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Bioorganic & Medicinal Chemistry 13 (2005) 4022–4036
Bioorganic & Medicinal Chemistry

Discovery of an *N*-(2-aminopyridin-4-ylmethyl)nicotinamide derivative: a potent and orally bioavailable NCX inhibitor

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Received 3 March 2005; revised 30 March 2005; accepted 30 March 2005
Available online 25 April 2005

Abstract—Ca^{2+} overload in myocardial cells is responsible for arrhythmia. Sodium–calcium exchanger (NCX) inhibitors are more effective than sodium–hydrogen exchanger (NHE) inhibitors with regard to modulation of Ca^{2+} overload, because NCX inhibitors can directly inhibit the influx of Ca^{2+} into cells. NCX is an attractive target for the treatment of heart failure and ischemia-reperfusion. We have designed and synthesized a series of *N*-(2-aminopyridin-4-ylmethyl)nicotinamide derivatives, based on compound **5**. We have discovered a novel NCX inhibitor (**23h**) with an IC_{50} value of 0.12 μM against reverse NCX. The inhibitory activities of our NCX inhibitors against cytochrome P450 were also evaluated. The effects on heart failure and the pharmacokinetic profile of compound **23h** are discussed.

1. Introduction

The sodium–calcium exchanger (NCX) plays an important role in calcium handling in cardiac myocytes.[1] In the setting of heart failure and myocardial ischemia-reperfusion, NCX can lead to calcium overload.[2–9] Calcium overload via NCX can contribute to the activation of an arrhythmogenic transient inward current, and can also be responsible for contractile dysfunction. Approaches that inhibit NCX could have potential anti-arrhythmic effects in pathophysiological states, such as heart failure or myocardial ischemia-reperfusion. NCX typically functions in the forward mode but can also function in the reverse mode. The reverse mode of NCX is more important in relation to the induction of calcium overload. Consequently, selective inhibition of the reverse NCX mode 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 beneficial in treating the above diseases.[10,11]

Two types of structures have been identified as NCX inhibitors. They are quinazoline derivatives, such as SM-15811 (**1**),[12] and benzyloxyphenyl derivatives, such as KB-R7943 (**2**)[13] (Fig. 1). A series of benzyloxyphenyl derivatives, SEA0400 (**3**),[14] and compounds **4**[15] and **5**[16] have also been reported. Although, potent orally bioavailable reverse NCX inhibitors are desirable, none has been reported to date. We have previously reported nicotinamide derivatives with reverse NCX inhibitory activity.[16–18] We have now succeeded in discovering potent and orally bioavailable reverse NCX inhibitors based on **5**; in addition, we were able to find reverse NCX inhibitors with less potent inhibitory activity against cytochrome P450 (CYP). Herein, we wish to report the results of our work on the synthesis and structure–activity relationships (SAR) of the nicotinamide derivatives, and their biological activities and pharmacokinetics.

2. Chemistry

The synthesis of nicotinamide derivatives **23a–n** is shown in Scheme 1. Compound **8** was prepared from isonicotinonitrile (**6**) by alkylation with 2-iodopropane, followed by catalytic reduction of the cyano group. Compound **9** was converted into amino derivative **10**

Keywords: Sodium–calcium exchanger; NCX; Anti-arrhythmics.
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doi:10.1016/j.bmc.2005.03.052

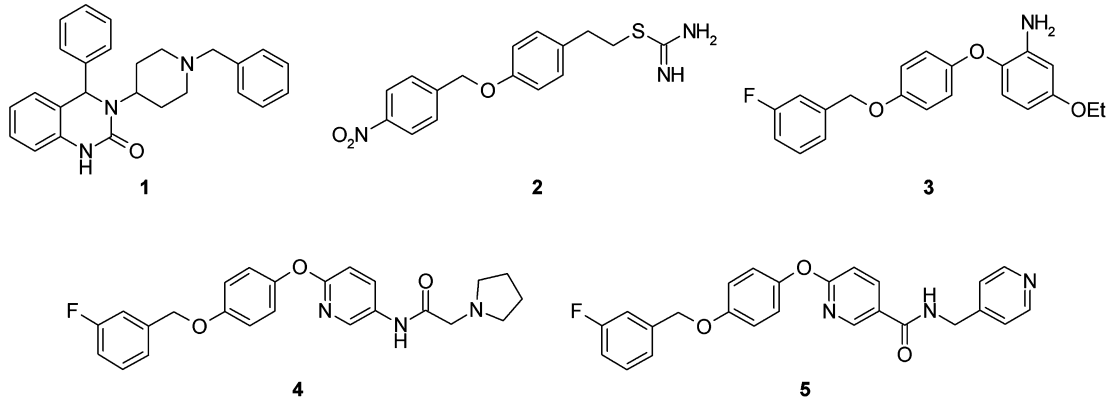
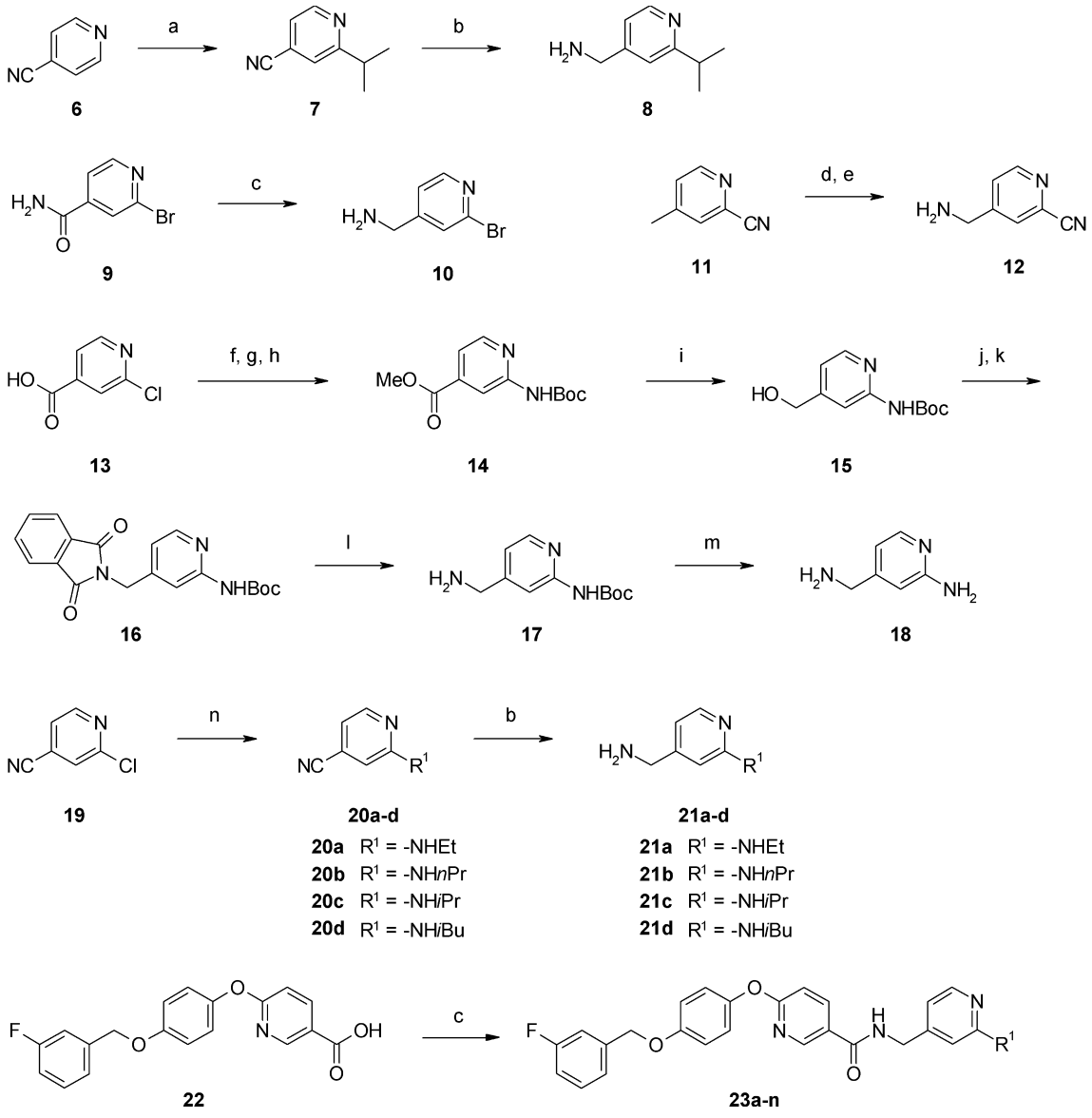


Figure 1. Several inhibitors of sodium–calcium exchanger: (**1**) SM-15811; (**2**) KB-R7943; (**3**) SEA0400; (**4**) patented compound of JP11092454; (**5**) nicotinamide derivative.



Scheme 1. Reagents and conditions: (a) 2-iodopropane, H_2O_2, $FeSO_4{\cdot}7H_2O$, concd H_2SO_4, DMSO–H_2O; (b) H_2, Raney Ni, EtOH–28% aqueous NH_3; (c) BH_3, THF, reflux; (d) NBS, benzoylperoxide, *hv*, CCl_4, reflux; (e) 28% aqueous NH_3, THF, 50 °C; (f) 28% aqueous NH_3, in steel tube, 220 °C; (g) concd H_2SO_4, MeOH, reflux; (h) Boc_2O, *t*-BuOH, 50 °C; (i) $CaCl_2$, $NaBH_4$, EtOH; (j) $MeSO_2Cl$, Et_3N, THF; (k) potassium 1,3-dioxo-1,3-dihydroisoindol-2-ide, DMF, 60 °C; (l) $H_2NNH_2{\cdot}H_2O$, MeOH–$CHCl_3$; (m) concd HCl, dioxane; (n) R^1H, *i*-Pr_2NEt, THF, in steel tube, 120–130 °C.

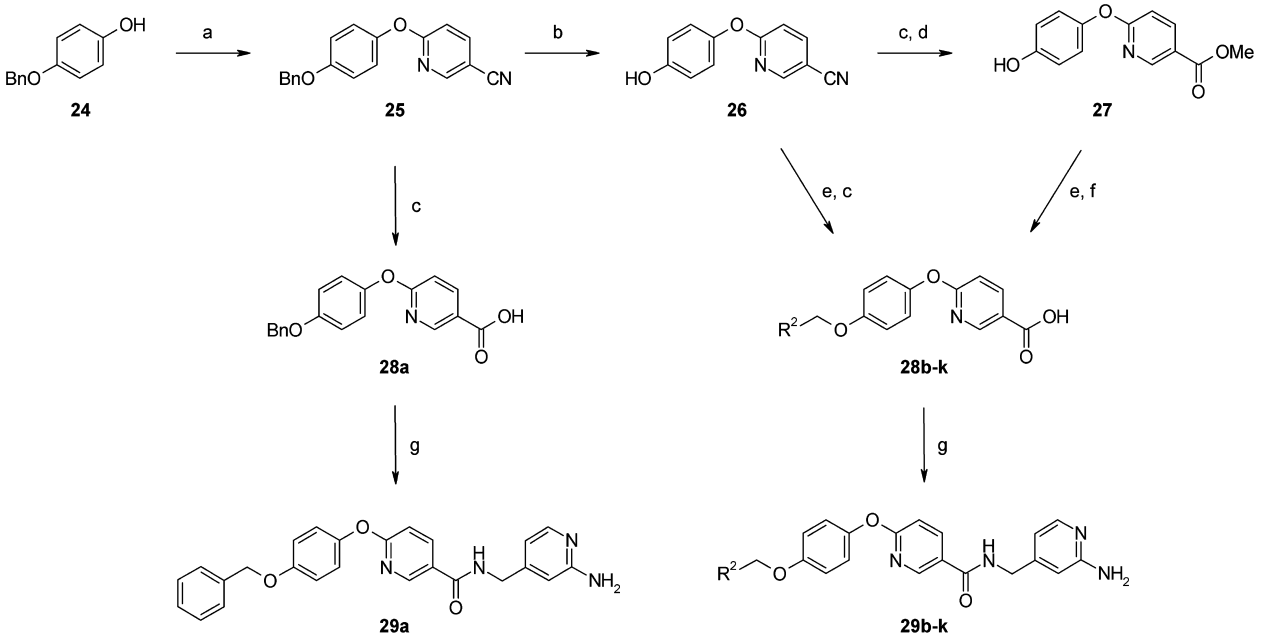
via reduction with borane. Compound **12** was produced from 4-methylpyridine-2-carbonitrile (**11**) by bromination of the methyl group with *N*-bromosuccinimide (NBS), followed by alkylation with ammonia. 2-Chloroisonicotinic acid (**13**) was treated with ammonia, followed by protection of the carboxyl group to form the methyl ester derivative. Protection of the amino group with a *tert*-butoxy carbonyl group gave **14**. Since compound **14** had both ester and carbamate groups, strong reductive reagents such as lithium aluminum hydride could not be used. Therefore, to reduce the ester group selectively, compound **14** was reduced by calcium borohydride to afford compound **15**. Compound **15** was converted to a mesylate intermediate by the introduction of a nitrogen atom into the alcohol group. In the next step, an isoindole-1,3(2*H*)-dione group was introduced into the intermediate with the potassium salt of 1*H*-isoindole-1,3(2*H*)-dione to give compound **16**. Deprotection of the phthaloyl group of **16** was performed using hydrazine to form **17** in good yield. Desired intermediate **18** was obtained via deprotection of the *tert*-butoxy carbonyl group with hydrochloride. Various intermediate amines **21a–d** were obtained from 2-chloroisonicotinonitrile (**19**) by *ipso*-condensation with the corresponding alkylamine, followed by reduction of cyano groups. Desired compounds **23a–n** were prepared by condensation of compound **22**[16] with the corresponding amines (**8**, **10**, **12**, **18**, and **21a–d**).

