Discovery of *N*-(3-{4-[(3-Fluorobenzyl)oxy]phenoxy}propyl)-2-pyridin-4-ylacetamide as a Potent and Selective Reverse NCX Inhibitor

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In the setting of heart failure and myocardial ischemia-reperfusion, the sodium–calcium exchanger (NCX) can lead to calcium overload, which is responsible for contractile dysfunction and arrhythmia. NCX is an attractive target for treatment in heart failure and myocardial ischemia-reperfusion. We have designed and synthesized a series of benzyloxyphenyl derivatives based on compound 3. These derivatives have been evaluated for their inhibitory activity against both the reverse and forward modes of NCX. We have discovered a novel potent and selective reverse NCX inhibitor (12) with an IC_{50} value of 0.085 μM against reverse NCX.

Key words sodium–calcium exchanger (NCX); anti-arrhythmics; transporter

Intracellular Ca^{2+} is of primary importance in the pathogenesis of ischemia and reperfusion injury in the myocardium. Recent studies have suggested that a massive Ca^{2+} influx may occur as a consequence of Na^+–Ca^{2+} exchange *via* the sodium–calcium exchanger (NCX) during reperfusion which, in turn, may be caused by an accumulation of Na^+ *via* the sodium–hydrogen exchanger (NHE) during ischemia.[1,2] This results in an intracellular Ca^{2+} overload, the detrimental effects of which include myocardial contracture, stunning, necrosis, and reperfusion arrhythmia.[3—9] NCX functions in both reverse and forward modes, and it is well known that an overactive reverse NCX causes Ca^{2+} overload. Inhibition of reverse NCX overactivity would effectively block this overload and prevent damage to the myocardium in ischemia-reperfusion. Therefore, reverse NCX inhibitors are currently considered to be beneficial in treating disease states.[10,11] Recently, quinazoline derivatives[12] and a series of benzyloxyphenyl derivatives[13—16] have been identified as NCX inhibitors. KB-R7943 (**1**) is one of the most widely known NCX inhibitors, and is used as a tool in heart and renal failure models,[17,18] and SEA0400 (**2**) is well known as a potent reverse NCX inhibitor that is efficacious in myocardial ischemia-reperfusion injury[19—22] (Fig. 1). We have recently discovered reverse NCX inhibitors, such as **3**, which we have reported elesewhere.[16,23,24] To create potent and selective reverse NCX inhibitors, we have now designed a novel class of NCX inhibitors based on **3**, and in this paper describe the results of our work on the synthesis and structure–activity relationships (SAR) of this novel class of benzyloxyphenyl derivatives.

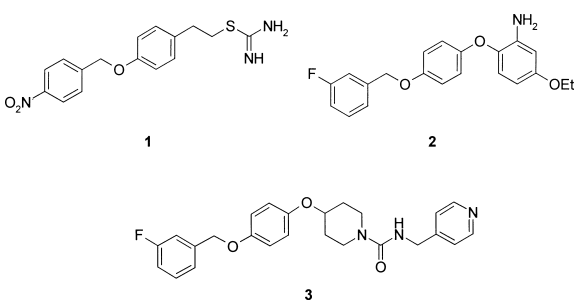

Fig. 1. Several Inhibitors of Sodium–Calcium Exchanger (**1**) KB-R7943; (**2**) SEA0400; (**3**) YM-252077

Chemistry

Compounds **4a**—**e** were converted into compounds **5a**—**e** *via* *O*-alkylation with 1-(bromomethyl)-3-fluorobenzene. Desired compounds **6a**—**e** were prepared from **5a**—**e** by condensation with pyridin-4-ylmethylamine as shown in Chart 1. Intermediate **4e** was synthesized from **7** *via* *S*-alkylation with the corresponding alkylbromide. Compounds **9a**—**c** were obtained by *O*-alkylation of **8**[15] with the corresponding alkylbromides followed by hydrolysis of the ester group. Compounds **10a**—**c** were afforded by condensation with **9a**—**c** and pyridin-4-ylmethylamine. Compound **8** was converted into a compound whose amino group was protected with a phthaloyl group, followed by deprotection of the phthaloyl group with hydrazine to give compound **11**. Compound **12** was afforded *via* condensation of **11** with pyridin-4-ylacetic acid. Compound **13** was prepared by *O*-alkylation of **8** with *tert*-butyl(2-bromoethyl)carbamate followed by deprotection of the *tert*-butoxycarbonyl group. Desired compound **14** was obtained from **8** *via* condensation with bis(trichloromethyl)carbonate and pyridin-4-ylmethylamine.

Results and Discussion

In order to measure the inhibitory effect of the synthesized compounds on the reverse mode of NCX activity, an Na^+-dependent Ca^{2+} influx assay was performed according to reported protocols, using ^{45}Ca and CCL39 cells stably expressing NCX1.1.[13,23] The inhibitory effect on the forward mode of NCX activity was assayed by a cell necrosis assay, which also used NCX1.1-expressing CCL39 cells.[23,25] The inhibitory potencies of our novel compounds were thus evaluated in both reverse and forward NCX assays. These compounds were then compared to reference compounds KB-R7943 (**1**), SEA0400 (**2**) and compound **3**.

