Structure–activity relationship study of 4-(thiazol-5-yl)benzoic acid derivatives as potent protein kinase CK2 inhibitors

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Abstract

Two classes of modified analogs of 4-(thiazol-5-yl)benzoic acid-type CK2 inhibitors were designed. The

Introduction

Protein kinase CK2 (previously known as casein kinase 2) is a ubiquitous, highly pleiotropic serine/threonine-specific protein

1. Introduction

protein kinase.1,2 It is often present as a heterotetramer composed of two catalytic α subunits (α or α′) and two regulatory β subunits in

various combinations.3,4 The CK2α′ subtype is exclusively found in brain and testis, whereas the CK2α subtype is ubiquitously

expressed.5 CK2 is a key regulator of various cellular events, including signal transduction, transcriptional control, apoptosis,

and cell cycle.6–8 More importantly, overexpression and elevated activity of CK2 are closely related to many human diseases,9–13

including cancers of the breast,9 lung,10 and pancreas,11 leukemia,12 and glomerulonephritis.13 CK2 is therefore an

important pharmacological target.14–16

Inhibition of CK2 decreases cellular proliferation and induces apoptosis in cancer cells. Several types of CK2 inhibitors, including

natural products, have been reported, such as emodine,17 4,5,6,7-tetramethyl-1H-benzo[d]imidazole (TBB),18 (5-oxo-5,6-dihydroindolo

[1,2-a]quinazolin-7-yl)acetic acid (IQA),19 CX-4945 (orally bioavailable inhibitor, currently undergoing clinical trials for
cancer treatment),20,21 and CC-479122,23 (Fig. 1). Recently, our group identified CK2 inhibitors with a phenylazole scaffold

through virtual screening of a compound database, based on the crystal structure of the CK2α–AMP–PNP complex.24 Structural

optimization of the azole and amide moieties led to identification of amido-substituted thiadiazol- and pyrazolyl-benzoic acid deriva-
tives 1 and 2.25 We then investigated the introduction of ring fusion for conformational restriction, to obtain the planar horse-

shoe-shaped conformation required for binding of CC-4791 to CK2α,26,27 and to reduce entropic loss during binding. Benzindazole
derivative 328 and indolopyrazole 4,29 derived from the pyrazole-based inhibitor 2, were identified as potent CK2 inhibitors. Further

structural optimization was achieved by modification of the benzoic acid moiety. In this study, the effects of replacement of

benzoic acid with a pyridine- or pyridazine-carboxylic acid and introduction of hydrophobic substituents were investigated to

obtain further structure–activity relationship information.

2. Results and discussion

2.1. Design

We designed the aza analogs 5a–c (Fig. 3) based on binding affinity predictions of CK2α–inhibitor complexes by the thermody-
namic integration (TI) method using Amber.30 The calculations

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suggested that replacement of the carbon atom at the 2- or 3-position of the benzoic acid moiety of 1 with a nitrogen atom (X or Y = N: 5a and 5b) would increase the affinities five- to ten-fold. Replacement of both carbon atoms with nitrogen atoms (X = Y = N: 5c) might further improve the binding affinity. We also expected that the aqueous solubilities of the aza analogs would be better than that of the parent benzoic acid because of additional hydrophilic interactions of the pyridine-type nitrogen(s). Our findings that nicotinic acid is a potential CK2 binder support this design concept.31

Many CK2 inhibitors have common substructures, including sp²-hybridized heteroatom(s) (C or O) and acidic functional group(s) (CO₂H, ArOH, or triazole) (Figs. 1 and 2). Our previous studies showed that these motifs in CC-4791 and the compound 1 are important for the formation of hydrogen bonds with the backbone amide group (Vali116) or electrostatic interaction with Lys68 of CK2 (Fig. 4).25–27 Our inhibitors 1–4 lack the hydrophobic group present in CX-4945 (chlorophenyl group) and CC-4791 (indazole ring bearing a cyclopentylamino group). Binding mode analyses of CX-4945 and CC-4791 suggested that these groups occupy the hydrophobic pocket of CK2; this helps to improve the binding efficacy and decrease the desolvation energy. Based on these findings, we examined the introduction of ring fusion or a hydrophobic group into the benzoic acid moiety. Naphthoic acid analogs 6 and 3-substituted benzoic acid derivatives 7a–d (R = OPh, OBn, CH₂OPh, or OCH₂CH₂Ph) were designed (Fig. 3).