The synthesis of *N*-(2-aminopyridin-4-ylmethyl)nicotinamide derivatives **29a–k** is summarized in Scheme 2. Compound **25** was afforded by condensation of **24** and 6-chloronicotinonitrile. Removal of the benzyl group of compound **25** using pentamethylbenzene and trifluoroacetic acid gave **26**. Hydrolysis of the cyano group of **26**, followed by protection with carboxylic acid afforded methyl ester derivative **27**. Desired compound **29a** was prepared from **25** by hydrolysis, followed by amidation with **18**. Compounds **28b–k** were obtained from compound **26** or **27** by *O*-alkylation with the corresponding halide, followed by hydrolysis of cyano or ester groups. Desired compounds **29b–k** were prepared by condensation of carboxylic acid intermediates **28b–k** and 4-(aminomethyl)pyridin-2-amine **18**.

3. Results and discussion

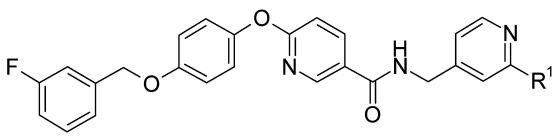
In order to measure the inhibitory effect of the synthesized compounds on reverse mode NCX activity, a Na^+-dependent Ca^{2+} influx assay was performed according to reported protocols, using ^{45}Ca and CCL39 cells that stably express NCX1.1.[13,17] The inhibitory effect on forward mode NCX activity was assayed by a cell necrosis assay using the same cells.[17,19] The inhibitory potencies of our novel compounds were thus evaluated in both reverse and forward NCX assays. To measure the oral activities of the synthesized compounds, ex vivo assays were performed using plasma. The compounds were then compared to reference compounds KB-R7943 (**2**), SEA0400 (**3**), **4**, and **5**. To screen out compounds that inhibit cytochrome P450 enzymes (CYP), to avoid any potential drug–drug interactions, selected NCX inhibitors were also subjected to CYP 1A2, 2C9, 2C19, 2D6, and 3A4 inhibition studies.[20] Mexiletine was evaluated for CYP inhibitory activity as a reference compound for heart failure treatment agents.

We previously reported that the pyridine ring system is the best ring system for inhibition of reverse NCX,[16] and compound **5** was reported as a potent reverse



Scheme 2. Reagents and conditions: (a) 6-chloronicotinonitrile, *t*-BuOK, DMF, 100 °C; (b) pentamethylbenzene, TFA; (c) 5 M NaOH, EtOH, 100 °C; (d) concd H_2SO_4, MeOH; (e) R^2CH_2Br, K_2CO_3, CH_3CN, 80 °C; (f) NaOH, MeOH–THF, 50 °C; (g) WSC·HCl, HOBt, 4-(aminomethyl)pyridin-2-amine, DMF.

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



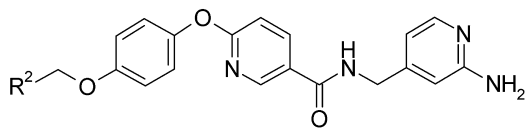
Compd	R^1	^{45}Ca influx[a] IC_{50} (μM)[c]	Cell necrosis[b] EC_{50} (μM)[c]	Selectivity[d]	Ex vivo[e] (inhibition %)
23a	Me	0.47	61	130	50 ± 13
23b	*i*-Pr	1.3	17	13	NT[f]
23c	F	1.1	80	73	5.1 ± 0.6
23d	Cl	0.43	39	91	NT[f]
23e	Br	0.94	29	31	15 ± 3
23f	CN	1.0	19	19	NT[f]
23g	OMe	1.2	>100	>83	NT[f]
23h	NH_2	0.12	28	230	66 ± 2
23i	NHMe	0.72	>100	>140	28 ± 2
23j	NHEt	0.97	97	100	36 ± 5
23k	NH*n*-Pr	3.4	NT[f]	—	NT[f]
23l	NH*i*-Pr	1.8	>100	>55	NT[f]
23m	NH*i*-Bu	7.1	NT[f]	—	NT[f]
23n	NMe_2	1.1	64	59	NT[f]
5	H	0.22	19	86	58 ± 4
4		0.94	34	36	12 ± 4
					54 ± 17[g]
					48 ± 11[h]
SEA0400 (**3**)		0.29	98	340	44 ± 11
KB-R7943 (**2**)		5.1	24	4.7	9.4 ± 5[g]

[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] Inhibitory activity for reverse NCX in ex vivo assay after 2 h with oral administration at 30 mg/kg: % inhibition in in vitro assay by ^{45}Ca influx compared with plasma control (mean ± SEM).
[f] Not tested.
[g] 100 mg/kg p.o.
[h] 10 mg/kg i.v.

NCX inhibitor. Compound **5** also has better oral activity than reference compounds **2**–**4** (Table 1). Unfortunately, **5** shows significant inhibitory activity against CYP 2C9, 2C19, 2D6, and 3A4, as shown in Table 3. For this reason, novel reverse NCX inhibitors with more acceptable levels of CYP[21] inhibitory activity are needed. We planned to synthesize more potent orally active NCX inhibitors with no CYP inhibitory activity based on **5**. We previously found that the 3-position in the phenyl ring system is optimal for the introduction of substituents designed to increase inhibitory activity against reverse NCX.[18] The results we obtained prompted us to introduce several substituents into the pyridine ring system to create novel NCX inhibitors. The structure–activity relationships of novel nicotinamide derivatives are shown in Table 1. Compounds **23a** (Me) and **23b** (*i*-Pr) were found to be less potent than compound **5**. The introduction of halogen atoms such as fluoro (**23c**), chloro (**23d**), and bromo (**23e**) also reduced potency. Derivatives containing cyano (**23f**) and methoxy (**23g**) groups were also approximately 5-fold less potent than **5** but the introduction of an amino group (**23h**) increased inhibitory activity against reverse NCX by 2-fold. The IC_{50} value of **23h** was 0.12 μM, showing it to be more potent than SEA0400 (**3**). Its selectivity was also increased 2.7-fold when compared to **5**, and compound **23h** showed better oral activity than **5**. Based on **23h**, the substituents were replaced with several alkyl amino groups (**23i**–**n**). These compounds, more hydrophobic than **23h**, were less potent. Compounds **23a**, **23d**, and **23h** were evaluated in CYP assays (Table 3). Although **23a** and **23d** displayed less potent inhibitory activities against CYP 2C9, 2C19, and 2D6 than **5**, IC_{50} values were not at acceptable levels. CYP inhibitory activity of **23h** was found significantly diminished and at an acceptable level. Thus, the introduction of an amino group at the 2-position of the pyridine ring gave a compound with a much reduced level of CYP inhibitory activity, while inhibitory activity against reverse NCX, selectivity, and oral activity were increased.

Next, we optimized the 3-fluorophenyl moiety (Table 2). To study the effect of the 3-fluoro group, this substituent was removed (**29a**). While compound **29a** had slightly reduced inhibitory activity against reverse NCX, its selectivity for reverse NCX was increased compared with **23h**. Its oral activity was slightly reduced compared to **23h**. To investigate the best position for substituents in the phenyl ring, a fluoro or methyl group was introduced into the 2- or 3- or 4-position. Compounds **29b** and **29c**, with 2-fluoro or 4-fluoro groups, respectively, were slightly less potent inhibitors of reverse NCX than

Table 2. Inhibitory activity of nicotinamide derivatives against the sodium–calcium exchanger



Compd	R^2	^{45}Ca influx[a] IC_{50} (μM)[c]	Cell necrosis[b] EC_{50} (μM)[c]	Selectivity[d]	Ex vivo[e] (inhibition %)
29a	C_6H_4–	0.20	100	500	60 ± 3
29b	2-F-C_6H_4–	0.22	94	420	33 ± 2
29c	4-F-C_6H_4–	0.22	14	63	71 ± 6
29d	2-Me-C_6H_4–	0.32	9.3	29	30 ± 4
29e	3-Me-C_6H_4–	0.21	12	57	NE[f]
29f	4-Me-C_6H_4–	0.37	>100	>270	NE[f]
29g	3-Cl-C_6H_4–	0.30	>100	>330	52 ± 12
29h	3-CN-C_6H_4–	0.31	15	48	94 ± 1
29i	3-NO_2-C_6H_4–	0.25	15	60	62 ± 8
29j	3-CF_3-C_6H_4–	0.27	3.3	12	60 ± 0.3
29k	3-Thienyl–	0.16	9.4	59	NE[f]
23h	3-F-C_6H_4–	0.12	28	230	66 ± 2

For footnotes a–e refer to Table 1.
[f] Not effective.

Table 3. Inhibitory activity against cytochrome P450

Compd	IC_{50} (μM)				
	CYP 1A2[a]	CYP 2C9[a]	CYP 2C19[a]	CYP 2D6[a]	CYP 3A4[b]
23a	>50	1.9	5.8	>50	<0.1
23d	>50	1.0	3.5	14	19
23h	>50	37	46	>50	>15[c]
29a	>50	>50	>50	>50	>50
29c	>50	>50	>50	>50	12
29h	>50	8.2	26	>50	4.2
29i	>50	2.8	10	>50	2.5
29j	>50	1.9	14	50	4.2
5	>50	<0.1	<0.1	<0.1	0.57
Mexiletine	2.2	>50	>50	2.8	>50

[a] Substrate is 3-cyano-7-ethoxycoumarin.
[b] Substrate is resorufin benzyl ether.
[c] Substrate is simvastatin.

23h with a 3-fluoro group. Among compounds **29d–f** with methyl groups, **29e**, a 3-methyl derivative, was slightly more potent than **29d** and **29f**. These results suggested that the 3-position of the phenyl ring is optimal for the introduction of substituents designed to increase for reverse NCX inhibitory activity. This prompted us to introduce other substituents into the 3-position of the phenyl ring. Compounds **29g** (Cl), **29h** (CN), **29i** (NO_2) and **29j** (CF_3) were slightly less potent inhibitors of reverse NCX than **23h**. Compounds **29h–j** had good oral activity, but their selectivity was significantly reduced. Compound **29k**, with a 3-thienyl ring, showed similar inhibitory activity against reverse NCX as that of **23h**, but had reduced selectivity and no oral activity. The orally active compounds **29a**, **29c**, and **29h–j** were evaluated for CYP inhibitory activity (Table 3). Among them, **29a** and **29c** retained acceptable levels of CYP inhibition. Compounds **29h–j** showed more potent inhibitory activity against CYP 2C9, 2C19, and 3A4 than **23h**. Considering together inhibitory activity against reverse NCX, selectivity, oral activity, and effects on CYP enzymes, the better compounds in the present series are **23h** and **29c**.

