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Synthesis and structure–activity relationships of benzyloxyphenyl derivatives as a novel class of NCX inhibitors: effects on heart failure

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Abstract—In the context of heart failure and myocardial ischemia reperfusion, the activity of the sodium–calcium exchanger can lead to calcium overload, which in turn can lead to contractile dysfunction and arrhythmia. Therefore, NCX is an attractive target for treatment of heart failure and myocardial ischemia reperfusion. We have designed and synthesized a series of benzyloxyphenyl derivatives as potential NCX inhibitors, based on compound **4**. These derivatives have been evaluated for their inhibitory activity against both the reverse and forward modes of NCX, and two novel potent NCX inhibitors (**7i**, **10a**) were discovered. Compound **7i** was evaluated for its efficacy on ouabain-induced tonotropy and arrhythmia in a heart-failure model.

1. Introduction

The sodium–calcium exchanger (NCX) is a transporter that controls the Ca^{2+} concentration in myocytes.[1] In myocardial ischemia, the Na^{+} concentration in myocytes is raised via the sodium–hydrogen exchanger (NHE).[2,3] This causes NCX-mediated efflux of Na^{+} ions to the extracellular fluid and influx of Ca^{2+} ions into cells, thus inducing calcium overload in ischemia reperfusion. Such calcium overload causes necrosis of myocytes, and is responsible for contractile dysfunction and arrhythmia.[4–7] NCX typically functions in the forward mode, but the reverse mode has a more important role with regard to calcium overload. Consequently, selective inhibition of reverse NCX function could provide a novel therapeutic approach to the prevention and treatment of reperfusion arrhythmias, aberrant myocardial contracture, and necrosis. Indeed, reverse mode NCX inhibitors are currently considered to be beneficial in treating these disease states.[8,9]

A number of compounds, including the quinazoline derivative, **1**[10] and several benzyloxyphenyl derivatives (**2**–**4**),[11–13] have been reported to be NCX inhibitors (Fig. 1). Among these, KB-R7943 (**2**) has been shown to be therapeutically effective for ischemia-reperfusion injury. In a previous paper, we reported the synthesis of a series of 6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide derivatives, and studied the SAR of these derivatives.[13] *N*-Benzyl-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide (**4**) was found to be a highly potent and selective inhibitor of reverse NCX activity. In the present paper, we have designed further novel and more potent reverse NCX inhibitors, based on compound **4**, and we describe the synthesis, biological activities and SAR of a novel class of benzyloxyphenyl derivatives.

2. Chemistry

Syntheses of the novel NCX inhibitors are summarized in Schemes 1 and 2. In Scheme 1, compound **6** was obtained from **5**[13] by hydrolysis of the cyano group. Compounds **7a** and **7c**–**i** were prepared from **6** by amidation with various amines. Compound **7b** was afforded by condensation of **6** and (1-trityl-1*H*-imidazol-4-yl)methylamine,

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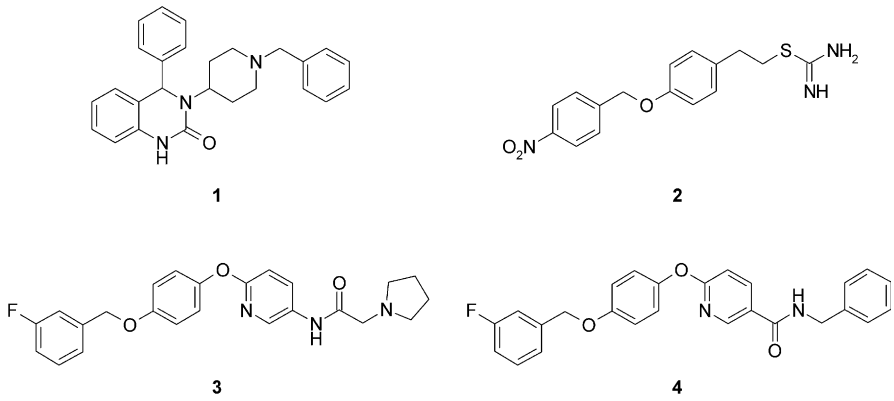
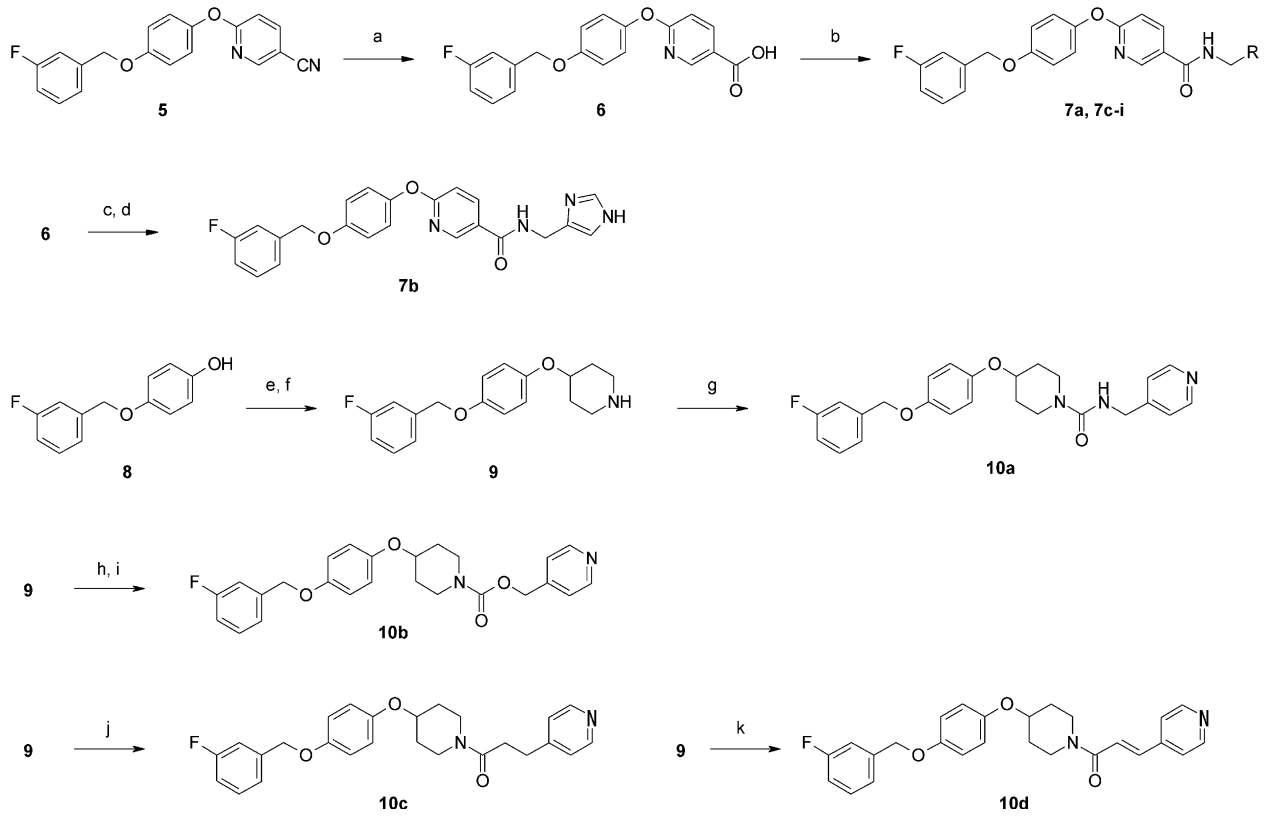


