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5,6,7,8-Tetrahydropyrido[4,3-*d*]pyrimidines as novel class of potent and highly selective CaMKII inhibitors

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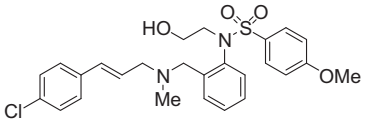
ABSTRACT

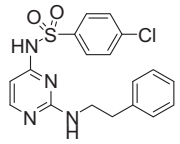
A novel series of 5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines containing substituted phenyl sulfonamide are synthesized and evaluated for their inhibitory activity against CaMKII. Substituents on the phenyl group had significant impact on CaMKII inhibition, in particular, the inhibitory activity of **8p** was 25-fold higher than that of KN-93, a known CaMKII inhibitor. Michaelis–Menten analysis of a representative compound suggested that the synthesized pyrimidines are calmodulin non-competitive inhibitors. Finally, **8p** exhibited more than 100-fold higher selectivity for CaMKII over five types of off-target kinases.

Calcium (Ca^{2+}) signaling is known as a critical component of biological pathways. Ca^{2+} is also an important second messenger for multiple intracellular processes, including apoptosis, ion channel and cell cycle regulation, and cellular response to oxidative stress.[1] Ca^{2+}/calmodulin-dependent protein kinases (CaMKs), which are ubiquitous serine/threonine protein kinases that are activated by Ca^{2+}-bound calmodulin and classified into three subtypes (I, II, and IV), modulate many cellular functions in response to intracellular Ca^{2+} levels.[2] CaMKII, a member of CaMKs family, assembles into a complex of dodecamers with four isoforms (α, β, γ, and δ), and each subunit is comprised of three main parts; catalytic, regulatory, and association domains.[3] Upon binding Ca^{2+}/calmodulin in the presence of ATP/Mg^{2+}, CaMKII undergoes a rapid autophosphorylation on Thr^{286}/Thr^{287} located within the autoinhibitory domain. The activated CaMKII maintains considerable enzyme activity even without Ca^{2+}/calmodulin.[4] This autophosphorylation has been reported to cause a dramatic increase in the affinity of the enzyme for Ca^{2+}/calmodulin.[5] CaMKII is well established for its effects on modulating synaptic plasticity and processes like learning and memory.[6] In addition, CaMKII plays a role in osteoclast differentiation and bone resorption,[7] and active CaMKII is known to enhance proliferation and cytotoxic activity of T cells.[8]

As calmodulin-competitive inhibitor **1** (KN-93, Fig. 1)[9] and autocamtide-2-related inhibitory peptide (AIP)[10] have been reported as well-known CaMKII inhibitors, we considered CaMKII to be a good target for the development of anti-inflammatory agents. Recently, we launched a HTS campaign to find novel structural CaMKII inhibitors, and compound **2** was identified as a hit compound



1 (KN-93)



2 (HTS hit compound)

Figure 1. Structures of 1, a representative CaMKII inhibitor, and **2**, a hit compound identified by HTS.

(Fig. 1).[11] The IC_{50} of **2** toward CaMKII was evaluated as 11 μM, approximately sevenfold weaker than that of **1**. Based on compound **2** we conducted a structure–activity relationship (SAR) study, and herein report the design, synthesis, and biological activity of a novel series of pyrimidyl sulfonamides.

The general synthesis of the pyrimidines **6**–**9** are shown in Scheme 1. Compounds **6**–**8** were prepared by simple replacement reaction of the dichloride **3** with appropriate nucleophiles. The first replacement reaction with the corresponding benzenesulfonamides (**5**) proceeded completely at position 4 of the pyrimidine ring. Compound **3** is commercially available or can be prepared easily according to a synthetic methods in the literature.[12] Catalytic reduction of the benzyl (Bn) group in compound **8**, followed by reductive amination using formaldehyde and $NaBH(OAc)_3$ afforded the N-methylated compound **9**.

