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Synthesis and biological evaluation of 3-substituted-benzofuran-2-carboxylic esters as a novel class of ischemic cell death inhibitors

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ABSTRACT

A series of 3-substituted-benzofuran-2-carboxylic esters was synthesized and evaluated for biological activity as ischemic cell death inhibitors in H9c2 cells and rat primary cardiac myocytes under conditions of oxygen and glucose deprivation. The introduction of a sulfur atom at the three-position substituent of the benzofuran ring markedly improved ischemic cell death inhibitory potency. In particular, 3-[2-(4-nitro-phenylsulfanyl)-acetylamino]-benzofuran-2-carboxylic acid ester (**10**) (EC_{50} = 0.532 μM, cell death = 6.18%) and 4-chloro-3-[3-(pyridin-2-ylsulfanyl)-propionylamino]-benzofuran-2-carboxylic ester (**18**) (EC_{50} = 0.557 μM, cell death = 7.02%) were shown to be the most potent in this series of benzofuran analogs.

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Ischemia, a symptom of reduced blood supply to organs or tissues, is caused by contraction or occlusion of the blood vessels.[1] Once ischemia occurs, subsequent reperfusion causes various sequelae, due to damaged nerve cells.[2] Ischemia is frequently associated with coronary artery diseases, cardiovascular diseases, angina pectoris, headache, or other symptoms related to such reduced blood supply, which ultimately leads to necrosis underlying the cells or tissues involved.[3] Although the mechanisms of cell death after cerebral ischemia and myocardial ischemia are not yet fully understood, Fas and FasL are known to be important factors in the pathology of ischemic stroke[4–7] and ischemic heart failure.[8,9] Recent studies have shown that Fas contributes to cell death after ischemic injury.[10] The interaction of Fas with its ligand allows the formation of a death-inducing signaling complex (DISC), which includes Fas, FADD,[11] FAF1,[12] and caspase-8.[13] Following Fas-DISC formation, caspase-8 is activated, leading to programmed cell death. Inhibitors of the Fas-mediated cell death pathway have been shown to be effective in animal models of ischemic stroke,[10] traumatic brain injury,[14] and myocardial hypertrophy.[15] Herein, we summarized 3-substituted-benzofuran-2-carboxylic esters found by high-throughput screening assay as inhibitors of the Fas-mediated cell death pathway. For lead optimization of inhibitors of ischemic cell death, we synthesized 3-substituted-benzofuran-2-carboxylic ester analogs. We describe the synthetic pathway and biological activity of a series of 3-substituted-benzofuran-2-carboxylic ester analogs as inhibitors of ischemic cell death. The simple synthetic route of 3-substituted-benzofuran-2-carboxylic esters analogs is shown in Scheme 1.[16]

The 3-amino-benzofuran analogs **1**, as key intermediates, were prepared by cyclization of corresponding 2-fluorobenzonitriles with methyl glycolate. The 3-amino benzofuran analogs **2** were obtained by reaction of **1** with each bromoacyl halide in the presence of TEA in THF. Alternatively, these compound were also obtained by a coupling reaction of the respective bromoalkyl acid with *N*,*N*′-diisopropylcarbodiimide (DIC) in CH_2Cl_2. The 3-(2-substituted-acethylamino) benzofuran analogs **3**–**17** with variously substituted aryl groups were synthesized through the two synthetic pathways.

The benzofuran derivatives with S and NH groups at the Y-position of **3**–**11**, **13**–**14**, and **16** were synthesized by nucleophilic substitution reaction of compound **2** with corresponding thiophenol, phenol or aniline compounds. The compound **17** was prepared by nucleophilic substitution reaction using 2-mercaptopyridine. On the other hand, the benzofuran derivatives containing O and CH_2 groups at the Y-position of **12** and **15** were prepared from 3-amino-benzofuran analogs **1** with substituted carboxylic acids by coupling reaction.

The 3-[3-(pyridin-2-ylsulfanyl)-propionylamino]-benzofuran analogs **18**–**19** were prepared from the corresponding bromide compounds **2** through a sequence of reactions including HBr elimination and 1,4-Micheal addition of 2-mercaptopyridine to α,β-unsaturated ketones.