On the basis of the in vitro studies described above, we selected compound **23h** and evaluated its efficacy in an ouabain-induced tonotropy and arrhythmia model of heart failure.[16] The effects of compound **23h** and lead compound **5** on the tonotropic effects of ouabain and on the time for the ouabain-induced onset of arrhythmia in isolated guinea pig atria were evaluated. With regard to the tonotropic effect, compound **23h** was slightly more potent than **5**, as shown in Table 4. The time to onset of

Table 4. Effective concentrations of compounds **23h** and reference compound **5** on tonotropic effects of ouabain in guinea pig isolated atria

Compd	EC_{50} (μM)
23h	0.68
5	0.95

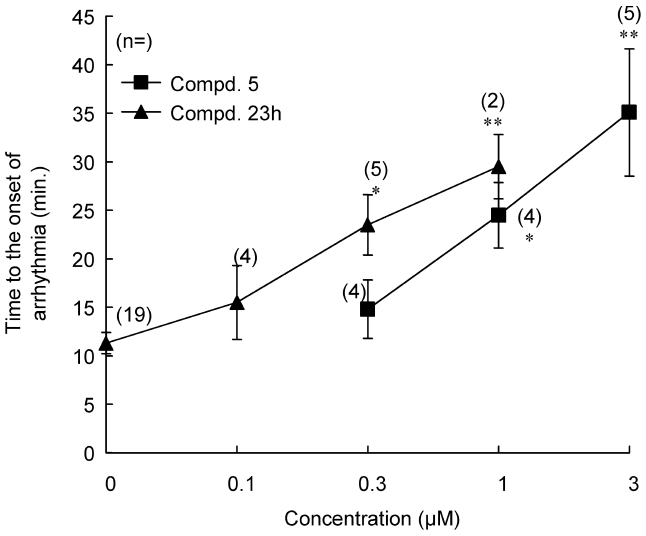


Figure 2. Effects of compounds **5** and **23h** on the onset of arrhythmia induced by ouabain in isolated guinea pig isolated atria. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control (Dunnett's test). Each value is the mean ± SEM of at least two experiments.

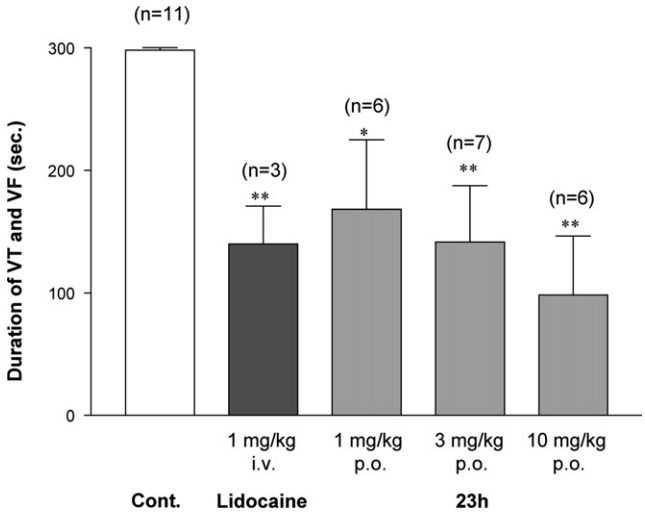


Figure 3. The effect of compound **23h** on the duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) in myocardial ischemia-reperfusion in rat. Lidocaine (1 mg/kg) was given intravenously as a bolus 5 min before coronary artery occlusion, and **23h** (1, 3, and 10 mg/kg) was given orally 2 h before coronary artery occlusion. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control (Dunnett's test). Each value is the mean ± SEM of 3–11 experiments.

arrhythmia was delayed in a concentration-dependent manner following treatment with compound **23h**, and compound **23h** was more effective than **5**, as shown in Figure 2. Therefore, compound **23h** was confirmed to have efficacy against ouabain-induced tonotropy and the onset of arrhythmia, and was also found clearly to be more potent than compound **5** in the model.

Finally, compound **23h** was subjected to further pharmacological evaluation. Compound **23h** was orally administered to rats, and its effects on ventricular tachycardia (VT) and ventricular fibrillation (VF) were examined in the ischemia-reperfusion setting. The effects of compound **23h** were concentration-dependent (Fig. 3) with an ED_{50} value of 2.9 mg/kg. The effect of compound **23h** at the ED_{50} value was similar to that of lidocaine after a single intravenous dose of 1 mg/kg. Orally administered **23h** showed potent anti-arrhythmic efficacy in the ischemia-reperfusion setting. Compound **23h** was further investigated and its pharmacokinetic profile measured in rats after a single intravenous dose of 1 mg/kg. The plasma concentration–time curve is shown in Figure 4. The experiment was used to calculate a plasma half-life for **23h** of 2.1 h with a clearance of 2.7 L/h/kg. In addition, the volume of distribution was 2.4 L/kg, suggesting significant accessibility to peripheral compartments. Compound **23h** plasma concentration was also measured after single-dose oral administration of 3 mg/kg. The dose resulted in a maximum plasma concentration (C_{max}) of 182 ng/mL, allowing bioavailability to be calculated as a dose-adjusted ratio of the area under the curve of **23h** after intravenous and oral administration; the bioavailability was calculated to be 24%. Compound **23h** was the most potent orally bioavailable inhibitor of reverse NCX. *N*-(2-Aminopyridin-4-ylmethyl)nicotinamide derivatives, as

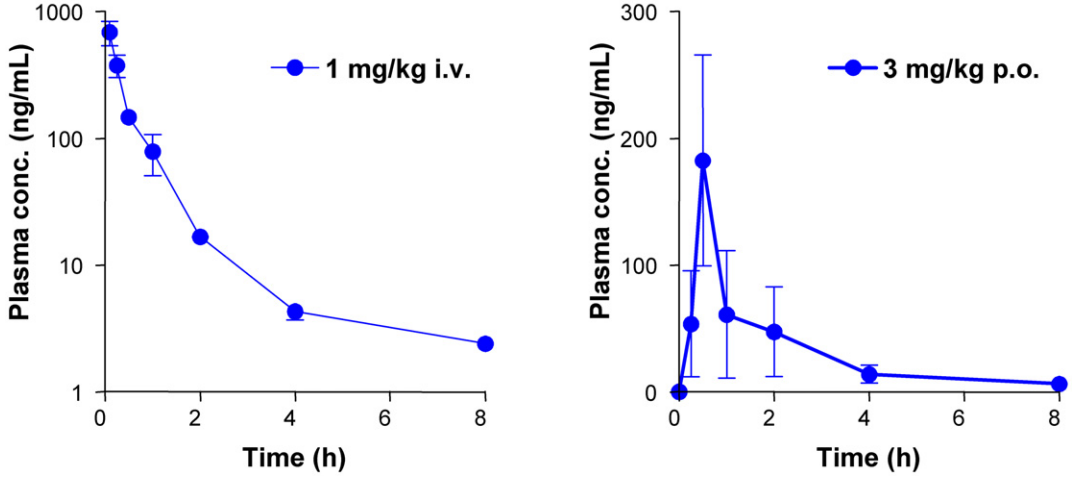


Figure 4. In vivo pharmacokinetic profiles of **23h**. Compound **23h** was dosed to rats intravenously or orally and plasma samples were drawn at the time points indicated (Mean ± SD, $n = 3$).

represented by **23h**,[22] are promising candidates for anti-arrhythmic drugs in the setting of ischemia-reperfusion and the prevention of heart failure or arrhythmia.

4. Conclusion

A series of *N*-(2-aminopyridin-4-ylmethyl)nicotinamide derivatives have been prepared and evaluated for their inhibitory activity against the reverse and forward modes of NCX. By modifying pyridine ring substituents, we have found an amino group in the pyridine ring to enhance reverse NCX inhibitory activity, selectivity, and oral activity. Compound **23h** (YM-281956) has an IC_{50} value of 0.12 μM against reverse NCX and is more potent than SEA0400 (**3**). We have also partially overcome the unwanted CYP inhibitory activity of some of the active compounds by introducing an amino group at the 2-position of the pyridine ring of template **5**. Compound **23h** was evaluated for its effects on VT and VF in the ischemia-reperfusion setting. The ED_{50} value of **23h** on oral administration was 2.9 mg/kg. Compound **23h** was found to be a potent orally bioavailable inhibitor of reverse NCX and a treatment agent for heart failure. We suggest that *N*-(2-aminopyridin-4-ylmethyl)nicotinamide derivatives, as represented by compound **23h**, are promising candidates for anti-arrhythmic drugs in the ischemia-reperfusion setting and as prophylactic anti-arrhythmic agents.

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. 2-Isopropylisonicotinonitrile (7). To the mixture of isonicotinonitrile (**6**) (2.08 g, 20 mmol), DMSO (140 mL), and concd H_2SO_4 (1.11 mL, 20 mmol), $FeSO_4{\cdot}H_2O$ (1.11 g, 4.00 mmol), and 31% H_2O_2 in H_2O (6.59 mL, 60 mmol) was added 2-iodopropane (5.99 mL, 60 mmol) at room temperature. The mixture was stirred at room temperature for 1 h. The mixture was quenched with H_2O (100 mL) and basified with 1 M NaOH. The mixture was extracted with AcOEt. The organic layer was dried and concentrated in vacuo. The residue was chromatographed over silica gel eluting with hexane–AcOEt (1:0–4:1 by volume) to give **7** as a light yellow oil (703 mg, 24%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32 (6H, d, J = 7.0 Hz), 3.05–3.20 (1H, m), 7.33 (1H, d, J = 1.5 Hz), 7.34 (1H, d, J = 1.5 Hz), 7.39–7.41 (1H, m), 8.71 (1H, dd, J = 5.0, 0.7 Hz); MS (FAB) m/z 146 M^+.

5.1.2. 1-(2-Isopropylpyridin-4-yl)methanamine (8). The mixture of 2-isopropylisonicotinonitrile (**7**) (690 mg, 4.72 mmol), EtOH (10 mL), 28% aqueous NH_3 (1 mL) and Raney Ni (0.5 mL) was stirred at room temperature under H_2 (3 kgf/cm^2) for 17 h. The catalyst was filtered though a Celite and the filtrate was concentrated in vacuo. The residue was chromatographed over silica gel eluting with $CHCl_3$–MeOH (98:2–92:8 by volume) to give **8** as a light yellow oil (320 mg, 45%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (6H, d, J = 7.0 Hz), 2.99–3.14 (1H, m), 3.89 (2H, s), 7.04–7.08 (1H, m), 7.13 (1H, s), 8.47 (1H, d, J = 5.1 Hz); MS (FAB) m/z 151 $(M+H)^+$.

5.1.3. 1-(2-Bromopyridin-4-yl)methanamine (10). To the mixture of 2-bromoisonicotinamide (**9**) (503 mg, 2.50 mmol) and THF (5 mL) was added borane THF complex (7.5 mL, 7.5 mmol) at 0 °C. The mixture was stirred at 70 °C for 4 h, and was quenched with MeOH (5 mL) and 1 M NaOH (5 mL) at 0 °C. The mixture was stirred at 70 °C for 30 min. The mixture was partitioned between $CHCl_3$ and saturated NaCl. The organic layer was dried and concentrated in vacuo. The residue was chromatographed over silica gel eluting with $CHCl_3$–MeOH (98:2–96:4 by volume) to give **10** as a light yellow syrup (238 mg, 51%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.73 (2H, s), 7.38 (1H, d, J = 5.1 Hz), 7.62 (1H, s), 8.27 (1H, d, J = 5.1 Hz); MS (FAB) m/z 189 $(M+H)^+$.

