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

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In the setting of heart failure and myocardial ischemia-reperfusion, the sodium–calcium exchanger (NCX) can lead to calcium overload, which is responsible for contractile dysfunction and arrhythmia. NCX is an attractive target for treatment in heart failure and myocardial ischemia-reperfusion. We have designed and synthesized a series of benzylxylophenyl derivatives based on compound 3. These derivatives have been evaluated for their inhibitory activity against both the reverse and forward modes of NCX. We have discovered a novel potent and selective reverse NCX inhibitor (12) with an IC50 value of 0.085 μM against reverse NCX.

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

Intracellular Ca2+ is of primary importance in the pathogenesis of ischemia and reperfusion injury in the myocardium. Recent studies have suggested that a massive Ca2+ influx may occur as a consequence of Na+-Ca2+ exchange via the sodium–calcium exchanger (NCX) during reperfusion, which, in turn, may be caused by an accumulation of Na+ via the sodium–hydrogen exchanger (NHE) during ischemia.1,2) This results in an intracellular Ca2+ overload, the detrimental effects of which include myocardial contracture, stunning, necrosis, and reperfusion arrhythmia.3–9) NCX functions in both reverse and forward modes, and it is well known that an overactive reverse NCX causes Ca2+ overload. Inhibition of reverse NCX overactivity would effectively block this overload and prevent damage to the myocardium in ischemia-reperfusion. Therefore, reverse NCX inhibitors are currently considered to be beneficial in treating disease states.10,11) Recently, quinazoline derivatives12) and a series of benzylxylophenyl derivatives13–16) have been identified as NCX inhibitors, and is used as a tool in heart and renal failure models.17,18) and SEA0400 (2) is well known as a potent reverse NCX inhibitor that is efficacious in myocardial ischemia-reperfusion injury19–22) (Fig. 1). We have recently discovered reverse NCX inhibitors, such as 3, which we have reported elsewhere.16,23,24) To create potent and selective reverse NCX inhibitors, we have now designed a novel class of NCX inhibitors based on 3, and in this paper describe the results of our work on the synthesis and structure–activity relationships (SAR) of this novel class of benzylxylophenyl derivatives.

Chemistry

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

Results and Discussion

In order to measure the inhibitory effect of the synthesized compounds on the reverse mode of NCX activity, an Na+-dependent Ca2+ influx assay was performed according to reported protocols, using 45Ca and CCL39 cells stably expressing NCX1.13,23) The inhibitory effect on the forward mode of NCX activity was assayed by a cell necrosis assay, which also used NCX1.1-expressing CCL39 cells.3,25) The inhibitory potencies of our novel compounds were thus evaluated in both reverse and forward NCX assays. These compounds were then compared to reference compounds KB-79743 (1), SEA0400 (2) and compound 3.

The structure–activity relationships of our novel series of NCX inhibitors are summarized in Tables 1 and 2. We have reported a SAR of the nicotinamide part.16) Compound...
3, with an N-(pyridin-4-ylmethyl)piperidine-1-carboxamide structure, had a potent inhibitory activity against reverse NCX with an IC\textsubscript{50} value of 0.22 μM. To create novel structures in the position of the piperidine-1-carboxamide moiety, we attempted to change the piperidine-1-carboxamide linker into an alkyl chain structure. To examine the effect of the length of the alkyl-linker on reverse NCX inhibitory activity, we evaluated compounds with several different lengths of structure between the phenoxy and pyridine parts (Table 1). Compounds 6a and 6b, with a linker length of 5 atoms between the phenoxy part and the pyridine ring produced an extreme reduction in reverse NCX inhibitory activity compared to compound 3. A compound with a 6-atoms linker (6c) had similar inhibitory activity as 3. When we changed the linker length to 7 atoms (10a), the inhibitory activity was increased. This prompted us to introduce longer linkages, with 8 atoms (10b) and 9 atoms (10c) between the phenoxy part and the pyridine ring. However, the inhibitory activities of 10b and 10c were reduced with increasing linker length. Compound 10a showed the most potent inhibitory activity, with an IC\textsubscript{50} value of 0.10 μM against reverse NCX (Table 1). The results indicated that linker length is important for inhibitory activity against reverse NCX, and also prompted us to introduce another linker connecting the phenoxy part with the pyridine ring as shown in Table 2. Replacements of –O– (10a) with –C– (6d) and –S– (6e) slightly reduced inhibitory activity against reverse NCX. Compound 12, with an inverse amide to compound 10a, also showed slightly increased inhibitory activity over 10a with an IC\textsubscript{50} value of 0.085 μM against reverse NCX. Compound 12 was 3-fold more potent than SEA0400 (2), and also showed higher selectivity. Replacement of the amide linkage with a urea linkage (14) resulted in a 2.6-fold decrease in inhibitory potency against reverse NCX compared to 10a. Each of these derivatives with an alkyl chain linker showed higher selectivity than 3.

