Synthesis of Novel Benzo-Fused Heteroaryl Derivatives as Ca^{2+}/Calmodulin-Dependent Protein Kinase II Inhibitors

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Based on the structure activity relationship of 2-(4-phenoxybenzoyl)-5-hydroxyindole (1), a novel structural class of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) inhibitors were synthesized. We show in this study that the acidic proton at the *N*(1)-position of the indole moiety is not essential for CaMKII inhibitory activity. Among the synthesized compounds, we found the benzofuran and benzothiazole derivative as promising scaffolds for the developement of potent CaMKII inhibitors. In particular, compounds 8 and 14 inhibited CaMKII with IC_{50} values of 24 nM and 32 nM, respectively.

Key words Ca^{2+}/calmodulin-dependent protein kinase II; inhibitor; kinase; indole; calmodulin

Calcium (Ca^{2+}) is an important intracellular messenger, controlling a diverse range of cellular processes, such as apoptosis, ion channel and cell cycle regulation, and cellular response to oxidative stress.[1,2] A rise in intracellular Ca^{2+} concentration leads to binding of Ca^{2+} ions to calmodulin (CaM), which in turn binds to and activates Ca^{2+}/CaM-dependent protein kinases (CaMKs). CaMKs, which are ubiquitous serine/threonine kinases classified into three subtypes (I, II, and IV), modulate many cellular functions in response to changes in intracellular Ca^{2+} levels.[3,4] CaMKII, a member of the CaMKs family, assembles into a complex of dodecamers with four isoforms (α, β, γ, and δ), having each a subunit composed of three main parts; catalytic, regulatory and association domains.[5–7] Upon binding to Ca^{2+}/CaM in the presence of ATP/Mg^{2+}, CaMKII undergoes a rapid autophosphorylation at the Thr^{286}/Thr^{287} located within the autoinhibitory domain. The transformed CaMKII maintains considerable enzyme activity even without Ca^{2+}/CaM.[8–11] This autophosphorylation has been reported to cause a dramatic increase in the affinity of the enzyme for Ca^{2+}/CaM.[12] CaMKII is well known for its modulating effects on synaptic plasticity and other processes like learning and memory.[13] In addition, CaMKII plays a role in osteoclasts differentiation and bone resorption,[14] and active CaMKII is known to enhance T cells proliferation and cytotoxic activity.[15]

The CaM-competitive inhibitor KN-93[16] and the autocamtide-2-related inhibitory peptide (AIP)[17] are well-known CaMKII inhibitors. Recent studies have also reported a number of Ca^{2+}/CaM antagonists[18] and CaM non-competitive inhibitors.[19] Based on these reports, we considered CaMKII to be a good target for the development of anti-inflammatory agents.

Our work started with high throughput screening against CaMKII. A description of this approach, which led to the discovery of **1** and its subsequent preliminary optimization to **2** and **3** (Fig. 1), was recently published.[20,21] We showed that a hydroxyl group in the indole moiety of **1** is essential for CaMKII inhibition and indicated that the effect of an acidic proton at the *N*(1)-position of the indole moiety was under examination. Here, we report in detail on our findings, in particular the structure–activity relationship (SAR) of a series of benzofuran and benzothiazole derivatives.

Chemistry The synthetic routes to the benzofuran and benzothiazole compounds are shown in Charts 1 and 2. The benzofuran derivatives **5**–**8** were prepared from the 4-phenoxyacetophenone **4**, which was treated with bromine to give a phenacyl bromide. Subsequent condensation with 2-hydroxy-5-methoxybenzaldehyde under basic conditions[22] furnished the benzofuran compound **5**. Deprotection of the methyl group of **5**, followed by bromination afforded compounds **7** and **8** (Chart 1).

The benzothiazole derivatives **12**–**14** were prepared from **9**, which was reacted with $SOCl_2$ to provide **10**. According to a reported method,[23] the anion of 4-phenoxyphenylacetonitrile was reacted with the chloride **10** to afford the diarylacetonitrile. Oxidation of the acetonitrile moiety with sodium peroxide and aqueous NH_4OAc solution gave **11**. Demethylation of **11**, followed by bromination of **12** afforded compounds **13** and **14** (Chart 2).

