–5 °C. The reaction mixture was allowed to reach room temperature and stirred for 3 h. The mixture was poured into ice/water (50 mL) and stirred for 15 min. The precipitate was filtered off and the solid was washed with cold 1 : 1 AcOH–H_2O (5 × 30 mL), then with cold H_2O until neutral pH (5 × 50 mL) and dried under reduced pressure until constant weight to yield the products **3** (12.8 g, 96%) as pale yellow powder. See ESI file for characterization data.†

Synthesis of compound 4. A solution of the dibromo compound **3** (8.00 g, 20.4 mmol) in 250 mL of anhydrous THF was placed in a N_2 atmosphere and cooled to 0 °C. A solution of *n*-butyllithium (21.4 mL, 2.5 M in hexanes, 2.1 equiv.) was added very quickly (addition time: 7 s) to the rapidly stirred solution using a syringe with a large gauge needle. The reaction mixture was stirred for 10 min, and solvent was removed. The residue was partitioned between AcOEt and H_2O, and the organic phase was dried with Ma_2SO_4 and evaporated to furnish the crude product, which was chromatographed on silica gel (petroleum ether 60–90 °C : ethyl acetate = 20 : 1) to afford the products **4** (2.53 g, 77%) as white crystals. See ESI file for characterization data.†

Synthesis of compound 5. To a solution of the tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran **4** (2.53 g, 15.6 mmol) in anhydrous THF (150 mL) was added *n*-butyllithium (10.0 mL, 2.5 M in hexanes, 1.6 equiv.) by syringe at −78 °C in a N_2 atmosphere. The mixture was stirred for 30 min. The external cool bath was replaced by an ice/water bath and the reaction mixture was stirred at 0–5 °C. Upon completion of the reaction (4 h), DMF

(3.6 mL, 46.8 mmol) was added and the mixture was stirred for a further 16 h while the temperature was allowed to increase slowly to room temperature. Then 0.5 M HCl (125 mL) was added at 0 °C to quench the reaction and the mixture was stirred 15 min. The resulting mixture was extracted with AcOEt (3 × 100 mL), the organic phases were combined and washed with H_2O (3 × 50 mL) until neutral pH and finally with brine (2 × 50 mL). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to yield crude product, which was chromatographed on silica gel (petroleum ether 60–90 °C : ethyl acetate = 3 : 1) to afford the products **5** (1.98 g, 67%) as yellow powder. See ESI file for characterization data.†

Synthesis of compound 6. To a stirred solution of tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-8-carboxaldehyde **5** (1.98 g, 10.4 mmol) in MeOH (50 mL) at 0 °C was added $NaBH_4$ (0.40 g, 10.4 mmol) in small portions over a period of 20 minutes, 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 before rotary evaporation. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether 60–90 °C : ethyl acetate = 1 : 1) to afford the products **6** (1.99 g, 99%) as white powder. See ESI file for characterization data.†

Synthesis of compounds 8–11. To a solution of tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-8-methanol **6** (192 mg, 1 mmol) in dichloromethane (30 mL) was added methanesulfonyl chloride (1.5 mmol) and triethylamine (2 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 2 h. After quenching the reaction with water (30 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 and imidazole or substituted imidazole (3 mmol) was stirred in toluene (15 mL) at reflux for 8–12 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–ethyl acetate = 3 : 1 → 1 : 1) to afford **8–11** in 68–79% yield (two steps) as yellow or white powder.

Compound 8. Yield 73%. Yellow powder, mp 116–118 °C. IR ν_{max} (cm^{-1}): 3434, 3108, 2972, 2925, 2852, 1616, 1499, 1454, 1323, 1235, 1061, 936, 819, 742, 646. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.60 (1H, s), 7.03 (1H, s), 6.70 (1H, s), 6.91 (1H, s), 4.98 (2H, s), 4.59 (4H, t, J = 8.7 Hz), 3.10 (4H, t, J = 8.7 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 158.40, 137.54, 128.79, 120.59, 119.42, 118.30, 102.18, 72.52, 39.84, 29.53. HRMS (ESI-TOF) m/z calcd for $C_{14}H_{15}N_2O_2$ $[M + 1]^+$ 243.1128, found 243.1127.