Conclusion
A series of benzyloxophenyl derivatives have been prepared and evaluated for their inhibitory activities against the reverse and forward modes of NCX. By modifying the piperidine-1-carboxamide moiety, we found that a compound containing a N-propyl-2-pyridin-4-ylacetamide (12), rather than a N-(pyridin-4-ylmethyl)piperidine-1-carboxamide (3), had enhanced reverse NCX inhibitory activity and increased selectivity. Compound 12 (YM-270951) had an IC\textsubscript{50} value of 0.085 μM against reverse NCX and higher selectivity. The reverse NCX inhibitory activity of 12 was approximately 3-times greater than that of SEA0400 (2). This study could
between CHCl₃ and H₂O. The organic layer was dried and concentrated with P2O₅. The residue was purified by column chromatography on silica gel (hexane : AcOEt = 1:0—4:1) to give 4e as a colorless oil (276 mg, 92%).

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

<table>
<thead>
<tr>
<th>Compd.</th>
<th>L</th>
<th>45Ca influx Æ</th>
<th>Cell necrosis Æ</th>
<th>Selectivity Æ</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>0.29</td>
<td>98</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td>5.6</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>6c</td>
<td>0.25</td>
<td>50</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>10a</td>
<td>0.10</td>
<td>43</td>
<td>430</td>
<td></td>
</tr>
<tr>
<td>10b</td>
<td>0.23</td>
<td>&gt;100</td>
<td>&gt;430</td>
<td></td>
</tr>
<tr>
<td>10c</td>
<td>0.63</td>
<td>63</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.22</td>
<td>27</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

SEA0400 (2) 5.1 24 4.7
KB-R7943 (1) 10b 0.10 43 430

a) Activity against the NCX1.1 expressed in CCL39 cells. ÆCa 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) IC₅₀ values and EC₅₀ values were determined in a single experimental run in triplicate. d) Ratio of EC₅₀ value of cell necrosis and IC₅₀ value of ÆCa influx. e) Not tested.

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

<table>
<thead>
<tr>
<th>Compd.</th>
<th>L</th>
<th>45Ca influx Æ</th>
<th>Cell necrosis Æ</th>
<th>Selectivity Æ</th>
</tr>
</thead>
<tbody>
<tr>
<td>6d</td>
<td>0.17</td>
<td>34</td>
<td>200</td>
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</tr>
<tr>
<td>6e</td>
<td>0.20</td>
<td>48</td>
<td>240</td>
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<tr>
<td>12</td>
<td>0.085</td>
<td>42</td>
<td>490</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.26</td>
<td>40</td>
<td>150</td>
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<tr>
<td>10a</td>
<td>0.10</td>
<td>43</td>
<td>430</td>
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<tr>
<td>3</td>
<td>0.22</td>
<td>27</td>
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<td></td>
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</table>

For footnotes a—d) refer to Table 1.

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

Experimental

Chemistry Melting points were determined with a Yanaco MP-500D melting point apparatus or a Buchi B-545 melting point apparatus and are uncorrected. 1H-NMR spectra were recorded on a JEOL JNM-LA300 or a JEOL-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 Na₂SO₄.

Ethyl 4-{4-(Hydroxyphenyl)sulfonyl}butanoate (4e) To a mixture of 4-sulfanylphenol (7) (1.26 g, 10.0 mmol) in MeCN (30 ml) were added K₂CO₃ (2.07 g, 15 mmol) and ethyl 4-bromobutanoate (1.57 ml, 11.0 mmol) and 1H-NMR (300 MHz, DMSO-d₆) δ 7.46 (1H, m), 12.93 (1H, br s); MS (FAB) m/z 288 (M+). Compounds 6a–e were obtained as a colorless oil (2.22 g, 92%). 1H-NMR (300 MHz, DMSO-d₆) δ 7.12—7.19 (1H, m), 7.24—7.30 (2H, m), 7.40—7.46 (1H, m), 12.07 (1H, s); MS (FAB) m/z 274 (M+).