Results and Discussion

The inhibitory activity against CaMKII of the synthesized compounds is summarized in Table 1.[24] According to the results of our previous paper,[20] a substitution at the *N*(1)-position of the indole moiety should be well tolerated. As expected, the benzofuran **6** showed moderate inhibitory activity against CaMKII, confirming the assumption that the proton of the indole at the 1-position is not essential for activity. However, like the indole derivatives, the methoxy compound **5**, as

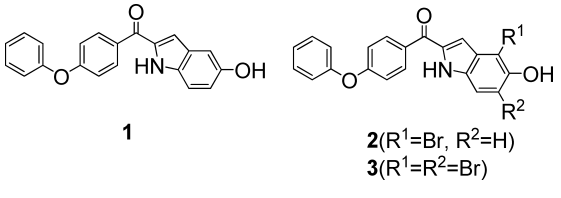

Fig. 1. Structures of **1**, **2**, and **3**

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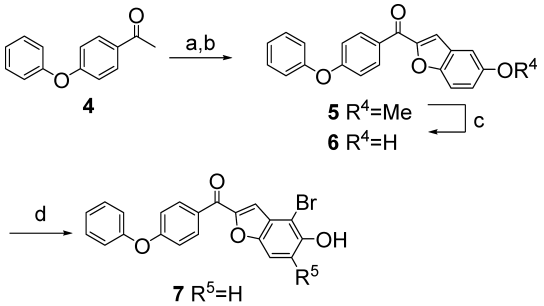
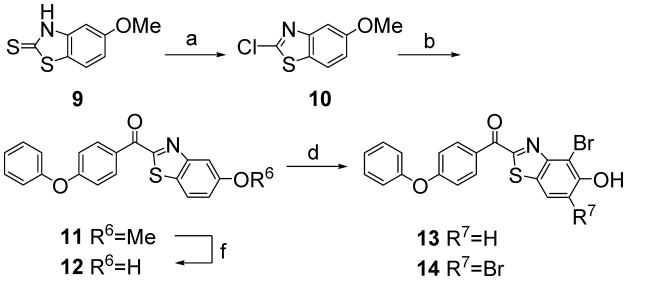


Reagents and conditions: (a) Br_2, AcOH, $CHCl_3$, rt, 1 h; (b) 2-hydroxy-5-methoxybenzaldehyde, K_2CO_3, MeCN, 70°C, 5 h, 73% in two steps; (c) BBr_3, $CHCl_3$, rt, 1 h, 83%. (d) NBS, THF, 0°C to rt, 1 h, **7**: 53%, **8**: 30%.

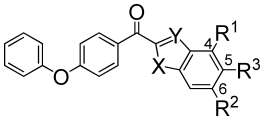
Chart 1



(a) $SOCl_2$, DMF(cat.), 70°C, 10 h, 70%; (b) NaHMDS, 4-phenoxyphenylacetonitrile, THF, rt, 3 h then NH_4OAc, Na_2O_2, rt, overnight, 51%; (c) BBr_3, CH_2Cl_2, rt, overnight, 65%; (d) NBS, THF, −10°C to rt, 1 h, **13**: 31%, **14**: 21%.