Compound 9. Yield 68%. White powder, mp 133–134 °C. IR ν_{max} (cm^{-1}): 3421, 2961, 2911, 2852, 1617, 1464, 1432, 1369, 1328, 1265, 1131, 1059, 974, 931, 757, 637. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.00 (1H, d, J = 1.2 Hz), 6.91 (1H, s), 6.81 (1H, d, J = 1.2 Hz), 4.88 (2H, s), 4.58 (4H, t, J = 8.7 Hz), 3.10 (4H, t, J = 8.7 Hz), 2.48 (3H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 158.39, 144.73, 126.51, 120.46, 119.92, 118.31, 102.30, 72.42, 39.13, 29.53, 12.95. HRMS (ESI-TOF) m/z calcd for $C_{15}H_{17}N_2O_2$ $[M + 1]^+$ 257.1284, found 257.1280.

Compound 10. Yield 79%. Yellow powder, mp 179–181 °C. IR ν_{max} (cm^{-1}): 3432, 3052, 2962, 2908, 1616, 1474, 1368, 1245, 1193, 1057, 1009, 936, 761. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.10 (1H, s), 7.74 (1H, dd, J = 7.2, 1.8 Hz), 7.70 (1H, dd, J = 7.2, 1.8 Hz), 7.28–7.19 (2H, m), 6.87 (1H, s), 5.21 (2H, s), 4.60 (4H, t, J = 8.7 Hz), 3.07 (4H, t, J = 8.7 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 158.49, 144.17, 143.61, 133.91, 122.50, 121.67, 120.61, 119.87, 118.37, 110.37, 101.58, 72.59, 38.10, 29.48. HRMS (ESI-TOF) m/z calcd for $C_{18}H_{17}N_2O_2$ $[M + 1]^+$ 293.1284, found 293.1279.

Compound 11. Yield 68%. Yellow powder, mp 184–185 °C. IR ν_{max} (cm^{-1}): 3430, 3023, 2960, 1619, 1457, 1359, 1223, 1125, 1054, 937, 854, 763. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.98 (1H, s), 7.49 (1H, s), 7.45 (1H, s), 6.87 (1H, s), 5.15 (2H, s), 4.60 (4H, t, J = 8.7 Hz), 3.07 (4H, t, J = 8.7 Hz), 2.38 (3H, s), 2.34 (3H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 158.49, 143.43, 142.24, 132.42, 131.41, 130.41, 120.52, 119.86, 118.33, 110.62, 101.77, 72.52, 38.02, 29.53, 20.72, 20.21. HRMS (ESI-TOF) m/z calcd for $C_{20}H_{21}N_2O_2$ $[M + 1]^+$ 321.1597, found 321.1596.

Synthesis of compounds 12–47. A mixture of tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran–imidazole hybrids **8–11** (0.2 mmol) and phenacyl bromides or alkyl bromides (0.24 mmol) was stirred in toluene (5 mL) at reflux 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 **12–47** in 64–99% 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.

Docking calculations. Compounds **43** and **34** were docked into PI3Kγ [from the complex between PI3K and 4-amino-2-methyl-*N*-(1*H*-pyrazol-3-yl)quinazoline-8-carboxamide, PDB code 3PRZ] using AutoDock (Version 4.0). A grid of 118, 126, and 126 points in the x, y, and z directions was constructed centered on 8.0, −7.0, and 8.0. We used a grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant for the energetic map calculations. Docking simulations of the compounds were carried out using the Lamarckian genetic algorithm and through a protocol with an initial population of 150 randomly placed individuals, a maximum number of 250 million energy evaluations, a mutation rate of 0.02, a crossover rate of 0.8, and an elitism value of 1. Fifty independent docking runs were carried out for each compound, and the resulting conformations that differed by 1.0 Å in positional root-mean-square deviation (rmsd) were clustered together. Cluster analysis was performed by selecting the most populated cluster,

which in all cases coincided with the one endowed with the best energy.

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