5.1.4. 4-(Aminomethyl)pyridine-2-carbonitrile (12). The mixture of 4-methylpyridine-2-carbonitrile (**11**) (1.60 g, 13.5 mmol), CCl_4 (60 mL), NBS (2.64 g, 14.9 mmol), and benzoylperoxide (40 mg) was irradiated by a 300 W lamp (National, PRS-300 W) under reflux. The mixture was filtrated and the filtrate was concentrated in vacuo. The residue was chromatographed over silica gel eluting with hexane–AcOEt (10:1 by volume) to give 4-(bromomethyl)pyridine-2-carbonitrile (250 mg). The intermediate was dissolved in THF (5 mL). To the mixture was added 28% aqueous NH_3 (5 mL), and the mixture was stirred at 50 °C for 2 h. The mixture was concentrated in vacuo. The residue was chromatographed over silica gel eluting with $CHCl_3$–MeOH (20:1 by volume) to give **12** as a pale yellow oil (40 mg, 2.2% in two steps): ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.81 (2H, s), 7.68–7.70 (1H, m), 7.99–8.00 (1H, m), 8.67 (1H, d, J = 6.4 Hz); MS (FAB) m/z 134 $(M+H)^+$.

5.1.5. Methyl 2-[(*tert*-butoxycarbonyl)amino]isonicotinate (14). The mixture of 2-chloroisonicotinic acid (**13**) (8.57 g, 55.0 mmol) and 28% aqueous NH_3 (76.5 mL, 550 mmol) was stirred at 240 °C for 22 h in a steel tube. The mixture was cooled at room temperature and was concentrated in vacuo to give crude 2-aminoisonicotinic acid as a white solid. To the mixture of crude 2-aminoisonicotinic acid and MeOH (100 mL) was added concd H_2SO_4 (10 mL) at 0 °C. The mixture was stirred at 75 °C

for 18 h. The mixture was cooled at room temperature and half the volume of solvent was removed under reduced pressure. MeOH (10 mL) and toluene (10 mL) were added to the mixture. The mixture was concentrated in vacuo. The azeotrope was performed two times. To the residue were added AcOEt (5 mL) and Et_2O (50 mL). The precipitate was filtered to give a mixture of methyl 2-aminoisonicotinate and methyl 2-oxo-1,2-dihydropyridine-4-carboxylate (**31**)[23] (100:13) as a beige powder (6.61 g, 70%): mp 141–142 °C; ^{1}H NMR of methyl 2-aminoisonicotinate (400 MHz, DMSO-d_6) δ 3.84 (3H, s), 6.28 (2H, s), 6.88 (1H, dd, J = 5.4, 1.5 Hz), 6.96 (1H, s), 8.05 (1H, d, J = 5.4 Hz); MS (FAB) m/z 153 $(M+H)^+$. Anal. Calcd for $C_7H_8N_2O_2{\cdot}0.13C_7H_7NO_3$ (mixture of methyl 2-aminoisonicotinate and methyl 2-oxo-1,2-dihydropyridine-4-carboxylate): C, 55.22; H, 5.22; N, 17.34. Found: C, 55.11; H, 5.09; N, 17.36.

To the mixture of methyl 2-aminoisonicotinate and methyl 2-oxo-1,2-dihydropyridine-4-carboxylate (7.53 g, 43.8 mmol) and *t*-BuOH (60 mL) was added a *t*-BuOH (15 mL) solution of Boc_2O (13.0 g, 59.4 mmol) at room temperature. The mixture was stirred at 60 °C for 19 h. After cooling at room temperature, the precipitate was filtered and washed with *t*-BuOH to give **14** as a beige powder (10.4 g, 94%): mp 181–183 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49 (9H, s), 3.32 (3H, s), 7.45 (1H, d, J = 4.9, 1.5 Hz), 8.33 (1H, s), 8.43 (1H, d, J = 4.9 Hz), 10.11 (1H, s); MS (FAB) m/z 253 $(M+H)^+$. Anal. Calcd for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.10. Found: C, 56.93; H, 6.20; N, 11.48.

5.1.6. *tert*-Butyl [4-(hydroxymethyl)pyridin-2-yl]carbamate (15). To the mixture of methyl 2-[(*tert*-butoxycarbonyl)amino]isonicotinate (**14**) (9.30 g, 36.9 mmol) and EtOH (140 mL) were added $CaCl_2$ (6.14 g, 55.3 mmol) and $NaBH_4$ (4.19 g, 110.7 mmol) at 0 °C. The mixture was stirred at room temperature for 4 h. To the mixture were added H_2O (140 mL) and 2-butanone (140 mL) at 0 °C. The precipitate was filtered through a Celite and washed with 2-butanone (four times). The filtrate was washed with aqueous NaCl. The organic layer was concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH = 99:1–96:4) to give **15** as a colorless solid (7.44 g, 90%): mp 137–138 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.47 (9H, s), 4.50 (2H, d, J = 5.9 Hz), 5.38 (1H, t, J = 5.9 Hz), 6.94 (1H, d, J = 4.9 Hz), 7.80 (1H, s), 8.14 (1H, d, J = 4.9 Hz), 9.64 (1H, s); MS (FAB) m/z 225 $(M+H)^+$. Anal. Calcd for $C_{11}H_{16}N_2O_3$: C, 58.91; H, 7.19; N, 12.49. Found: C, 58.50; H, 7.16; N, 12.37.

5.1.7. *tert*-Butyl {4-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]pyridin-2-yl}carbamate (16). To the mixture of *tert*-butyl [4-(hydroxymethyl)pyridin-2-yl]carbamate (**15**) (7.44 g, 33.2 mmol) and THF (70 mL) were added Et_3N (5.52 mL, 39.6 mmol) and a THF (10 mL) solution of methanesulfonyl chloride (2.81 mL, 36.3 mmol) at 0 °C. The mixture was stirred at room temperature for 15 min. The mixture was partitioned between Et_2O (150 mL) and 1 M NaOH (100 mL). The organic layer was washed with aqueous NaCl and dried over Na_2SO_4. The layer was concentrated in vasuo to give crude {2-[(*tert*-butoxycarbonyl)amino]pyridin-4-yl}methyl methanesulfonate as a colorless solid. The crude product was diluted with DMF (80 mL). To the mixture was added potassium 1,3-dioxo-1,3-dihydroisoindol-2-ide (6.72 g, 36.3 mmol). The mixture was stirred at 50 °C for 10 min. DMF (30 mL) was added to the mixture. The mixture was stirred for 20 min. DMF (20 mL) was added to the mixture. The mixture was cooled at 0 °C. H_2O (300 mL) was added to the mixture. The precipitate was filtered to afford **16** as a colorless powder (11.24 g, 95%): mp 201–203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (9H, s), 4.77 (2H, s), 6.92 (1H, dd, J = 5.3, 1.4 Hz), 7.72 (1H, s), 7.87–7.90 (2H, m), 7.91–7.94 (2H, m), 8.16 (1H, d, J = 5.3 Hz), 9.74 (1H, s); MS (FAB) m/z 354 $(M+H)^+$. Anal. Calcd for $C_{19}H_{19}N_3O_4{\cdot}0.2H_2O$: C, 63.93; H, 5.48; N, 11.77. Found: C, 63.99; H, 5.40; N, 11.88.

5.1.8. *tert*-Butyl [4-(aminomethyl)pyridin-2-yl]carbamate (17). To the mixture of *tert*-butyl {4-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]pyridin-2-yl}carbamate 0.2 hydrate (**16**) (11.24 g, 31.5 mmol) and MeOH (90 mL), $CHCl_3$ (60 mL) was added $H_2NNH_2{\cdot}H_2O$ (7.69 mL, 158.5 mmol) at room temperature. The mixture was stirred at room temperature for 24 h. The precipitate was filtered and the filtrate was partitioned between $CHCl_3$ (150 mL) and 1 M NaOH (100 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH–NH_4OH = 100:0.9:0.1–100:3.6:0.4) to give **17** as a colorless solid (7.04 g, 100%): mp 131–132 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.47 (9H, s), 3.70 (2H, s), 6.99 (1H, dd, J = 4.9, 1.0 Hz), 7.78 (1H, s), 8.12 (1H, d, J = 4.9 Hz), 9.61 (1H, s, CONH); MS (FAB) m/z 224 $(M+H)^+$. Anal. Calcd for $C_{11}H_{17}N_3O_2$: C, 59.17; H, 7.67; N, 18.82. Found: C, 59.04; H, 7.64; N, 18.61.

5.1.9. 4-(Aminomethyl)pyridin-2-amine hydrochloride (18).[24] To the mixture of *tert*-butyl [4-(aminomethyl)pyridin-2-yl]carbamate (**17**) (7.04 g, 31.5 mmol) and dioxane (50 mL) was added concd HCl (26.3 mL, 315 mmol) at 0 °C. The mixture was stirred at room temperature for 8 h. The mixture was concentrated in vacuo. EtOH (60 mL) was added to the residue. The mixture was refluxed for 10 min and stirred at room temperature for 3 h. The precipitate was filtered and washed with EtOH to give **18** as a colorless powder (5.85 g, 95%): mp 290–292 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.08 (2H, s), 6.98–7.04 (2H, m), 8.02 (1H, d, J = 6.3 Hz), 8.33 (2H, br s), 8.87 (2H, br s), 14.15 (1H, br s); MS (FAB) m/z 124 $(M+H)^+$. Anal. Calcd for $C_6H_9N_3{\cdot}2HCl$: C, 36.75; H, 5.65; N, 21.43; Cl, 36.16. Found: C, 36.55; H, 5.48; N, 21.37; Cl, 36.33.

5.1.10. 2-(Ethylamino)isonicotinonitrile (20a). The mixture of 2-chloroisonicotinonitrile (**19**) (1.6 g, 11.5 mmol) and 2 M $EtNH_2$ in THF (30 mL, 60 mmol) was stirred at 120 °C in a steel tube for 3 h. The mixture was concentrated in vacuo. The residue was partitioned between AcOEt and H_2O. The organic layer was dried and

concentrated in vacuo. The residue was chromatographed over silica gel eluting with hexane–AcOEt (4:1–3:1 by volume) to give **20a** as a pale yellow solid (834 mg, 49%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (3H, t, J = 7.2 Hz), 3.22–3.29 (2H, m), 6.75 (1H, dd, J = 5.2, 1.6 Hz), 6.77 (1H, s), 7.05 (1H, br s), 8.15 (1H, d, J = 5.2 Hz); MS (FAB) m/z 148 $(M+H)^+$.

5.1.11. 2-(Propylamino)isonicotinonitrile (20b). The mixture of 2-chloroisonicotinonitrile (**19**) (1.5 g, 10.8 mmol), n-propylamine (4.4 mL, 54.0 mmol), and i-Pr_2NEt (3.8 mL, 21.6 mmol) was stirred at 130 °C in a steel tube for 25 h. The mixture was concentrated in vacuo. The residue was partitioned between AcOEt and H_2O. The organic layer was dried, concentrated in vacuo. The residue was chromatographed over silica gel eluting with hexane–AcOEt (5:1–4:1 by volume) to give **20b** as a pale yellow solid (450 mg, 41%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (3H, t, J = 7.3 Hz), 1.44–1.54 (2H, m), 3.13–3.25 (1H, m), 6.74 (1H, dd, J = 4.9, 1.4 Hz), 6.79 (1H, s), 7.10 (1H, br s), 8.14 (1H, d, J = 4.9 Hz); MS (FAB) m/z 162 $(M+H)^+$.

5.1.12. 2-(Isopropylamino)isonicotinonitrile (20c). Compound **20c** was prepared from **19** by a procedure similar to that described for **20b**. Compound **20c** was obtained as a pale yellow oil (15%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.13 (6H, d, J = 6.4 Hz), 3.96–4.02 (1H, m), 6.73 (1H, d, J = 5.2 Hz), 6.75 (1H, s), 6.93 (1H, d, J = 5.2 Hz), 8.14 (1H, d, J = 5.2 Hz); MS (FAB) m/z 162 $(M+H)^+$.