The structure–activity relationships of our novel series of NCX inhibitors are summarized in Tables 1 and 2. We have reported a SAR of the nicotinamide part.[16] Compound

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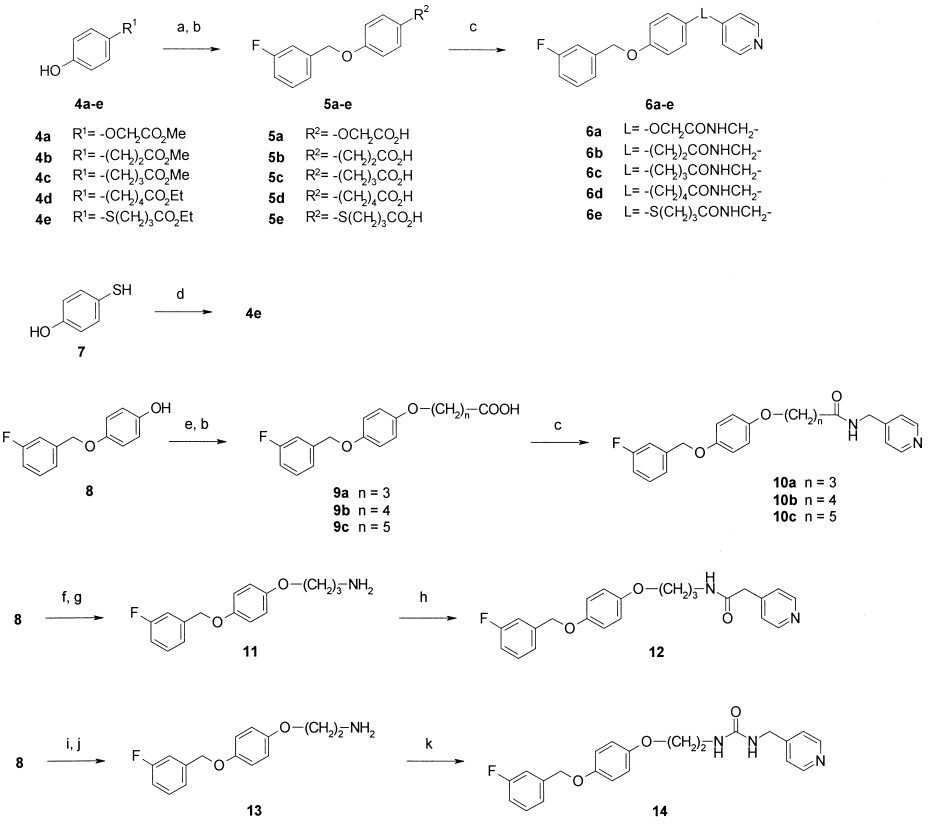


Reagents and conditions: (a) 1-(bromomethyl)-3-fluorobenzene, K_2CO_3, DMF; (b) NaOH, MeOH; (c) pyridin-4-ylmethylamine, WSC · HCl, HOBt, THF; (d) $Br(CH_2)_3CO_2Et$, K_2CO_3, MeCN; (e) $Br\text{-}(CH_2)_n\text{-}CO_2R$ (n = 3—5, R = Me, or Et), K_2CO_3, CH_3CN; (f) 2-(3-bromopropyl)-1*H*-isoindole-1,3(2*H*)-dione, K_2CO_3, CH_3CN; (g) $H_2NNH_2{\cdot}H_2O$, MeOH-$CHCl_3$; (h) pyridin-4-ylacetic acid, WSC·HCl, HOBt, THF; (i) *tert*-butyl (2-bromoethyl)carbamate, K_2CO_3, CH_3CN, 80 ºC; (j) HCl, AcOEt; (k) bis(trichloromethyl)carbonate, Et_3N, THF, then pyridin-4-ylmethylamine.

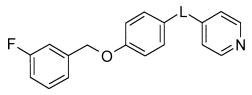
Chart 1. Preparation of Compounds **6a**—**e**, **10a**—**c**, **12** and **14**