5-{4-[[3-Fluorobenzyl]oxy]phenyl}pentanoic Acid (5b) Compound 5b was prepared from 4b by a procedure similar to that described for 5a. Compound 5b was obtained as a colorless solid (89% in 2 steps): 1H-NMR (400 MHz, DMSO-d₆) δ 2.75—2.82 (2H, m), 2.92 (2H, s), 6.92 (2H, d, J = 8.8 Hz), 7.11—7.17 (3H, m), 7.24—7.30 (2H, m), 7.40—7.46 (1H, m), 12.07 (1H, s); MS (FAB) m/z 274 (M+).

For footnotes a—d) refer to Table 1.
3-[4-(3-Fluorobenzyl)oxy]phenyl-N-(pyridin-4-ylmethyl)propanamide Hydrochloride (6b) Compound 6b was prepared from 5b by a procedure similar to that described for 6a. Compound 6b was obtained as a colorless solid (42%); mp 109—112 °C; 1H-NMR (400 MHz, DMSO-d6) δ 1.76—1.88 (2H, m), 2.23 (2H, t, J = 7.8 Hz), 2.48—2.55 (2H, m), 4.50 (2H, J = 5.9 Hz), 5.10 (2H, s), 6.93 (2H, d, J = 8.8 Hz), 7.09—7.19 (1H, m), 7.24—7.30 (2H, m), 7.41—7.47 (1H, m), 7.82 (2H, d, J = 6.8 Hz), 8.68, 8.74 (1H, m), 8.76 (2H, d, J = 6.3 Hz); MS (FAB) m/z 367 (M+H). Anal. Calc. for C23H21N2O2F· HCl: C, 65.91; H, 5.53; N, 6.99; F, 4.74; MS (FAB) m/z 372 (M+). Anal. Calc. for C23H20N2O2F· HCl: C, 65.91; H, 5.53; N, 6.99; F, 4.74; MS (FAB) m/z 372 (M+). Anal. Calc. for C22H21N2O2F· HCl: C, 66.58; H, 5.23; N, 6.97; F, 4.58; Cl, 8.54. Found: C, 66.2; H, 5.55; N, 6.95; F, 4.75; Cl, 8.60.

4-[3-(Fluorobenzyl)oxy]phenyl-N-(pyridin-4-ylmethyl)butanamide Hydrochloride (6c) Compound 6c was prepared from 5c by a procedure similar to that described for 6a. Compound 6c was obtained as a colorless solid (52%); mp 155—158 °C; 1H-NMR (400 MHz, DMSO-d6) δ 1.82—1.88 (2H, m), 2.36 (2H, t, J = 7.8 Hz), 2.93—2.97 (2H, m), 5.07 (2H, s), 8.66 (2H, d, J = 8.8 Hz), 9.02 (2H, d, J = 9.3 Hz), 8.68 (2H, d, J = 6.8 Hz), 9.02 (2H, d, J = 9.3 Hz). Anal. Calc. for C23H22N2O2F· HCl: C, 63.99; H, 5.63; N, 6.51; F, 4.29; MS (FAB) m/z 372 (M+H). Anal. Calc. for C22H21N2O2F· HCl: C, 66.58; H, 5.23; N, 6.97; F, 4.58; Cl, 8.54. Found: C, 66.5; H, 5.55; N, 6.95; F, 4.75; Cl, 8.60.

4-[3-(Fluorobenzyl)oxy]phenyl-N-(pyridin-4-ylmethyl)butanamide Hydrochloride (6d) Compound 6d was prepared from 5e by a procedure similar to that described for 6a. Compound 6d was obtained as a colorless solid (99%); mp 110—112 °C; 1H-NMR (400 MHz, DMSO-d6) δ 2.14—2.21 (2H, m), 2.60—2.67 (2H, m), 3.86—3.93 (2H, m), 5.06 (2H, s), 5.68 (2H, d, J = 9.2 Hz), 6.93 (2H, d, J = 9.2 Hz). Anal. Calc. for C21H20N2O2F· HCl: C, 68.45; H, 5.44; N, 6.99; F, 4.29; MS (FAB) m/z 372 (M+H). Anal. Calc. for C20H18N2O2F· HCl: C, 66.49; H, 5.23; N, 6.97; F, 4.58; Cl, 8.54. Found: C, 66.5; H, 5.55; N, 6.95; F, 4.75; Cl, 8.60.