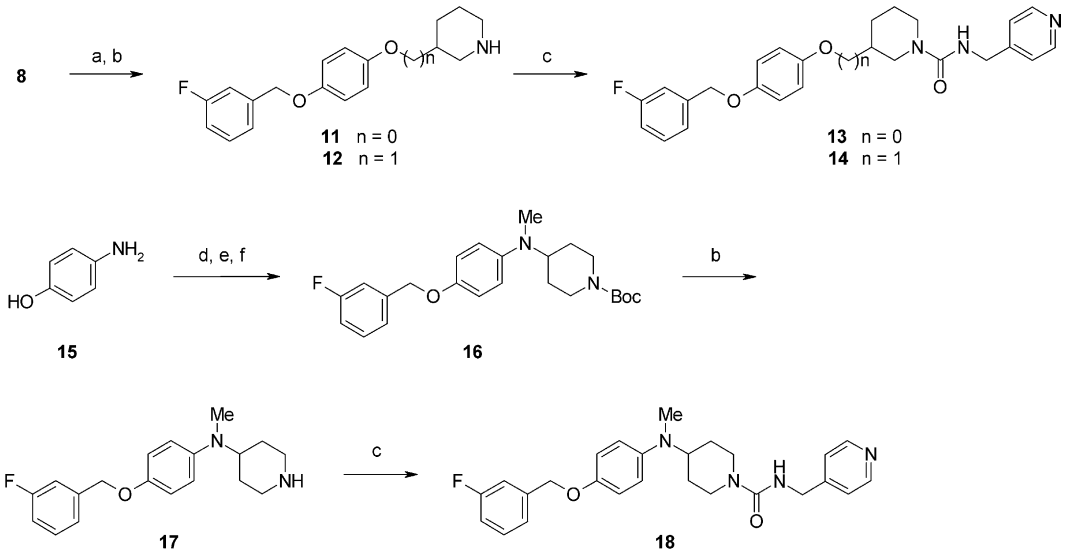
Figure 1. Several inhibitors of sodium–calcium exchanger. (**1**) SM-15811; (**2**) KB-R7943; (**3**) patented compound of JP11092454; (**4**) nicotinamide derivative.



Scheme 1. Reagents and conditions: (a) 5 M NaOH, EtOH, reflux; (b) WSC·HCl, HOBt, RCH_2NH_2, THF; (c) WSC·HCl, HOBt, (1-trityl-1*H*-imidazol-4-yl)methylamine, THF; (d) AcOH, 70 °C; (e) PPh_3, *t*-butyl 4-hydroxypiperidine-1-carboxylate, DEAD, THF; (f) HCl, AcOEt–MeOH; (g) bis(trichloromethyl)carbonate, Et_3N, THF, then pyridin-4-ylmethylamine; (h) methyl chloroformate, Et_3N, THF; (i) pyridin-4-ylmethanol, NaH, toluene, reflux; (j) WSC·HCl, HOBt, 3-(pyridin-4-yl)propanoic acid, THF; (k) WSC·HCl, HOBt, (2*E*)-3-(pyridin-4-yl)acrylic acid, THF.

followed by removal of the trityl group. Mitsunobu condensation of the starting material **8**[12] and *t*-butyl 4-hydroxypiperidine-1-carboxylate, followed by deprotection of the *t*-butoxycarbonyl group with hydrochloric acid, produced the intermediate amine **9**, which on treatment with bis(trichloromethyl)carbonate and pyridin-4-ylmethylamine yielded the desired compound **10a**. Compound **10b** was prepared from intermediate amine **9** by condensation with methyl chloroformate, followed by a substitution reaction with pyridin-4-ylmethanol. Amide condensation of intermediate **9** with the corresponding amines afforded compounds **10c** and **10d**, respectively.

Mitsunobu condensation of compound **8** and *t*-butyl 3-hydroxypiperidine-1-carboxylate, followed by deprotection of the *t*-butoxycarbonyl group with hydrochloric acid, afforded intermediate amine **11**, as shown in Scheme 2. Compound **12** was prepared from **8** by a procedure similar to that used for compound **11**. Desired compounds **13** and **14** were obtained from **11** and **12**,



Scheme 2. Reagents and conditions: (a) DEAD, PPh_3, *t*-butyl 3-hydroxypiperidine-1-carboxylate, or *t*-butyl 3-hydroxymethylpiperidine-1-carboxylate, THF; (b) HCl, AcOEt–MeOH; (c) bis(trichloromethyl)carbonate, Et_3N, THF, then pyridin-4-ylmethylamine; (d) 1-(bromomethyl)-3-fluorobenzene, *t*-BuOK, DMF; (e) *t*-butyl 4-oxopiperidine-1-carboxylate, $NaBH(OAc)_3$, AcOH; (f) formaldehyde, $NaBH(OAc)_3$, AcOH.

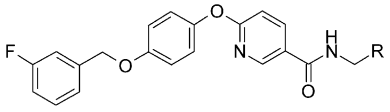
respectively, by amide condensation. *O*-alkylation of **15** with 1-(bromomethyl)-3-fluorobenzene, followed by reductive alkylations with *t*-butyl 4-oxopiperidine-1-carboxylate and formaldehyde, afforded **16**. Compound **16** was converted to **17** by deprotection of the *t*-butoxycarbonyl moiety with hydrochloric acid. Urea condensation of **17** with pyridin-4-ylmethylamine gave desired compound **18**.

3. Results and discussion

In order to measure the inhibitory effect of the synthesized compounds on reverse mode NCX activity, a Na^+-dependent Ca^{2+} influx assay was performed according to reported protocols, using ^{45}Ca and CCL39 cells that stably express NCX1.1.[11,13] The inhibitory effect on forward mode NCX activity was assayed using a cell necrosis assay, in which NCX1.1-expressing CCL39 cells were also used.[13,14] The inhibitory potencies of our novel compounds were thus evaluated in both reverse and forward NCX assays. Results for these compounds were then compared to those for KB-R7943 (**2**) and compounds **3** and **4**. The effects of selected compounds on the tonotropic effects of ouabain and the time for ouabain-induced arrhythmia onset in isolated guinea pig atria were also tested.

The structure–activity relationships of our novel series of NCX inhibitors are summarized in Tables 1 and 2.