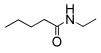
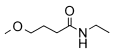
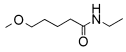
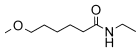
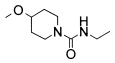
3, with an *N*-(pyridin-4-ylmethyl)piperidine-1-carboxamide structure, had a potent inhibitory activity against reverse NCX with an IC_{50} value of 0.22 μM. To create novel structures in the position of the piperidine-1-carboxamide moiety, we attempted to change the piperidine-1-carboxamide linker into an alkyl chain structure. To examine the effect of the length of the alkyl-linker on reverse NCX inhibitory activity, we evaluated compounds with several different lengths of structure between the phenoxy and pyridine parts (Table 1). Compounds **6a** and **6b**, with a linker length of 5 atoms between the phenoxy part and the pyridine ring produced an extreme reduction in reverse NCX inhibitory activity compared to compound **3**. A compound with a 6-atoms linker (**6c**) had similar inhibitory activity as **3**. When we changed the linker length to 7 atoms (**10a**), the inhibitory activity was increased. This prompted us to introduce longer linkages, with 8 atoms (**10b**) and 9 atoms (**10c**) between the phenoxy part and the pyridine ring. However, the inhibitory activities of **10b** and **10c** were reduced with increasing linker length. Compound **10a** showed the most potent inhibitory activity, with an IC_{50} value of 0.10 μM against reverse NCX (Table 1). The results indicated that linker length is important for inhibitory activity against reverse NCX, and also prompted us to introduce another linker connecting the phenoxy part with the pyridine ring as shown in Table 2. Replacements of –O– (**10a**) with –C– (**6d**) and –S– (**6e**) slightly reduced inhibitory activity against reverse NCX. Compound **12**, with an inverse amide to compound **10a**, also showed slightly increased inhibitory activity over **10a** with an IC_{50} value of 0.085 μM against reverse NCX. Compound **12** was 3-fold more potent than SEA0400 (**2**), and also showed higher selectivity. Replacement of the amide linkage with a urea linkage (**14**) resulted in a 2.6-fold decrease in inhibitory potency against reverse NCX compared to **10a**. Each of these derivatives with an alkyl chain linker showed higher selectivity than **3**.

Conclusion

A series of benzyloxyphenyl derivatives have been prepared and evaluated for their inhibitory activities against the reverse and forward modes of NCX. By modifying the piperidine-1-carboxamide moiety, we found that a compound containing a *N*-propyl-2-pyridin-4-ylacetamide (**12**), rather than a *N*-(pyridin-4-ylmethyl)piperidine-1-carboxamide (**3**), had enhanced reverse NCX inhibitory activity and increased selectivity. Compound **12** (YM-270951) had an IC_{50} value of 0.085 μM against reverse NCX and higher selectivity. The reverse NCX inhibitory activity of **12** was approximately 3-times greater than that of of SEA0400 (**2**). This study could

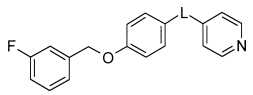
Table 1. Inhibitory Activity of Compounds **6a**—**c** and **10a**—**c** against the Sodium–Calcium Exchanger

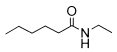
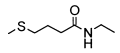
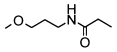
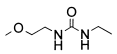
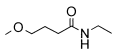
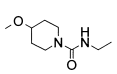


Compd.	L	^{45}Ca influx[a] IC_{50} (μM)[c]	Cell necrosis[b] EC_{50} (μM)[c]	Selectivity[d]
6a		17	NT[e]	—
6b		5.6	NT[e]	—
6c		0.25	50	200
10a		0.10	43	430
10b		0.23	>100	>430
10c		0.63	63	100
3		0.22	27	120
SEA0400 (**2**)		0.29	98	340
KB-R7943 (**1**)		5.1	24	4.7

a) Activity against the NCX1.1 expressed in CCL39 cells. ^{45}Ca influx reflects NCX inhibitory activity in the reverse mode. *b*) Activity against the NCX1.1 expressed in CCL39 cells. Cell necrosis reflects NCX inhibitory activity in the forward mode. *c*) IC_{50} values and EC_{50} values were determined in a single experimental run in triplicate. *d*) Ratio of EC_{50} value of cell necrosis and IC_{50} value of ^{45}Ca influx. *e*) Not tested.

Table 2. Inhibitory Activity of Compounds **6d**, **6e**, **12** and **14** against the Sodium–Calcium Exchanger



Compd.	L	^{45}Ca influx[a] IC_{50} (μM)[c]	Cell necrosis[b] EC_{50} (μM)[c]	Selectivity[d]
6d		0.17	34	200
6e		0.20	48	240
12		0.085	42	490
14		0.26	40	150
10a		0.10	43	430
3		0.22	27	120

For footnotes *a*—*d*) refer to Table 1.

provide a novel approach to more potent reverse NCX inhibitors with higher selectivity.

Experimental

Chemistry Melting points were determined with a Yanaco MP-500D melting point apparatus or a Buchi B-545 melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-LA300 or a JNM-EX400 spectrometer and the chemical shifts are expressed in δ (ppm) values with tetramethylsilane as an internal standard (in NMR description, s=singlet, d=doublet, t=triplet, m=multiplet, and br=broad peak). Mass spectra were recorded on a Hitachi M-80 or a JEOL JMS-LX2000 spectrometer. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and were within ±0.4% of theoretical values. Drying of organic solutions during workup was done over anhydrous Na_2SO_4.

