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Small-molecular inhibitors of Ca^{2+}-induced mitochondrial permeability transition (MPT) derived from muscle relaxant dantrolene

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ABSTRACT

A structure consisting of substituted hydantoin linked to a 5-(halophenyl)furan-2-yl group via an amide bond was identified as a promising scaffold for development of low-molecular-weight therapeutic agents to treat vascular dysfunction, including ischemia/reperfusion injury. Among the compounds synthesized, 5-(3,5-dichlorophenyl)-*N*-{2,4-dioxo-3-[(pyridin-3-yl)methyl]imidazolidin-1-yl}-2-furamide (**17**) possessed the most potent inhibitory activity against Ca^{2+}-induced mitochondrial swelling. The structural development, synthesis and structure–activity relationship of these compounds are described.

1. Introduction

The mitochondrial permeability transition (MPT) is a rapid permeabilization of the mitochondrial inner membrane, which leads to mitochondrial swelling, dysfunction and cell death.[1–3] It is thought to play an important role in ischemia/reperfusion injury in a variety of pathological conditions, including trauma,[4,5] heart attack[6,7] and stroke.[6,8–10] MPT is a consequence of opening of the MPT pore, and one of the components of this pore has been identified as the peptidylpropyl *cis–trans* isomerase (PPIase) cyclophilin D (CypD).[11] It has been reported that cyclosporin A (CsA, Fig. 1) is a CypD inhibitor,[12] and therefore CsA may inhibit MPT and show cytoprotective effects in various diseases. Indeed, CsA was found to ameliorate ischemia/reperfusion injury in cellular and animal models.[13–16] However, although CsA is already in clinical use as a potent immunosuppressant, it is associated with serious adverse effects, including neurotoxicity and nephrotoxicity.[17–19] Further, CsA is a lipophilic, cyclic undecapeptide with a molecular weight of over 1200,[20] so modification to isolate the CypD-inhibitory activity would be difficult. Therefore, there is a need for low-molecular-weight CypD inhibitors as candidate drugs to treat ischemia/reperfusion injury.

To address this issue, we focused on dantrolene (**1**,[21] Fig. 2), an aminohydantoin derivative condensed with a nitro-substituted arylfuran component that has been used clinically to treat malignant hyperthermia, neuroleptic malignant syndrome, heat stroke, and muscle spasticity.[22] Although dantrolene (**1**) primarily acts on skeletal muscles, it also shows protective effects against ischemic damage to neurons and myocardial tissues.[23–25] The major target of dantrolene was reported to be ryanodine receptor RyR1,[26] but direct effects on mitochondria were also reported,[27] suggesting the existence of a mitochondrial target of dantrolene. On the other hand, compound **3** (Fig. 2) was reported to show an inhibitory effect on HIV-1 replication through binding to cyclophilin A (CypA).[28] We were struck by the similarity between the hydantoin-type structure of dantrolene and its major metabolite **2**,[29] and the oxalyl diamide structure of **3**, both of which seemed analogous to parabanic acid (also known as oxalyl urea) (Fig. 3). Based on (a) the CypA-inhibitory activity of **3**,[28] (b) the existence of a mitochondrial target of dantrolene,[27] (c) the reported 75% identity

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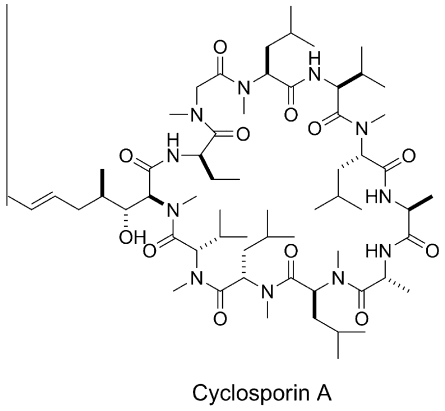


Figure 1. Structure of cyclosporin A (CsA).

in the PPIase domain between CypA and CypD,[30] and (d) the structural similarity of dantrolene and **3** noted above, we speculated that dantrolene might inhibit CypD and consequently inhibit MPT. Indeed, we found that dantrolene inhibited Ca^{2+}-induced mitochondrial swelling, which we employed as a surrogate of MPT.

In this paper, we describe the synthesis of a series of small-molecular inhibitors of Ca^{2+}-induced MPT with dantrolene-derived structures consisting of substituted hydantoin linked to a 5-(halophenyl)furan-2-yl group via an amide bond, based on the rationale described above. We also report the results of assay of their MPT-inhibitory activity and analysis of their structure–activity relationship.

2. Results and discussion

The X-ray crystal structure of CypD in the complex with CsA,[31] a cyclic peptide consisting of hydrophobic residues, shows two characteristic gorge areas around the CsA-binding site. One is surrounded with hydrophobic amino acid residues such as Phe and Leu. The other is surrounded with polar amino acid residues such as Ser, Thr, and Gln. On the basis of the design rationale described in the introduction, together with the above structural and chemical information, we designed and synthesized a series of molecules possessing chloro-substituted phenylfurans as a hydrophobic core bound to hydantoin at the 1-position via an imino or amido linkage, and having a 3-aminobenzyl group as a hydrogen-bond acceptor and/or donor at the 3-position of hydantoin (**8**–**11**). Compounds with a methyl group at the 3-position of hydantoin (**4**–**7**) were also synthesized for comparison.

The synthetic routes are shown in Schemes 1 and 2. Briefly, 3,5-dichloroaniline was coupled with 2-furaldehyde by Meerwein arylation reaction to give 5-(3,5-dichlorophenyl)-2-furaldehyde (**19b**). Compound **19b** and commercially available 5-(3-chlorophenyl)-2-furaldehyde (**19a**) were condensed with 1-aminohydantoin hydrochloride. The resulting imino compounds **20a** and **20b** were N-alkylated with methyl iodide or *N*-Boc-3-aminobenzyl bromide[32] under basic conditions to give **4**, **5**, **21a**, and **21b**. Finally, deprotection of the amino group of **21a** and **21b** with HCl gave **8** and **9**. As for the amido-type compounds, aldehydes **19a** and **19b** were oxidized under Pinnick oxidation conditions to give carboxylic acids **22a** and **22b**, which were condensed with 1-aminohydantoin hydrochloride by using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[33] as a condensation

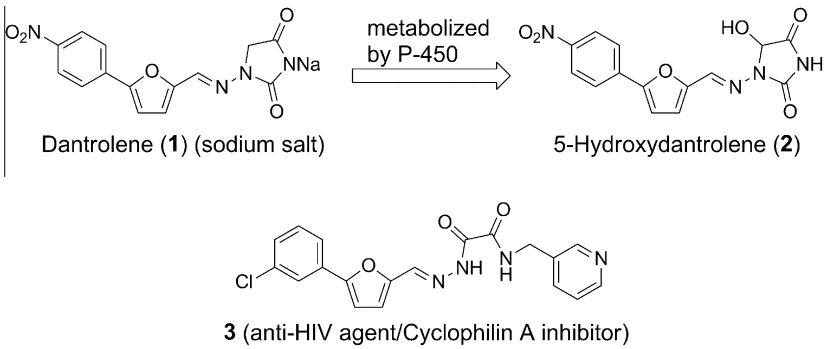


Figure 2. Structures of dantrolene (**1**), 5-hydroxydantrolene (**2**), and cyclophilin inhibitor (**3**).