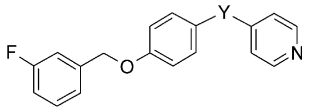
Table 1. Inhibitory activity of benzyloxyphenyl derivatives against the sodium–calcium exchanger

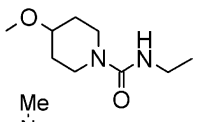
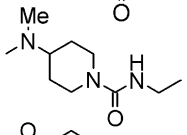
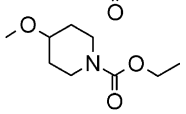
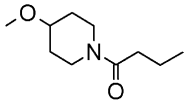
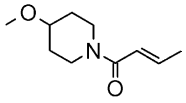
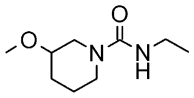
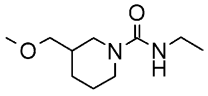
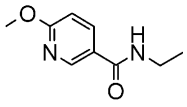


Compound	R	^{45}Ca influx[a] IC_{50} (μM)[c]	Cell necrosis[b] EC_{50} (μM)[c]	Selectivity[d]
7a	2-Furyl	1.3	>100	>76
7b	4-Imidazolyl	0.70	35	50
7c	4-Thiazolyl	0.81	64	79
7d	4-Pyrimidinyl	0.97	69	71
7e	2-Pyrimidinyl	3.9	NT[e]	—
7f	2-Pyrazinyl	3.9	NT[e]	—
7g	2-Pyridyl	1.7	NT[e]	—
7h	3-Pyridyl	1.1	>30	>27
7i	4-Pyridyl	0.22	19	86
4	Phenyl	0.79	>100	>120
KB-R7943 (**2**)		5.1	24	4.7
3		0.94	34	36

[a] Activity at the NCX1.1 expressed in CCL39 cells. ^{45}Ca influx mean NCX inhibitory activity for reverse mode.
[b] Activity at the NCX1.1 expressed in CCL39 cells. Cell necrosis mean NCX inhibitory activity for 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 benzyloxyphenyl derivatives against the sodium–calcium exchanger



Compound	Y	^{45}Ca influx[a] IC_{50} (μM)[c]	Cell necrosis[b] EC_{50} (μM)[c]	Selectivity[d]
10a		0.22	27	120
18		4.7	NT[e]	—
10b		0.38	24	63
10c		0.60	43	72
10d		2.7	50	19
13		5.9	NT[e]	—
14		3.9	NT[e]	—
7i		0.22	19	86
4		0.79	>100	>120

[a] Activity at the NCX1.1 expressed in CCL39 cells. ^{45}Ca influx mean NCX inhibitory activity for reverse mode.
[b] Activity at the NCX1.1 expressed in CCL39 cells. Cell necrosis mean NCX inhibitory activity for 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.

Initially, the phenyl ring of compound **4** was replaced with several heterocyclic ring systems, such as furyl, thiazole, pyrimidine, and pyridine rings (**7a**–**i**) (Table 1). Compounds with five-membered heterocycles (furyl, imidazolyl, and thiazolyl derivatives) had similar inhibitory activities against reverse NCX activity, compared to **4**. These results indicate that five-membered heterocycles seem to be tolerated for inhibition of reverse NCX activity. For six-membered heterocycles, the 4-pyrimidinyl derivative (**7d**) was as potent as compound **4**, but the inhibitory activities of the 2-pyrimidinyl (**7e**), and 2-pyrazinyl derivatives (**7f**) were five times less potent than **4**. The 2-pyridyl derivative (**7g**) showed an approximately 2-fold loss of reverse NCX inhibitory activity, but the 3-pyridyl derivative (**7h**) was equipotent to **4**. Furthermore, the 4-pyridyl derivative (**7i**) showed a 4-fold increase in activity compared to **4**. The results for pyridine derivatives (**7g**–**i**) suggest that the position of the hetero atom in the heterocyclic ring seems to be important for inhibition of reverse NCX activity, and also suggest that the N atom of the 4-pyridyl ring (**7i**) may be behaving as a H-bond acceptor. Among the heterocyclic derivatives, compound **7i** was the most potent against reverse NCX activity, with an IC_{50} value of 0.22 μM. Hence, the 4-pyridyl ring system was found to be the best ring system for inhibition of reverse NCX function.

KB-R7943 (**2**) does not have a pyridine ring connected to the benzyloxyphenyl moiety. This prompted us to change the nicotinamide moiety in **7i** into several aliphatic cyclic linkers, as shown in Table 2. Replacement of the nicotinamide by a 4-piperidino ring (**10a**) maintained inhibition of reverse NCX function, compared to **7i**. In contrast, compound **18**, in which the –O– link connecting the phenoxy group to the piperidine ring

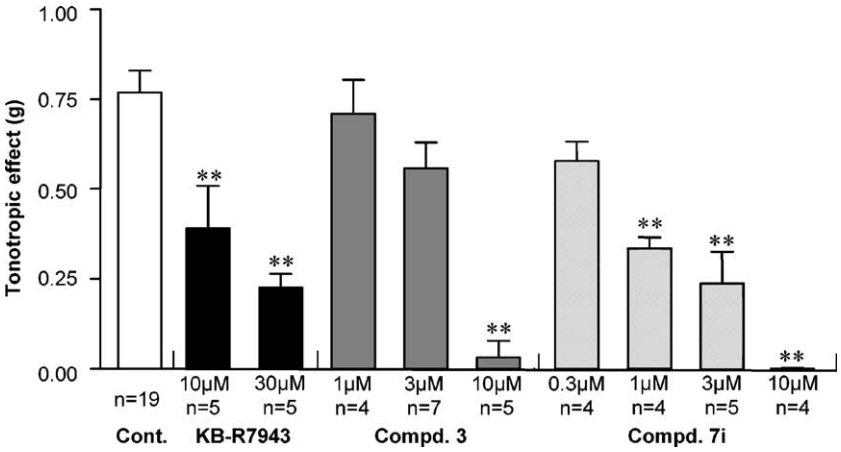


Figure 2. Effects of compounds KB-R7943 (**2**), **3**, and **7i** on tonotropic effects of ouabain in guinea pig isolated atria. $*P < 0.05$, $**P < 0.01$ versus control (Dunnett's test). Each value is the mean ± s.e.mean of at least four experiments.

was changed into an –N(Me)– link, had reduced inhibitory activity for reverse NCX, indicating that the basic nature of the linker in **18** was not tolerated. The derivatives with carbamate (**10b**) and amide (**10c**) linkages also showed slightly weaker inhibitory activity. The acrylamide derivative (**10d**), which has a planar structure from the amide to the pyridine, showed a 4-fold loss of inhibitory activity, compared to compound **10c**, suggesting that such a planar structure is conformationally unfavorable for inhibition. Compounds **13** and **14**, which both contain a 3-piperidino ring, rather than a 4-piperidino ring, also showed a dramatic decrease in inhibitory potency. Collectively, these results indicate that the structure of the linker is important for activity.