Ethyl 4-[(4-Hydroxyphenyl)sulfanyl]butanoate (4e) To a mixture of 4-sulfanylphenol (**7**) (1.26 g, 10.0 mmol) in MeCN (30 ml) were added K_2CO_3 (2.07 g, 15 mmol) and ethyl 4-bromobutanoate (1.57 ml, 11.0 mmol) at room temperature. The mixture was stirred for 1 h and partitioned between $CHCl_3$ and H_2O. The organic layer was dried and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane : AcOEt=1 : 0—4 : 1) to give **4e** as a colorless oil (2.22 g, 92%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.25 (3H, t, J=7.1 Hz), 1.83—1.94 (2H, m), 2.44 (2H, t, J=7.1 Hz), 2.84 (2H, t, J=7.1 Hz), 4.12 (2H, q, J=7.1 Hz), 5.17 (1H, s), 6.77 (2H, d, J=8.6 Hz), 7.30 (2H, d, J=8.7 Hz); MS (FAB) *m/z* 240 $(M)^+$.

{4-[(3-Fluorobenzyl)oxy]phenoxy}acetic Acid (5a) A mixture of methyl (4-hydroxyphenoxy)acetate (**4a**) (500 mg, 2.74 mmol), K_2CO_3 (455 mg, 3.29 mmol) in DMF (5 ml) was added 1-(bromomethyl)-3-fluorobenzene (0.35 ml, 2.85 mmol) at room temperature. The mixture was stirred at room temperature for overnight. Water was added to the mixture and appeared precipitate was collected to give methyl {4-[(3-fluorobenzyl)oxy]phenoxy}acetate as a colorless solid (797 mg, 100%). The mixture of methyl {4-[(3-fluorobenzyl)oxy]phenoxy}acetate (747 mg, 2.57 mmol) in MeOH (15 ml) and 1 M NaOH (3.09 ml) was stirred at 60 °C for 3 h. To the mixture was added 1 M HCl. The precipitate was collected and washed with H_2O to afford **5a** as a colorless solid (637 mg, 90%): ^{1}H-NMR (400 MHz, DMSO-d_6) δ 4.58 (2H, s), 5.06 (2H, s), 6.84 (2H, d, J=9.3 Hz), 6.93 (2H, d, J=9.3 Hz), 7.11—7.18 (1H, m), 7.23—7.29 (2H, m), 7.39—7.46 (1H, m), 12.93 (1H, br s); MS (FAB) *m/z* 275 $(M-H)^-$.

3-{4-[(3-Fluorobenzyl)oxy]phenyl}propanoic Acid (5b) Compound **5b** was prepared from **4b** by a procedure similar to that described for **5a**. Compound **5b** was obtained as a colorless solid (89% in 2 steps): ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.45—2.52 (2H, m), 2.75 (2H, t, J=7.5 Hz), 5.09 (2H, s), 6.92 (2H, d, J=8.8 Hz), 7.11—7.18 (3H, m), 7.24—7.30 (2H, m), 7.40—7.46 (1H, m), 12.07 (1H, s); MS (FAB) *m/z* 274 $(M)^+$.

4-{4-[(3-Fluorobenzyl)oxy]phenyl}butanoic Acid (5c) Compound **5c** was prepared from **4c** by a procedure similar to that described for **5a**. Compound **5c** was obtained as a colorless solid (89% in 2 steps): ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.72—1.80 (2H, m), 2.19 (2H, t, J=7.4 Hz), 2.49—2.55 (2H, m), 5.10 (2H, s), 6.93 (2H, d, J=8.5 Hz), 7.10 (2H, d, J=8.5 Hz), 7.10—7.18 (1H, m), 7.24—7.30 (2H, m), 7.40—7.47 (1H, m), 12.03 (1H, s); MS (FAB) *m/z* 288 $(M)^+$.

5-{4-[(3-Fluorobenzyl)oxy]phenyl}pentanoic Acid (5d) Compound **5d** was prepared from **4d** by a procedure similar to that described for **5a**. Compound **5d** was obtained as a colorless powder (94% in 2 steps): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.45—1.57 (4H, m), 2.15—2.25 (2H, m), 2.48—2.54 (2H, m), 5.09 (2H, s), 6.91 (2H, d, J=8.5 Hz), 7.10 (2H, d, J=8.5 Hz), 7.13—7.19 (1H, m), 7.23—7.30 (2H, m), 7.39—7.47 (1H, m), 11.98 (1H, br s); MS (FAB) *m/z* 301 $(M)^-$.

4-({4-[(3-Fluorobenzyl)oxy]phenyl}sulfanyl)butanoic Acid (5e) Compound **5e** was prepared from **4e** by a procedure similar to that described for **5a**. Compound **5e** was obtained as a colorless solid (95% in 2 steps): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.63—1.75 (2H, m), 2.26 (2H, t, J=7.2 Hz), 2.85 (2H, t, J=7.2 Hz), 5.11 (2H, s), 6.99 (2H, d, J=8.8 Hz), 7.12—7.20 (1H, m), 7.24—7.35 (4H, m), 7.39—7.48 (1H, m); MS (FAB) *m/z* 319 $(M)^-$.