The ischemic cell death inhibitory activities of the synthesized compounds were determined by staining of nuclei with DAPI or

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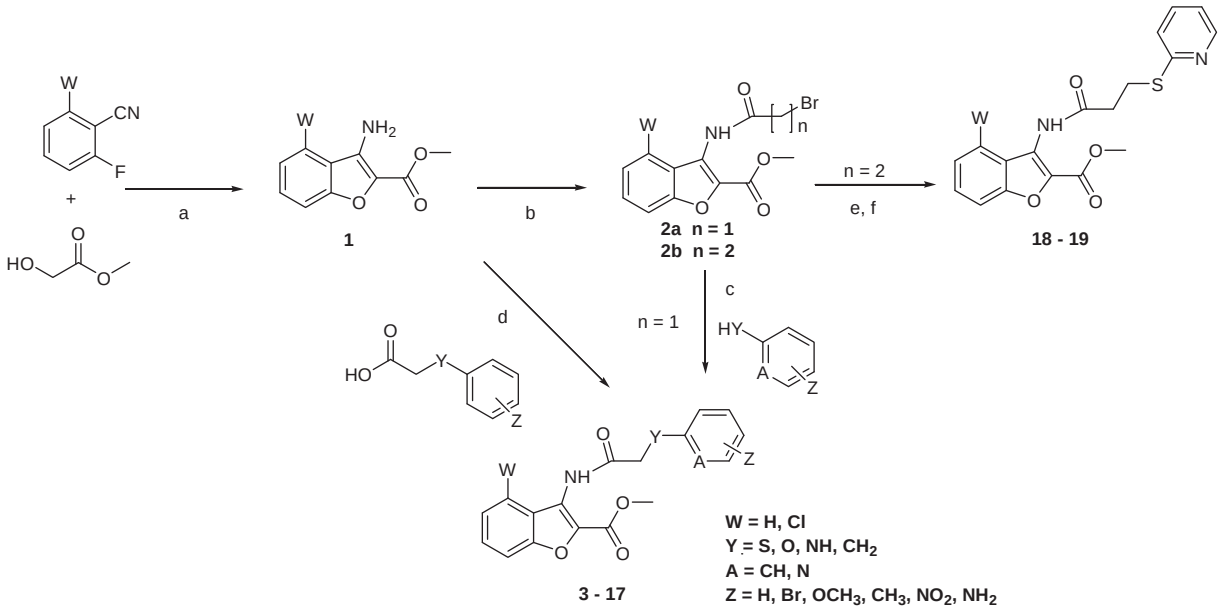


Scheme 1. (a) methyl glycolate, K_2CO_3, DMF, 100 °C, 40–50%; (b) (i) bromoacyl halide, NEt_3, THF, rt or (ii) bromoalkyl acid, *N,N′*-diisopropylcarbodiimide (DIC), CH_2Cl_2, rt, 50–90%; (c) NEt_3, THF, 0 °C–rt, 20–91%; (d) *N,N′*-diisopropylcarbodiimide (DIC), CH_2Cl_2, rt, 25–90%; (e) NEt_3, THF, rt, 90–95%; (f) 2-mercaptopyridine, NEt_3, THF, 0 °C–rt, 80–90%.

lactate dehydrogenase (LDH) release after oxygen and glucose deprivation (OGD)-induced cell death in H9c2 cells[17–20] (Fig. 1).

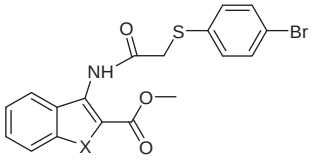
Initially, we compared benzofuran and benzothiophene analogs for their inhibitory activity against ischemic cell death. The benzofuran compound **3** with oxygen at X showed stronger in vitro ischemic cell death inhibitory activity than benzothiophene compound **4** (Table 1).

From initial biological results, the 3-(3-substituted-propionylamino)-benzofuran-2-carboxylic esters and 3-(2-substituted-acethylamino)-benzofuran-2-carboxylic esters were synthesized. The biological inhibitory results are shown in Table 2.

The 4-substituted derivative **3** with a bromo group at Ar_1 showed markedly increased ischemic cell death inhibitory activity, compared with unsubstituted and 2- or 3-substituted derivatives **5**–**7**. Remarkably, **10** (cell death = 6.18% at 10 μM) with an electron withdrawing NO_2 group at the *para* position was much more potent than most of the other analogs in this series and three-fold more potent than compound **9** with 4-methyl group.

Generally, the introduction of an O, NH, or CH_2 group at Y reduced the inhibitory functions, compared with benzofuran derivatives containing S at Y. The benzofuran derivatives **16**–**17** with Cl at W were more potent than the unsubstituted derivatives. Replacement of the 4-bromophenyl group at Ar_1 in **16** with a

Table 1
In vitro efficacy against ischemic cell death of H9c2 cells by the effects of substituent on benzofuran and benzothiophene.