2.2. Synthesis

The aza analogs 5a–c were prepared from 5-bromothiazol-2-amino hydrochloride 8 (Scheme 1). A reported procedure32 was used to protect the amino group of 8 with Boc and 2-(trimethylsilyl)ethoxymethyl (SEM) groups. Conversion of 9 to the corresponding pinacol (pin) borane by treatment with n-BuLi and i-PrOBpin, followed by Suzuki–Miyaura cross coupling with aryl halides 10a–c bearing a methoxycarbonyl group at the 4-position, afforded the coupling products 11a–c. Removal of the Boc and SEM groups, acylation, and hydrolysis gave the desired aza analogs 5a–c.

The synthesis of 3-substituted benzoic acid derivatives 7a–d is shown in Scheme 2. Commercially available thiazol-2-amino 13 was converted to stannane 14 through acylation of the amino group and regioselective lithiation–stannylation at the 5-position. Migita–Kosugi–Stille cross coupling of 14 with halides 15a–d followed by hydrolysis of the ester gave the substituted benzoic acid

![Figure 1. Representative CK2 inhibitors.](image)

![Figure 2. CK2 inhibitors developed by our group.](image)

![Figure 3. Design of CK2 inhibitors 5, 6, and 7.](image)

![Figure 4. Superposition of CX-4945 (blue), CC-4791 (yellow), and inhibitor 1 (magenta) at ATP binding site. PDBIDs for CK2 complexes with CX-4945, CC-4791, and inhibitor 1 are 3PE1, 3AT3, and 5B0X, respectively.](image)

![Scheme 1. Synthesis of aza analogs 5a–c.](image)
derivatives 7a–d. Other substituted derivatives, i.e., 6 and 17a–h, were prepared in a similar manner (see Supplementary data).

2.3. CK2 inhibitory and cell antiproliferative activities

The in vitro inhibitory activities of the aza analogs 5a–c toward two subtypes of the catalytic subunit of CK2 are shown in Table 1. As predicted by the TI calculations, 5a–c showed highly potent inhibitory activities toward CK2α (IC50 = 0.014–0.017 μM) and CK2α' (0.0046–0.010 μM). These are comparable to the activities of CX-4945 and the parent compound 1, but significant improvements were not observed. However, 5a–c did not inhibit proliferation of lung cancer cells A549 at 30 μM. This is partly because the basic pyridine or pyridazine moiety decreases the membrane permeabilities of 5a–c.13

We next focused on benzoic acid derivatives bearing an additional hydrophobic moiety (Table 2). Replacement of the benzene ring of 1 by a naphthalene ring to give 6 significantly decreased the inhibitory activity toward both CK2α (IC50 = 1.3 μM) and CK2α' (0.28 μM). In contrast, introduction of an alkoxy group retained the potent inhibitory activities toward CK2: the phenoxy (7a), benzyloxy (7b), phenoxymethyl (7c), and 2-phenylethoxy (7d) derivatives were highly potent toward CK2α (IC50 = 0.015–0.028 μM) and CK2α' (0.010–0.012 μM). Promising cell antiproliferative activities against A549 were observed with 7a (IC50 = 4.7 μM), 7b (1.5 μM), and 7d (3.6 μM). Encouraged by these results, we further optimized the structure of the 3-substituted benzoic acid derivatives. The 3-benzyloxy derivative 7b was selected as the lead compound for further optimization, because of its higher cell antiproliferative activity and ease of derivatization because of the wide availability of substituted benzyl halides.

A series of 3-benzyloxy-substituted analogs 17a–h were prepared by Migita–Kosugi–Stille cross coupling using a variety of substituted 4-iodobenzoate derivatives of type 15 (see Supplementary data). The biological activities of 17a–h are summarized in Table 3. Among the chlorinated benzyl ethers 17a–c, the inhibitory activities of the 2-chloro derivative 17a toward CK2α (IC50 = 0.016 μM) and CK2α' (0.0098 μM) were higher than those of 7b. The inhibitory activities of 4-chloro derivative 17c toward CK2α (IC50 = 0.026 μM) and CK2α' (0.019 μM) were lower than those of 17a. For a reason that is unclear, 3-chloro derivative 17b showed significantly lower CK2 inhibitory activities [IC50 (CK2α) = 0.33 μM; IC50 (CK2α') = 0.16 μM] and cell antiproliferative activ-

Table 1
CK2 inhibitory and cell antiproliferative activities of pyridine- and pyridazine-carboxylic acid derivatives 5a–c