2-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyridin-4-ylmethyl)acetamide Hydrochloride (6a) To a mixture of pyridin-4-ylmethylamine (108 mg, 1.00 mmol), 1-hydroxybenzotriazole (HOBt) (68 mg, 0.50 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSC·HCl) (211 mg, 1.10 mmol) and THF (5 ml) was added **5a** (276 mg, 1.00 mmol) at room temperature. The mixture was stirred for 2 h. The reaction mixture was quenched by saturated $NaHCO_3$. The mixture was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried and concentrated *in vacuo* to give light yellow solid. The residue was purified by column chromatography on silica gel ($CHCl_3$: MeOH=98 : 2) to afford a free base of **6a** (256 mg). The compound was dissolved in AcOEt, CH_3CN and MeOH, the mixture was treated with 4 M HCl/AcOEt (0.210 ml, 0.838 mmol) and was recrystallized to give **6a** as a colorless solid (182 mg, 45%): mp 154—157 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 4.57 (2H, s), 4.58 (2H, s), 5.09 (2H, s), 6.93—7.01 (4H, m), 7.12—7.19 (1H, m), 7.24—7.32 (2H, m),

7.41—7.47 (1H, m), 7.80 (2H, d, J=6.3 Hz), 8.79 (2H, d, J=6.4 Hz), 8.96 (1H, t, J=6.3 Hz); MS (FAB) m/z 367 $(M+H)^+$. *Anal.* Calcd for $C_{21}H_{19}N_2O_3F \cdot 0.95HCl$: C, 62.90; H, 5.01; N, 6.99; F, 4.74; Cl, 8.40. Found: C, 63.12; H, 5.04; N, 7.16; F, 4.56; Cl, 8.44.

3-{4-[(3-Fluorobenzyl)oxy]phenyl}-*N*-(pyridin-4-ylmethyl)propanamide Hydrochloride (6b) Compound **6b** was prepared from **5b** by a procedure similar to that described for **6a**. Compound **6b** was obtained as a colorless solid (70%): mp 176—177 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.48—2.54 (2H, m), 2.80 (2H, t, J=7.5 Hz), 4.49 (2H, d, J=3.8 Hz), 5.11 (2H, s), 6.94 (2H, d, J=8.8 Hz), 7.11—7.19 (3H, m), 7.25—7.32 (2H, m), 7.41—7.47 (1H, m), 7.70 (2H, d, J=6.4 Hz), 8.68—8.74 (1H, m), 8.76 (2H, d, J=6.3 Hz); MS (FAB) m/z 365 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{21}N_2O_2F \cdot HCl$: C, 65.91; H, 5.53; N, 6.99; F, 4.74; Cl, 8.84. Found: C, 65.62; H, 5.55; N, 6.95; F, 4.75; Cl, 8.60.

4-{4-[(3-Fluorobenzyl)oxy]phenyl}-*N*-(pyridin-4-ylmethyl)butanamide Hydrochloride (6c) Compound **6c** was prepared from **5c** by a procedure similar to that described for **6a**. Compound **6c** was obtained as a colorless solid (42%): mp 109—112 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.76—1.88 (2H, m), 2.23 (2H, t, J=7.8 Hz), 2.48—2.55 (2H, m), 4.50 (2H, d, J=5.9 Hz), 5.10 (2H, s), 6.93 (2H, d, J=8.8 Hz), 7.09—7.19 (3H, m), 7.24—7.30 (2H, m), 7.41—7.47 (1H, m), 7.82 (2H, d, J=6.3 Hz), 8.68 (1H, t, J=5.9 Hz), 8.82 (2H, d, J=6.9 Hz); MS (FAB) m/z 379 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{23}N_2O_2F \cdot HCl$: C, 66.58; H, 5.83; N, 6.75; F, 4.58; Cl, 8.54. Found: C, 66.44; H, 5.91; N, 6.73; F, 4.42; Cl, 8.52.

***N*-(4-{4-[(3-Fluorobenzyl)oxy]phenyl}butyl)-2-pyridin-4-ylacetamide Oxalate (6d)** To a mixture of pyridin-4-ylmethylamine (108 mg, 1.00 mmol), 1-hydroxybenzotriazole (HOBt) (68 mg, 0.50 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSC·HCl) (211 mg, 1.10 mmol) and THF (6 ml) was added **5d** (302 mg, 1.00 mmol) at room temperature. The mixture was stirred for 3 h. The reaction mixture was quenched by saturated $NaHCO_3$. The mixture was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried and concentrated *in vacuo* to give light yellow solid. The residue was purified by column chromatography on silica gel ($CHCl_3$: MeOH=98 : 2—96 : 4) to afford a free base of **6d** (371 mg). The compound was dissolved in MeOH. To the mixture was added oxalic acid (94 mg, 1.04 mmol). The mixture was concentrated *in vacuo*, the residue was recrystallized from AcOEt–CH_3CN (5 : 1) to give **6d** as a colorless powder (284 mg, 59%): mp 128—130 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.49—1.47 (4H, m), 2.16—2.22 (2H, m), 2.48—2.54 (2H, m), 4.29 (2H, d, J=5.9 Hz), 5.09 (2H, s), 6.92 (2H, d, J=8.3 Hz), 7.10 (2H, d, J=8.3 Hz), 7.12—7.18 (1H, m), 7.24—7.30 (4H, m), 7.40—7.49 (1H, m), 8.34—8.44 (1H, m), 8.47—8.53 (2H, m); MS (FAB) m/z 393 $(M+H)^+$. *Anal.* Calcd for $C_{24}H_{25}N_2O_2F \cdot (CO_2H)_2$: C, 64.72; H, 5.64; N, 5.81; F, 3.94. Found: C, 64.65; H, 5.57; N, 5.75; F, 4.06.