Compounds	X	Cell death (%, 10 μM)	
		DAPI	LDH
3	O	10.59 ± 0.14[#]	32.39 ± 5.44[#]
4	S	13.92 ± 1.08[#]	41.31 ± 0.82[#]

Data are means ± SEM of three independent experiments.
[#] $P < 0.001$ versus control.

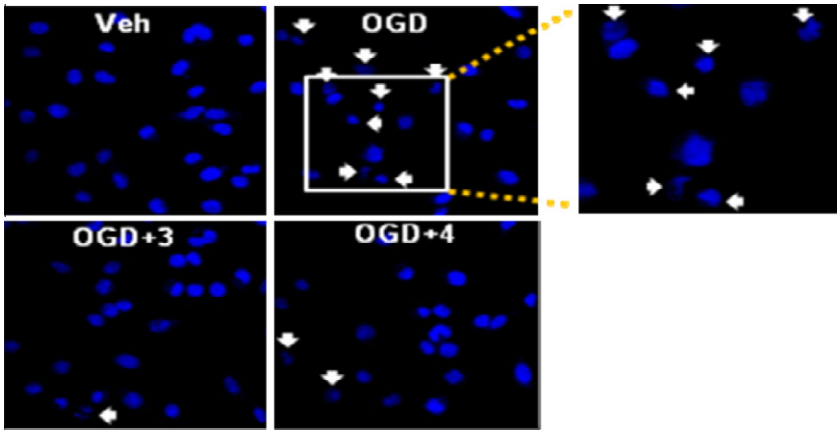
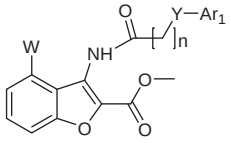


Figure 1. Nuclear morphological changes upon ischemic insult in H9c2 cells. DAPI (400×) images were acquired using an inverted Zeiss LSM510 META confocal microscope. Arrows indicate dead cells.

Table 2
In vitro efficacy against ischemic cell death of H9c2 cells by the effects of substituent on 3-(3-substituted-propionylamino)-benzofuran-2-carboxylic esters and 3-(2-substituted-acethylamino)-benzofuran-2-carboxylic esters



Compounds	W	*n*	Y	Ar_1	Cell death (%, 10 μM)	
					DAPI	LDH
Control					3.93 ± 0.75	15.26 ± 1.54
Vehicle					36.04 ± 0.46$^{\#}$	73.40 ± 6.32$^{\#}$
KR-31378					15.54 ± 0.77$^{\#}$	43.33 ± 2.06$^{\#}$
3	H	1	S	4-Bromophenyl	10.59 ± 0.14$^{\#}$	32.39 ± 5.44*
5	H	1	S	phenyl	15.97 ± 0.83$^{\#}$	40.93 ± 3.58$^{\#}$
6	H	1	S	3-Bromophenyl	16.85 ± 2.92$^{\#}$	39.51 ± 2.64$^{\#}$
7	H	1	S	2-Bromophenyl	13.63 ± 0.54$^{\#}$	41.01 ± 0.74$^{\#}$
8	H	1	S	4-Methoxyphenyl	9.73 ± 0.81$^{\#}$	40.34 ± 2.45$^{\#}$
9	H	1	S	4-Methylphenyl	21.57 ± 1.85$^{\#}$	56.39 ± 5.54$^{\#}$
10	H	1	S	4-Nitrophenyl	6.18 ± 0.88*	30.21 ± 2.16$^{\#}$
11	H	1	S	4-Aminophenyl	16.54 ± 0.77$^{\#}$	40.15 ± 0.61$^{\#}$
12	H	1	O	4-Bromophenyl	13.17 ± 0.17$^{\#}$	36.17 ± 0.76$^{\#}$
13	H	1	NH	4-Bromophenyl	22.37 ± 1.60$^{\#}$	60.32 ± 3.33$^{\#}$
14	H	1	NH	Phenyl	21.04 ± 1.67$^{\#}$	62.76 ± 1.03$^{\#}$
15	H	1	CH_2	Phenyl	15.94 ± 4.76*	38.72 ± 3.66$^{\#}$
16	Cl	1	S	4-Bromophenyl	8.94 ± 2.10*	35.48 ± 5.05*
17	Cl	1	S	2-Pyridyl	7.33 ± 0.35$^{\#}$	25.59 ± 3.80†
18	Cl	2	S	2-Pyridyl	7.02 ± 0.75*	29.46 ± 1.37$^{\#}$
19	H	2	S	2-Pyridyl	11.55 ± 1.35$^{\#}$	36.07 ± 1.01†

Data are means ± SEM of three independent experiments. $^{\dagger}P$ <0.05, $^{*}P$ <0.01, $^{\#}P$ <0.001 versus control.