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>Y</th>
<th>IC50a (μM)</th>
<th>CC50a (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CK2α</td>
<td>CK2α'</td>
</tr>
<tr>
<td>CX-4945</td>
<td></td>
<td></td>
<td>0.019</td>
<td>0.011</td>
</tr>
<tr>
<td>1</td>
<td>CH</td>
<td>CH</td>
<td>0.020</td>
<td>0.011</td>
</tr>
<tr>
<td>5a</td>
<td>N</td>
<td>CH</td>
<td>0.017</td>
<td>0.0046</td>
</tr>
<tr>
<td>5b</td>
<td>CH</td>
<td>N</td>
<td>0.014</td>
<td>0.010</td>
</tr>
<tr>
<td>5c</td>
<td>N</td>
<td>N</td>
<td>0.014</td>
<td>0.0096</td>
</tr>
</tbody>
</table>

a IC50 values were derived from the dose–response curves generated from duplicate data points of the CK2 kinase assay.

b CC50 values were determined by the MTS assay against A549 cells after 72 h exposure to the compound.

Table 2
CK2 inhibitory and cell antiproliferative activities of benzoic acid derivatives 6 and 7a–d

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>IC50a (μM)</th>
<th>CC50a (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CK2α</td>
<td>CK2α'</td>
</tr>
<tr>
<td>CX-4945</td>
<td></td>
<td>0.019</td>
<td>0.011</td>
</tr>
<tr>
<td>1</td>
<td>H</td>
<td>0.020</td>
<td>0.011</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>1.3</td>
<td>0.28</td>
</tr>
<tr>
<td>7a</td>
<td>OPh</td>
<td>0.015</td>
<td>0.010</td>
</tr>
<tr>
<td>7b</td>
<td>OBn</td>
<td>0.019</td>
<td>0.012</td>
</tr>
<tr>
<td>7c</td>
<td>CH2OPh</td>
<td>0.028</td>
<td>0.012</td>
</tr>
<tr>
<td>7d</td>
<td>OCH2CH2Ph</td>
<td>0.015</td>
<td>0.011</td>
</tr>
</tbody>
</table>

a IC50 values were derived from the dose–response curves generated from duplicate data points of the CK2 kinase assay.

b CC50 values were determined by the MTS assay against A549 cells after 72 h exposure to the compound.

c IC50 values were determined by the MTS assay against A549 cells after 72 h exposure to the compound.

Table 3
CK2 inhibitory and cell antiproliferative activities of benzyloxy-substituted derivatives 17a–h

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ar</th>
<th>IC50a (μM)</th>
<th>CC50a (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CK2α</td>
<td>CK2α'</td>
</tr>
<tr>
<td>CX-4945</td>
<td></td>
<td>0.019</td>
<td>0.011</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0.020</td>
<td>0.011</td>
</tr>
<tr>
<td>7b</td>
<td></td>
<td>0.019</td>
<td>0.012</td>
</tr>
<tr>
<td>17a</td>
<td>C6H5(2-CI)</td>
<td>0.016</td>
<td>0.0098</td>
</tr>
<tr>
<td>17b</td>
<td>C6H5(3-CI)</td>
<td>0.33</td>
<td>0.16</td>
</tr>
<tr>
<td>17c</td>
<td>C6H5(4-CI)</td>
<td>0.026</td>
<td>0.019</td>
</tr>
<tr>
<td>17d</td>
<td>C6H5(2-F)</td>
<td>0.014</td>
<td>0.0088</td>
</tr>
<tr>
<td>17e</td>
<td>C6H5(2-Br)</td>
<td>0.017</td>
<td>0.014</td>
</tr>
<tr>
<td>17f</td>
<td>C6H5(2-OMe)</td>
<td>0.014</td>
<td>0.013</td>
</tr>
<tr>
<td>17g</td>
<td>C6H5(2-CN)</td>
<td>0.016</td>
<td>0.011</td>
</tr>
<tr>
<td>17h</td>
<td>2-Pyridyl</td>
<td>0.015</td>
<td>0.0095</td>
</tr>
</tbody>
</table>

a IC50 values were derived from the dose–response curves generated from duplicate data points of the CK2 kinase assay.

b CC50 values were determined by the MTS assay against A549 cells after 72 h exposure to the compound.