4-({4-[(3-Fluorobenzyl)oxy]phenyl}sulfanyl)-*N*-(pyridin-4-ylmethyl)-butanamide Hydrochloride (6e) Compound **6e** was prepared from **5e** by a procedure similar to that described for **6a**. Compound **6e** was obtained as a colorless powder (57%): mp 101—103 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.72—1.82 (2H, m), 2.36 (2H, t, J=7.3 Hz), 2.87 (2H, t, J=7.3 Hz), 4.50 (2H, d, J=5.4 Hz), 5.12 (2H, s), 7.00 (2H, d, J=8.8 Hz), 7.13—7.19 (1H, m), 7.25—7.35 (4H, m), 7.41—7.48 (1H, m), 7.82 (2H, d, J=6.9 Hz), 8.70—8.77 (1H, m), 8.81 (2H, d, J=6.3 Hz); MS (FAB) m/z 411 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{23}N_2O_2SF \cdot HCl$: C, 60.11; H, 5.57; N, 6.10; F, 4.13; Cl, 7.71; S, 6.98. Found: C, 59.91; H, 5.44; N, 6.07; F, 3.92; Cl, 7.65; S, 6.98.

4-{4-[(3-Fluorobenzyl)oxy]phenoxy}butanoic Acid (9a) To a mixture of 4-[(3-fluorobenzyl)oxy]phenol **8** (436 mg, 2.00 mmol), K_2CO_3 (332 mg, 2.40 mmol) in CH_3CN (10 ml) was added ethyl 4-bromobutanoate (0.315 ml, 2.20 mmol) at room temperature. The mixture was stirred at 80 °C for 26 h. The mixture was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane : AcOEt=1 : 0—4 : 1) to give ethyl 4-{4-[(3-fluorobenzyl)oxy]phenoxy}butanoate as a colorless oil (630 mg, 95%). The mixture of the intermediate (620 mg, 1.87 mmol) in 1 M NaOH (2.8 ml, 2.8 mmol) and EtOH (5 ml) was stirred at room temperature for 4 h. The mixture was concentrated *in vacuo*. 1 M HCl was added to the residue. The precipitate was collected to afford **9a** as a colorless powder (537 mg, 94%): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.84—1.95 (2H, m), 2.32—2.40 (2H, m), 3.87—3.94 (2H, m), 5.06 (2H, s), 6.85 (2H, d, J=9.1 Hz), 6.93 (2H, d, J=9.1 Hz), 7.10—7.18 (1H, m), 7.22—7.30 (2H, m), 7.38—7.47 (1H, m), 12.11 (1H, s); MS (FAB) m/z 304 $(M)^-$.

5-{4-[(3-Fluorobenzyl)oxy]phenoxy}pentanoic Acid (9b) Compound **9b** was prepared from **8** by a procedure similar to that described for **9a**. Compound **9b** was obtained as a colorless powder (92% in 2 steps): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.58—1.73 (4H, m), 2.23—2.30 (2H, m), 3.86—3.92 (2H, m), 5.06 (2H, s), 6.85 (2H, d, J=9.2 Hz), 6.93 (2H, d, J=9.2 Hz), 7.10—7.18 (1H, m), 7.22—7.30 (2H, m), 7.38—7.47 (1H, m); MS (FAB) m/z 317 $(M)^-$.

6-{4-[(3-Fluorobenzyl)oxy]phenoxy}hexanoic Acid (9c) Compound **9c** was prepared from **8** by a procedure similar to that described for **9a**. Compound **9c** was obtained as a colorless powder (92% in 2 steps): ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.33—1.46 (2H, m), 1.49—1.60 (2H, m), 1.62—1.73 (2H, m), 2.18—2.26 (2H, m), 3.84—3.91 (2H, m), 5.06 (2H, s), 6.84 (2H, d, J=9.2 Hz), 6.93 (2H, d, J=9.2 Hz), 7.10—7.18 (1H, m), 7.22—7.30 (2H, m), 7.38—7.47 (1H, m), 11.99 (1H, br s); MS (FAB) m/z 331 $(M)^-$.