On the basis of the in vitro study described above, we selected compound **7i**[15] and evaluated its efficacy in an ouabain-induced tonotropic and arrhythmia model of heart failure.[16] The effects of compound **7i** and reference compounds KB-R7943 (**2**) and **3** on the tonotropic effects of ouabain and on the time for ouabain-induced onset of arrhythmia in isolated guinea pig atria were evaluated. The results are shown in Figures 2 and 3. The tonotropic effect of ouabain was lowered in a concentration-dependent manner by compounds KB-R7943 (**2**), **3**, and **7i**. The effect of **7i** was approximately 10-times greater than that of KB-R7943 (**2**), and **7i** was also more potent than **3**, as shown in Figure 2. The time to the onset of arrhythmia following treatment with compound **7i** was delayed in a concentration-dependent manner, and compound **7i** was more effective than KB-R7943 (**2**) and **3**, as shown in Figure 3. Hence, compound **7i** was confirmed to have efficacy against ouabain-induced tonotropy and arrhythmia onset, and was found to be more potent than KB-R7943 (**2**) and compound **3** in this particular heart failure model.

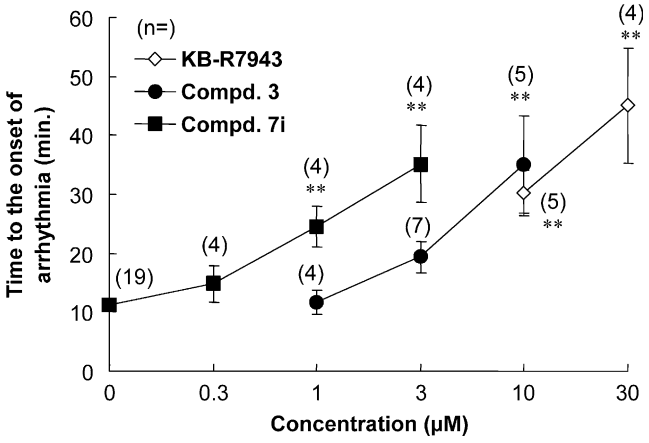


Figure 3. Effects of compounds KB-R7943 (**2**), **3**, and **7i** on the onset of arrhythmia induced by ouabain in guinea pig isolated atria. $*P < 0.05$, $**P < 0.01$ versus control (Dunnett's test). Each value is the mean ± s.e.mean of at least four experiments.

4. Conclusion

A series of benzyloxyphenyl derivatives have been prepared and evaluated for their inhibitory activity against reverse and forward mode NCX functions, following their design based on compound **4**. We found that a compound containing a 4-pyridyl ring (**7i**), rather than a phenyl ring, had enhanced reverse NCX inhibitory activity. Compound **10a**, in which the pyridine ring was changed into a piperidine ring, had the same inhibitory activity as **7i**, and both **7i** and **10a** were potent NCX inhibitors, with IC_{50} values of 0.22 μM against reverse NCX activity. Compound **7i** also showed efficacy against tonotropic effect of ouabain and ouabain-induced arrhythmia onset in a heart failure model using isolated atria from guinea pigs. Further optimization studies to discover orally active and potent inhibitors of reverse NCX activity, based on compound **7i**, will be reported in future publications.

5. Experimental

5.1. Chemistry

Melting points were determined with a Yanaco MP-500D melting point apparatus or a Büchi 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.

5.1.1. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}nicotinic acid (6). To the mixture of 6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinonitrile (**5**) (12.0 g, 37.5 mmol), EtOH (80 mL), and 5 M NaOH (75 mL, 375 mmol) was stirred at 100 °C for 1.5 h. After cooling at room temperature, the mixture was concentrated in vacuo. To the mixture was added 1 M HCl at 0 °C. The precipitate was filtered and dried in vacuo to afford compound **6** as a beige powder (12.6 g, 99%): ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.15 (2H, s), 7.04–7.20 (6H, m), 7.27–7.33 (2H, m), 7.42–7.49 (1H, m), 8.26 (1H, dd, J = 8.6, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 13.16 (1H, br s); MS (FAB) m/z 340 $(M+H)^+$.

5.1.2. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(2-furylmethyl)nicotinamide (7a). To the mixture of furfurylamine (78 mg, 0.80 mmol), compound **6** (285 mg, 0.84 mmol), HOBt (54 mg, 0.40 mmol), and THF (5 mL) was added WSC·HCl (169 mg, 0.880 mmol) at 0 °C. The mixture was stirred at room temperature for 2 h. The mixture was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried, concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 1:0–98:2) to give **7a** as a beige solid (316 mg). This material was crystallized from hexane–AcOEt to give **7a** as a colorless powder (185 mg, 55%): mp 100–101 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.47 (2H, d, J = 5.3 Hz), 5.15 (2H, s), 6.28 (1H, d, J = 2.9 Hz), 6.38–6.41 (1H, m), 7.02–7.13 (5H, m), 7.14–7.20 (1H, m), 7.28–7.33 (2H, m), 7.42–7.49 (1H, m), 7.57–7.59 (1H, m), 8.25 (1H, dd, J = 8.5, 2.5 Hz), 8.60 (1H, d, J = 2.5 Hz), 9.02 (1H, t, J = 5.9 Hz); MS (FAB) m/z 419 $(M+H)^+$. Anal. Calcd for $C_{24}H_{19}N_2O_4F$: C, 68.89; H, 4.58; N, 6.70; F, 4.54. Found: C, 68.75; H, 4.54; N, 6.67; F, 4.54.

5.1.3. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(1*H*-imidazol-4-ylmethyl)nicotinamide hydrochloride (7b). To the mixture of (1-trityl-1*H*-imidazol-4-yl)methylamine (407 mg, 1.20 mmol), compound **6** (407 mg, 1.20 mmol), HOBt (81 mg, 0.60 mmol) and THF (6 mL), DMF (1 mL) was added WSC·HCl (253 mg, 1.32 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h, and was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried, concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 98:2–96:4) to give 6-{4-[(3-fluorobenzyl)oxy]phenoxy}-*N*-[(1-trityl-1*H*-imidazol-4-yl)methyl]nicotinamide as a beige form (746 mg, 94%). The intermediate was dissolved in 90% AcOH (10 mL). The mixture was stirred at 70 °C for 1 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. The residue was recrystallized from $CHCl_3$. This material was converted to its hydrochloride salt by treating it with 4 M HCl/AcOEt (0.256 mL). The crude salt was crystallized from EtOH–AcOEt to give **7b** as a colorless powder (341 mg, 68%): mp 207–221 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.54 (2H, d, J = 4.9 Hz), 5.15 (2H, s), 7.04–7.14 (5H, m), 7.14–7.22 (1H, m), 7.28–7.35 (2H, m), 7.43–7.50 (1H, m), 7.51 (1H, s), 8.26 (1H, dd, J = 8.8, 2.2 Hz), 8.67 (1H, d, J = 2.2 Hz), 9.04 (1H, s), 9.30 (1H, t, J = 5.9 Hz), 14.52 (2H, br s); MS (FAB) m/z 419 $(M+H)^+$. Anal. Calcd for $C_{23}H_{19}N_4O_3SF$·HCl: C, 60.73; H, 4.43; N, 12.32; F, 4.18; Cl, 7.79. Found: C, 60.59; H, 4.41; N, 12.36; F, 4.07; Cl, 7.90.