Chart 2

Table 1. Inhibitory Activity of Fused Heteroaryl Derivatives against CaMKII



Compd.	X	Y	R^1	R^2	R^3	CaMKII IC_{50} (μM)
1	NH	CH	H	H	OH	0.61
2	NH	CH	Br	H	OH	0.040
3	NH	CH	Br	Br	OH	0.012
5	O	CH	H	H	OMe	>10
6	O	CH	H	H	OH	1.1
7	O	CH	Br	H	OH	0.26
8	O	CH	Br	Br	OH	0.024
12	S	N	H	H	OH	1.1
13	S	N	Br	H	OH	0.17
14	S	N	Br	Br	OH	0.032

a no-acidic proton compound, showed diminished CaMKII inhibitory activity. Introduction of a bromo group into **6** resulted in improved activity, suggesting that benzofuran derivatives can be potent CaMKII inhibitors. Elsewhere, the mono-bromo compound **7** showed better membrane permeability than the indole **2** (data not shown). The benzothiazole **12** also showed moderate CaMKII inhibitory activity, suggesting that a thiazole moiety can be well tolerated. Finally, the mono-bromo compound **13** and di-bromo compound **14** showed strong CaMKII inhibitory activity, suggesting that benzothiazole derivatives can also be potent CaMKII inhibitors.

Conclusion

In this study, we clarified the effect of an acidic proton at the *N*(1)-position of the indole moiety of **1** on CaMKII inhibition by synthesizing and evaluating a series of benzofuran and benzothiazole derivatives. We found that the acidic proton of the NH group is not essential for CaMKII inhibitory activity. We also found that some of the synthesized compounds can serve as promising scaffolds for the discovery of potent CaMKII inhibitors. In particular, the di-bromo compounds **8** and **14**, which showed strong CaMKII inhibitory activity with IC_{50} values of 24 nM and 32 nM, respectively, and are therefore worthy of further optimization.

Experimental

Melting points (mp) were determined on an electrothermal apparatus without correction. IR spectra were recorded on a JEOL JIR-SPX60 spectrometer. NMR spectra were recorded on a JEOL JNM-LA300 spectrometer. Chemical shifts (δ) are given in parts per million, and tetramethylsilane (TMS) was used as the internal standard for spectra obtained in DMSO-d_6 and $CDCl_3$. All J values are given in Hz. Mass spectra were recorded on a Waters ACQUITY UPLC/MS system. Elemental analysis was performed on a CE Instrument EA1110 and a Yokokawa analytical system IC7000. Reagents and solvents were used as obtained from commercial suppliers without further purification. Column chromatography was carried out using a Yamazen W-prep system, and performed using prepacked silica gel. Reaction progress was determined by TLC analysis on silica gel coated glass plate. Visualization was done with UV light (254 nm). All reactions were carried out under a nitrogen atmosphere unless otherwise mentioned.

(5-(Methoxy)benzofuran-2-yl)(4-phenoxyphenyl)methanone (5) To a solution of **4** (500 mg, 2.36 mmol) in $CHCl_3$ (5.9 mL) and AcOH (5.9 mL) was added Br_2 (377 mg, 2.36 mmol) in $CHCl_3$ (0.5 mL). The mixture was stirred at room temperature for 1 h and the solvent was evaporated under reduced pressure. According to a literature procedure, the residue was dissolved in MeCN (11.8 mL), and 2-hydroxy-5-methoxybenzaldehyde (359 mg, 2.36 mmol) and K_2CO_3

(978 mg, 7.08 mmol) were added. The mixture was stirred at 70°C for 5 h and cooled. The mixture was diluted with EtOAc and washed with water and brine. The organic fraction was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc–hexane) and crystallized from EtOAc to provide compound **5** (592 mg, 73%) as a white solid.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 3.80 (3H, s), 7.10–7.30 (7H, m), 7.46–7.51 (2H, m), 7.65–7.70 (2H, m), 8.05 (2H, m); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ: 55.6, 104.4, 113.0, 116.4, 117.2, 118.3, 120.1, 124.9, 127.4, 130.4, 131.2, 131.9, 150.3, 152.3, 154.9, 156.2, 161.4, 181.8; MS (electrospray ionization (ESI)) *m/z* 345 (M+1); *Anal.* Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.68; H, 4.66.