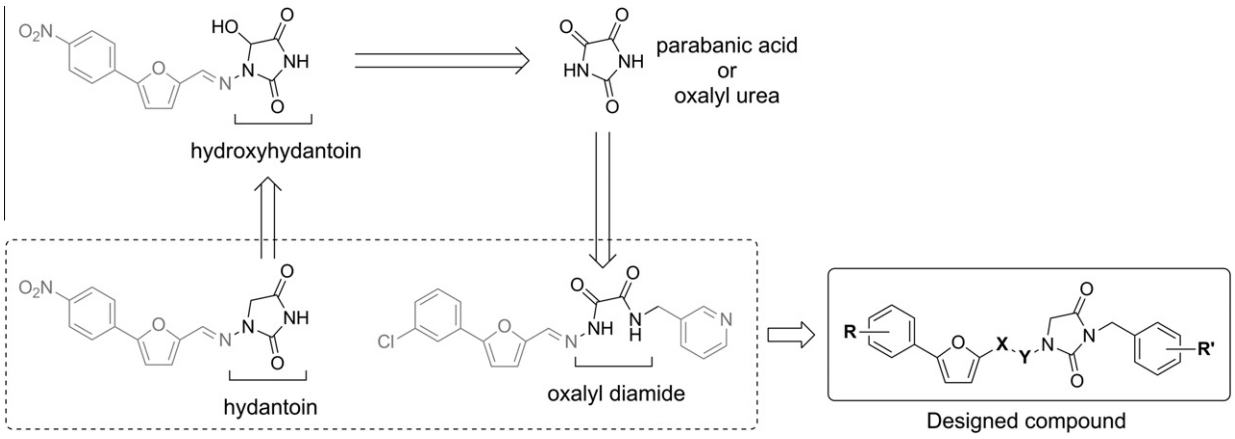
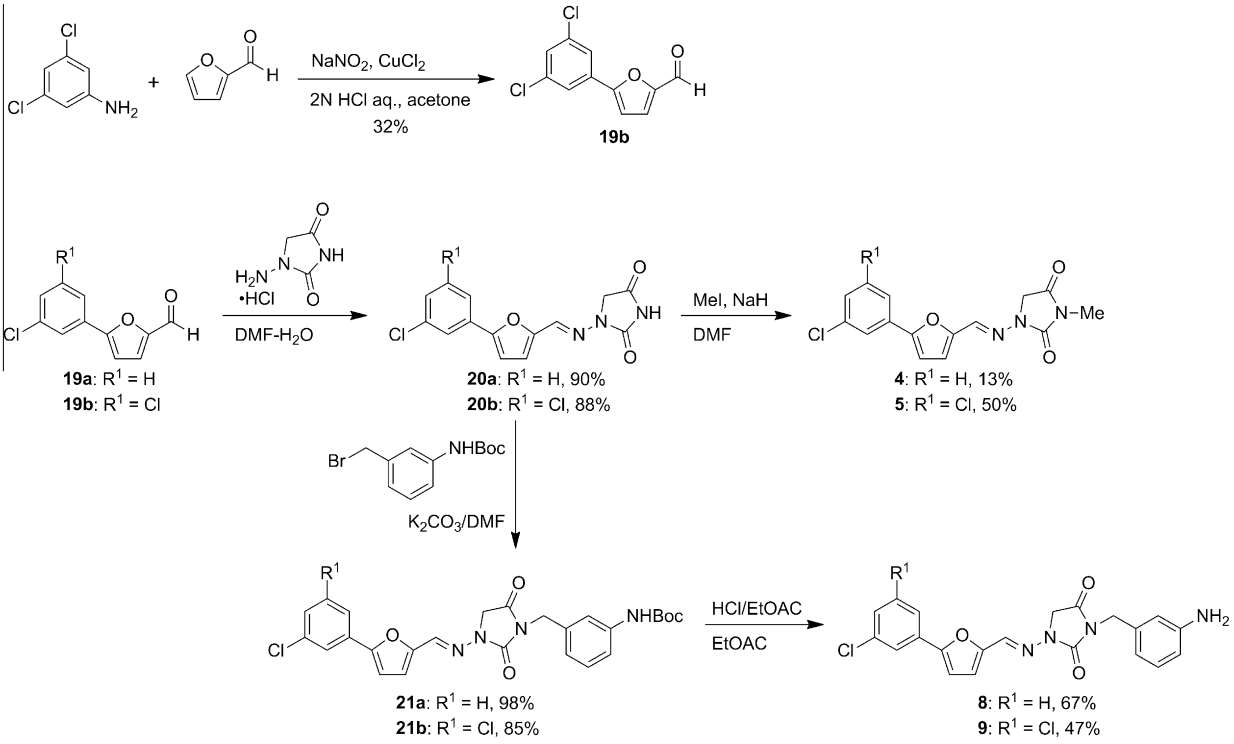
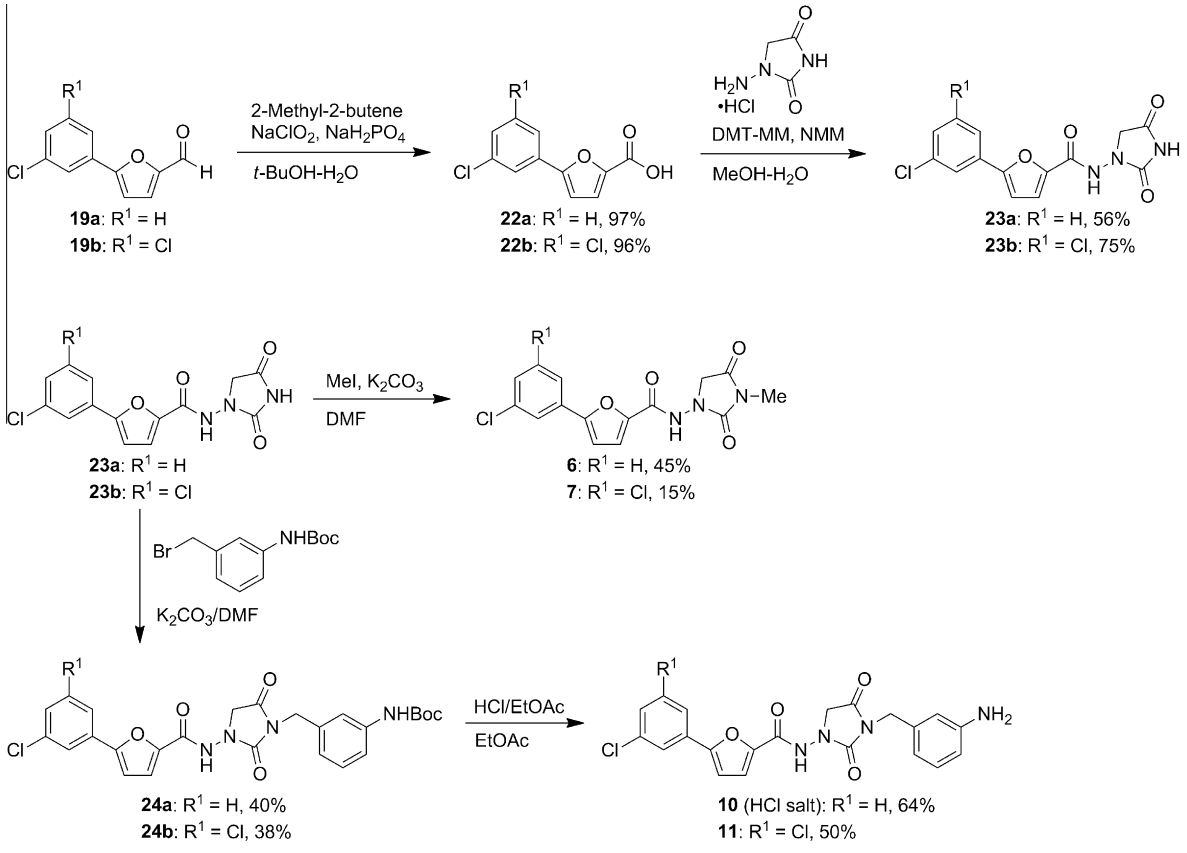


Figure 3. Illustration of the design strategy employed in this study (see text).



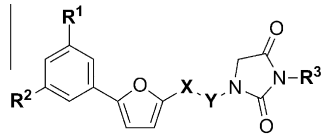
Scheme 1. Synthesis of hydantoin analogs with imino linkage (**4**, **5**, **8**, and **9**).



Scheme 2. Synthesis of hydantoin analogs with amido linkage (**6**, **7**, **10**, and **11**).

agent and *N*-methylmorpholine (NMM) as a base. The resulting amido compounds **23a** and **23b** were N-alkylated under the same conditions as described for the imino compounds to afford **6**, **7**, **10**, and **11**.

Table 1
Effects of linkage structure (**X**–**Y**) and substituents (**R**^{1}, **R**^{2}, and **R**^{3}) of hydantoin analogs on the inhibition of Ca^{2+}-induced mitochondrial swelling