5.1.4. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(1,3-thiazol-4-ylmethyl)nicotinamide hydrochloride (7c). Compound **7c** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7c** was obtained as a colorless amorphous (66%): ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.62 (2H, d, J = 5.9 Hz), 5.15 (2H, s), 7.06–7.21 (6H, m), 7.28–7.34 (2H, m), 7.43–7.51 (2H, m), 8.28 (1H, dd, J = 8.8, 2.5 Hz), 8.65 (1H, d, J = 2.5 Hz), 9.08 (1H, d, J = 2.0 Hz), 9.16 (1H, t, J = 5.5 Hz); MS (FAB) m/z 436 $(M+H)^+$. Anal. Calcd for $C_{23}H_{18}N_4O_3F$·0.7HCl·0.8H_2O: C, 58.11; H, 4.30; N, 8.84; F, 4.00; Cl, 5.22; S, 6.74. Found: C, 57.95; H, 4.13; N, 8.84; F, 3.97; Cl, 5.42; S, 6.76.

5.1.5. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyrimidin-4-ylmethyl)nicotinamide (7d). Compound **7d** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7d** was obtained as a colorless solid (74%): mp 138–139 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.56 (2H, d, J = 5.9 Hz), 5.15 (2H, s), 7.05–7.21 (6H, m), 7.28–7.34 (2H, m), 7.43–7.49 (2H, m), 8.29 (1H, dd, J = 8.8, 2.4 Hz), 8.67 (1H, d, J = 2.4 Hz), 8.73 (1H, d, J = 5.4 Hz), 9.11 (1H, s), 9.25 (1H, t, J = 6.1 Hz); MS (FAB) m/z 431 $(M+H)^+$. Anal. Calcd for $C_{24}H_{19}N_4O_3F$: C, 66.97; H, 4.45; N, 13.02; F, 4.41. Found: C, 67.11; H, 4.57; N, 13.20; F, 4.24.

5.1.6. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyrimidin-2-ylmethyl)nicotinamide hydrobromide (7e). Compound **7e** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7e** was obtained as an orange powder (54%): mp 161–166 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.67 (2H, d, J = 5.9 Hz), 5.16 (2H, s), 7.04–7.21 (6H, m), 7.28–7.34 (2H, m), 7.41–7.50 (2H, m), 8.29 (1H, dd, J = 8.5, 2.4 Hz), 8.66 (1H, d, J = 2.4 Hz), 8.79 (2H, d, J = 4.9 Hz), 9.16 (1H, t, J = 5.8 Hz); MS (FAB) m/z 431 $(M+H)^+$. Anal. Calcd for $C_{24}H_{19}N_4O_3F$·2HBr·0.1H_2O: C, 48.52; H, 3.60; N, 9.43; F, 3.20; Br, 26.96. Found: C, 48.30; H, 3.43; N, 9.41; F, 3.24; Br, 26.71.

5.1.7. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyrazin-2-ylmethyl)nicotinamide (7f). Compound **7f** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7f** was obtained as a colorless powder

(69%): mp 135–136 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.62 (2H, d, J = 5.9 Hz), 5.15 (2H, s), 7.05–7.14 (5H, m), 7.14–7.21 (1H, m), 7.28–7.33 (2H, m), 7.42–7.49 (1H, m), 8.28 (1H, dd, J = 8.8, 2.4 Hz), 8.54 (1H, d, J = 2.4 Hz), 8.57–8.61 (1H, m), 8.63–8.66 (2H, m), 9.23 (1H, t, J = 5.8 Hz); MS (FAB) m/z 431 $(M+H)^+$. Anal. Calcd for $C_{24}H_{19}N_4O_3F$: C, 66.97; H, 4.45; N, 13.02; F, 4.41. Found: C, 67.10; H, 4.40; N, 12.93; F, 4.23.

5.1.8. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyridin-2-ylmethyl)nicotinamide hydrochloride (7g). Compound **7g** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7g** was obtained as a colorless solid (167 mg, 45%): mp 160–163 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.78 (2H, d, J = 5.4 Hz), 7.06–7.12 (5H, m), 7.12–7.21 (1H, s), 7.28–7.34 (2H, m), 7.42–7.49 (1H, m), 7.76–7.87 (2H, m), 8.30–8.38 (2H, m), 8.70 (1H, d, J = 2.4 Hz), 8.75–8.78 (1H, m), 9.53 (1H, t, J = 5.4 Hz); MS (FAB) m/z 430 $(M+H)^+$. Anal. Calcd for $C_{25}H_{20}N_3O_3F$·HCl: C, 64.45; H, 4.54; N, 9.02; F, 4.08; Cl, 7.61. Found: C, 64.23; H, 4.46; N, 9.00; F, 4.12; Cl, 7.59.

5.1.9. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyridin-3-ylmethyl)nicotinamide hydrochloride (7h). Compound **7h** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7h** was obtained as a colorless powder (75%): mp 178–185 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.64 (2H, d, J = 5.6 Hz), 5.15 (2H, s), 7.05–7.13 (5H, m), 7.14–7.21 (1H, m), 7.28–7.34 (2H, m), 7.42–7.49 (1H, m), 7.93–7.98 (1H, m), 8.30 (1H, dd, J = 8.8, 2.5 Hz), 8.44 (1H, d, J = 7.8 Hz), 8.67 (1H, d, J = 2.5 Hz), 8.79 (1H, d, J = 5.3 Hz), 8.87 (1H, s), 9.45 (1H, t, J = 5.6 Hz); MS (FAB) m/z 430 $(M+H)^+$. Anal. Calcd for $C_{25}H_{20}N_3O_3F$·HCl: C, 64.45; H, 4.54; N, 9.02; F, 4.08; Cl, 7.61. Found: C, 64.35; H, 4.51; N, 9.04; F, 3.88; Cl, 7.65.