(5-Hydroxybenzofuran-2-yl)(4-phenoxyphenyl)methanone (6) To a solution of **5** (200 mg, 0.476 mmol) in $CHCl_3$ (2.4 mL) was added BBr_3 in CH_2Cl_2 (1.0 M, 1.40 mL, 1.40 mmol) at 0°C. The mixture was stirred at room temperature for 1 h and quenched with saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine. The organic fraction was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc–hexane) to provide compound **6** (130 mg, 83%) as a yellow amorphous.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 5.05 (1H, s), 7.02–7.13 (6H, m), 7.20–7.25 (1H, m), 7.40–7.54 (4H, m), 8.09 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 107.1, 113.1, 115.7, 117.3, 117.8, 120.3, 124.8, 127.8, 130.1, 131.3, 132.0, 151.1, 152.3, 153.3, 155.3, 162.1, 182.9; IR (attenuated total reflection (ATR)) 1621 cm^{-1}; MS (ESI) *m/z* 331 (M+1); *Anal.* Calcd for $C_{21}H_{14}O_4$: C, 75.33; H, 4.36. Found: C, 75.35; H, 4.33.

(4-Bromo-5-hydroxybenzofuran-2-yl)(4-phenoxyphenyl) methanone (7) and (4,6-Dibromo-5-hydroxybenzofuran-2-yl)(4-phenoxyphenyl)methanone (8) To an ice cooled solution of **6** (150 mg, 0.454 mmol) in tetrahydrofuran (THF) (3.0 mL) was added *N*-bromosuccinimide (NBS) (105 mg, 0.590 mmol). The mixture was stirred at room temperature for 1 h and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc–hexane) and crystallized from EtOAc to provide compound **7** (99.3 mg, 53%) as a yellow solid. Compound **8** (67.6 mg, 30%) was obtained as a yellow amorphous.

7: mp=123–124°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 5.48 (1H, s), 7.08–7.14 (4H, m), 7.19–7.23 (2H, m), 7.41–7.51 (4H, m), 8.10 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 100.8, 112.6, 115.1, 117.3, 117.4, 120.4, 124.8, 128.5, 130.1, 131.0, 132.0, 149.4, 150.1, 153.4, 155.2, 162.3, 182.4; IR (ATR) 3317, 1630 cm^{-1}; MS (ESI) *m/z* 409 (M+1); *Anal.* Calcd for $C_{21}H_{13}BrO_4$: C, 61.63; H, 3.20. Found: C, 61.60; H, 3.17.

8: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 5.87 (1H, s), 7.08–7.14 (4H, m), 7.21–7.26 (1H, m), 7.41–7.46 (3H, m), 7.77 (1H, s), 8.08 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 100.9, 111.0, 115.1, 115.5, 117.3, 117.5, 120.4, 124.9, 128.9, 130.1, 130.8, 132.0, 146.4, 149.3, 153.6, 155.2, 162.5; IR (ATR) 1637 cm^{-1}; MS (ESI) *m/z* 489 (M+1); *Anal.* Calcd for $C_{21}H_{12}Br_2O_4 0.5H_2O$: C, 50.74; H, 2.64. Found: C, 50.40; H, 2.45.

2-Chloro-5-methoxybenzo[*d*]thiazole (10) To a solution of **9** (1.00 g, 5.07 mmol) in $SOCl_2$ (5.0 mL) was added *N*,*N*-dimethylformamide (DMF) (2 drops). The mixture was stirred at 70°C for 10 h and cooled. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel ($CHCl_3$) to provide compound **10** (705 mg, 70%) as a white solid.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 3.88 (3H, s), 7.05 (1H, m), 7.44 (1H, m), 7.62 (1H, m); MS (ESI) *m/z* 200 (M+1).

(5-Methoxybenzo[*d*]thiazol-2-yl)(4-phenoxyphenyl)methanone (11) According to a literature procedure, a mixture of **10** (600 mg, 3.01 mmol) and 4-phenoxyphenylacetonitrile (755 mg, 3.61 mmol) and sodium hexamethyldisilazide in toluene (0.99 M, 7.59 mL, 7.51 mmol) in THF (30 mL) was stirred at room temperature for 3 h. Then, to the mixture was added saturated NH_4OAc solution (20 mL) and Na_2O_2 (937 mg, 12.0 mmol). The mixture was stirred at room temperature overnight and quenched with 10% $NaHSO_3$ solution. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine. The organic fraction was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc–hexane) to provide compound **11** (559 mg, 51%) as a yellow solid.