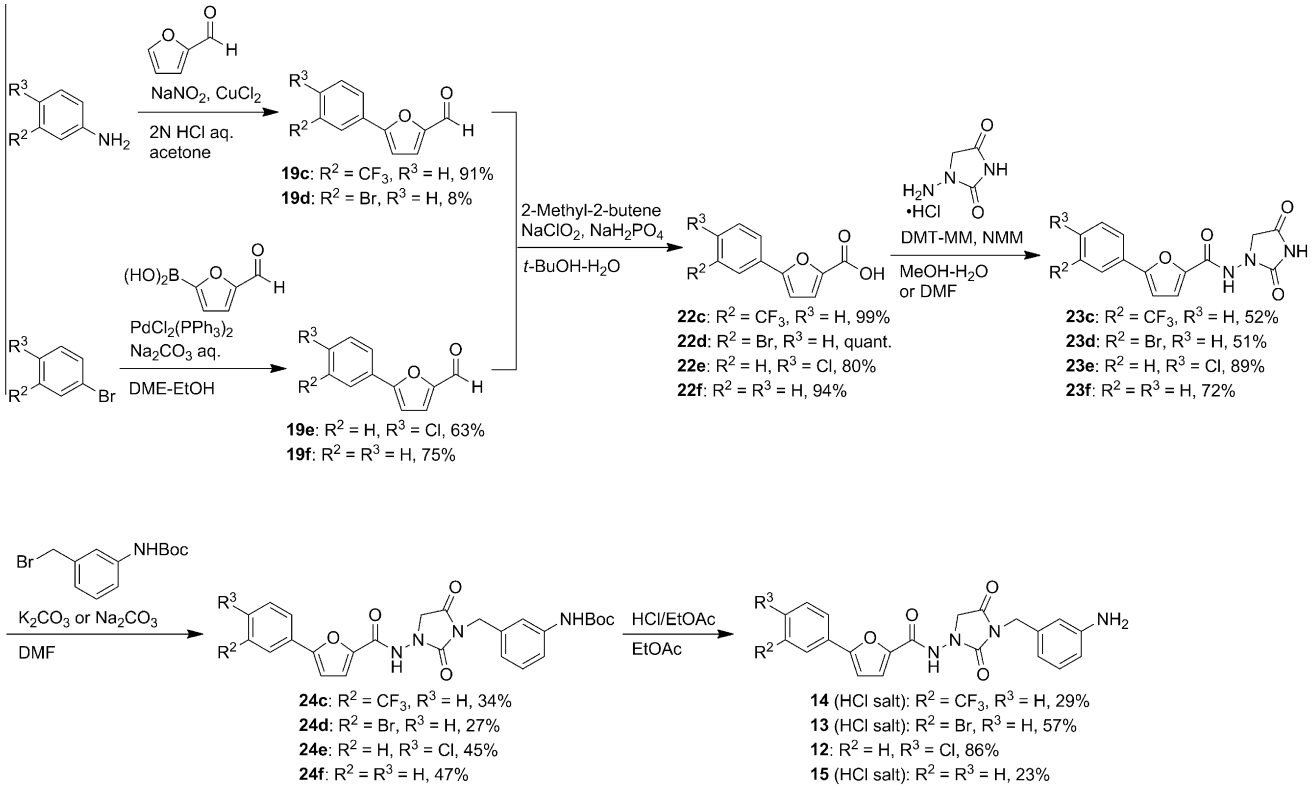
Compd	R^1	R^2	R^3	X–Y	Inhibition ratio[a] (%)
Dantrolene (**1**)	4-NO_2		Na	HC=N	13
4	H	Cl	CH_3	HC=N	6.0
5	Cl	Cl	CH_3	HC=N	23
6	H	Cl	CH_3	CO–NH	2.0
7	Cl	Cl	CH_3	CO–NH	8.5
8	H	Cl	*m*-$NH_2C_6H_4CH_2$	HC=N	14
9	Cl	Cl	*m*-$NH_2C_6H_4CH_2$	HC=N	14
10	H	Cl	*m*-$NH_2C_6H_4CH_2$	CO–NH	16
11	Cl	Cl	*m*-$NH_2C_6H_4CH_2$	CO–NH	35

[a] The inhibitory activity of test compounds was evaluated as inhibition (%) by measuring the change of absorbance at 540 nm in MPT assay in the presence of the test compound at 25 μM, 40 min after incubation. In the absence of test compound, −$CaCl_2$ is equivalent to 100% inhibition and +$CaCl_2$ is equivalent to 0% inhibition (see Fig. 4).

MPT in isolated mitochondria is a well-characterized Ca^{2+}-induced phenomenon that results in large-amplitude swelling, which can be monitored based on the decrease of absorbance at 540 nm. Therefore, we adopted MPT assay to evaluate the biological activity of our compounds. MPT-inhibitory activity was evaluated in terms of Ca^{2+}-induced mitochondrial swelling in the presence and absence of the synthesized compounds. In isolated mitochondria from HL-60 cells, $CaCl_2$ induced a rapid decrease of OD_{540}, which was inhibited by 5 μM CsA (a known MPT inhibitor), indicating that this assay is suitable for detection of MPT-inhibitory activity. Dantrolene (**1**) also inhibited Ca^{2+}-induced mitochondrial swelling at 25 μM in this assay system. Based on these results, inhibition by the synthesized compounds (**4**–**11**) and dantrolene (**1**) was determined at 40 min after $CaCl_2$ treatment (Table 1). Among compounds **4**–**11**, the 3,5-dichlorophenyl compounds all exhibited similar or higher inhibitory activity than the 3-chlorophenyl compounds, suggesting that an increase of the hydrophobicity around the phenyl group bound to the furan ring is favorable for the activity. As for linkage structure, among compounds with a methyl group at the 3-position of hydantoin, the imino-linked compounds tended to be more potent than the amido-linked compounds. On the other hand, compounds with a 3-aminobenzyl group at the 3-position of hydantoin exhibited the opposite tendency, that is, the amido-linked compounds were more potent than the imino-linked compounds. These results suggest that the site to which these compounds bind can accommodate and interact with a polar group beyond the 3-position of hydantoin.

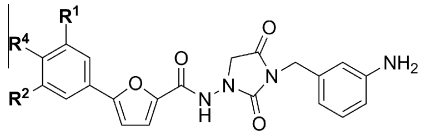
Focusing on amido-linked compound **11**, which showed the greatest Ca^{2+}-induced mitochondrial swelling-inhibitory activity among these compounds, the effect of substituents on the phenyl ring was examined. Several compounds halogenated on the phenyl ring were prepared in a manner similar to that shown in Scheme 2, with some modifications[34] as shown in Scheme 3, and their mitochondrial swelling-inhibitory activities were measured at a fixed concentration of 50 μM (Table 2). Compounds with a bromo group and trifluoromethyl group, **13** and **14**, showed decreased activity in comparison with the chlorinated compounds **11** and **12**. In contrast, dehalogenated compound **15** showed almost the same activity as chlorinated compound **12**. These results suggest that the effect of steric hindrance around the phenyl ring is greater than that of hydrophobicity.

Next, the effect of N-substituents of hydantoin was examined. Compounds bearing an anilyl or a pyridyl group were synthesized in a manner similar to that shown in Scheme 2, as illustrated in



Scheme 3. Synthesis of hydantoin analogs **12**–**15**.

Table 2
Effects of halogenated phenyl group combining to furanyl group of hydantoin analogs on the inhibition of Ca^{2+}-induced mitochondrial swelling



Compd	R^1	R^2	R^4	Inhibition ratio[a] (%)
11	Cl	Cl	H	48 (35) [b]
12	H	H	Cl	20
13	H	Br	H	11
14	H	CF_3	H	6.5[b]
15	H	H	H	19

[a] The inhibitory activity of test compounds was evaluated as inhibition (%) by measuring the change of absorbance at 540 nm in MPT assay in the presence of the test compound at 50 μM, 40 min after incubation. In the absence of test compound, $-CaCl_2$ is equivalent to 100% inhibition and $+CaCl_2$ is equivalent to 0% inhibition (see Fig. 4).
[b] At 25 μM..

Scheme 4. The N-aminobenzylated compounds **11** and **16** showed similar mitochondrial swelling-inhibitory activity with inhibition rates of 46–48% (Table 3). On the other hand, *N*-pyridylmethylated compounds **17** and **18** showed quite different behaviors, that is, the 3-pyridylmethyl compound **17** was a potent inhibitor, while the 4-pyridylmethyl compound **18** was markedly less potent. The reason for these differences among the four compounds is thought to be related to the characteristics of the amino group. Generally speaking, primary amines possess both hydrogen bond-donating and -accepting abilities, while tertiary amines, including heterocyclic aromatic amine such as pyridine, act only as hydrogen bond acceptors. Thus, in the case of aminobenzylated **11** and **16**, hydrogen bond-donating ability of the amino group may predominate, whereas **17** and **18** possess only hydrogen bond-accepting ability. From the viewpoint of interaction of the compounds with amino acid residues of their binding site, interaction via hydrogen bond donation is rather non-specific, whereas hydrogen bond-accepting interaction is much more specific. This may explain why the 3-pyridylmethyl compound **17** showed the most potent inhibitory activity (71% after 40 min incubation).

Finally, we compared the effect of **17** with that of CsA. As shown in Figure 4 and **17** at 50 μM showed similar inhibitory activity to CsA at 5 μM. Thus, the MPT-inhibitory activity of dantrolene has been greatly increased by the structural conversion to **17**.

The mechanism of action of these compounds remains to be examined. Although the design of our compounds, including **17**, was partly based on the idea that they might inhibit MPT via inhibition of CypD, we cannot rule out the possibility that they have a different target(s). This possibility may be supported by the unexpected finding that one of the lead compounds, the CypA inhibitor **3**, did not show MPT-inhibitory activity (data not shown). For example, it has recently been reported that a channel (also known as mitochondrial ryanodine receptor, mRyR) having a function similar to RyR1 is expressed on the mitochondrial inner membrane.[35] On the other hand, our previous structure–activity relationship study of the inhibitory effect of dantrolene analogs on RyR1 opening demonstrated that replacement of the imino bond of dantrolene (**1**) with an amide bond resulted in a marked decrease of the activity (data not shown). Thus, it seems unlikely that the MPT-inhibitory activities of our compounds involve inhibition of mRyR function. We are currently examining the interaction between our compounds and CypD. But, whether our compounds act on CypD or not, they represent a novel type of MPT inhibitor, and appear to be candidate therapeutic agents for ischemia/reperfusion injury.