5.1.10. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyridin-4-ylmethyl)nicotinamide hydrochloride (7i). Compound **7i** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7i** was obtained as a colorless powder (69%): mp 158–162 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.74 (2H, d, J = 5.8 Hz), 5.15 (2H, s), 7.06–7.14 (5H, m), 7.14–7.21 (1H, m), 7.28–7.34 (2H, m), 7.43–7.49 (1H, m), 7.96 (2H, d, J = 6.5 Hz), 8.34 (1H, dd, J = 8.8, 2.5 Hz), 8.72 (1H, d, J = 2.5 Hz), 8.84 (2H, d, J = 6.3 Hz), 9.58 (1H, t, J = 5.8 Hz); MS (FAB) m/z 430 $(M+H)^+$. Anal. Calcd for $C_{25}H_{20}N_3O_3F$·HCl·0.25H_2O: C, 63.83; H, 4.61; N, 8.93; F, 4.04; Cl, 7.54. Found: C, 64.05; H, 4.47; N, 8.92; F, 4.74; Cl, 7.41.

5.1.11. 4-{4-[(3-Fluorobenzyl)oxy]phenoxy}piperidine hydrochloride (9). To the mixture of 4-[(3-fluorobenzyl)-oxy]phenol (**8**) (770 mg, 3.53 mmol), PPh_3 (1.20 g, 4.59 mmol), and THF (10 mL) was added a mixture of DEAD (0.723 mL, 4.59 mmol), *t*-butyl 4-hydroxypiperidine-1-carboxylate (853 mg, 4.24 mmol), and THF (10 mL) at 0 °C. The mixture was stirred at room temperature for 1 day. The mixture was partitioned between AcOEt and H_2O. The organic layer was washed with brine and dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 1:0–4:1) to give *t*-butyl 4-{4-[(3-fluorobenzyl)oxy]phenoxy}piperidine-1-carboxylate as a colorless oil (1.37 g, 97%). The mixture of the intermediate (1.36 g, 3.39 mmol), 4 M HCl in AcOEt (4.23 mL, 16.9 mmol), and MeOH (10 mL) was stirred at room temperature. The mixture was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried and concentrated in vacuo to give a crude beige solid (650 mg). The residue was dissolved in AcOEt (10 mL). To the mixture was added 4 M HCl in AcOEt (0.650 mL, 2.59 mmol) at 0 °C. The precipitate was collected to give **9** as a colorless powder (640 mg, 56%): mp 199–200 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.75–1.86 (2H, m), 2.01–2.10 (2H, m), 2.95–3.07 (2H, m), 3.16–3.24 (2H, m), 4.46–4.54 (1H, m), 5.07 (2H, s), 6.95 (4H, s), 7.11–7.18 (1H, m), 7.23–7.29 (2H, m), 7.39–7.47 (1H, m), 8.98 (2H, br s); MS (FAB) m/z 302 $(M+H)^+$. Anal. Calcd for $C_{18}H_{20}NO_2F$·HCl: C, 64.00; H, 6.27; N, 4.15; F, 5.62; Cl, 10.49. Found: C, 63.98; H, 6.28; N, 4.15; F, 5.61; Cl, 10.51.

5.1.12. 4-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyridin-4-ylmethyl)piperidine-1-carboxamide hydrochloride (10a). To the mixture of bis(trichloromethyl) carbonate (119 mg, 0.40 mmol) and THF (5 mL) was added a THF (5 mL) solution of 4-{4-[(3-fluorobenzyl)oxy]phenoxy}piperidine (301 mg, 1.00 mmol) and Et_3N (0.209 mL, 1.50 mmol) at 0 °C. The mixture was stirred at room temperature for 70 min. To the mixture were added a THF (5 mL) solution of pyridin-4-ylmethylamine (130 mg, 1.20 mmol) and Et_3N (0.167 mL, 1.20 mmol) at 0 °C. The mixture was stirred at room temperature for 16 h. The mixture was partitioned between CH_3Cl 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 = 98:2–96:4) to give the free base of **10a** as a brown syrup (290 mg, 67%). This material was converted to its hydrochloride salt by treating it with hydrochloride in AcOEt–MeOH. The mixture was concentrated in vacuo. The residue was recrystallized from AcOEt–EtOH–CH_3CN to give **10a** as a light brown powder (95 mg, 20%): mp 175–183 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.46–1.56 (2H, m), 1.84–1.94 (2H, m), 3.13–3.23 (2H, m), 3.68–3.77 (2H, m), 4.40–4.49 (3H, m), 5.07 (2H, m), 6.90–6.96 (4H, m), 7.12–7.18 (1H, m), 7.23–7.30 (2H, m), 7.40–7.47 (1H, m), 7.50–7.55 (1H, m), 7.87 (2H, t, J = 6.9 Hz), 8.81 (2H, d, J = 6.9 Hz); MS (FAB) m/z 436 $(M+H)^+$. Anal. Calcd for $C_{25}H_{26}N_3O_3F$·HCl·0.3H_2O: C, 62.90; H, 5.83; N, 8.80; F, 3.98; Cl, 7.43. Found: C, 62.82; H, 5.76; N, 8.94; F, 3.96; Cl, 7.30.

5.1.13. Pyridin-4-ylmethyl 4-{4-[(3-fluorobenzyl)oxy]phenoxy}piperidine-1-carboxylate hydrochloride (10b). To the mixture of 4-{4-[(3-fluorobenzyl)oxy]phenoxy}piperidine hydrochloride (**9**) (406 mg, 1.20 mmol), Et_3N (0.201 mL, 1.44 mmol), and THF (5 mL) was added the THF (2 mL) solution of methyl chloroformate (0.102 mL, 1.44 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min. The mixture was partitioned between AcOEt and aqueous HCl. The organic layer was washed with aqueous NaOH, dried, and concentrated in vacuo. The residue was purified by column

chromatography on silica gel ($CHCl_3$/MeOH = 99:1) to give methyl 4-{4-[(3-fluorobenzyl)oxy]phenoxy}piperidine-1-carboxylate as a light yellow oil (445 mg). The material was added to the mixture of pyridin-4-ylmethanol (313 mg, 2.88 mmol), 60% NaH (46 mg, 1.16 mmol) and toluene (10 mL) at room temperature. The mixture was refluxed for 2 days. After cooling at room temperature, the mixture was partitioned between $CHCl_3$ and brine. The organic layer was dried, concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 99:1) to give the free base of **10b** as a light yellow syrup (235 mg). The material was converted to its hydrochloride salt by treating it with hydrochloride in AcOEt. The mixture was concentrated in vacuo. The residue was recrystallized from AcOEt–CH_3CN to give **10b** as a colorless powder (100 mg, 18% for two steps): mp 155–162 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.51–1.69 (2H, m), 1.87–2.00 (2H, m), 3.22–3.47 (2H, m), 3.64–3.91 (2H, m), 4.45–4.52 (1H, m), 5.07 (2H, s), 5.38 (2H, s), 6.91–6.97 (4H, m), 7.12–7.18 (1H, m), 7.24–7.30 (2H, m), 7.40–7.47 (1H, m), 7.93 (2H, t, J = 6.9 Hz), 8.87 (2H, t, J = 6.9 Hz); MS (FAB) m/z 437 $(M+H)^+$. Anal. Calcd for $C_{25}H_{25}N_2O_4F$·HCl: C, 63.49; H, 5.54; N, 5.92; F, 4.02; Cl, 7.50. Found: C, 63.48; H, 5.53; N, 5.98; F, 3.97; Cl, 7.56.