4-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyridin-4-ylmethyl)butanamide Hydrochloride (10a) Compound **10a** was prepared from **9a** by a procedure similar to that described for **6a**. Compound **10a** was obtained as a colorless powder (77%): mp 144—146 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.90—2.00 (2H, m), 2.80—2.41 (2H, m), 3.91 (2H, t, J=6.3 Hz), 4.52 (2H, d, J=5.9 Hz), 5.07 (2H, s), 6.85 (2H, d, J=9.3 Hz), 6.94 (2H, d, J=9.3 Hz), 7.11—7.18 (1H, m), 7.23—7.30 (2H, m), 7.39—7.47 (1H, m), 7.83 (2H, d, J=6.4 Hz), 8.73—8.83 (3H, m); MS (FAB) m/z 395 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{23}N_2O_3F \cdot HCl$: C, 64.11; H, 5.61; N, 6.50; F, 4.41; Cl, 8.23. Found: C, 63.99; H, 5.63; N, 6.51; F, 4.29; Cl, 8.26.

5-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyridin-4-ylmethyl)pentanamide Hydrochloride (10b) Compound **10b** was prepared from **9b** by a procedure similar to that described for **6a**. Compound **10b** was obtained as a beige powder (79%): mp 110—112 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.66—1.72 (4H, m), 2.26—2.34 (2H, m), 3.88—3.93 (2H, m), 4.51 (2H, d, J=5.9 Hz), 5.06 (2H, s), 6.85 (2H, d, J=8.8 Hz), 6.93 (2H, d, J=8.8 Hz), 7.11—7.18 (1H, m), 7.23—7.29 (2H, m), 7.39—7.46 (1H, m), 7.83 (2H, d, J=6.8 Hz), 8.69—8.74 (1H, m), 8.81 (2H, d, J=6.9 Hz); MS (FAB) m/z 409 $(M+H)^+$. *Anal.* Calcd for $C_{24}H_{25}N_2O_3F \cdot HCl$: C, 64.79; H, 5.89; N, 6.30; F, 4.27; Cl, 7.97. Found: C, 64.68; H, 5.83; N, 6.21; F, 4.27; Cl, 8.09.

6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyridin-4-ylmethyl)hexanamide Hydrochloride (10c) Compound **10c** was prepared from **9c** by a procedure similar to that described for **6a**. Compound **10c** was obtained as a beige powder (68%): mp 125—127 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.36—1.46 (2H, m), 1.55—1.73 (4H, m), 2.22—2.28 (2H, m), 3.89 (2H, t, J=6.3 Hz), 4.51 (2H, d, J=5.8 Hz), 5.06 (2H, s), 6.84 (2H, d, J=9.2 Hz), 6.93 (2H, d, J=9.2 Hz), 7.11—7.18 (1H, m), 7.22—7.30 (2H, m), 7.39—7.46 (1H, m), 7.82 (2H, d, J=6.3 Hz), 8.66—8.72 (1H, m), 8.80 (2H, d, J=6.3 Hz); MS (FAB) m/z 423 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{27}N_2O_3F \cdot HCl$: C, 65.42; H, 6.15; N, 6.10; F, 4.14; Cl, 7.72. Found: C, 65.27; H, 6.14; N, 6.09; F, 4.21; Cl, 7.69.

(3-{4-[(3-Fluorobenzyl)oxy]phenoxy}propyl)amine (11) To a mixture of 4-[(3-fluorobenzyl)oxy]phenol **8** (655 mg, 3.0 mmol) and K_2CO_3 (829 mg, 6.0 mmol) in MeCN (10 ml) was added 2-(3-bromopropyl)-1*H*-isoindole-1,3(2*H*)-dione (1.05 g, 3.90 mmol) at room temperature. The mixture was stirred at 80 °C. H_2O was added to the mixture. The precipitate was collected by filtration, washed with H_2O and dried *in vacuo* to give 2-(3-{4-[(3-fluorobenzyl)oxy]phenoxy}propyl)-1*H*-isoindole-1,3(2*H*)-dione as a light brown powder (1.21 g, 99%). The mixture of 2-(3-{4-[(3-fluorobenzyl)oxy]phenoxy}propyl)-1*H*-isoindole-1,3(2*H*)-dione (1.21 g, 2.98 mmol) and hydrazine hydrate (448 mg, 8.94 mmol) in MeOH (10 ml) and $CHCl_3$ (20 ml) was stirred at room temperature for overnight. The precipitate was filtered and washed with $CHCl_3$. The filtrate was washed with aqueous NaOH and brine and dried, concentrated *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$: MeOH=88 : 12) to give **11** as a beige solid (539 mg, 66%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.85—1.96 (2H, m), 2.87—2.93 (2H, m), 3.97—4.04 (2H, m), 5.01 (2H, s), 6.83 (2H, d, J=9.2 Hz), 6.89 (2H, d, J=9.2 Hz), 6.96—7.04 (1H, m), 7.12—7.21 (2H, m), 7.21—7.28 (1H, m); MS (FAB) m/z 276 $(M+H)^+$.