3. Conclusion

We have designed and synthesized a series of small-molecular inhibitors of Ca^{2+}-induced MPT based on a structural analysis of dantrolene (**1**), CypA inhibitor **3** and CypD, and examined their structure–function relationship. Among them, compound **17** was the most potent inhibitor of Ca^{2+}-induced MPT, its efficacy at 50 μM concentration being similar to that of CsA at 5 μM. Further structure–activity relationship studies based on **17** are under way, and work is also in progress to identify the molecular target(s) of these compounds.

4. Experimental

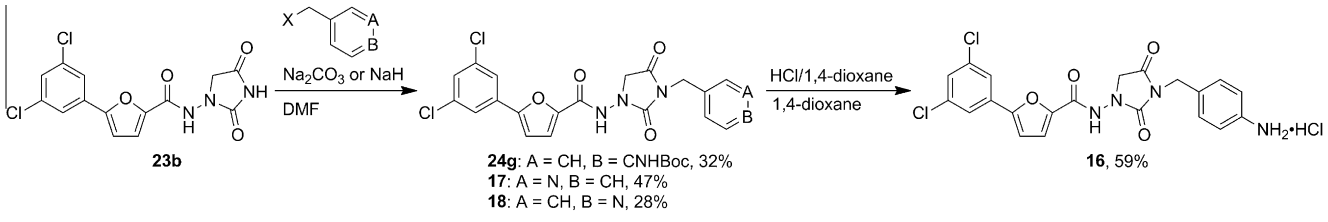
4.1. Chemistry

4.1.1. General

^{1}H NMR spectra were recorded on a JEOL JNMGX500 (500 MHz) spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) relative to chloroform or tetramethylsilane as an internal reference. Coupling constants are given in Hz. The abbreviations s, d, t, dd, dt, br, br s, and m signify singlet, doublet, triplet, double doublet, double triplet, broad, broad singlet, and multiplet, respectively. Fast atom bombardment mass spectra (FAB-MS) and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS-DX303 spectrometer with *m*-nitrobenzyl alcohol matrix. Melting points (Mp) were determined by using a Yanagimoto hot-stage melting point apparatus. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Science, The University of Tokyo, and were within ±0.4% of theoretical values.

4.1.2. Synthesis of 5-aryl-2-furaldehydes by Meerwein arylation reaction

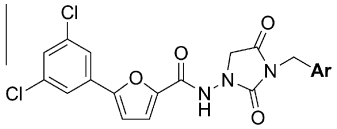
4.1.2.1. 5-(3,5-Dichlorophenyl)-2-furaldehyde (19b): General procedure A. A mixture of 3,5-dichloroaniline (1.94 g, 12.0 mmol) and 2 N HCl (45 mL) was heated at 110 °C to get a clear solution. This was cooled to 0 °C, and to it was added a solution of $NaNO_2$ (829 mg, 12.0 mmol) in cold H_2O (3.0 mL) at 0 °C. The mixture was stirred for 20 min at 0 °C, then a solution of 2-furaldehyde (1.15 g, 12.0 mmol) in cold acetone (3.0 mL) was added to it at 0 °C. Stirring was continued for 15 min at 0 °C, then a solution of $CuCl_2$ (408 mg, 2.40 mmol) in cold H_2O (3.0 mL) was added at 0 °C. After

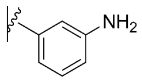
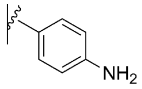


23b
24g: A = CH, B = CNHBoc, 32%
17: A = N, B = CH, 47%
18: A = CH, B = N, 28%
16, 59%

Scheme 4. Synthesis of hydantoin analogs **16**–**18**.

Table 3
Effects of aminobenzyl and azabenzyl group substituted at 1-position of hydantoin analogs on the inhibition of Ca^{2+}-induced mitochondrial swelling



Compd	Ar	Inhibition ratio[a] (%)
11		48
16		46
17		71
18		8.7

[a] The inhibitory activity of test compounds was evaluated as inhibition (%) by measuring the change of absorbance at 540 nm in MPT assay in the presence of the test compound at 50 μM, 40 min after incubation. In the absence of test compound, $-CaCl_2$ is equivalent to 100% inhibition and $+CaCl_2$ is equivalent to 0% inhibition (see Fig. 4).

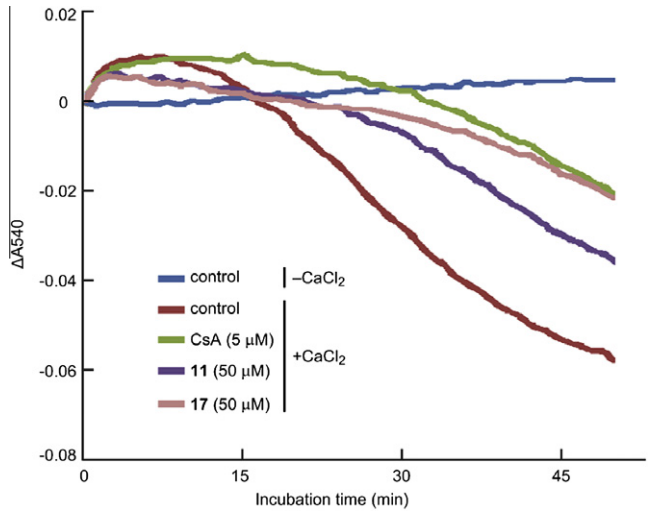


Figure 4. Mitochondrial swelling induced by $CaCl_2$ (250 μM) in the absence or presence of CsA (5 μM), **11** and **17** (50 μM).

further stirring for 14 h at room temperature, the reaction mixture was filtered and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The resulting residue was purified by silica gel chromatography (hexane/EtOAc = 15:1) to afford **19b** (920 mg, 32%) as a yellow solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.64 (1H, s), 7.91 (2H, d, J = 1.8 Hz), 7.69 (1H, t, J = 1.8 Hz), 7.67 (1H, d, J = 4.2 Hz), 7.52 (1H, d, J = 4.2 Hz); MS (FAB) m/z 241 $(M+H)^+$.

4.1.2.2. 5-[3-(Trifluoromethyl)phenyl]-2-furaldehyde (19c). Prepared from 3-(trifluoromethyl)aniline in accordance with procedure A. Yellow solid (91%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.67 (1H, s), 8.03 (1H, s), 7.98 (1H, d, J = 7.9 Hz), 7.63 (1H, d, J = 7.9 Hz), 7.56 (1H, t, J = 7.9 Hz), 7.32 (1H, d, J = 3.6 Hz), 6.91 (1H, d, J = 3.6 Hz); MS (FAB) m/z 241 $(M+H)^+$.

4.1.2.3. 5-(3-Bromophenyl)-2-furaldehyde (19d). Prepared from 3-bromoaniline in accordance with procedure A. Orange oil (8%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.66 (1H, s) 7.95 (1H, t, J = 1.8 Hz), 7.73 (1H, dd, J = 7.8, 1.8 Hz), 7.50 (1H, dt, J =7.8, 1.8 Hz), 7.31 (1H, d, J = 3.6 Hz), 7.30 (1H, t, J = 7.8 Hz), 6.84 (1H, d, J = 3.6 Hz); MS (FAB) m/z 251 $(M+H)^+$.

4.1.3. Synthesis of 5-aryl-2-furaldehydes by Suzuki–Miyaura cross-coupling reaction

4.1.3.1. 5-(4-Chlorophenyl)-2-furaldehyde (19e): General procedure B. To a solution of 1-bromo-4-chlorobenzene (478 mg, 2.50 mmol) in a mixture of DME (7.5 mL) and EtOH (5.0 mL) were added $PdCl_2(PPh_3)_2$ (36.5 mg, 52.0 μmol), 5-formyl-2-furanboronic acid (365 mg, 2.61 mmol), and 2 M aqueous Na_2CO_3 solution. The reaction mixture was stirred for 2 h at 60 °C, then cooled to room temperature, and volatile materials were removed under reduced pressure. To the obtained residue was added H_2O and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/EtOAc = 5:1) to afford **19e** (323 mg, 63%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.64 (1H, s), 7.73 (2H, d, J = 8.5 Hz), 7.40 (2H, d, J = 8.5 Hz), 7.30 (1H, d, J = 3.6 Hz), 6.81 (1H, d, J = 3.6 Hz); MS (FAB) m/z 207 $(M+H)^+$.

4.1.3.2. 5-Phenyl-2-furaldehyde (19f). Prepared from bromobenzene in accordance with procedure B. Orange oil (75%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.64 (1H, s), 7.81 (2H, d, J = 7.3 Hz), 7.45–7.36 (3H, m), 7.30 (1H, d, J = 3.6 Hz), 6.83 (1H, d, J = 3.6 Hz); MS (FAB) m/z 173 $(M+H)^+$.

