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Structure and activity relationship of 2-(substituted benzoyl)-hydroxyindoles as novel CaMKII inhibitors

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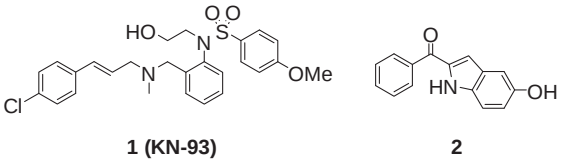
ABSTRACT

A series of novel 2-substituted-5-hydroxyindoles were synthesized and evaluated for their inhibitory activity against CaMKII. Structure and activity relationship results indicated that potent inhibitory activity could be achieved by modification at the *para*-position of the phenyl ring of the high throughput screening hit compound **2**. Among the prepared compounds, we identified **14** as a novel CaMKII inhibitor with an activity stronger than that of KN-93, a known CaMKII inhibitor.

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 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 intracellular Ca^{2+} levels.[3,4] CaMKII, a member of 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] CaMKII is well established for its modulating effects on synaptic plasticity and processes like learning and memory.[8] In addition, CaMKII plays a role in osteoclast differentiation and bone resorption,[9] and active CaMKII is known to enhance proliferation and cytotoxic activity of T cells.[10]

CaM-competitive inhibitor KN(93) (**1**)[11] and autocamtide-2-related inhibitory peptide (AIP)[12] are well-known CaMKII inhibitors. Recently, Ca^{2+}/CaM antagonists[13] and CaM non-competitive inhibitors[14] were reported. We considered CaMKII to be a good target for anti-inflammatory agents. In search for potent CaMKII inhibitors, we have recently run a high throughput screening (HTS) campaign of our library compounds and found compound **2**[15] as a hit with a novel structure (Fig. 1). The inhibitory activity of **2** against CaMKII was sixfold weaker than that of **1** (**2**; IC_{50} = 10 μM vs **1**; IC_{50} = 1.6 μM). Based on the structure of compound **2**, we initiated a structure and activity relationship (SAR) study to improve the inhibitory activity for CaMKII. Here, we report the synthesis and biological evaluation of a novel series of 2-(substituted benzoyl)-hydroxyindoles.

The synthetic routes for the indole compounds are described in Schemes 1 and 2. The various indole compounds were prepared from the commercially available indoles **3–5**. According to the reported method,[16] NH group in **3–5** was protected by using carbon dioxide, followed by treatment with *tert*-butyllithium to afford 2-lithium carbanion. Nucleophilic addition of the carbanion to an appropriate benzaldehyde (R^2–C_6H_4CHO), followed by oxidation of the hydroxyl group with MnO_2 produced the 2-acylindole derivatives. The 2-acylindole derivatives except **16–18** underwent deprotection of *tert*-butyldimethylsilyl (TBS) group with tetrabutylammonium fluoride (TBAF) to afford **6–15**. Hydrogenation of compound **18**, which was prepared in a similar manner above, gave the key intermediate **19** in good yield. Mitsunobu reaction of **19**



1 (KN-93) **2**

Figure 1. Structures of **1** and HTS hit compound **2**.