***N*-(3-{4-[(3-Fluorobenzyl)oxy]phenoxy}propyl)-2-pyridin-4-ylacetamide Hydrochloride (12)** Compound **12** was prepared from **11** by a procedure similar to that described for **6a**. Compound **12** was obtained as a slightly pink powder (93%): mp 143—145 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.33—3.40 (2H, m), 3.91 (2H, t, J=5.6 Hz), 4.47 (2H, d, J=5.3 Hz), 5.07 (2H, s), 6.55—6.65 (1H, m), 6.88 (2H, d, J=9.3 Hz), 6.92—6.97 (3H, m), 7.11—7.18 (1H, m), 7.23—7.29 (2H, m), 7.40—7.47 (1H, m), 7.84 (2H, d, J=6.3 Hz), 8.81 (2H, d, J=6.4 Hz); MS (FAB) m/z 395 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{23}N_2O_3F \cdot HCl$: C, 64.11; H, 5.61; N, 6.50; F, 4.41; Cl, 8.23. Found: C, 64.01; H, 5.53; N, 6.47; F, 4.30; Cl, 8.09.

(2-{4-[(3-Fluorobenzyl)oxy]phenoxy}ethyl)amine (13) A mixture of *tert*-butyl(2-bromoethyl)carbamate (1.0 g, 4.5 mmol), 4-[(3-fluorobenzyl)-

oxy]phenol **8** (655 mg, 3.00 mmol) and K_2CO_3 (1.24 g, 9.00 mmol) in MeCN (20 ml) was stirred at 80 °C for over night. The mixture was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel ($CHCl_3$: MeOH=99 : 1) to give *tert*-butyl (2-{4-[(3-fluorobenzyl)oxy]phenoxy}ethyl)carbamate as a light syrup (604 mg). The mixture of *tert*-butyl (2-{4-[(3-fluorobenzyl)oxy]phenoxy}ethyl)-carbamate (604 mg, 1.67 mmol) and AcOEt (5 ml) and 4 M AcOEt solution of HCl (2.1 ml, 8.36 mmol) was stirred at room temperature for 6.5 h. The mixture was concentrated *in vacuo*. The residue was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried and concentrated *in vacuo* to give **13** as a beige syrup (448 mg, 57% in 2 steps): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.06 (2H, d, J=5.1 Hz), 3.94 (2H, d, J=5.1 Hz), 5.01 (2H, s), 6.84 (2H, d, J=9.2 Hz), 6.89 (2H, d, J=9.3 Hz), 6.96—7.04 (1H, m), 7.12—7.21 (2H, m), 7.29—7.38 (1H, m); MS (FAB) m/z 262 $(M+H)^+$.

***N*-(2-{4-[(3-Fluorobenzyl)oxy]phenoxy}ethyl)-*N'*-(pyridin-4-ylmethyl)urea Hydrochloride (14)** To a mixture of bis(trichloromethyl)carbonate (190 mg, 0.64 mmol) in THF (5 ml) was added a mixture of **13** (430 mg, 1.60 mmol), Et_3N (0.335 ml, 2.40 mmol) in THF (5 ml) at 0 °C. The mixture was stirred at room temperature for 1 h. To the mixture was added a mixture of 4-aminomethylpyridine (208 mg, 1.92 mmol), Et_3N (0.268 ml, 1.92 mmol) in THF (5 ml) at 6 °C. The mixture was stirred at room temperature for 1 h. The reaction mixture was quenched by saturated $NaHCO_3$. The mixture was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried and concentrated *in vacuo* to give light yellow solid. The residue was purified by column chromatography on silica gel ($CHCl_3$: MeOH=92 : 8) to afford a free base of **14** (260 mg). The compound was dissolved in AcOEt (7 ml) and MeOH (5 ml), the mixture was treated with 4 M HCl/AcOEt (0.197 ml, 0.789 mmol) and concentrated *in vacuo*. The residue was recrystallized from AcOEt–CH_3CN (5 : 2) to give **14** as a beige powder (231 mg, 33%): mp 115—116 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.33—3.40 (2H, m), 3.91 (2H, t, J=5.6 Hz), 4.47 (2H, d, J=5.3 Hz), 5.07 (2H, s), 6.55—6.65 (1H, m), 6.88 (2H, d, J=9.3 Hz), 6.92—6.97 (3H, m), 7.11—7.18 (1H, m), 7.23—7.29 (2H, m), 7.40—7.47 (1H, m), 7.84 (2H, d, J=6.3 Hz), 8.81 (2H, d, J=6.4 Hz); MS (FAB) m/z 396 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{22}N_3O_3F \cdot HCl \cdot H_2O$: C, 58.73; H, 5.60; N, 9.34; F, 4.22; Cl, 7.88. Found: C, 58.79; H, 5.36; N, 9.54; F, 4.14; Cl, 7.85.

Pharmacology The methods of ^{45}Ca influx assay and Cell necrosis assay were described in previous report.[23)]

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