4.1.4. Synthesis of 5-aryl-2-furancarboxylic acid by Pinnick oxidation reaction

4.1.4.1. 5-(3-Chlorophenyl)-2-furancarboxylic acid (22a): General procedure C. To a solution of 5-(3-chlorophenyl)-2-furaldehyde (**19a**) (206 mg, 1.00 mol) in a mixture of *tert*-BuOH (37.5 mL) and H_2O (7.5 mL) was added NaH_2PO_4 (213 mg, 1.50 mmol), 2-methyl-2-butene (0.78 mL, 7.50 mmol), and $NaClO_2$ (228 mg, 2.50 mmol) and the mixture was stirred for 11 h at room temperature. The volatile materials were removed under reduced pressure, and then H_2O and 1 N HCl were added. The mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated to afford **22a** (214 mg, 97%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (1H, s) 7.66 (1H, dd, J = 7.2, 1.8 Hz), 7.38 (1H, d, J = 3.6 Hz), 7.35 (1H, t, J = 7.3 Hz), 7.32 (1H, dd, J = 7.3, 1,8 Hz), 6.79 (1H, d, J = 3.6 Hz); MS (FAB) m/z 223 $(M+H)^+$.

4.1.4.2. 5-(3,5-Dichlorophenyl)-2-furancarboxylic acid (22b). Prepared from **19b** in accordance with procedure C. White solid (96%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (2H, d, J = 1.8 Hz), 7.37 (1H, d, J = 3.6 Hz), 7.33 (1H, t, J = 1.8 Hz), 6.81 (1H, d, J = 3.6 Hz); MS (FAB); m/z 257 $(M+H)^+$.

4.1.4.3. 5-[3-(Trifluoromethyl)phenyl]-2-furancarboxylic acid (22c). Prepared from **19c** in accordance with procedure C. White solid (99.7%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (1H, s) 7.97 (1H, d, J = 7.9 Hz), 7.60 (1H, d, J = 7.9 Hz), 7.55 (1H, t, J = 7.9 Hz), 7.39 (1H, d, J = 3.6 Hz), 6.85 (1H, d, J = 3.6 Hz); MS (FAB) m/z 257 $(M+H)^+$.

4.1.4.4. 5-(3-Bromophenyl)-2-furancarboxylic acid (22d). Prepared from **19d** in accordance with procedure C. White solid (quant.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (1H, t, J = 1.8 Hz), 7.71 (1H, dd, J = 7.9, 1.2 Hz), 7.48 (1H, dd, J = 7.9,

1.2 Hz), 7.39 (1H, d, J = 3.6 Hz), 7.29 (1H, t, J = 7.9 Hz), 6.79 (1H, d, J = 3.6 Hz); MS (FAB) m/z 267 $(M+H)^+$.

4.1.4.5. 5-(4-Chlorophenyl)-2-furancarboxylic acid (22e). Prepared from **19e** in accordance with procedure C. White solid (80%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (2H, d, J = 8.5 Hz), 7.40 (2H, d, J = 8.5 Hz), 7.37 (1H, d, J = 3.6 Hz), 6.76 (1H, d, J = 3.6 Hz); MS (FAB) m/z 223 $(M+H)^+$.

4.1.4.6. 5-Phenyl-2-furancarboxylic acid (22f). Prepared from **19f** in accordance with procedure C. White solid (94%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (2H, d, J = 7.3 Hz), 7.44–7.34 (4H, m), 6.77 (1H, d, J = 3.6 Hz); MS (FAB) m/z 189 $(M+H)^+$.

4.1.5. Synthesis of 5-aryl-2-furaldimines

4.1.5.1. 1-{[5-(3-Chlorophenyl)furfurylidene]amino}imidazolidine-2,4-dione (20a): General procedure D. To a solution of 5-(3-chlorophenyl)-2-furaldehyde (**19a**) (127 mg, 500 μmol) in DMF (1.0 mL) was added 1-aminohydantoin hydrochloride (79.4 mg, 525 μmol) and the mixture was stirred for 5 h at room temperature. The reaction was quenched by the addition of H_2O, and the resulting precipitate was collected by filtration with suction and washed with H_2O on a funnel. The collected solid was dried in vacuo to afford **20a** (166 mg, 90%) as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.28 (1H, s), 7.82 (2H, d, J = 1,8 Hz), 7.73 (1H, d, J = 7.9 Hz), 7.73 (1H, s), 7.49 (1H, d, J = 7.9 Hz), 7.40 (1H, dd, J = 7.9, 1.8 Hz), 7.26 (1H, J = 3.6 Hz), 6.97 (1H, J = 3.6 Hz), 4.35 (2H, s); MS (FAB) m/z 304 $(M+H)^+$.

4.1.5.2. 1-{[5-(3,5-Dichlorophenyl)furfurylidene]amino}imidazolidine-2,4-dione (20b). Prepared from **19b** in accordance with procedure D. Pale orange solid (88%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.30 (1H, s), 7.79 (2H, d, J = 1,8 Hz), 7.73 (1H, s), 7.57 (1H, t, J = 1,8 Hz), 7.38 (1H, d, J = 4.2 Hz), 6.98 (1H, J = 4.2 Hz), 4.32 (2H, s); MS (FAB) m/z 338 $(M+H)^+$.

4.1.6. Synthesis of 5-aryl-2-furamides

4.1.6.1. 5-(3-Chlorophenyl)-*N*-(2,4-dioxoimidazolidin-1-yl)-2-furamide (23a): General procedure E. To a mixture of *N*-methylmorpholine (14.5 μL, 115 μmol), DMT-MM[33] (54.0 mg, 192 μmol), and **22a** (21.4 mg, 96.1 μmol) in MeOH (1.0 mL) was added a solution of 1-aminohydantoin hydrochloride (16.0 mg, 105 μmol) in H_2O (0.1 mL), and the mixture was stirred at room temperature. Stirring was continued for 19 h, then H_2O was added to the reaction mixture, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The resulting residue was purified by silica gel chromatography (hexane/EtOAc = 1:1) to afford **23a** (17.2 mg, 56%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.33 (1H, s) 10.99 (1H, s), 8.10 (1H, d, J = 1.8 Hz), 7.90 (1H, d, J = 7.9 Hz), 7.50 (1H, t, J = 7.9 Hz), 7.44 (1H, dd, J = 7.9, 1.8 Hz), 7.36 (1H, d, J = 3.6 Hz), 7.30 (1H, d, J = 3.6 Hz); 4.16 (2H, s), MS (FAB) m/z 320 $(M+H)^+$.

4.1.6.2. 5-(3,5-Dichlorophenyl)-*N*-(2,4-dioxoimidazolidin-1-yl)-2-furamide (23b). Prepared from **22b** in accordance with procedure E. White solid (75%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.35 (1H, s) 11.00 (1H, s), 8.10 (2H, d, J = 1.8 Hz), 7.63 (1H, t, J = 1.8 Hz), 7.43 (1H, d, J = 3.6 Hz), 7.38 (1H, d, J = 3.6 Hz), 4.17 (2H, s); MS (FAB) m/z 354 $(M+H)^+$.

4.1.6.3. 5-[3-(Trifluoromethyl)phenyl]-*N*-(2,4-dioxoimidazolidin-1-yl)-2-furamide (23c). Prepared from **22c** in accordance with procedure E. White solid (52%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.35 (1H, s), 11.06 (1H, s), 8.35 (1H, s), 8.24 (1H, d, J = 7.3 Hz), 7.75 (1H, d, J = 8.5 Hz), 7.72 (1H, dd, J = 8.5, 7.3 Hz), 7.40 (1H, d, J = 3.7 Hz), 7.39 (1H, d, J = 3.7 Hz), 4.18 (2H, s); MS (FAB) m/z 354 $(M+H)^+$.

4.1.6.4. 5-(3-Bromophenyl)-*N*-(2,4-dioxoimidazolidin-1-yl)-2-furamide (23d). Prepared from **22d** in accordance with procedure E. White solid (51%); ^{1}H NMR (500 MHz, methanol-d_4) δ 8.11 (1H, s), 7.83 (1H, d, J = 7.9 Hz), 7.53 (1H, d, J = 7.9 Hz), 7.36 (1H, t, J = 7.9 Hz), 7.32 (1H, d, J = 3.6 Hz), 7.05 (1H, d, J = 3.6 Hz), 4.21 (2H, s); MS (FAB) m/z 364 $(M+H)^+$.

4.1.6.5. 5-(4-Chlorophenyl)-*N*-(2,4-dioxoimidazolidin-1-yl)-2-furamide (23e). Prepared from **22e** in accordance with procedure E. White solid (89%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.32 (1H, s), 10.95 (1H, s), 7.97 (2H, d, J = 8.4 Hz), 7.56 (2H, d, J = 8.4 Hz), 7.36 (1H, d, J = 3.6 Hz), 7.23 (1H, d, J = 3.6 Hz), 4.15 (2H, s); MS (FAB) m/z 320 $(M+H)^+$.