^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 3.92 (3H, s), 7.07–7.25 (6H, m), 7.39–7.46 (2H, m), 7.64 (1H, m), 7.85 (1H, m), 8.59–8.63 (2H, m); MS (ESI) *m/z* 362 (M+1).

(5-Hydroxybenzo[*d*]thiazol-2-yl)(4-phenoxyphenyl)methanone (12) To a solution of **11** (100 mg, 0.277 mmol) in CH_2Cl_2 (2.0 mL) was added BBr_3 in CH_2Cl_2 (1.0 M, 0.830 mL, 0.830 mmol) at 0°C. The mixture was stirred at room temperature overnight and quenched with saturated $NaHCO_3$ solution. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine. The organic fraction was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc–hexane) and crystallized from EtOAc to provide compound **12** (62.6 mg, 65%) as a yellow solid.

mp=140–141°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 4.34 (1H, s), 7.06–7.16 (5H, m), 7.20–7.26 (1H, m), 7.40–7.45 (2H, m), 7.61 (1H, d, J=2.2 Hz), 7.85 (1H, d, J=8.8 Hz), 8.58 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 109.9, 117.1, 118.1, 120.5, 122.7, 124.9, 129.2, 129.3, 130.1, 133.8, 155.0, 155.1, 155.3, 163.0, 168.8, 183.7; IR (ATR) 1633 cm^{-1}; MS (ESI) *m/z* 348 (M+1); *Anal.* Calcd for $C_{20}H_{13}NO_3S$: C, 69.15; H, 3.77; N, 4.03. Found: C, 69.14; H, 3.84; N, 4.16.

(4-Bromo-5-hydroxybenzo[*d*]thiazol-2-yl)(4-phenoxyphenyl)methanone (13) and (4,6-Dibromo-5-hydroxybenzo[*d*] thiazol-2-yl)(4-phenoxyphenyl)methanone (14) To an ice cooled solution of **12** (200 mg, 0.576 mmol) in THF (4.0 mL) was added NBS (103 mg, 0.576 mmol). The mixture was stirred at room temperature for 1 h and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc–hexane) to provide compound **13** (77.2 mg, 31%) as a yellow solid and compound **14** (61.4 mg, 21%) as a yellow solid.

13: mp=161–162°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 5.83 (1H, s), 7.08–7.15 (4H, m), 7.22–7.31 (2H, m), 7.41–7.46 (2H, m), 7.82 (1H, d, J=8.8 Hz), 8.73 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 104.4, 117.1, 117.7, 120.6, 121.6, 124.9, 128.9, 129.7, 130.1, 134.1, 152.5, 155.1, 163.2, 169.5, 182.6; IR (ATR) 1631 cm^{-1}; MS (ESI) *m/z* 426 (M+1); *Anal.* Calcd for $C_{20}H_{12}BrNO_3S \cdot 0.5H_2O$: C, 55.18; H, 3.01; N, 3.22. Found: C,

55.52; H, 3.10; N, 3.35.

14: mp=164–165°C. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 6.17 (1H, s), 7.08–7.15 (3H, m), 7.22–7.27 (1H, m), 7.41–7.46 (2H, m), 8.12 (1H, s), 8.73 (2H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 104.7, 111.7, 117.1, 120.6, 124.1, 125.0, 128.7, 130.0, 130.1, 134.1, 149.0, 152.3, 155.0, 163.4, 169.8, 182.3; IR (ATR) 1626 cm^{-1}; MS (ESI) *m/z* 506 (M+1); *Anal.* Calcd for $C_{20}H_{11}Br_2NO_3S$: C, 47.65; H, 2.19; N, 2.77. Found: C, 47.67; H, 2.18; N, 2.89.

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