4-[3-(Fluorobenzyl)oxy]phenyl-N-(pyridin-4-ylmethyl)pentanamide Hydrochloride (10a) Compound 10a was prepared from 9a by a procedure similar to that described for 6a. Compound 10a was obtained as a colorless powder (77%); mp 144—146 °C; 1H-NMR (400 MHz, DMSO-d6) δ 1.90—2.00 (2H, m), 2.80—2.84 (2H, m), 3.91 (2H, d, J = 6.9 Hz), 5.06 (2H, s), 5.68 (2H, d, J = 9.2 Hz), 6.93 (2H, d, J = 9.2 Hz). Anal. Calc. for C21H20N2O2F· HCl: C, 64.01; H, 5.63; N, 6.95; F, 4.29; Cl, 8.26.

6-[4-[3-(Fluorobenzyl)oxy]phenyl]hexanamide Hydrochloride (10b) Compound 10b was prepared from 9b by a procedure similar to that described for 6a. Compound 10b was obtained as a colorless powder (68%); mp 125—127 °C; 1H-NMR (400 MHz, DMSO-d6) δ 1.76—1.98 (2H, m), 2.26—2.30 (2H, m), 2.75—2.87 (2H, m), 3.87—4.04 (2H, m), 5.03 (2H, s), 6.70 (2H, d, J = 10.9 Hz), 6.78 (2H, d, J = 10.9 Hz). Anal. Calc. for C20H18N2O2F· HCl: C, 64.01; H, 5.63; N, 6.95; F, 4.29; Cl, 8.26.

N-(4-[3-(Fluorobenzyl)oxy]phenyl)propylamine (11) To a mixture of 4-[3-(fluorobenzyl)oxy]phenol (8) (436 mg, 2.00 mmol), K2CO3 (332 mg, 2.40 mmol) in CHCl3 (10 ml) was added ethyl 4-bromobutanoate (0.315 ml, 2.20 mmol) at room temperature. The mixture was stirred at 80 °C for 24 h. The mixture was partitioned between CHCl3 and aqueous NaOH. The organic layer was dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane: AcOEt = 1:0—1:4) to give ethyl 4-[3-(fluorobenzyl)oxy]phenylbutanoate as a colorless oil (630 mg, 95%). The mixture of the intermediate (620 mg, 1.87 mmol) in 1M NaOH (2.8 ml, 2.8 mmol) and EtOH (5 ml) was stirred at room temperature for 4 h. The mixture was concentrated in vacuo. 1M HCl was added to the residue. The precipitate was collected to afford 9a as a colorless powder (537 mg, 94%): 1H-NMR (300 MHz, DMSO-d6) δ 1.84—1.95 (2H, m), 2.32—2.40 (2H, m), 3.87—3.94 (2H, m), 5.06 (2H, s), 6.85 (2H, d, J = 9.1 Hz), 6.93 (2H, d, J = 9.1 Hz), 7.10—7.18 (1H, m), 7.22—7.30 (2H, m), 7.38—7.47 (1H, m), 12.11 (1H, s); MS (FAB) m/z 304 (M+).
oxy]phenol 8 (655 mg, 3.00 mmol) and K₂CO₃ (1.24 g, 9.00 mmol) in MeCN (20 ml) was stirred at 80 °C for over night. The mixture was partitioned between CHCl₃ and aqueous NaOH. The organic layer was dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CHCl₃:MeOH=99:1) to give tert-butyl (2-[4-[(3-fluorobenzyl)oxy]phenoxo]ethyl)carbamate as a light syrup (604 mg).

To a mixture of bis(trichloromethyl)carbomate (190 mg, 0.64 mmol) in THF (5 ml) was added a mixture of HCl (2.1 ml, 8.36 mmol) was stirred at room temperature for 6.5 h. The mixture was partitioned between CHCl₃ and aqueous NaOH. The organic layer was dried and concentrated in vacuo to give a free base of [3-(fluorobenzyl)oxy]phenoxy]ethyl)carbamate as a light syrup (448 mg, 57% in 2 steps): 1H-NMR (CDCl₃) δ 3.06 (2H, d, J=6.4 Hz), 8.81 (2H, d, J=8.8 Hz), 6.89 (2H, d, J=6.8 Hz); MS (FAB) m/z 262 (M+H)⁺.

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References and Notes