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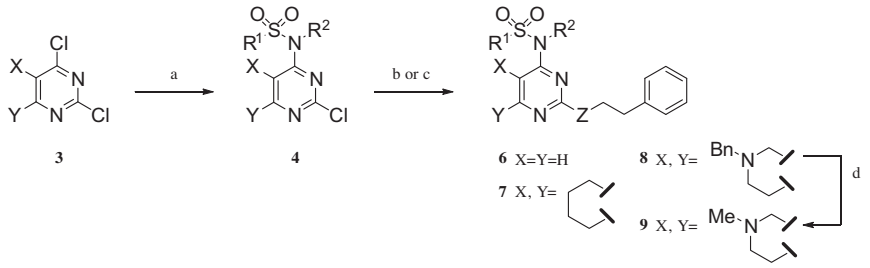


Scheme 1. Synthesis of pyrimidines. Reagents and conditions: (a) $R^2NHSO_2R^1$ (**5**), K_2CO_3, NMP, 100 °C; (b) $PhCH_2CH_2NH_2$, K_2CO_3, NMP, 100 °C; (c) $PhCH_2CH_2OH$, NaH, THF, rt; (d) (1) 10%—Pd/C, H_2 (0.45 MPa), CF_3CO_2H, MeOH, rt; (2) HCHO, $NaBH(OAc)_3$, dichloroethane, MeOH, rt.

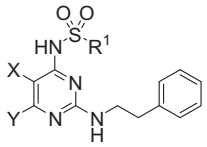
First, we investigated the effects of various pyrimidine moieties on CaMKII inhibition. The inhibitory activity of compounds **2**, **6a**,**b**, **7**, **8a**,**b**, and **9** for CaMKII are shown in Table 1. The results of monocyclic pyrimidines (**2** and **6a**,**b**) suggested that the phenyl group shown as R^1 into the sulfonamide was important for CaMKII inhibition. We then focused our efforts on conversion of the mono-cyclic pyrimidine to a fused-pyrimidine. The tetrahydroquinazoline compound **7** abolished CaMKII inhibition compared to the HTS hit compound **2**. Therefore, we introduced a basic amine into position 6 on the tetrahydroquinazoline ring based on the tertiary amino moiety in **1**. Among the tetrahydropyrido[3,4-*d*]pyrimidine compounds, **8a**,**b** exhibited inhibitory activity with single micro molar IC_{50}, while compound **9** showed no activity. These findings suggest that the benzyl group at the nitrogen atom of the piperidine moiety could be important in filling the hydrophobic region of the enzyme. As compound **8b** exhibited potent activity threefold that of **8a**, we conducted a detailed SAR study on the phenyl group substituents (R^1).

Next, we turned our attention to the effects of different substituents on both the phenyl moiety (R^1) and the nitrogen atom (R^2). The results for compounds **8a**–**q** are summarized in Table 2. Although the methyl compound **8c** had an activity comparable to that of the hydrogen compound **8a**, the propyl compound **8d** showed a complete loss of activity. These results indicate that steric hindrance produced a significant impact on CaMKII inhibition. In order to optimize the substituent on the phenyl moiety (R^1), we chose the chlorine atom as electron-withdrawing group and the methyl group as electron-donating one. The order of potency was *meta* (**8f**) > *ortho* (**8e**) > *para* (**8b**) in chlorine atom substituted compounds, whereas *ortho* (**8l**) was better than *meta* (**8m**) or *para* (**8n**) in methyl substitution. As the suitable position was different with the chlorine atom and the methyl group, we combined these findings and made bis-substituted compounds. As expected, compound **8p**,[13] considered as the best bis-substituted compound, showed potent activity much higher than that of single substituted compounds, including **8f** and **8l**. Compound **8q**, with a methyl group not in the best position, gave over 12-fold weaker activity than **8p**. Unfortunately, compound **8o**, which had 4-methoxybenzensulfonamide as a common partial structure with **1**, exhibited moderate inhibitory activity (IC_{50} = 3.2 μM).