5.1.14. 4-[3-(4-{4-[(3-Fluorobenzyl)oxy]phenoxy}piperidin-1-yl)-3-oxopropyl]pyridine hydrochloride (10c). Compound **10c** was prepared from **9** by a procedure similar to that described for **7a**. Compound **10c** was obtained as a beige powder (68%): mp 159–165 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.39–1.50 (1H, m), 1.50–1.61 (1H, m), 1.78–1.96 (2H, m), 2.87 (2H, t, J = 7.3 Hz), 3.09 (2H, t, J = 7.3 Hz), 3.17–3.26 (1H, m), 3.27–3.37 (1H, m), 3.65–3.76 (1H, m), 3.78–3.88 (1H, m), 4.43–4.52 (1H, m), 5.07 (2H, s), 6.88–6.97 (4H, m), 7.12–7.18 (1H, m), 7.23–7.29 (2H, m), 7.39–7.47 (1H, m), 7.96 (2H, d, J = 6.3 Hz), 8.80 (2H, d, J = 6.3 Hz); MS (FAB) m/z 435 $(M+H)^+$. Anal. Calcd for $C_{26}H_{27}N_2O_3F$·HCl: C, 66.31; H, 5.99; N, 5.95; F, 4.03; Cl, 7.53. Found: C, 66.07; H, 5.87; N, 5.97; F, 4.07; Cl, 7.47.

5.1.15. 4-[(1*E*)-3-(4-{4-[(3-Fluorobenzyl)oxy]phenoxy}-piperidin-1-yl)-3-oxoprop-1-en-1-yl]pyridine hydrochloride (10d). Compound **10d** was prepared from **9** by a procedure similar to that described for **7a**. Compound **10d** was obtained as a beige powder (52%): mp 185–199 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.51–1.68 (2H, m), 1.88–2.02 (2H, m), 3.37–3.46 (1H, m), 3.55–3.64 (1H, m), 3.90–4.05 (2H, m), 4.49–4.57 (1H, m), 5.07 (2H, s), 6.95 (4H, m), 7.12–7.18 (1H, m), 7.24–7.30 (2H, m), 7.40–7.47 (1H, m), 7.60 (1H, d, J = 15.6 Hz), 7.85 (1H, d, J = 15.6 Hz), 8.29 (2H, d, J = 5.4 Hz), 8.89 (2H, d, J = 6.9 Hz); MS (FAB) m/z 433 $(M+H)^+$. Anal. Calcd for $C_{26}H_{25}N_2O_3F$·HCl: C, 66.59; H, 5.59; N, 5.97; F, 4.05; Cl, 7.56. Found: C, 66.40; H, 5.68; N, 5.98; F, 3.93; Cl, 7.56.

5.1.16. 3-{4-[(3-Fluorobenzyl)oxy]phenoxy}piperidine (11). Compound **11** was prepared from **8** by a procedure similar to that described for **9**. Compound **11** was obtained as an orange liquid (44% for two steps): ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.34–1.50 (2H, m), 1.56–1.69 (1H, m), 1.90–2.04 (1H, m), 2.38–2.48 (2H, m), 2.62–2.79 (1H, m), 2.97–3.03 (1H, m), 4.01–4.12 (1H, m), 5.05 (2H, s), 6.83–6.95 (4H, m), 7.09–7.19 (1H, m), 7.20–7.30 (2H, m), 7.37–7.47 (1H, m); MS (FAB) m/z 302 $(M+H)^+$.

5.1.17. 3-({4-[(3-Fluorobenzyl)oxy]phenoxy}methyl)piperidine hydrochloride (12). Compound **12** was prepared from **8** by a procedure similar to that described for **9**. Compound **12** was obtained as a white powder (76% for two steps): ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.24–1.40 (1H, m), 1.63–1.87 (3H, m), 2.14–2.30 (1H, m), 2.63–2.85 (2H, m), 3.16–3.26 (1H, m), 3.27–3.35 (1H, m), 3.74–3.83 (1H, m), 3.84–3.91 (1H, m), 5.07 (2H, s), 6.87 (2H, d, J = 9.2 Hz), 6.95 (2H, d, J = 9.2 Hz), 7.10–7.19 (1H, m), 7.22–7.29 (2H, m), 7.39–7.47 (1H, m), 8.91–9.25 (2H, m); MS (FAB) m/z 316 $(M+H)^+$.

5.1.18. 3-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(pyridin-4-ylmethyl)piperidine-1-carboxamide (13). Compound **13** was prepared from **11** by a procedure similar to that described for **10a**. Compound **13** was obtained as a colorless solid (29%): mp 148–152 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.40–1.53 (1H, m), 1.53–1.66 (1H, m), 1.65–1.78 (1H, m), 1.93–2.04 (1H, m), 3.05–3.18 (2H, m), 3.52–3.60 (1H, m), 3.85–3.93 (1H, m), 4.13–4.21 (1H, m), 4.27 (2H, d, J = 5.8 Hz), 5.07 (2H, s), 6.90 (2H, d, J = 9.3 Hz), 6.93 (2H, d, J = 9.3 Hz), 7.11–7.18 (1H, m), 7.19–7.32 (5H, m), 7.39–7.46 (1H, m), 8.47–8.56 (2H, m); MS (FAB) m/z 436 $(M+H)^+$. Anal. Calcd for $C_{25}H_{26}N_3O_3F·C_2H_2O_4$: C, 61.71; H, 5.37; N, 8.00; F, 3.62. Found: C, 61.60; H, 5.25; N, 7.99; F, 3.66.

5.1.19. 3-({4-[(3-Fluorobenzyl)oxy]phenoxy}methyl)-*N*-(pyridin-4-ylmethyl)piperidine-1-carboxamide (14). Compound **14** was prepared from **12** by a procedure similar to that described for **10a**. Compound **14** was obtained as a colorless solid (24%): mp 129–134 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.23–1.46 (2H, m), 1.59–1.68 (1H, m), 1.78–1.90 (2H, m), 2.63–2.71 (1H, m), 2.78–2.86 (1H, m), 3.73–3.87 (3H, m), 4.00–4.07 (1H, m), 4.25 (2H, d, J = 5.9 Hz), 5.06 (2H, s), 6.87 (2H, d, J = 9.2 Hz), 6.93 (2H, d, J = 9.2 Hz), 7.11–7.18 (2H, m), 7.23–7.30 (4H, m), 7.39–7.46 (1H, m), 8.40–8.60 (2H, m); MS (FAB) m/z 450 $(M+H)^+$. HRMS calcd for $C_{26}H_{28}N_3O_3F$ 450.2153. Found: 450.2166.