4.1.6.6. 5-Phenyl-*N*-(2,4-dioxoimidazolidin-1-yl)-2-furamide (23f). Prepared from **22f** in accordance with procedure E. White solid (72%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (1H, s), 10.90 (1H, s), 7.93 (2H, d, J = 7.9 Hz), 7.48 (2H, dd, J = 7.9, 7.3 Hz), 7.39 (1H, t, J = 7.3 Hz), 7.36 (1H, d, J = 3.7 Hz), 7.18 (1H, d, J = 3.7 Hz), 4.16 (2H, s); MS (FAB) m/z 286 $(M+H)^+$.

4.1.7. N-Alkylation of hydantoin with NaH as a base

4.1.7.1. 1-{[5-(3-Chlorophenyl)furfurylidene]amino}-3-methylimidazolidine-2,4-dione (4): General procedure F. To a solution of **20a** (39.8 mg, 131 μmol) in DMF (1.0 mL) was added NaH (6.3 mg, 0.14 mmol) at 0 °C, and the mixture was stirred for 10 min at room temperature. It was cooled to 0 °C, then iodomethane (16.5 μL, 262 μmol) was added to it and stirring was continued for 6 h at room temperature. The reaction was quenched by the addition of H_2O, and the resulting precipitate was collected by filtration with suction and washed with H_2O on a funnel. The collected solid was recrystallized from EtOH to afford **4** (5.6 mg, 13%) as a yellow solid. Mp 236–237 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.82 (1H, d, J = 1.8 Hz), 7.79 (1H, s), 7.73 (1H, d, J = 7.9 Hz), 7.49 (1H, t, J = 7.9 Hz), 7.40 (1H, dd, J = 7.9, 1.2 Hz), 7.26 (1H, d, J = 3.6 Hz), 6.98 (1H, d, J = 3.6 Hz), 4.37 (2H, s), 2.93 (3H, s); MS (FAB) m/z 318 $(M+H)^+$; HRMS (FAB) calcd for $C_{15}H_{13}ClN_3O_3$ 318.0645, found 318.0652 $(M+H)^+$.

4.1.7.2. 1-{[5-(3,5-Dichlorophenyl)furfurylidene]amino}-3-methylimidazolidine-2,4-dione (5). Prepared from **20b** in accordance with procedure F. Pale yellow solid (50%); Mp 191–192 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (1H, s), 7.79 (2H, d, J = 1.8 Hz), 7.58 (1H, d, J = 1,8 Hz), 7.39 (1H, d, J = 3.6 Hz), 7.01 (1H, d, J = 3.6 Hz), 4.37 (2H, s), 2.93 (3H, s); MS (FAB) m/z 352 $(M+H)^+$ Anal. Calcd for $C_{15}H_{11}Cl_2N_3O_3 \cdot 1/5H_2O$; C, 50.64; H, 3.23; N, 11.81. Found: C, 50.63; H, 3.19; N, 11.67.

4.1.7.3. 5-(3,5-Dichlorophenyl)-*N*-{2,4-dioxo-3-[(pyridin-3-yl)methyl]imidazolidin-1-yl}-2-furamide (17). Prepared from **23b** and 3-(chloromethyl)pyridine hydrochloride instead of iodomethane in accordance with procedure F. White solid (47%); Mp 254–254 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.91 (1H, s), 8.67 (1H, s), 8.54 (1H, d, J = 4.8 Hz), 7.74 (1H, d, J = 7.8 Hz), 7.50 (2H, d, J = 1.8 Hz), 7.32 (1H, t, J = 1.8 Hz), 7.27 (1H, dd, J = 7.8, 4.8 Hz), 7.23 (1H, d, J = 3.6 Hz), 6.76 (1H, d, J = 3.6 Hz), 4.76 (2H, s), 4.23 (2H, s); MS (FAB) m/z 445 $(M+H)^+$ Anal. Calcd for $C_{20}H_{14}Cl_2N_4O_4$: C, 53.95; H, 3.17; N, 12.58. Found: C, 53.66; H, 3.21; N, 12.30.

4.1.8. N-Alkylation of hydantoin with carbonates as base

4.1.8.1. 5-(3-Chlorophenyl)-*N*-(2,4-dioxo-3-methylimidazolidin-1-yl)-2-furamide (6): General procedure G. To a solution of **23a** (25.5 mg, 80.0 μmol) in DMF (1.0 mL) was added K_2CO_3

(6.2 mg, 45 μmol) at 0 °C under an argon atmosphere, and the mixture was stirred for 10 min at room temperature. To this was added iodomethane (5.2 μL, 84 μmol) at 0 °C, and the whole was stirred for 4 h at room temperature. The reaction was quenched by the addition of H_2O and the resulting mixture was extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 3:2) to afford **6** (12.1 mg, 45%) as a white solid. Mp 228–229 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.05 (1H, s), 7.70 (1H, d, J = 1.8 Hz), 7.32 (1H, dd, J = 7.2, 1.8 Hz), 7.38–7.33 (2H, m) 7.19 (1H, d, J = 3.6 Hz), 6.71 (1H, d, J = 3.6 Hz), 4.24 (2H, s), 3.15 (3H, s); MS (FAB) m/z 334 $(M+H)^+$; HRMS (FAB) calcd for $C_{15}H_{13}ClN_3O_4$ 334.0595, found 334.0601 $(M+H)^+$.

4.1.8.2. 5-(3,5-Dichlorophenyl)-*N*-(2,4-dioxo-3-methylimidazolidin-1-yl)-2-furamide (7). Prepared from **23b** in accordance with procedure G. White solid (15%); Mp 226–228 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.05 (1H, s), 7.54 (2H, d, J = 7.3 Hz), 7.32 (1H, t, J = 1.8 Hz), 7.13 (1H, d, J = 3.6 Hz), 6.70 (1H, d, J = 3.6 Hz),, 4.22 (2H, s), 3.13 (3H, s); MS (FAB) m/z 367 $(M+H)^+$; HRMS (FAB) calcd for $C_{15}H_{12}Cl_2N_3O_4$ 368.0205, found 368.0209 $(M+H)^+$.

4.1.8.3. 3-[3-(*tert*-Butoxycarbonylamino)benzyl]-1-{[5-(3-chlorophenyl)furfurylidene]amino}imidazolidine-2,4-dione (21a). Prepared from **20a** and 3-(*tert*-butoxycarbonylamino)benzyl bromide[32] instead of iodomethane in accordance with procedure G. Pale yellow solid (98%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (1H, s) 7.70 (2H, t, J = 1.8 Hz), 7.59 (1H, d, J = 7.3 Hz), 7.42 (1H, d, J = 7.3 Hz), 7.31 (1H, t, J = 7.9 Hz), 7.25 (2H, m), 7.09 (1H, d, J = 7.9 Hz), 6.88 (1H, d, J = 3.6 Hz), 6.75 (1H, d, J = 3.6 Hz), 6.45 (1H, s), 4.70 (2H, s), 4.24 (2H, s), 1.48 (9H, s); MS (FAB) m/z 509 $(M+H)^+$.

4.1.8.4. 3-[3-(*tert*-Butoxycarbonylamino)benzyl]-1-{[5-(3,5-dichlorophenyl)furfurylidene]amino}imidazolidine-2,4-dione (21b). Prepared from **20b** and 3-(*tert*-butoxycarbonylamino)benzyl bromide[32] instead of iodomethane in accordance with procedure G. White solid (85%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (1H, s) 7.58 (2H, d, J = 1.8 Hz), 7.42–7.38 (1H, m), 7.31 (1H, s), 7.26 (1H, d, J = 1.8 Hz), 7.43 (1H, d, J = 7.3 Hz), 7.09 (1H, d, J = 7.3 Hz), 6.89 (1H, d, J = 3.6 Hz), 6.77 (1H, d, J = 3.6 Hz), 6.45 (1H, s), 4.70 (2H, s), 4.24 (2H, s), 1.48 (9H, s); MS (FAB) m/z 543 $(M+H)^+$.

4.1.8.5. *N*-{3-[3-(*tert*-Butoxycarbonylamino)benzyl]-2,4-dioxoimidazolidin-1-yl}-5-(3-chlorophenyl)-2-furamide (24a). Prepared from **23a** and 3-(*tert*-butoxycarbonylamino)benzyl bromide[32] instead of iodomethane in accordance with procedure G. White solid (40%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (1H, s) 7.66 (1H, d, J = 1.8 Hz), 7.55 (1H, dd, J = 7.3, 1.8 Hz), 7.40 (1H, m), 7.34 (1H, t, J = 7.3 Hz), 7.32 (1H, s), 7.34–7.22 (3H, m), 7.04 (1H, d, J = 7.3 Hz), 6.75 (1H, d, J = 3.6 Hz), 6.51 (1H, s), 4.70 (2H, s), 4.20 (2H, s), 1.49 (9H, s); MS (FAB) m/z 525 $(M+H)^+$.