5.1.13. 2-(Isobutylamino)isonicotinonitrile (20d). Compound **20d** was prepared from **19** by a procedure similar to that described for **20b**. Compound **20d** was obtained as a pale yellow solid (29%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (6H, d, J = 6.4 Hz), 1.76–1.86 (1H, m), 3.07 (2H, t, J = 6.4 Hz), 6.73 (1H, d, J = 5.2 Hz); MS (FAB) m/z 176 $(M+H)^+$.

5.1.14. 4-(Aminomethyl)-*N*-ethylpyridin-2-amine (21a). The mixture of 2-(ethylamino)isonicotinonitrile (**20a**) (830 mg, 5.7 mmol), Raney Ni (2.0 g), and MeOH (60 mL), and 28% aqueous NH_3 (60 mL) was stirred at room temperature under H_2 (3.4 kgf/cm^2) for 3 h. The mixture was filtered through a Celite pad and the filtrate was concentrated in vacuo. The residue was chromatographed over silica gel eluting with $CHCl_3$–MeOH (100:1–100:10 by volume) to give **21a** as a pale yellow oil (680 mg, 79%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (3H, t, J = 7.2 Hz), 3.18–3.26 (2H, m), 3.55 (2H, s), 6.27 (1H, br s), 6.39 (1H, s), 6.41 (1H, d, J = 5.2 Hz), 7.83 (1H, d, J = 5.2Hz); MS (FAB) m/z 152 $(M+H)^+$.

5.1.15. 4-(Aminomethyl)-*N*-propylpyridin-2-amine (21b). Compound **21b** was prepared from **20b** by a procedure similar to that described for **21a**. Compound **21b** was obtained as a pale yellow oil (46%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (3H, t, J = 7.2 Hz), 1.45–1.56 (2H, m), 3.13–3.18 (2H, m), 3.55 (2H, s), 6.29–6.32 (1H, m), 6.39–6.42 (2H, m), 7.82 (1H, d, J = 4.8 Hz); MS (FAB) m/z 166 $(M+H)^+$.

5.1.16. 4-(Aminomethyl)-*N*-isopropylpyridin-2-amine (21c). Compound **21c** was prepared from **20c** by a procedure similar to that described for **21a**. Compound **21c** was obtained as a pale yellow oil (88%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (6H, d, J = 6.4 Hz), 3.54 (2H, s), 3.91–4.01 (1H, m), 6.10 (1H, d, J = 7.6 Hz), 6.37–6.41 (2H, m), 7.83 (1H, d, J = 4.8 Hz); MS (FAB) m/z 166 $(M+H)^+$.

5.1.17. 4-(Aminomethyl)-*N*-isobutylpyridin-2-amine (21d). Compound **21d** was prepared from **20d** by a procedure similar to that described for **21a**. Compound **21d** was obtained as a pale yellow oil (77%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (6H, d, J = 6.8 Hz), 1.76–1.87 (1H, m), 3.02 (2H, t, J = 6.4 Hz), 3.54 (2H, s), 6.33–6.43 (3H, m), 8.46 (1H, d, J = 5.2 Hz); MS (FAB) m/z 180 $(M+H)^+$.

5.1.18. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-[(2-methylpyridin-4-yl)methyl]nicotinamide hydrochloride (23a). To the mixture of 6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinic acid (**22**) (574 mg, 1.69 mmol) and 1-hydroxybenzotriazole (HOBt) (114 mg, 0.84 mmol), DMF (4 mL), and 4-aminomethyl-2-methylpyridine (259 mg, 2.12 mmol) was added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSC·HCl) (324 mg, 1.69 mmol) at room temperature. The mixture was stirred overnight. The mixture was partitioned between AcOEt and H_2O. The organic layer was washed with brine and dried over $MgSO_4$. The residue was purified by column chromatography on silica gel (CH_3Cl–MeOH = 50:1–30:1) to give the free base of **23a** (151 mg). This material was converted to its hydrochloride salt by treating it with hydrochloride in THF. To the mixture was added Et_2O. The precipitate was collected to give **23a** as a white solid (18%): mp 173–175 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.70 (3H, s), 4.68 (2H, d, J = 5.8 Hz), 5.16 (2H, s), 7.05–7.14 (5H, m), 7.14–7.21 (1H, m), 7.28–7.34 (2H, m), 7.43–7.49 (1H, m), 7.75 (1H, d, J = 5.9 Hz), 7.80 (1H, s), 8.32 (1H, dd, J = 8.8, 2.5 Hz), 8.68 (1H, d, J = 6.4 Hz), 8.70 (1H, d, J = 2.4 Hz), 9.43–9.50 (1H, m); MS (FAB) m/z 444 $(M+H)^+$. Anal. Calcd for $C_{26}H_{22}N_3O_3F$·HCl: C, 65.07; H, 4.83; N, 8.76; F, 3.96; Cl, 7.39. Found: C, 64.92; H, 4.90; N, 8.62; F, 3.91; Cl, 7.15.

5.1.19. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-[(2-isopropylpyridin-4-yl)methyl]nicotinamide oxalate (23b). Compound **23b** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23b** was obtained as a beige amorphous (47%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22 (6H, d, J = 6.8 Hz), 2.95–3.07 (1H, m), 4.50 (2H, d, J = 5.8 Hz), 5.15 (2H, s), 7.04–7.20 (7H, m), 7.25 (1H, s), 7.28–7.34 (2H, m), 7.42–7.49 (1H, m), 8.28 (1H, dd, J = 8.3, 2.4 Hz), 8.43 (1H, d, J = 5.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 9.15 (1H, t, J = 6.0 Hz); MS (FAB) m/z 472 $(M+H)^+$. Anal. Calcd for $C_{28}H_{26}N_3O_3F$·$(CO_2H)_2$: C, 64.16; H, 5.03; N, 7.48; F, 3.38. Found: C, 64.32; H, 5.17; N, 7.66; F, 3.33.

5.1.20. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-[(2-fluoropyridin-4-yl)methyl]nicotinamide hydrobromide (23c). Compound **23c** was prepared from **22** by a procedure

similar to that described for **23a**. Compound **23c** was obtained as a beige powder (78%): mp 140–147 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.45 (2H, d, J = 5.3 Hz), 5.15 (2H, s), 7.05–7.14 (6H, m), 7.14–7.21 (1H, m), 7.27–7.34 (3H, m), 7.42–7.49 (1H, m), 8.18 (1H, d, J = 5.4 Hz), 8.28 (1H, dd, J = 8.8, 2.5 Hz), 8.65 (1H, d, J = 2.5 Hz), 9.21 (1H, t, J = 5.8 Hz); MS (FAB) m/z 448 $(M+H)^+$. Anal. Calcd for $C_{25}H_{19}N_3O_3F_2$·2HBr: C, 56.83; H, 3.82; N, 7.95; F, 7.19; Br, 15.12. Found: C, 56.74; H, 3.83; N, 7.86; F, 7.19; Br, 15.43.

5.1.21. *N*-[(2-Chloropyridin-4-yl)methyl]-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide (23d). Compound **23d** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23d** was obtained as a white solid (65%): mp 111–112 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.52 (2H, d, J = 5.8 Hz), 5.15 (2H, s), 7.05–7.21 (6H, m), 7.28–7.36 (3H, m), 7.42–7.49 (2H, m), 8.28 (1H, dd, J = 8.8, 2.4 Hz), 8.35 (1H, d, J = 4.9 Hz), 8.65 (1H, d, J = 2.4 Hz), 9.19 (1H, t, J = 5.8 Hz); MS (FAB) m/z 464 $(M+H)^+$. Anal. Calcd for $C_{25}H_{19}N_3O_3FCl$: C, 64.73; H, 4.13; N, 9.06; F, 4.10; Cl, 7.64. Found: C, 64.78; H, 4.03; N, 9.15; F, 4.37; Cl, 7.62.

5.1.22. *N*-[(2-Bromopyridin-4-yl)methyl]-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide dihydrobromide (23e). Compound **23e** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23e** was obtained as a white solid (19%): mp 160–175 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.50 (2H, d, J = 5.8 Hz), 5.15 (2H, s), 7.05–7.21 (6H, m), 7.28–7.34 (2H, m), 7.36–7.40 (1H, m), 7.42–7.49 (1H, m), 7.57 (1H, s), 8.28 (1H, dd, J = 8.3, 2.4 Hz), 8.33 (1H, d, J = 4.9 Hz), 8.66 (1H, d, J = 2.0 Hz), 9.21 (1H, t, J = 6.0 Hz); MS (FAB) m/z 508, 510 $(M+H)^+$. Anal. Calcd for $C_{25}H_{19}N_3O_3FBr$·2HBr·0.2H_2O: C, 44.57; H, 3.20; N, 6.24; F, 2.82; Br, 35.58. Found: C, 44.73; H, 3.10; N, 6.21; F, 2.74; Br, 35.36.

5.1.23. *N*-[(2-Cyanopyridin-4-yl)methyl]-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide hydrobromide (23f). Compound **23f** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23f** was obtained as a beige powder (78%): mp 156–166 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.15 (2H, s), 7.05–7.13 (5H, m), 7.14–7.21 (1H, m), 7.28–7.34 (2H, m), 7.43–7.48 (1H, m), 7.66 (1H, dd, J = 5.4, 2.0 Hz), 7.98 (1H, s), 8.28 (1H, dd, J = 8.4, 2.4 Hz), 8.66 (1H, d, J = 2.4 Hz), 8.68 (1H, d, J = 4.4 Hz), 9.22 (1H, t, J = 5.8 Hz); MS (FAB) m/z 455 $(M+H)^+$. Anal. Calcd for $C_{26}H_{19}N_4O_3F$·HBr: C, 58.33; H, 3.77; N, 10.47; F, 3.55. Found: C, 58.63; H, 3.64; N, 10.49; F, 3.67.

5.1.24. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-[(2-methoxypyridin-4-yl)methyl]nicotinamide (23g). Compound **23g** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23g** was obtained as a beige powder (19%): mp 89–90 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.82 (3H, s), 4.45 (2H, d, J = 5.9 Hz), 5.15 (2H, s), 6.70 (1H, s), 6.92 (1H, d, J = 5.4 Hz), 7.04–7.14 (5H, m), 7.14–7.21 (1H, m), 7.28–7.34 (2H, m), 7.43–7.49 (1H, m), 8.09 (1H, d, J = 5.3 Hz), 8.27 (1H, dd, J = 8.7, 2.4 Hz), 8.64 (1H, d, J = 2.4 Hz), 9.13 (1H, t, J = 6.1 Hz); MS (FAB) m/z 460 $(M+H)^+$. Anal. Calcd for $C_{26}H_{22}N_3O_4F$: C, 67.97; H, 4.83; N, 9.15; F, 4.13. Found: C, 68.05; H, 4.74; N, 9.14; F, 4.18.

5.1.25. *N*-[(2-Aminopyridin-4-yl)methyl]-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide (23h). Compound **23h** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23h** was obtained as a white powder (60%): mp 182–183 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (2H, d, J = 5.6 Hz), 5.15 (2H, s), 5.85 (2H, br s), 6.34 (1H, s), 6.40–6.41 (1H, m), 7.06–7.13 (5H, m), 7.15–7.20 (1H, m), 7.29–7.32 (2H, m), 7.43–7.49 (1H, m), 7.81 (1H, d, J = 5.2 Hz), 8.27 (1H, dd, J = 8.8, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 9.05 (1H, t, J = 5.6 Hz); MS (FAB) m/z 445 $(M+H)^+$. Anal. Calcd for $C_{25}H_{21}N_4O_3F$: C, 67.56; H, 4.76; N, 12.61; F, 4.27. Found: C, 67.56; H, 4.76; N, 12.61; F, 4.37.