5.1.20. *tert*-Butyl 4-[{4-[(3-fluorobenzyl)oxy]phenyl}(methyl)amino]piperidine-1-carboxylate (16). To the mixture of 4-aminophenol **15** (1.09 g, 10.0 mmol) and DMF (40 mL) were added *t*-BuOK (1.35 g, 12.0 mmol) and 1-(bromomethyl)-3-fluorobenzene (1.23 mL, 10.0 mmol) at 0 °C. The mixture was stirred at room temperature for 2 h. H_2O was added to the mixture at 0 °C. The mixture was partitioned between AcOEt and brine. The organic layer was dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/AcOEt = 1:0–1:1) to give {4-[(3-fluorobenzyl)-oxy]phenyl}amine as a yellow oil (1.87 g, 86%). To the mixture of {4-[(3-fluorobenzyl)oxy]phenyl}amine (869 mg, 4.00 mmol) and AcOH (10 mL) were added *t*-butyl

4-oxopiperidine-1-carboxylate (797 mg, 4.00 mmol) and $NaBH(OAc)_3$ (1.02 g, 4.80 mmol) at 10 °C. The mixture was stirred at room temperature for 4 h. The mixture was concentrated in vacuo. The residue was partitioned between $CHCl_3$ and 5 M 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 *t*-butyl 4-({4-[(3-fluorobenzyl)oxy]phenyl}amino)piperidine-1-carboxylate as a yellow syrup (1.62 g). To the mixture of *t*-butyl 4-({4-[(3-fluorobenzyl)oxy]phenyl}amino)piperidine-1-carboxylate as a yellow syrup (1.62 g) and AcOH (10 mL) was added 35% formaldehyde in H_2O (1.72 g, 20.0 mmol) and $NaBH(OAc)_3$ (1.70 g, 8.00 mmol) at 10 °C. The mixture was stirred at room temperature for 18 h. The mixture was concentrated in vacuo. The residue was partitioned between $CHCl_3$ and 5 M 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 **16** as a yellow syrup (1.33 g, 80% for two steps): ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44–1.50 (3H, m), 1.46 (9H, s), 1.67–1.76 (2H, m), 2.70 (3H, s), 2.70–2.79 (2H, m), 3.42–3.55 (1H, m), 4.12–4.25 (1H, m), 5.00 (2H, s), 6.81 (2H, d, J = 9.2 Hz), 6.89 (2H, d, J = 9.2 Hz), 6.97–7.03 (1H, m), 7.12–7.21 (2H, m), 7.29–7.37 (1H, m); MS (FAB) m/z 414 M^+.

5.1.21. *N*-{4-[(3-Fluorobenzyl)oxy]phenyl}-*N*-methylpiperidin-4-amine hydrochloride (17). To the mixture of **16** (1.32 g, 3.17 mmol) and MeOH (10 mL) was added 4 M AcOEt solution of HCl (3.97 mL, 15.9 mmol) at 0 °C. The mixture was stirred at room temperature for overnight and concentrated in vacuo. The residue was crystallized from solvent of AcOEt and CH_3CN, MeOH to afford **17** as a beige powder (1.03 g, 84%): ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.69–2.07 (3H, m), 2.30–2.44 (1H, m), 2.78–2.92 (2H, m), 2.98–3.08 (3H, m), 3.27–3.48 (2H, m), 3.77–3.90 (1H, m), 5.16 (2H, s), 7.11–7.25 (3H, m), 7.30 (2H, d, J = 8.3 Hz), 7.41–7.49 (1H, m), 7.50–7.79 (2H, m), 8.69 (1H, br s), 9.26 (1H, br s), 13.43 (1H, br s); MS (FAB) m/z 315 $(M+H)^+$.

5.1.22. 4-[{4-[(3-Fluorobenzyl)oxy]phenyl}(methyl) amino]-*N*-(pyridin-4-ylmethyl)piperidine-1-carboxamide (18). Compound **18** was prepared from **17** by a procedure similar to that described for **10a**. Compound **18** was obtained as a light yellow powder (17%): mp 75–77 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.42–1.55 (2H, m), 1.55–1.63 (2H, m), 2.63 (3H, s), 2.73–2.83 (2H, m), 3.59–3.69 (1H, m), 4.03–4.12 (2H, m), 4.25 (2H, d, J = 5.3 Hz), 5.04 (2H, s), 6.83 (2H, d, J = 8.8 Hz), 6.89 (2H, d, J = 8.8 Hz), 7.10–7.21 (2H, m), 7.22–7.29 (4H, m), 7.39–7.46 (1H, m), 8.45–8.52 (2H, m); MS (FAB) m/z 449 $(M+H)^+$. Anal. Calcd for $C_{26}H_{29}N_4O_2F \cdot 1.4H_2O$: C, 65.92; H, 6.77; N, 11.83; F, 4.01. Found: C, 65.83; H, 6.40; N, 11.59; F, 3.92.

6. Pharmacology

6.1. ^{45}Ca influx assay and cell necrosis assay

The methods were described in previous report.[13]

6.1.1. Effects on tonotropic effects of ouabain and the onset of arrhythmia induced by ouabain in guinea pig isolated atria.[16] Toxic effects of ouabain on hearts have partly been explained by its action on the reverse mode of Na^+/Ca^{2+} exchangers playing in the cardiac myocytes. The influx of Ca^{2+} induced by the reverse mode of Na^+/Ca^{2+} exchangers have been thought to produce cardiac injuries triggering arrhythmia and/or diastolic dysfunctions. These compounds were thus tested on the cardiac diastolic dysfunctions and arrhythmia in the guinea pig atria evoked by ouabain at its toxic dose (3 μM). Isolated whole atria of male guinea pigs (350–450 g) were bathed in warmed (37 °C) and gassed (95% O_2 + 5% CO_2) Tyrode solution containing (in mM): NaCl 137, KCl 6, $CaCl_2$ 1.82, $MgCl_2$ 1.05, NaH_2PO_4 0.417, $NaHCO_3$ 11.5, and glucose 5.5. One end of the muscle was attached to a rigid support and the other end was attached to a force–displacement transducer (Nihon Kohden SB-1T) via a silk suture in the 30 mL bath. Each tissue was placed under 0.5 g tension. Following stabilization of spontaneous beating of the preparations for about 45 min with replacing the solution every 15 min, compounds were added. Ouabain (3 μM) was added 45 min after the addition of the compounds. The concentration of atria was recorded continuously for 90 min after the addition of ouabain. Beating rates were counted from the signals of the contraction measured.

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15. Both compounds **7i** and **10a** were found to be potent NCX inhibitors with IC_{50} values of 0.22 μM against the reverse NCX mode. While compound **10a** was slightly better than compound **7i** in terms of its selectivity for reverse NCX activity versus forward NCX activity, compound **7i** was better than compound **10a** with regard to oral activity. Since our goal is to create orally-active NCX inhibitors, compound **7i** was selected as the test compound in the ouabain-induced heart failure model.
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