c IC50 values were determined by the MTS assay against A549 cells after 72 h exposure to the compound.
Highly potent inhibitory activities against CK2 were observed with 2-halophenyl derivatives 17d (fluorine) and 17e (bromine). Other 2-substituted derivatives, including 2-methoxyphenyl (17f), 2-cyanophenyl (17g), and 2-pyridyl (17h) derivatives showed potent CK2 inhibitory activities [IC50 (CK2α) = 0.014–0.016 μM; IC50 (CK2β) = 0.0095–0.013 μM], although no antiproliferative activities were detected with 17g and 17h at 30 μM. All the 2-halo derivatives and the 2-methoxy-substituted one showed good antiproliferative activities against A549 (CC50 = 1.5–3.3 μM). Docking simulation of the potent inhibitors 17a, 17e, and 17f suggested that these derivatives bind to CK2 at ATP binding site in a similar manner to CX-4945, CC-4791, and the inhibitor 1 (Fig. S1, Supplementary data). As we expected, the substituted phenyl group of 17 newly introduced in this study occupies the hydrophobic pocket of CK2.

2.4. Aqueous solubility

The aqueous solubilities of representative inhibitors were evaluated (Table 4). The 2-aza (5a) and 3-aza analogs (5b) are five to 33 times more soluble (14–90 μg/mL) in phosphate buffer at pH 7.4 than 1 (2.7 μg/mL). The introduction of two nitrogen atoms (5c) significantly improves the solubility (1.025 mg/mL). In contrast, the introduction of 2-methoxybenzyl ether (17f) does not have a positive effect on the aqueous solubility (1.4 μg/mL). As described above, the aza analogs 5a–c showed no antiproliferative activities at 30 μM. The 2-halo- (17a, 17d, and 17e) and 2-methoxy- (17f) benzyl ether derivatives, which have antiproliferative activities three to six times more potent than that of the parent compound 1, are promising potent CK2 inhibitors, although the solubility problem needs to be solved.

3. Conclusions

Modification of the benzoic acid moiety of 4-(thiazol-5-yl)benzoic acid-type CK2 inhibitor was investigated. As the TI calculations predicted, replacement of the benzoic acid with pyridine-or pyrazidine-carboxylic acid maintained the highly potent inhibitory activities toward CK2. However, no antiproliferative activities at 30 μM were observed with these aza analogs. In contrast, the introduction of 2-halo- and 2-methoxy-substituted benzoxyl groups into the benzoic acid moiety was highly effective in improving the antiproliferative activity, leading to the identification of promising CK2 inhibitor candidates.

4. Experimental

4.1. General methods

1H NMR spectra were recorded using a JEOL AL-500 spectrometer at 500 MHz frequency. Chemical shifts are reported in δ (ppm) relative to MeSi (in CDCl3) as internal standard. 13C NMR spectra were recorded using a JEOL AL-500 and referenced to the residual CHCl3 signal. IR spectra were obtained on a JASCO FT/IR-4100 spectrometer. Exact mass (HRMS) spectra were recorded on a JMS-HX/ HX 110A mass spectrometer or Shimadzu LC–ESI-IT-TOF-MS equipment (ESI). Melting points were measured by a hot stage melting points apparatus (uncorrected). For column chromatography, Wakogel C-300E (Wako), Chromatorex NH-DM1020 (Fuji Sylva), or Aluminum oxide 90 (Mercck-Millipore) was employed. Several benzoic acid derivatives (7a, 17b, and 17g) were further purified by HPLC [Cosmosil SC18-ARI column (20 × 250 mm, Nacalai Tesque, Inc.); water/acetonitrile containing 0.1% NH3; linear gradient; flow rate of 8 mL/min; UV detector at 254 nm] to afford their ammonia salts as lyophilized powders.