5.1.26. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-{[2-(methylamino)pyridin-4-yl]methyl}nicotinamide (23i). Compound **23i** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23i** was obtained as a white powder (78%): mp 119–121 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.74 (3H, d, J = 4.8 Hz), 4.34 (2H, d, J = 5.6 Hz), 5.15 (2H, s), 6.36 (1H, s), 6.43 (1H, d, J = 5.3 Hz), 6.52 (1H, br s), 7.04–7.13 (5H, m), 7.14–7.20 (1H, m), 7.28–7.32 (2H, m), 7.42–7.49 (1H, m), 7.89 (1H, d, J = 5.4 Hz), 8.26 (1H, dd, J = 8.5, 2.5 Hz), 8.64 (1H, d, J = 2.5 Hz), 9.02–9.09 (1H, m); MS (FAB) m/z 459 $(M+H)^+$. Anal. Calcd for $C_{26}H_{23}N_4O_3F$·0.2H_2O: C, 67.58; H, 5.10; N, 12.12; F, 4.11. Found: C, 67.57; H, 4.98; N, 12.12; F, 4.35.

5.1.27. *N*-{[2-(Ethylamino)pyridin-4-yl]methyl}-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide (23j). Compound **23j** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23j** was obtained as a white powder (74%): mp 93–95 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, t, J = 7.3 Hz), 3.20–3.28 (2H, m), 4.36 (2H, d, J = 5.9 Hz), 5.15 (2H, s), 6.46 (1H, s), 6.48 (1H, d, J = 5.9 Hz), 7.05–7.13 (5H, m), 7.14–7.20 (1H, m), 7.28–7.33 (2H, m), 7.43–7.49 (1H, m), 7.86 (1H, d, J = 5.8 Hz), 8.27 (1H, dd, J = 8.8, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 9.10 (1H, t, J = 5.9 Hz); MS (FAB) m/z 473 $(M+H)^+$. Anal. Calcd for $C_{27}H_{25}N_4O_3F$·H_2O: C, 66.11; H, 5.55; N, 11.42; F, 3.87. Found: C, 66.28; H, 5.21; N, 11.42; F, 4.22.

5.1.28. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-{[2-(propylamino)pyridin-4-yl]methyl}nicotinamide (23k). Compound **23k** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23k** was obtained as a white powder (69%): mp 109–111 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (3H, t, J = 7.6 Hz), 1.44–1.54 (2H, m), 3.12–3.18 (2H, m), 4.32 (2H, d, J = 5.3 Hz), 5.15 (2H, s), 6.34 (1H, s), 6.37 (1H, d, J = 5.3 Hz), 6.44–6.49 (1H, m), 7.05–7.13 (5H, m), 7.14–7.21 (1H, m), 7.28–7.34 (2H, m), 7.43–7.49 (1H, m), 7.86 (1H, d, J = 5.4 Hz), 8.26 (1H, dd, J = 8.8, 2.5 Hz), 8.64 (1H, d, J = 2.5 Hz), 9.05 (1H, t, J = 6.0 Hz); MS (FAB) m/z 487 $(M+H)^+$. Anal. Calcd

for $C_{28}H_{27}N_4O_3F$: C, 69.12; H, 5.59; N, 11.52; F, 3.90. Found: C, 69.06; H, 5.55; N, 11.57; F, 3.96.

5.1.29. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-{[2-(isopropylamino)pyridin-4-yl]methyl}nicotinamide hydrochloride (23l). Compound **23l** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23l** was obtained as a white powder (85%): mp 116–118 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.20 (6H, d, J = 6.4 Hz), 3.90–3.99 (1H, m), 4.47 (2H, d, J = 5.4 Hz), 5.16 (2H, s), 6.79 (1H, d, J = 6.4 Hz), 6.85 (1H, s), 7.06–7.13 (5H, m), 7.14–7.21 (1H, m), 7.28–7.34 (2H, m), 7.43–7.49 (1H, m), 7.83 (1H, d, J = 6.8 Hz), 8.30 (1H, d, J = 8.8, 2.4 Hz), 8.66–8.72 (2H, m), 9.33 (1H, t, J = 5.8 Hz); MS (FAB) m/z 487 $(M+H)^+$. Anal. Calcd for $C_{28}H_{27}N_4O_3F{\cdot}HCl{\cdot}1.4H_2O$: C, 60.53; H, 5.51; N, 10.08; F, 3.42; Cl, 8.93. Found: C, 60.58; H, 5.50; N, 10.08; F, 3.30; Cl, 9.20.

5.1.30. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-{[2-(isobutylamino)pyridin-4-yl]methyl}nicotinamide (23m). Compound **23m** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23m** was obtained as a white powder (40%): mp 114–115 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (6H, d, J = 6.8 Hz), 1.72–1.84 (1H, m), 3.02 (2H, t, J = 6.4 Hz), 4.32 (2H, d, J = 5.3 Hz), 5.15 (2H, s), 6.33–6.38 (2H, m), 6.48–6.54 (1H, m), 7.04–7.07 (5H, m), 7.07–7.21 (1H, m), 7.24–7.27 (2H, m), 7.41–7.44 (1H, m), 7.81–7.83 (1H, m), 8.27 (1H, dd, J = 8.8, 2.5 Hz), 8.65 (1H, d, J = 2.5 Hz), 9.04 (1H, t, J = 5.9 Hz); MS (FAB) m/z 501 $(M+H)^+$. Anal. Calcd for $C_{29}H_{29}N_4O_3F$: C, 69.58; H, 5.84; N, 11.19; F, 3.80. Found: C, 69.55; H, 5.81; N, 11.23; F, 3.87.

5.1.31. *N*-{[2-(Dimethylamino)pyridin-4-yl]methyl}-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide (23n). Compound **23n** was prepared from **22** by a procedure similar to that described for **23a**. Compound **23n** was obtained as a white solid (51%): mp 123–124 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.99 (6H, s), 4.40 (2H, d, J = 5.8 Hz), 5.15 (2H, s), 6.50 (1H, d, J = 5.4 Hz), 6.55 (1H, s), 7.04–7.20 (6H, m), 7.28–7.34 (2H, m), 7.42–7.49 (1H, m), 8.00 (1H, d, J = 5.3 Hz), 8.26 (1H, dd, J = 8.3, 2.4 Hz), 8.63 (1H, d, J = 2.4 Hz), 9.07 (1H, t, J = 5.8 Hz); MS (FAB) m/z 473 $(M+H)^+$. Anal. Calcd for $C_{27}H_{25}N_4O_3F$: C, 68.63; H, 5.33; N, 11.86; F, 4.02. Found: C, 68.49; H, 5.58; N, 11.96; F, 4.30.

5.1.32. 6-[4-(Benzyloxy)phenoxy]nicotinonitrile (25). The mixture of 4-(benzyloxy)phenol **24** (3.00 g, 15.0 mmol), DMF (20 mL), *t*-BuOK (2.02 g, 18.0 mmol), and 6-chloronicotinonitrile (2.18 g, 15.8 mmol) was stirred at 100 °C for 3.5 h. The mixture was poured into H_2O (200 mL) at 0 °C. The precipitate was filtered and washed with H_2O to give **25** as a light brown powder (4.47 g, 99%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.12 (2H, s), 7.05–7.20 (5H, m), 7.32–7.48 (5H, m), 8.28 (1H, dd, J = 8.6, 2.4 Hz), 8.63 (1H, d, J = 2.4 Hz); MS (FAB) m/z 303 $(M+H)^+$.

5.1.33. 6-(4-Hydroxyphenoxy)nicotinonitrile (26). The mixture of 6-[4-(benzyloxy)phenoxy]nicotinonitrile **25** (21.6 g, 68.7 mmol), pentamethylbenzene (20.4 g, 137 mmol), and trifluoroacetic acid (120 mL) was stirred at room temperature for 8 h. The mixture was concentrated in vacuo. Toluene was added to the residue. The mixture was concentrated in vacuo. To the residue was added $CHCl_3$ (50 mL) and H_2O (50 mL). The precipitate was collected to give **26** as a beige powder (13.7 g, 94%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.79–6.81 (2H, m), 6.97–7.00 (2H, m), 7.13 (1H, d, J = 8.6 Hz), 8.26 (1H, dd, J = 8.6, 2.4 Hz), 8.63 (1H, t, J = 1.2 Hz), 9.47 (1H, s); MS (FAB) m/z 313 $(M+H)^+$.

5.1.34. Methyl 6-(4-hydroxyphenoxy)nicotinate (27). The mixture of 6-(4-hydroxyphenoxy)nicotinonitrile (**26**) (2.12 g, 10.0 mmol), EtOH (20 mL), and 5 M NaOH (20.0 mL, 100 mmol) was stirred at 100 °C for 1 h. The mixture was concentrated in vacuo to half the volume. To the residue was added 1 M HCl. The precipitate was filtered and washed with H_2O to give 6-(4-hydroxyphenoxy)nicotinic acid as a beige powder (2.18 g, 94%). $SOCl_2$ (3.44 mL, 47.2 mmol) was added to MeOH (30 mL) at −78 °C. To the mixture was added 6-(4-hydroxyphenoxy)nicotinic acid (2.18 g, 9.43 mmol) at −78 °C. The mixture was stirred at room temperature for 37 h. The mixture was concentrated in vacuo. To the residue were added $CHCl_3$ (30 mL) and saturated $NaHCO_3$ at 0 °C. The mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried and concentrated in vacuo. The residue was recrystallized from AcOEt–hexane to give **27** as a beige powder (1.50 g, 65%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (3H, s), 6.80 (2H, d, J = 8.8 Hz), 6.98 (2H, d, J = 8.8 Hz), 7.02 (1H, d, J = 8.8 Hz), 8.26 (1H, dd, J = 8.4, 2.4 Hz), 8.68 (1H, t, J = 1.2 Hz), 9.44 (1H, s); MS (FAB) m/z 246 $(M+H)^+$.

5.1.35. 6-[4-(Benzyloxy)phenoxy]nicotinic acid (28a). The mixture of **25** (544 mg, 1.80 mmol), EtOH (5 mL), and 5 M NaOH (3.60 mL, 18 mmol) was stirred at 100 °C for 105 min. The mixture was concentrated in vacuo. The residue was acidified with 1 M HCl at 0 °C. The precipitate was filtered and washed with H_2O to give **28a** as a white powder (570 mg, 99%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.12 (2H, s), 7.04–7.13 (5H, m), 7.27–7.48 (5H, m), 8.26 (1H, dd, J = 8.4, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 13.15 (1H, s); MS (FAB) m/z 322 $(M+H)^+$.

5.1.36. 6-{4-[(2-Fluorobenzyl)oxy]phenoxy}nicotinic acid (28b). To the mixture of **26** (424 mg, 2.00 mmol), CH_3CN (10 mL), and K_2CO_3 (415 mg, 3.00 mmol) was added 1-(bromomethyl)-2-fluorobenzene (416 mg, 2.20 mmol) at room temperature. The mixture was stirred at 80 °C for 1 h. The mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was dried and concentrated in vacuo. The residue was recrystallized from EtOH (12 mL) to give 6-{4-[(2-fluorobenzyl)oxy]phenoxy}nicotinonitrile as a beige powder (555 mg, 87%). The mixture of the intermediate (555 mg, 1.73 mmol), EtOH (5 mL), and 5 M NaOH (3.47 mL, 17.3 mmol) was stirred at 100 °C for 90 min. The mixture was concentrated in vacuo. The residue was acidified with 1 M HCl. The precipitate was filtered and

washed with H_2O to give **28b** as a white powder (550 mg, 94%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.16 (2H, s), 7.05–7.15 (5H, m), 7.24–7.29 (2H, m), 7.41–7.47 (1H, m), 7.57–7.61 (1H, m), 8.26 (1H, dd, J = 8.4, 2.4 Hz), 8.65 (1H, d, J = 2.0 Hz), 13.16 (1H, s); MS (FAB) m/z 340 $(M+H)^+$.

5.1.37. 6-{4-[(4-Fluorobenzyl)oxy]phenoxy}nicotinic acid (28c). Compound **28c** was prepared from **26** by a procedure similar to that described for **28b**. Compound **28c** was obtained as a beige powder (87% in two steps): ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.10 (2H, s), 7.04–7.13 (5H, m), 7.21–7.26 (2H, m), 7.51–7.54 (2H, m), 8.26 (1H, dd, J = 8.8, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 13.15 (1H, s); MS (FAB) m/z 340 $(M+H)^+$.