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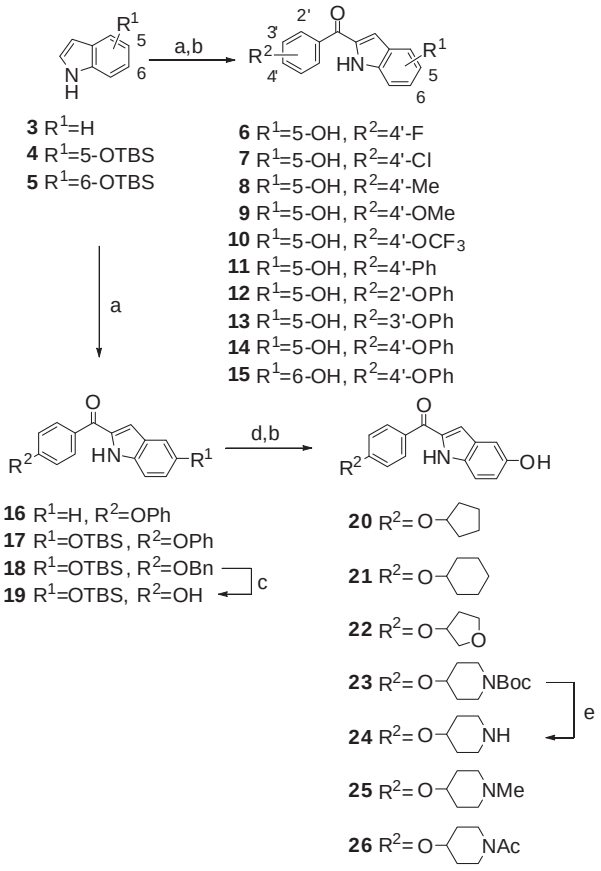
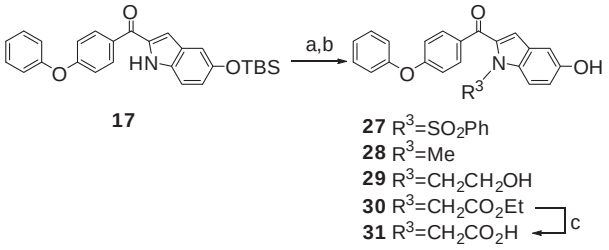


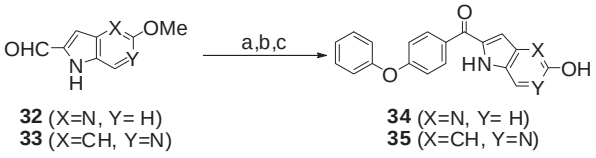
Scheme 1. Reagents and conditions: (a) (i) *n*-BuLi (1.2 equiv), CO_2(s), THF, −78 °C, 1 h; (ii) *t*-BuLi (1.1 equiv); (iii) R^2–C_6H_4CHO (1.1 equiv), −78 °C to rt, 2 h; (iv) MnO_2 (10 equiv), acetone, rt, overnight, 30–83%; (b) TBAF (1.1 equiv), THF, rt, 1 h, 45–95%; (c) 10% Pd/C (25 wt %), H_2, THF, EtOAc, rt, 4 h, 64%; (d) alcohols (1.5 equiv), PPh_3 (2.0 equiv), diisopropyl azodicarboxylate (2.0 equiv), THF, rt, overnight, 48–85%; (e) trifluoroacetic acid, $CHCl_3$, rt, overnight, 68%.



Scheme 2. Reagents and conditions: (a) R^3X (1.1 equiv), NaH (1.1 equiv), DMF, 0–50 °C, 4 h, 26–75% in two steps; (b) TBAF (1.1 equiv), THF, rt, 1 h; (c) 1 M NaOH aq (1.2 equiv), THF, MeOH, rt, overnight, 51%.

with an appropriate alcohol followed by deprotection of TBS and/or Boc afforded **20–26** (Scheme 1). Benzenesulfonylation or alkylation of **17** with the corresponding halides followed by deprotection of TBS group gave **27–30**, and then hydrolysis of the formed ethyl ester compound **30** gave compound **31** (Scheme 2).

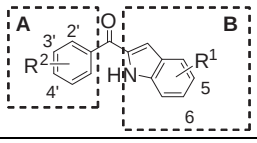
The synthetic route for the azaindole compounds **34** and **35** is shown in Scheme 3. Aldehyde **32** and **33** were prepared according to a reported method.[17] The aldehyde derivatives were reacted with 4-lithium diphenylether, which generated by halogen–lithium exchange reaction using *n*-BuLi. Oxidation of the formed hydroxyl group followed by deprotection of the methyl group with BBr_3 gave **34** and **35**.