n-BuLi (1.55 M solution in hexane; 0.484 mL, 0.75 mmol) was added slowly to a mixture of bromide 9 (204 mg, 0.5 mmol) and THF (5 mL) at −78 °C under argon. i-ProOPin (152 μL, 0.75 mmol) was added to the mixture, and the resulting mixture was then allowed to warm to 0 °C. 2NH4Cl was added to the mixture. The mixture was partitioned between EtOAc and brine, and the layers were separated. The organic layer was dried over MgSO4 and concentrated in vacuo to give a residual oil. A mixture of this crude borate, bromopyridine 10a (129 mg, 0.6 mmol), Pd(PPh3)4 (5.8 mg, 0.005 mmol), and K2CO3 (207 mg, 1.5 mmol) in DMF (5 mL) under argon was stirred at 80 °C overnight. After being cooled to room temperature, the solvent was removed under reduced pressure and the residue was diluted with CH2Cl2 and H2O. The organic phase was washed with brine and dried over MgSO4. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by flash chromatography over silica gel with EtOAc/hexane to give 11a (88.6 mg, 38%) as a white solid: mp 128–130 °C; 1H NMR (500 MHz, CDCl3, 40 °C) δ: −0.02 (5, 9H, Si(CH3)3), 0.95 (5, J = 8.3 Hz, 2H, CH2), 1.60 (5, 9H, C(CH3)3), 3.69 (5, J = 8.3 Hz, 2H, OCH2), 4.00 (5, 3H, OCH3), 5.57 (5, 2H, NCH3), 7.78 (5, 1H, Ar), 7.92 (dd, J = 8.0, 1.7 Hz, 1H, Ar), 8.11 (d, J = 8.0 Hz, 1H, Ar), 8.88 (dd, J = 1.7 Hz, 1H, Ar); 13C NMR (125 MHz, CDCl3, 40 °C) δ: −1.4 (3C), 18.2, 28.1 (3C), 52.9, 67.1, 75.7, 84.4, 125.4, 128.7, 131.6, 133.2, 136.1, 146.0, 146.5, 152.8, 161.8, 165.3; HRMS (FAB+) calcd for C22H27N5O7Si3 [M]+: 666.1826; found: 666.1832.

4.1.2. General procedure for deprotection and N-acylation: synthesis of methyl 5-[(2-(4-methoxybenzamido)thiazol-5-yl)]picolinate (12a)

HCl (4 N in dioxane; 8 mL) was added to a solution of 11a (233 mg, 0.5 mmol) in dioxane (2 mL), and the mixture was stirred at room temperature for 16 h. After evaporation of the solvent, a mixture of this crude product, 4-methoxybenzoyl chloride (101 μL, 0.75 mmol) and Et3N (208 μL, 3.0 mmol) in THF (10 mL) was stirred at 0 °C overnight. The solvent was removed under reduced pressure. The residue was dissolved in EtOAc and H2O.
The organic phase was washed with brine, and dried over MgSO₄. The filtrate was concentrated under reduced pressure, and the resulting residue was purified by flash chromatography over NH-silica gel with CHCl₃–MeOH to give 12a (160 mg, 86%) as a pale yellow solid: mp 263–265 °C; ¹H NMR (500 MHz, CDCl₃, 40 °C) δ: 3.90 (s, 3H, OCH₃), 4.03 (s, 3H, OCH₂), 7.03 (d, J = 8.6 Hz, 2H, Ar), 7.67 (s, 1H, Ar), 7.92–7.99 (m, 3H, Ar), 8.15 (d, J = 8.0 Hz, 1H, Ar), 8.92 (s, 1H, Ar), 10.77 (br s, 1H, NH); ¹³C NMR (125 MHz, CDCl₃, 40 °C) δ: 52.9, 55.6, 114.4 (2C), 124.0, 125.5, 128.2, 129.8 (2C), 131.4, 133.3, 135.6, 146.5, 146.6, 160.1, 163.8, 164.5, 165.3; HRMS (FAB⁺) calcd for C₁₇H₁₄N₃O₄S [M+H]⁺: 370.0856; found: 370.0861.

4.1.3. General procedure for hydrolysis: synthesis of 5-(2-(4-methoxybenzamido)thiazol-5-yl)picolinic acid (5a)

1 N LiOH (750 μL, 0.75 mmol) was added to a stirred mixture of 12a (92.3 mg, 0.25 mmol) in THF (5 mL) and water (5 mL), and the mixture was stirred for 24 h at room temperature. The mixture was acidified by 1 N HCl until pH <2, then cooled to 0 °C. The yellow precipitate was collected by filtration, washed with water, and dried under vacuum to give 5a (71.5 mg, 81%) as a yellow solid:

**References and notes**

32. Sleebs, B. E.; Street, I. P.; Bu, X.; Baell, J. B. Synthesis 2010, 1091.
33. The observed lower CC<sub>50</sub> values in cell proliferation assay compared with the IC<sub>50</sub> in the kinase assay can be partly attributed to far higher ATP concentration (1–10 mM) in cells than those in the kinase assay (5 μM). As described, the CC<sub>50</sub> values of the cell-based assay is also influenced by the membrane permeabilities of the compounds. For related discussions, see Ref. 20.