5.1.38. 6-{4-[(2-Methylbenzyl)oxy]phenoxy}nicotinic acid (28d). Compound **28d** was prepared from **26** by a procedure similar to that described for **28b**. Compound **28d** was obtained as a beige powder (66% in two steps): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.35 (3H, s), 5.10 (2H, s), 7.05–7.14 (5H, m), 7.19–7.28 (3H, m), 7.43 (1H, d, J = 7.6 Hz), 8.26 (1H, dd, J = 8.8, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 13.15 (1H, s); MS (FAB) m/z 336 $(M+H)^+$.

5.1.39. 6-{4-[(3-Methylbenzyl)oxy]phenoxy}nicotinic acid (28e). Compound **28e** was prepared from **26** by a procedure similar to that described for **28b**. Compound **28e** was obtained as a beige powder (52% in two steps): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.33 (3H, s), 5.08 (2H, s), 7.04–7.16 (6H, m), 7.24–7.34 (3H, m), 8.25 (1H, dd, J = 8.8, 2.0 Hz), 8.65 (1H, d, J = 2.0 Hz), 13.14 (1H, s); MS (FAB) m/z 336 $(M+H)^+$.

5.1.40. 6-{4-[(4-Methylbenzyl)oxy]phenoxy}nicotinic acid (28f). Compound **28f** was prepared from **26** by a procedure similar to that described for **28b**. Compound **28f** was obtained as a beige powder (48% in two steps): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (3H, s), 5.07 (2H, s), 7.02–7.06 (3H, m), 7.08–7.12 (2H, m), 7.21 (2H, d, J = 7.6 Hz), 7.35 (2H, d, J = 7.6 Hz), 8.25 (1H, dd, J = 8.8, 2.4 Hz), 8.64 (1H, d, J = 2.4 Hz); MS (FAB) m/z 336 $(M+H)^+$.

5.1.41. 6-{4-[(3-Chlorobenzyl)oxy]phenoxy}nicotinic acid (28g). Compound **28g** was prepared from **26** by a procedure similar to that described for **28b**. Compound **28g** was obtained as a beige powder (65% in two steps): ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.15 (2H, s), 7.04–7.15 (5H, m), 7.38–7.45 (3H, m), 7.54 (1H, s), 8.26 (1H, dd, J = 8.8, 2.4 Hz), 8.65 (1H, d, J = 1.2 Hz), 13.16 (1H, s); MS (FAB) m/z 356 $(M+H)^+$.

5.1.42. 6-{4-[(3-Cyanobenzyl)oxy]phenoxy}nicotinic acid (28h). To the mixture of methyl 6-(4-hydroxyphenoxy)nicotinate (**27**) (490 mg, 2.00 mmol), K_2CO_3 (553 mg, 4.00 mmol), and CH_3CN (10 mL) was added 3-(bromomethyl)benzonitrile (471 mg, 2.40 mmol) at room temperature. The mixture was stirred at 80 °C for 4.5 h. The mixture was partitioned between $CHCl_3$ and aqueous NaOH. The organic layer was dried and concentrated in vacuo. The residue was recrystallized from EtOH (10 mL) to give methyl 5-{4-[(3-cyanobenzyl)oxy]phenoxy}pyridine-2-carboxylate as a light orange powder (650 mg, 90%). The intermediate (650 mg) was dissolved in MeOH (5 mL), THF (5 mL), and 1 M NaOH (3.61 mL). The mixture was stirred at 50 °C for 30 min. The mixture was concentrated in vacuo. One molar HCl (10 mL ca.) was added to the mixture. The precipitate was collected and recrystallized from EtOH (8 mL) to give **28h** as a beige powder (58%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.19 (2H, s), 7.05–7.15 (5H, m), 7.64 (1H, t, J = 7.6 Hz), 7.83 (2H, dd, J = 8.0, 1.6 Hz), 7.95 (1H, s), 8.25 (1H, dd, J = 8.8, 2.4 Hz), 8.33–8.34 (1H, m), 8.65 (1H, d, J = 2.4 Hz), 13.15 (1H, br s); MS (FAB) m/z 347 $(M+H)^+$.

5.1.43. 6-{4-[(3-Nitrobenzyl)oxy]phenoxy}nicotinic acid (28i). Compound **28i** was prepared from **27** by a procedure similar to that described for **28h**. Compound **28i** was obtained as a beige powder (64% in two steps): ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.29 (2H, s), 7.05–7.16 (5H, m), 7.73 (1H, t, J = 8.0 Hz), 7.95 (1H, d, J = 7.2 Hz), 8.22 (1H, dd, J = 8.0, 1.6 Hz), 8.26 (1H, dd, J = 8.8, 2.4 Hz), 8.33–8.34 (1H, m), 8.65 (1H, d, J = 2.0 Hz), 13.15 (1H, br s); MS (FAB) m/z 367 $(M+H)^+$.

5.1.44. 6-(4-{[3-(Trifluoromethyl)benzyl]oxy}phenoxy)nicotinic acid (28j). Compound **28j** was prepared from **27** by a procedure similar to that described for **28h**. Compound **28j** was obtained as a white powder (91% in two steps): ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.24 (2H, s), 7.05–7.16 (5H, m), 7.64–7.84 (4H, m), 7.83 (2H, dd, J = 8.0, 1.6 Hz), 7.95 (1H, s), 8.26 (1H, dd, J = 8.8, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 13.16 (1H, br s); MS (FAB) m/z 390 $(M+H)^+$.

5.1.45. 6-[4-(3-Thienylmethoxy)phenoxy]nicotinic acid (28k). Compound **28k** was prepared from **27** by a procedure similar to that described for **28h**. Compound **28k** was obtained as a white powder (69% in two steps): ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.11 (2H, s), 7.04–7.13 (5H, m), 7.20 (1H, dd, J = 5.2, 1.2 Hz), 7.56–7.59 (2H, m), 7.95 (1H, s), 8.25 (1H, dd, J = 8.8, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 13.15 (1H, br s); MS (FAB) m/z 328 $(M+H)^+$.

5.1.46. *N*-[(2-Aminopyridin-4-yl)methyl]-6-[4-(benzyloxy)-phenoxy]nicotinamide (29a). Compound **29a** was prepared from **28a** by a procedure similar to that described for **23a**. Compound **29a** was obtained as a beige powder (73%): mp 199–200 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (2H, d, J = 6.0 Hz), 5.12 (2H, s), 5.84 (2H, br s), 6.34 (1H, s), 6.40–6.41 (1H, m), 7.05–7.12 (5H, m), 7.32–7.48 (5H, m), 7.81 (1H, d, J = 5.6 Hz), 8.27 (1H, dd, J = 8.8, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 9.05 (1H, t, J = 6.0 Hz); MS (FAB) m/z 427 $(M+H)^+$. Anal. Calcd for $C_{25}H_{22}N_4O_3$: C, 70.41; H, 5.20; N, 13.14. Found: C, 70.42; H, 5.09; N, 13.12.

5.1.47. *N*-[(2-Aminopyridin-4-yl)methyl]-6-{4-[(2-fluorobenzyl)oxy]phenoxy}nicotinamide (29b). Compound **29b** was prepared from **28b** by a procedure similar to that described for **23a**. Compound **29b** was obtained as a beige powder (61%): mp 115–118 °C; ^{1}H NMR

(400 MHz, DMSO-d_6) δ 4.38 (2H, d, J = 5.6 Hz), 5.16 (2H, s), 5.85 (2H, br s), 6.51–6.54 (4H, m), 7.06–7.13 (5H, m), 7.15–7.20 (1H, m), 7.24–7.29 (2H, m), 7.41–7.47 (1H, m), 7.56–7.61 (1H, m), 8.28 (1H, dd, J = 8.4, 2.4 Hz), 8.66 (1H, d, J = 2.4 Hz), 9.14 (1H, t, J = 5.6 Hz); MS (FAB) m/z 445 $(M+H)^+$. Anal. Calcd for $C_{25}H_{21}N_4O_3F$: C, 64.43; H, 5.06; N, 12.02; F, 4.08. Found: C, 64.45; H, 4.73; N, 12.09; F, 4.15.

5.1.48. *N*-[(2-Aminopyridin-4-yl)methyl]-6-{4-[(4-fluorobenzyl)oxy]phenoxy}nicotinamide (29c). Compound **29c** was prepared from **28c** by a procedure similar to that described for **23a**. Compound **29c** was obtained as a beige solid (73%): mp 199–200 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.34 (2H, d, J = 6.0 Hz), 5.10 (2H, s), 5.98 (2H, br s), 6.37 (1H, s), 6.43–6.44 (1H, m), 7.05–7.12 (5H, m), 7.21–7.26 (2H, m), 7.51–7.54 (2H, m), 7.81 (1H, d, J = 5.6 Hz), 8.27 (1H, dd, J = 8.4, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 9.07 (1H, t, J = 6.0 Hz); MS (FAB) m/z 445 $(M+H)^+$. Anal. Calcd for $C_{25}H_{21}N_4O_3F$: C, 67.02; H, 4.81; N, 12.50; F, 4.24. Found: C, 66.95; H, 4.66; N, 12.52; F, 4.19.

5.1.49. *N*-[(2-Aminopyridin-4-yl)methyl]-6-{4-[(2-methylbenzyl)oxy]phenoxy}nicotinamide (29d). Compound **29d** was prepared from **28d** by a procedure similar to that described for **23a**. Compound **29d** was obtained as a beige solid (73%): mp 119–121 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.35 (3H, s), 4.33 (2H, d, J = 5.6 Hz), 5.10 (2H, s), 5.84 (2H, br s), 6.34 (1H, s), 6.40–6.42 (1H, m), 7.06–7.13 (5H, m), 7.20–7.28 (3H, m), 7.43 (1H, d, J = 7.2 Hz), 8.28 (1H, dd, J = 8.4, 2.4 Hz), 8.66 (1H, d, J = 2.4 Hz), 9.08 (1H, t, J = 6.0 Hz); MS (FAB) m/z 441 $(M+H)^+$; HRMS: $(M+H)^+$ Calcd for $C_{26}H_{25}O_3N_4$, 441.1927. Found: 441.1947.

5.1.50. *N*-[(2-Aminopyridin-4-yl)methyl]-6-{4-[(3-methylbenzyl)oxy]phenoxy}nicotinamide (29e). Compound **29e** was prepared from **28e** by a procedure similar to that described for **23a**. Compound **29e** was obtained as a slightly yellow powder (72%): mp 177–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.33 (3H, s), 4.33 (2H, d, J = 6.0 Hz), 5.08 (2H, s), 5.87 (2H, br s), 6.34 (1H, s), 6.41–6.42 (1H, m), 7.05–7.16 (6H, m), 7.24–7.31 (3H, m), 7.81 (1H, d, J = 5.2 Hz), 8.27 (1H, dd, J = 8.4, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 9.06 (1H, t, J = 5.6 Hz); MS (FAB) m/z 441 $(M+H)^+$. Anal. Calcd for $C_{26}H_{24}N_4O_3$: C, 70.89; H, 5.49; N, 12.72. Found: C, 70.76; H, 5.43; N, 12.82.

5.1.51. *N*-[(2-Aminopyridin-4-yl)methyl]-6-{4-[(3-methylbenzyl)oxy]phenoxy}nicotinamide (29f). Compound **29f** was prepared from **28f** by a procedure similar to that described for **23a**. Compound **29f** was obtained as a beige powder (83%): mp 212–213 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.31 (3H, s), 4.33 (2H, d, J = 5.6 Hz), 5.07 (2H, s), 5.84 (2H, br s), 6.34 (1H, s), 6.40–6.42 (1H, m), 7.03–7.11 (5H, m), 7.21 (2H, d, J = 8.0 Hz), 7.35 (2H, d, J = 7.6 Hz), 7.81 (1H, d, J = 5.2 Hz), 8.27 (1H, dd, J = 8.8, 2.4 Hz), 8.64 (1H, d, J = 2.0 Hz), 9.04 (1H, t, J = 6.0 Hz); MS (FAB) m/z 441 $(M+H)^+$. Anal. Calcd for $C_{26}H_{24}N_4O_3$: C, 70.89; H, 5.49; N, 12.72. Found: C, 70.82; H, 5.50; N, 12.72.