2-pyridyl group **17** resulted in further enhanced the potency to inhibit ischemic cell death. Thus, benzofuran analogs with a Cl group at W and 2-pyridyl group at Ar_1 showed very potent inhibitory effects on ischemic cell death. A longer carbon chain at the 3-position in this series of benzofuran analogs influenced the inhibitory activity. The 4-chloro-3-[3-(pyridin-2-ylsulfanyl)-propionylamino]-benzofuran-2-carboxylic ester **18** showed better activity than 17. However, **19** with H at W was much less potent than Cl substituted analogs. The median effective concentration (EC_{50}) of compounds for protection of cells from ischemic death was determined by counting after staining of nuclei with DAPI after OGD in rat primary cardiac myocytes. KR-31378 was used as a standard to measure the EC_{50} of potent active compounds. KR-31378 has been reported to show antiapoptotic activities against ischemic injury[21] and has been assessed in vivo.[22]

As shown in Table 3, the EC_{50} values for the benzofuran derivatives indicated that they were generally more potent than KR-31378.

3-[2-(4-Nitro-phenylsulfanyl)-acetylamino]-benzofuran-2-carboxylic acid ester **10** (EC_{50} = 0.532 μM, cell death = 6.18% at 10 μM) and 4-chloro-3-[3-(pyridin-2-ylsulfanyl)-propionylamino]-benzofuran-2-carboxylic ester **18** (EC_{50} = 0.557 μM, cell death = 7.02% at 10 μM) were shown to be the most potent in this series of benzofuran analogs. However, compound **12** with oxygen at Y was much less potent than most of the other analogs in this series. These results indicated that sulfur at Y has an important effect on ischemic cell death inhibitory activity.

Table 3
In vitro efficacy against ischemic cell death in rat primary cardiac myocytes by the effects of substituent on 3-(3-substituted-propionylamino)-benzofuran-2-carboxylic esters and 3-(2-substituted-acethylamino)-benzofuran-2-carboxylic esters

Compounds	EC_{50} (μM)[a]
KR-31378	1.109
3	0.778
8	0.752
10	0.532
12	1.177
16	0.630
17	0.653
18	0.557
19	0.929

[a] Values are means of eight experiments.

In conclusion, we synthesized and evaluated a series of 3-substituted-benzofuran-2-carboxylic esters as a novel class of ischemic cell death inhibitors targeting Fas-mediated cell death pathway through cell-based screening. In particular, **10** and **18** were exhibited to be the most potent in vitro efficacies, superior to KR-31378, a potent K_{ATP}-channel opener. Additionally, the introduction of a sulfur atom at the 3-position of the benzofuran ring markedly improved ischemic cell death inhibitory potency. Further studies including analysis of in vivo efficacy as well as pharmacokinetic and metabolic studies are underway to identify as a novel ischemic cell death inhibitor.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.102.

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18. *Cell culture*
Rat heart derived H9c2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% antibiotics (penicillin/streptomycin) at 37 °C in humidified 95% air and 5% CO_2 atmosphere.
Induction of ischemia
To induce OGD, cells were exposed to glucose-free DMEM and then incubated in an anaerobic chamber (Forma Scientific) at 37 °C under an atmosphere of 5% CO_2, 10% H_2, and 85% N_2.
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20. **Measurement of cell death**
H9c2 cells were treated with 10 μM compounds for 30 min before and during OGD. For 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI) analysis, cells were fixed with 0.5% Triton X-100 in PBS for 15 min, stained with DAPI (2 μg/ml), and counted with the use of a fluorescence microscope (Zeiss). Three random fields of at least 300 cells in monolayer culture were scored to determine the percentage of cells undergoing cell death. DAPI images were examined on a Zeiss LSM510 META confocal microscope using 40× objective. To determine EC_{50} values, we prepared primary cultures of cardiac myocytes from the heart of 1-day-old Sprague–Dawley rats as described, and exposed the cells after culture for seven days to OGD for 6 h in the presence of the drugs at concentrations ranging from 100 nM–50 μM. For measurement of lactate dehydrogenase (LDH) activity in the extracellular medium, we used a CytoTox96 nonradioactive cytotoxicity assay (Promega). LDH release was expressed as a percentage of that induced by lysis of cells with 0.1% Triton X-100.
Statistical analysis
Data were expressed as mean ± SEM. Comparisons between groups of data were analyzed by student's *t*-test. Data analysis and graph generation were performed with Sigmaplot 10.0 (Chicago, IL, USA). A value of $P < 0.05$ was accepted as statistically significant.
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