4.1.8.6. *N*-{3-[3-(*tert*-Butoxycarbonylamino)benzyl]-2,4-dioxoimidazolidin-1-yl}-5-(3,5-dichlorophenyl)-2-furamide (24b). Prepared from **23b** and 3-(*tert*-butoxycarbonylamino)benzyl bromide[32] instead of iodomethane in accordance with procedure G. White solid (38%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.24 (1H, s) 7.51 (1H, s), 7.50 (1H, s), 7.34 (1H, s), 7.32 (1H, s), 7.29 (1H, t, J = 7.9 Hz), 7.18 (1H, t, J = 7.9 Hz), 7.11 (1H, d, J = 3.6 Hz), 7.00 (1H, d, J = 7.9 Hz), 6.67 (1H, s), 6.65 (1H, d, J = 3.6 Hz), 4.69 (2H, s), 4.11 (2H, s), 1.48 (9H, s); MS (FAB) m/z 558 $(M)^+$.

4.1.8.7. *N*-{3-[3-(*tert*-Butoxycarbonylamino)benzyl]-2,4-dioxoimidazolidin-1-yl}-5-[3-(trifluoromethyl)phenyl]-2-furamide (24c). Prepared from **23c** and 3-(*tert*-butoxycarbonylamino)benzyl bromide[32] instead of iodomethane in accordance with procedure G. White solid (34%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.35 (1H, s), 7.78 (1H, s), 7.77 (1H, d, J = 7.9 Hz), 7.56 (1H, d, J = 7.9 Hz), 7.43 (1H, t, J = 7.9 Hz), 7.35 (1H, br s), 7.34 (1H, s), 7.18 (1H, t, J = 7.9 Hz), 7.04 (1H, d, J = 3.6 Hz), 7.00 (1H, d, J = 7.9 Hz), 6.68 (1H, s), 6.64 (1H, d, J = 3.6 Hz), 4.68 (2H, s), 4.09 (2H, s), 1.48 (9H, s); MS (FAB) m/z 558 $(M)^+$.

4.1.8.8. 5-(3-Bromophenyl)-*N*-{3-[3-(*tert*-butoxycarbonylamino)benzyl]-2,4-dioxoimidazolidin-1-yl}-2-furamide (24d). Prepared from **23d** with 3-(*tert*-butoxycarbonylamino)benzyl bromide[32] and Na_2CO_3 instead of iodomethane and K_2CO_3 in accordance with procedure G. Pale pink solid (27%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.04 (1H, s) 7.76 (1H, t, J = 1.8 Hz), 7.55 (1H, d, J = 7.3, Hz), 7.43 (1H, d, J = 7.9, Hz), 7.38 (1H, d, J = 7.9, Hz), 7.30 (1H, s), 7.24–7.19 (2H, m), 7.11 (1H, d, J = 3.6 Hz), 7.02 (1H, d, J = 7.3 Hz), 6.64 (1H, s), 6.63 (1H, d, J = 3.6 Hz), 4.69 (2H, s), 4.12 (2H, s), 1.49 (9H, s); MS (FAB) m/z 569 $(M+H)^+$.

4.1.8.9. *N*-{3-[3-(*tert*-Butoxycarbonylamino)benzyl]-2,4-dioxoimidazolidin-1-yl}-5-(4-chlorophenyl)-2-furamide (24e). Prepared from **23e** with 3-(*tert*-butoxycarbonylamino)benzyl bromide[32] and Na_2CO_3 instead of iodomethane and K_2CO_3 in accordance with procedure G. White solid (45%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (1H, s), 7.57 (1H, d, J = 8.5 Hz), 7.39 (1H, d, J = 6.7 Hz), 7.37–7.30 (4H, m), 7.25–7.23 (1H, m), 7.16 (1H, t, J = 3.6 Hz), 7.03 (1H, d, J = 6.7 Hz), 6.64 (1H, d, J = 3.6 Hz), 6.55 (1H, s), 4.70 (2H, s), 4.16 (2H, s), 1.49 (9H, s); MS (FAB) m/z 525 $(M+H)^+$.

4.1.8.10. *N*-{3-[3-(*tert*-Butoxycarbonylamino)benzyl]-2,4-dioxoimidazolidin-1-yl}-5-phenyl-2-furamide (24f). Prepared from **23f** with 3-(*tert*-butoxycarbonylamino)benzyl bromide[32] and Na_2CO_3 instead of iodomethane and K_2CO_3 in accordance with procedure G. White solid (47%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.04 (1H, s), 9.38 (1H, s), 8.31 (1H, s), 7.93 (1H, d, J = 7.9 Hz), 7.50–7.47 (3H, m), 7.40 (1H, t, J = 7.9 Hz), 7.37 (1H, d, J = 3.6 Hz), 7.33 (1H, d, J = 7.9 Hz), 7.22 (1H, t, J = 7.9 Hz), 7.18 (1H, d, J = 3.6 Hz), 6.88 (1H, d, J = 7.9), 4.58 (2H, s), 4.28 (2H, s), 1.46 (9H, s); MS (FAB) m/z 491 $(M+H)^+$.

4.1.8.11. *N*-{3-[4-(*tert*-Butoxycarbonylamino)benzyl]-2,4-dioxoimidazolidin-1-yl}-5-(3,5-dichlorophenyl)-2-furamide (24g). Prepared from **23b** with 4-(*tert*-butoxycarbonylamino)benzyl bromide[36] and Na_2CO_3 instead of iodomethane and K_2CO_3 in accordance with procedure G. White solid (32%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.18 (1H, s), 9.35 (1H, s), 8.10 (2H, d, J = 1.8 Hz), 7.63 (1H, t, J = 1.8 Hz), 7.43 (1H, d, J = 3.6 Hz), 7.41 (1H, br s), 7.39 (2H, d, J = 3.6 Hz), 7.18 (2H, d, J = 3.6 Hz), 4.57 (2H, s), 4.28 (2H, s), 1.46 (9H, s); MS (FAB) m/z 558 $(M+H)^+$, 581 $(M+Na)^+$.

4.1.8.12. 5-(3,5-Dichlorophenyl)-*N*-{2,4-dioxo-3-[(pyridin-4-yl)methyl]imidazolidin-1-yl}-2-furamide (18). Prepared from **23b** with 4-(bromomethyl)pyridine and Na_2CO_3 instead of iodomethane and K_2CO_3 in accordance with procedure G. White solid (28%); Mp 275–277 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.26 (1H, s), 8.55 (2H, d, J = 5.4 Hz), 8.10 (2H, d, J = 1.8 Hz), 7.63 (1H, t, J = 1.8 Hz), 7.44 (1H, d, J = 3.6 Hz), 7.42 (1H, d, J = 3.6 Hz), 7.30 (2H, d, J = 5.4 Hz), 4.71 (2H, s), 4.36 (2H, s); MS (FAB) m/z 445$(M+H)^+$; HRMS (FAB) calcd for $C_{20}H_{15}Cl_2N_4O_4$ 445.0470, found 445.0469 $(M+H)^+$.

4.1.9. Deprotection of Boc-protected aminobenzyl group on hydantoin (1)

4.1.9.1. 3-(3-Aminobenzyl)-1-{[5-(3-chlorophenyl)furfurylidene]amino}imidazolidine-2,4-dione (8): General procedure H. To a solution of **21a** (29.1 mg, 57.1 μmol) in 1,4-dioxane

(1.8 mL) was added 4 M HCl in EtOAc (0.6 mL) at 0 °C. The mixture was stirred for 20 h at room temperature, then cooled to 0 °C, and the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution. Volatile materials were removed under reduced pressure. The remaining aqueous solution was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (hexane/EtOAc = 2:1) to afford **8** (15.6 mg, 67%) as a pale yellow solid. Mp 223–224 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (1H, s), 7.97 (1H, s), 7.79 (1H, s), 7.71 (1H, s), 7.68 (1H, d, J = 7.9 Hz), 7.60 (1H, t, J = 7.9 Hz), 7.40–7.23 (3H, m), 7.19 (1H, d, J = 7.3 Hz), 7.07 (1H, s), 6.82 (1H, d, J = 3.6 Hz), 6.75 (1H, d, J = 3.6 Hz), 4.78 (2H, s), 4.27 (2H, s); MS (FAB) m/z 409 $(M+H)^+$; HRMS (FAB) calcd for $C_{21}H_{18}ClN_4O_3$ 409.1067, found 409.1060 $(M+H)^+$.

4.1.9.2. 3-(3-Aminobenzyl)-1-{[5-(3,5-dichlorophenyl)furfurylidene]amino}imidazolidine-2,4-dione (9). Prepared from **21b** in accordance with procedure H. Pale yellow solid (47%); Mp 194.0–194.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (1H, s), 7.58 (2H, t, J = 1.8 Hz), 7.25 (1H, d, J = 1.8 Hz), 7.08 (1H, t, J = 7.9 Hz), 6.88 (1H, d, J = 3.6 Hz), 6.82 (1H, t, J = 7.9 Hz), 6.78 (1H, d, J = 3.6 Hz), 6.77 (1H, d, J = 1.8 Hz), 6.59 (1H, d, J = 7.9 Hz), 4.64 (2H, s), 4.23 (2H, s), 3.74–3.60 (2H, br); MS (FAB) m/z 443 $(M+H)^+$ Anal. Calcd for $C_{21}H_{16}Cl_2N_4O_3$: C, 56.90; H, 3.64; N, 12.64. Found: C, 57.20; H, 3.83; N, 12.16.