5.1.52. *N*-[(2-Aminopyridin-4-yl)methyl]-6-{4-[(3-chlorobenzyl)oxy]phenoxy}nicotinamide (29g). Compound **29g** was prepared from **28g** by a procedure similar to that described for **23a**. Compound **29g** was obtained as a beige powder (77%): mp 185–186 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (2H, d, J = 5.6 Hz), 5.15 (2H, s), 5.90 (2H, br s), 6.36 (1H, s), 6.41–6.43 (1H, m), 7.06–7.13 (5H, m), 7.39–7.47 (3H, m), 7.54 (1H, s), 7.81 (1H, d, J = 5.2 Hz), 8.27 (1H, dd, J = 8.4, 2.4 Hz), 8.65 (1H, d, J = 2.4 Hz), 9.06 (1H, t, J = 5.6 Hz); MS (FAB) m/z 461 $(M+H)^+$. Anal. Calcd for $C_{25}H_{21}N_4O_3Cl$: C, 65.15; H, 4.59; N, 12.16; Cl, 7.69. Found: C, 64.86; H, 4.46; N, 12.15; Cl, 7.98.

5.1.53. *N*-[(2-Aminopyridin-4-yl)methyl]-6-{4-[(3-cyanobenzyl)oxy]phenoxy}nicotinamide (29h). Compound **29h** was prepared from **28h** by a procedure similar to that described for **23a**. Compound **29h** was obtained as a beige powder (64%): mp 153–154 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (2H, d, J = 5.6 Hz), 5.19 (2H, s), 5.84 (2H, br s), 6.34 (1H, s), 6.40–6.42 (1H, m), 7.06–7.14 (5H, m), 7.64 (1H, t, J = 8.0 Hz), 7.80–7.84 (3H, m), 7.94 (1H, s), 8.27 (1H, dd, J = 8.4, 2.8 Hz), 8.33–8.34 (1H, m), 8.65 (1H, d, J = 2.4 Hz), 9.05 (1H, t, J = 6.0 Hz); MS (FAB) m/z 452 $(M+H)^+$. Anal. Calcd for $C_{26}H_{21}N_5O_3$: C, 69.17; H, 4.69; N, 15.51. Found: C, 68.88; H, 4.68; N, 15.53.

5.1.54. *N*-[(2-Aminopyridin-4-yl)methyl]-6-{4-[(3-nitrobenzyl)oxy]phenoxy}nicotinamide (29i). Compound **29i** was prepared from **28i** by a procedure similar to that described for **23a**. Compound **29i** was obtained as a light yellow powder (84%): mp 120–123 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (2H, d, J = 5.6 Hz), 5.29 (2H, s), 5.84 (2H, br s), 6.34 (1H, s), 6.40–6.42 (1H, m), 7.06–7.15 (5H, m), 7.73 (1H, t, J = 8.0 Hz), 7.81 (1H, d, J = 5.2 Hz), 7.95 (1H, d, J = 7.6 Hz), 8.21 (1H, dd, J = 8.0, 2.0 Hz), 8.27 (1H, dd, J = 8.8, 2.8 Hz), 8.33–8.34 (1H, m), 8.64 (1H, d, J = 2.4 Hz), 9.05 (1H, t, J = 6.0 Hz); MS (FAB) m/z 472 $(M+H)^+$. Anal. Calcd for $C_{25}H_{21}N_5O_5 \cdot 0.1H_2O$: C, 63.45; H, 4.51; N, 14.80. Found: C, 63.27; H, 4.38; N, 14.79.

5.1.55. *N*-[(2-Aminopyridin-4-yl)methyl]-6-(4-{[3-(trifluoromethyl)benzyl]oxy}phenoxy)nicotinamide (29j). Compound **29j** was prepared from **28j** by a procedure similar to that described for **23a**. Compound **29j** was obtained as a beige powder (65%): mp 140–141 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (2H, d, J = 5.6 Hz), 5.24 (2H, s), 5.85 (2H, br s), 6.34 (1H, s), 6.40–6.42 (1H, m), 7.06–7.14 (5H, m), 7.64–7.73 (2H, m), 7.79–7.84 (3H, m), 7.94 (1H, s), 8.27 (1H, dd, J = 8.4, 2.8 Hz), 8.65 (1H, d, J = 2.4 Hz), 9.06 (1H, t, J = 6.0 Hz); MS (FAB) m/z 495 $(M+H)^+$. Anal. Calcd for $C_{26}H_{21}N_4O_3F$: C, 63.15; H, 4.28; N, 11.33; F, 11.53. Found: C, 63.30; H, 4.15; N, 11.32; F, 11.83.

5.1.56. *N*-[(2-Aminopyridin-4-yl)methyl]-6-[4-(3-thienylmethoxy)phenoxy]nicotinamide (29k). Compound **29k** was prepared from **28k** by a procedure similar to that described for **23a**. Compound **29k** was obtained as a white powder (84%): mp 208–209 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.33 (2H, d, J = 5.6 Hz), 5.11 (2H, s),

5.84 (2H, br s), 6.34 (1H, s), 6.40–6.42 (1H, m), 7.05–7.12 (5H, m), 7.20 (1H, dd, $J = 5.2$, 1.2 Hz), 7.56–7.59 (2H, m), 7.81 (1H, d, $J = 5.2$ Hz), 7.94 (1H, s), 8.27 (1H, dd, $J = 8.8$, 2.4 Hz), 8.65 (1H, d, $J = 2.0$ Hz), 9.05 (1H, t, $J = 6.0$ Hz); MS (FAB) *m/z* 433 $(M+H)^+$. Anal. Calcd for $C_{23}H_{20}N_4O_3S$: C, 63.87; H, 4.66; N, 12.95; S, 7.41. Found: C, 64.08; H, 4.56; N, 12.87; S, 7.56.

5.1.57. Methyl 2-oxo-1,2-dihydropyridine-4-carboxylate (31).[22] The mixture of 2-methoxyisonicotinic acid (**30**) (550 mg, 3.59 mmol), 48% aqueous HBr (1 mL), and 20% HBr in AcOH (4 mL) was stirred at 80 °C for 22 h. The mixture was concentrated in vacuo to give 2-oxo-1,2-dihydropyridine-4-carboxylic acid as a beige solid. The intermediate was dissolved in MeOH (15 mL). To the mixture was added concd H_2SO_4 (1 mL). The mixture was stirred at 80 °C for 6 h. The mixture was partitioned between AcOEt and H_2O. The organic layer was dried and concentrated in vacuo. The residue was recrystallized from MeOH–AcOEt to give **31** as a white powder (96 mg, 17% in two steps): mp 212–214 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 3.84 (3H, s) 6.51 (1H, dd, $J = 6.8$, 1.6 Hz), 6.81 (1H, s), 7.52 (1H, d, $J = 6.8$ Hz), 11.98 (1H, br s, OH); MS (EI) *m/z* 153 M^+. Anal. Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15. Found: C, 54.73; H, 4.60; N, 9.16.

5.2. Pharmacology

5.2.1. ^{45}Ca influx assay and cell necrosis assay. The methods were described in previous report.[17]

5.2.2. Ex vivo. The inhibition of ^{45}Ca influx in ex vivo from plasma was determined as follows: Crj: CD(SD)IGS rats were dosed orally with the compound at 30 mg/kg. After 2 h, the plasmas were obtained and assayed for reverse NCX inhibitory activity as reported procedure.[17] The data are expressed as the degree of inhibitory activity for reverse NCX compared with the plasma control.

5.2.3. Effects on tonotropic effects of ouabain and the onset of arrhythmia induced by ouabain in guinea pig isolated atria. The methods were described in a previous report.[16]

5.2.4. Effect on myocardial ischemia-reperfusion. Male SD rats (200–500 g) were used in the experiment. Compounds were given by single oral administration to the rats. Two hours after the administration of compounds, the rats were anaesthetized by pentobarbital (60 mg/kg ip). A cannula was inserted into the trachea and the animals were ventilated with air using a ventilator. Subcutaneous peripheral limb electrodes were inserted and an electrocardiogram (ECG) was continuously recorded for the entire duration of the experiment. All rats underwent thoracotomy at the fifth left intercostal space, the pericardium was opened and a loose 6.0 braided silk suture was placed around the left anterior descending coronary artery at almost proximal position. To facilitate the successive removal of the suture, a small plastic ring was inserted in the silk thread below the knot. Applying tension to the ligature could then occlude the artery, and reperfusion was achieved by releasing the tension. Successful coronary artery occlusion was evidenced by regional cyanosis of the heart and ischemic ECG changes (ST-segment elevation). Reperfusion was indicated by recovery from cyanosis and ECG changes (reversal of ST-segment elevation).

Rats were allowed to equilibrate for 20 min to enable ECG values to stabilize. Ischemia was induced by tightening the threads of the coronary suture and was maintained for 30 min. Reperfusion was obtained by reopening the chest and cutting the ligature around the coronary artery. The duration of reperfusion was predetermined to 60 min. In the animals that did not survive the entire reperfusion period, reperfusion lasted until cessation of the cardiac activity as revealed in ECG recordings. To exclude that premature mortality of rats was caused by the surgical procedures or individual abnormalities, rats showing ECG signs of impaired cardiac function during the stabilization period before induction of ischemia or soon after the coronary artery ligature were excluded from the experiments.

5.2.5. Human cytochrome P450 enzyme inhibition assays. The methods were described in the report.[20]

Acknowledgments

We wish to thank Mr. Takayuki Sato for evaluating the inhibitory activity of compounds against the sodium–calcium exchanger. We would like to express our gratitude to Dr. Minoru Okada, Dr. Toshihiro Watanabe, and Dr. Tomo-Oki Satoh for helpful support in the preparation of this manuscript and are also grateful to Mr. Masashi Funatsu for measurements of experimental data.

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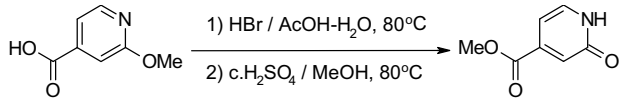
21. IC_{50} values above 10 μM against CYP 1A2, 2C9, 2C19, 2D6, and 3A4.
22. In the course of our drug development program, we performed several studies. The predicted bioavailability in man was 57 ± 7%, based on results in the rat. The inhibitory activity of **23h** toward hERG potassium channels had an IC_{50} value of over 100 μM, which indicates 400-times less potency than dofetilide in the ^{86}Rb efflux assay. An Ames test of **23h** was negative for mutagenicity. In addition, the effects of **23h** on other pharmacological sites were examined using PanlaboScreen, no remarkably potent inhibition was observed with **23h** (30 μM) in assays used to assess selectivity against major ion channel, receptor, enzyme, and transporter sites. In two assays, inhibitory activities were observed at an acceptable level: a calcium channel L-type, and a tachykinin NK1. These activities were observed at high concentrations compared to the IC_{50} value for reverse NCX inhibition of **23h** or the plasma concentration of **23h** in rats following oral administration at a dose of 3 mg/kg. Therefore, **23h** has sufficient selectivity for reverse NCX.
23. In order to confirm the structure of byproduct, another experiment was performed according to the below scheme to afford methyl 2-oxo-1,2-dihydropyridine-4-carboxylate (**31**)



30 **31**

24. On the basis of the middle scale experiment, we performed a large scale experiment. 2-Chloroisonicotinic acid (**13**) of starting material (538 g) was converted into the intermediate **14** (437 g) in three steps. Compound **15** (270 g) was given from **14**, and was converted into compound **17** (189 g) in three steps. Desired compound **18** (150 g) was afforded from **17**.