In the case of di-chlorophenyl compounds, *meta*- and *para*-substitution (**8g**) was better than *ortho*- and *meta*-substitution (**8h**). However, the tendency was different from that of **8p** or **8q**, suggesting that sterically small substituents might be better to place at the *ortho* position. As representative of the di-cyclic group, we prepared the naphthyl compounds **8i** and **8j**. The 2-naphtyl compound **8j** was more effective than the 1-naphtyl one **8i** (**8j**, IC_{50} = 0.16 μM). These findings were similar to those obtained with di-chlorophenyl compounds. Unfortunately, using oxygen as the linker atom (Z), gave decreased activity (**8j** vs **8k**). Among the compounds prepared, **8p** showed the most potent inhibitory activity for CaMKII with an IC_{50} value of 0.063 μM, that is, 170-fold improvement from compound **2**.

We next investigated CaMKII inhibition pattern of fused-pyrimidines. Kinetics analysis of CaMKII inhibition by compound **8j** using

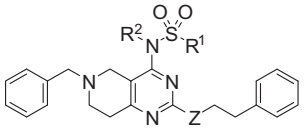
Table 1
Effects of pyrimidines on CaMKII



Compd	R^1	X, Y	CaMKII IC_{50}[a] (μM)
1			1.6[b]
2	4-Cl–C_6H_4	–H, –H	11
6a	Me	–H, –H	>60
6b	Bn	–H, –H	27
7	4-Cl–C_6H_4	–$CH_2CH_2CH_2CH_2$–	>60
8a	Ph	–$CH_2N(Bn)CH_2CH_2$–	9.7
8b	4-Cl–C_6H_4	–$CH_2N(Bn)CH_2CH_2$–	3.2
9	Ph	–$CH_2N(Me)CH_2CH_2$–	>10

[a] Compounds inhibitory activity for CaMKII enzyme.
[b] In-house data.

Table 2
Effects of 5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines on CaMKII



Compd	R^1	R^2	Z	CaMKII IC_{50}[a] μM
8a	Ph	H	NH	9.7
8c	Ph	Me	NH	9.3
8d	Ph	$CH_2CH_2CH_3$	NH	>30
8e	2-Cl–C_6H_4	H	NH	0.92
8f	3-Cl–C_6H_4	H	NH	0.42
8b	4-Cl–C_6H_4	H	NH	3.2
8g	2,3-Cl_2–C_6H_4	H	NH	1.0
8h	3,4-Cl_2–C_6H_4	H	NH	0.20
8i	1-Naphtyl	H	NH	3.3
8j	2-Naphtyl	H	NH	0.16
8k	2-Naphtyl	H	O	1.8
8l	2-Me–C_6H_4	H	NH	0.53
8m	3-Me–C_6H_4	H	NH	2.4
8n	4-Me–C_6H_4	H	NH	2.7
8o	4-OMe–C_6H_4	H	NH	3.2
8p	3-Cl–2-Me–C_6H_4	H	NH	0.063
8q	3-Cl–4-Me–C_6H_4	H	NH	0.76
1				1.6[b]
2				11

[a] Compound inhibitory activity for CaMKII enzyme.
[b] In-house data.