4.1.9.3. *N*-[3-(3-Aminobenzyl)-2,4-dioxoimidazolidin-1-yl]-5-(3,5-dichlorophenyl)-2-furamide (11). Prepared from **24b** in accordance with procedure H. White solid (50%); Mp 247.0–247.5 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (1H, s), 8.10 (2H, d, J = 1.8 Hz), 7.62 (1H, t, J = 1.8 Hz), 7.44 (1H, d, J = 3.7 Hz), 7.38 (1H, d, J = 3.7 Hz), 6.96 (1H, dd, J = 7.9, 7.3 Hz), 7.50 (1H, t, J = 1.8 Hz), 6.46 (1H, dd, J = 7.9, 1.8 Hz), 6.42 (1H, d, J = 7.3 Hz), 5.09 (2H, s), 4.48 (2H, s), 4.28 (2H, s); MS (FAB) m/z 459 $(M+H)^+$ Anal. Calcd for $C_{21}H_{16}Cl_2N_4O_4 \cdot 1/4\ H_2O$; C, 54.38; H, 3.59; N, 12.08. Found: C, 54.33; H, 3.49; N, 11.89.

4.1.9.4. *N*-[3-(3-Aminobenzyl)-2,4-dioxoimidazolidin-1-yl]-5-(4-chlorophenyl)-2-furamide (12). Prepared from **24e** in accordance with procedure H. Pale yellow solid (86%); Mp 187.0–187.5 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.07 (1H, s), 7.97 (2H, d, J = 8.5 Hz), 7.57 (2H, d, J = 8.5 Hz), 7.37 (1H, d, J = 3.6 Hz), 7.23 (1H, d, J = 3.6 Hz), 6.96 (1H, t, J = 7.9 Hz), 6.50 (1H, s), 6.46 (1H, d, J = 7.9 Hz), 6.42 (1H, d, 7.9 Hz), 5.09 (2H, s), 4.47 (2H, s), 4.26 (2H, s); MS (FAB) m/z 425$(M+H)^+$; HRMS (FAB) calcd for $C_{21}H_{18}ClN_4O_4$ 425.1017, found 425.1007 $(M+H)^+$.

4.1.10. Deprotection of Boc-protected aminobenzyl group on hydantoin (2)

4.1.10.1. *N*-[3-(3-Aminobenzyl)-2,4-dioxoimidazolidin-1-yl]-5-(3-chlorophenyl)-2-furamide hydrochloride (10): General procedure I. To a solution of **24a** (75.2 mg, 143 μmol) in EtOAc (1.4 mL) was added 4 N HCl in EtOAc (1.4 mL) at 0 °C, and the mixture was stirred for 3 h at room temperature. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution and the resulting mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was passed through a short silica plug (hexane/EtOAc = 1:1). The eluate was evaporated under reduced pressure, and the resulting residue was dissolved in EtOAc (0.9 mL). To this was added 4 N HCl in EtOAc (0.1 mL). The resulting precipitate was collected by filtration with suction, washed with EtOAc in the funnel, and dried under reduced pressure to afford **10** (42.1 mg, 64%) as a pale yellow solid. Mp 163–164 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.18 (1H, s), 8.10 (2H, t, J = 1.8 Hz), 7.51 (1H, t, J = 7.9 Hz), 7.45 (1H, dd, J = 7.9, 1.8 Hz), 7.38 (1H, d, J = 3.6 Hz), 7.36 (1H, t, J = 7.9 Hz), 7.32 (1H, d, J = 3.6 Hz), 7.01–6.90 (3H, m), 4.60 (2H, s), 4.28 (2H, s); MS (FAB) m/z 425 $(M+H)^+$ Anal. Calcd for $C_{21}H_{18}Cl_2N_4O_4$: C, 54.68; H, 3.93; N, 12.15. Found: C, 54.91; H, 4.20; N, 11.88.

4.1.10.2. *N*-[3-(3-Aminobenzyl)-2,4-dioxoimidazolidin-1-yl]-5-[3-(trifluoromethyl)phenyl]-2-furamide hydrochloride (14). Prepared from **24c** in accordance with procedure I. Pale yellow solid (29%); Mp 150.0–151.0 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.23 (1H, s), 8.34 (1H, s), 8.24 (1H, d, J = 7.9 Hz), 7.75 (1H, d, J = 7.9 Hz), 7.72 (1H, t, J = 7.9 Hz), 7.41–7.37 (3H, m), 7.15 (3H, m), 4.63 (2H, s), 4.17 (2H, s); MS (FAB) m/z 459 $(M+H)^+$ Anal. Calcd for $C_{22}H_{18}ClF_3N_4O_4 \cdot 1/4\ H_2O$; C, 52.92; H, 3.73; N, 11.22. Found: C, 52.88; H, 3.84; N, 11.15.

4.1.10.3. *N*-[3-(3-Aminobenzyl)-2,4-dioxoimidazolidin-1-yl]-5-(3-bromophenyl)-2-furamide hydrochloride (13). Prepared from **24d** in accordance with procedure I. Pale orange solid (57%); Mp 150.0–151.0 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.17 (1H, s), 8.23 (1H, t, J = 1.8 Hz), 7.94 (1H, d, J = 7.9 Hz), 7.58 (1H, dd, J = 7.9, 1.8 Hz), 7.44 (1H, d, J = 7.9 Hz), 7.38 (1H, d, J = 3.6 Hz), 7.35–7.32 (1H, m), 7.30 (1H, d, J = 3.6 Hz), 7.12–7.05 (3H, m), 4.64 (2H, s), 4.30 (2H, s); MS (FAB) m/z 469 $(M+H)^+$; HRMS (FAB) calcd for $C_{21}H_{18}BrN_4O_4$ 469.0511, found 469.0520 $(M+H)^+$.

4.1.10.4. *N*-[3-(3-Aminobenzyl)-2,4-dioxoimidazolidin-1-yl]-5-phenyl-2-furamide hydrochloride (15). Prepared from **24f** in accordance with procedure I. Pale orange solid (23%); Mp 144.0–145.0 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.02 (1H, s), 8.30 (1H, s), 7.93 (2H, d, J = 7.9 Hz), 7.49 (2H, t, J = 7.9 Hz), 7.40 (1H, d, J = 7.9 Hz), 7.37 (1H, d, J = 3.6 Hz), 7.19 (1H, d, J = 3.6 Hz), 6.96 (1H, t, J = 7.9 Hz), 6.50 (1H, s), 6.47 (1H, d, J = 7.9 Hz), 6.43 (1H, d, J = 7.9 Hz), 5.20–5.06 (2H, br), 4.60 (2H, s), 4.28 (2H, s); MS (FAB) m/z 391 $(M+H)^+$; HRMS (FAB) calcd for $C_{21}H_{19}N_4O_4$ 391.1406, found 391.1401 $(M+H)^+$.

4.1.10.5. *N*-[3-(4-Aminobenzyl)-2,4-dioxoimidazolidin-1-yl]-5-(3,5-dichlorophenyl)-2-furamide hydrochloride (16). Prepared from **24g** in accordance with procedure I using HCl/1,4-dioxane instead of HCl/EtOAc in the removal step of Boc group. Pale orange solid (59%); Mp 213.0–214.0 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.22 (1H, s), 9.75–8.75 (1H, br), 8.09 (2H, d, J = 1.8 Hz), 7.63 (1H, t, J = 1.8 Hz), 7.44 (1H, d, J = 3.6 Hz), 7.40 (1H, d, J = 3.6 Hz), 7.35 (2H, d, J = 7.9 Hz), 7.21 (2H, d, J = 7.9 Hz), 4.65 (2H, s), 4.29 (2H, s); MS (FAB) m/z 459 $(M+H)^+$; HRMS (FAB) calcd for $C_{21}H_{17}Cl_2N_4O_4$ 459.0627, found 459.0646 $(M+H)^+$.

4.2. Bioassay

4.2.1. Cell culture

HL-60 cells were maintained in RPMI1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 5% heat-inactivated fetal bovine serum (FBS). Cells were grown in a humidified incubator at 37 °C under 5% CO_2/95% air.

4.2.2. Mitochondrial swelling assay

Mitochondria were isolated from human leukemia HL-60 cells as described previously.[37] Mitochondria were incubated with test compounds at 30 °C (0.2 mg mitochondrial protein/mL, 20 mM Tris–MOPS, pH 7.4, 200 mM sucrose, 12.5 mM succinate, 2 μM rotenone, 10 μM EGTA) for 10 min. Then $CaCl_2$ solution was added (final concentration: 250 μM) and OD_{540} was monitored at 30 °C with a microplate reader (SpectraMax M2e, Molecular Devices Inc.).

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Supplementary data

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