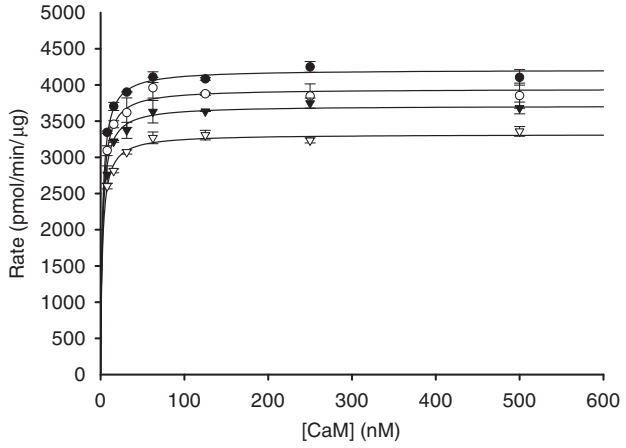


Figure 2. Michaelis–Menten plot of CaMKII inhibition by various concentrations (0 (●), 62.5 (○), 125 (▼), 250 (▽) μM) of compound **8j**. The inhibition kinetics analysis of CaMKII by compound **8j** were performed SigmaPlot Enzyme Kinetic Module. We calculated the goodness of fit to the enzyme inhibition model of competitive, non-competitive, and un-competitive following equations $V = V\mathrm{max}/\{1 + (K_m/S)(1 + I/K_i)\}$, $V = V_{max}/(1 + K_m/S)(1 + I/K_i)$, and $V = V_{max}/(1 + I/K_i + K_m/S)$, respectively ($V_{max}$: the maximum velocity. K_m: Michaelis constant for the varied substrate. S: concentration of the varied substrate. I: concentration of the test substance. K_i: inhibition constant.). The coefficient of determination of non-competitive was best and K_i value of **8j** was calculated with 930 nM.

Michaelis–Menten plot indicated that **8j** was a typical non-competitive inhibitor (Fig. 2) with K_i value calculated using analysis software as 926 nM.[14] This finding indicates that our compounds can be considered as calmodulin non-competitive inhibitors. Interestingly, compounds **1** and **8j** displayed different inhibition patterns in spite of their structural similarities (aryl-sulfonamide moiety). We speculate that the aminopyrimidine moiety in compound **8j** plays an important role in the inhibition pattern of CaMKII enzyme.

Finally, we examined the selectivity profile of **2**, **8j**, and **8p** against five types of kinase. The results are shown in Table 3. The selectivity of the 2-naphtyl compound **8j** against myosin light chain kinase (MLCK), which belongs to the CaM kinase family, was not good enough. Fortunately, compound **8p**, the most potent compound, showed significantly high selectivity against five off-target kinases, CaMKIV, MLCK, p38α, Akt1, and PKC.

In summary, we herein disclose a novel class of pyrimidine-based CaMKII inhibitors. Key SAR as well as the synthetic methods for these inhibitors are described. In particular, we found that compound **8p** inhibits CaMKII with an IC_{50} value of 0.063 μM, which is substantially superior to that of the known CaMKII inhibitor **1** (IC_{50} = 1.0 μM, Table 1). We therefore expect **8p** to be a novel lead compound in the development of treatments for inflammatory diseases, including rheumatoid arthritis.

Table 3
Kinase selectivity

Compd	CaMKII IC_{50}[a] (μM)	Kinase selectivity IC_{50} (μM)				
		CaMKIV	MLCK	p38α	Akt1	PKC
2	11	Nt[b]	51	Nt[b]	Nt[b]	Nt[b]
8j	0.16	>60	0.90	11	8.5	22
8p	0.063	>60	36	11	30	21

[a] Compounds inhibitory activity for CaMKII enzyme.
[b] Nt: not tested.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.005.

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13. Compound **8p**: mp 208–210 °C (dec); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 2.45 (3H, s), 2.47–2.53 (2H, m), 2.64 (2H, m), 2.69 (2H, br t, J = 7.0 Hz), 3.10 (2H, m), 3.17 (2H, br s), 3.67 (2H, br s), 6.62 (1H, br s), 7.02 (1H, t, J = 7.6 Hz), 7.17 (2H, m), 7.21 (1H, m), 7.30 (2H, m), 7.24–7.37 (5H, m), 7.44 (1H, dd, J = 7.6, 1.0 Hz), 7.89 (1H, dd, J = 7.6, 1.0 Hz), 11.25 (1H, br s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 16.8, 26.2, 34.2, 41.3, 47.8, 49.1, 61.0, 104.7, 125.8, 126.7, 127.1, 127.9, 128.0, 128.3, 130.6, 132.8, 134.3, 138.0, 138.8, 145.6, 151.1, 162.1; IR (ATR) 3307, 1664, 1626 cm^{-1}. Anal. Calcd for $C_{29}H_{30}ClN_5O_2S$: C, 63.55; H, 5.52; N, 12.78; S, 5.85; Cl, 6.47. Found: C, 63.20; H, 5.42; N, 12.55; S, 5.68; Cl, 6.29.
14. SigmaPlot for Windows Version 10.0.