Scheme 3. Reagents and conditions: (a) 4-bromodiphenylether (8.0 equiv), *n*-BuLi (8.2 equiv), THF, −10 °C, 2 h, 38–74%; (b) MnO_2 (10 equiv), $CHCl_3$, rt, 1 h, 50–81%; (c) BBr_3 (4.0 equiv), CH_2Cl_2, rt, 6 h, 12–71%.

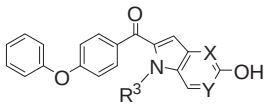
The inhibitory activity of the synthesized indole compounds toward CaMKII is shown in Table 1.[18,19] First, we examined the effect of a substituent on the phenyl moiety (A). An electron-donating or electron-withdrawing group was introduced to investigate for substitution at the *para* position, but no clear electronic effect was observed (**6–10**). However, the inhibitory activity for CaMKII was slightly improved in the *para* phenyl compound **11** compared to the hit compound **2** (**11**; IC_{50} = 6.4 μM). Surprisingly, the phenoxy compound **14**[20] demonstrated the most potent inhibitory activity with an IC_{50} value about 10-fold greater than that of **11** (**14**; IC_{50} = 0.61 μM). From these results we speculated that sterically large alkoxy substituents are favorable for better activity. Regard to the position of the phenoxy group in the phenyl ring (A), the *ortho* **12** and *meta* **13** showed decreased activity compared with *para* **14**, indicating that the substituent should be in the *para*-position. Based on these findings, we prepared and evaluated various alkoxy compounds at the *para*-position, in particular, those with a sterically large alkoxy group. Compounds **20** and **21** exhibited moderate activity with IC_{50} values in the single micromolar range, whereas compounds with small alkoxy groups, such as a methoxy (**9**) or trifluoromethoxy group (**10**), showed abolished activity. This confirmed our hypothesis that introduction of sterically large alkoxy substituents improves the activity. As both the phenoxy and cycloalkoxy groups are lipophilic, we therefore changed these

Table 1
Inhibitory activity of indole derivatives against CaMKII



Compd	R^1	R^2	CaMKII IC_{50} (μM)
1			1.6
2	5-OH	H	10
6	5-OH	4′-F	>10
7	5-OH	4′-Cl	>10
8	5-OH	4′-Me	8.8
9	5-OH	4′-OMe	>10
10	5-OH	4′-OCF_3	>10
11	5-OH	4′-Ph	6.4
12	5-OH	2′-OPh	1.8
13	5-OH	3′-OPh	1.7
14	5-OH	4′-OPh	0.61
15	6-OH	4′-OPh	2.3
16	H	4′-OPh	7.5
20	5-OH	4′-O-cyclopentyl	3.9
21	5-OH	4′-O-cyclohexyl	2.3
22	5-OH	4′-O-tetrahydrofuranyl	6.8
24	5-OH	4′-O-piperidinyl	>10
25	5-OH	4′-O-N-methylpiperidinyl	>10
26	5-OH	4′-O-N-acetylpiperidinyl	9.9

Table 2
Inhibitory activity of substituted 5-hydroxyindole derivatives against CaMKII



Compd	R^3	X	Y	CaMKII IC_{50} (μM)
14	H	CH	CH	0.61
27	SO_2Ph	CH	CH	>10
28	Me	CH	CH	1.3
29	CH_2CH_2OH	CH	CH	1.9
30	CH_2CO_2Et	CH	CH	0.77
31	CH_2CO_2H	CH	CH	1.8
34	H	N	CH	>10
35	H	CH	N	6.8
1				1.6

groups to find out whether substituent size or lipophilicity is important for the activity. Compound **22** where the carbon atom in the cyclopentyl ring was displaced with an oxygen atom, gave a twofold decreased activity. Conversion of the cyclohexyl ring to a piperidine or substituted piperidine resulted in a large drop in the activity (**21** vs **24–26**). These results indicate that lipophilic large substituents are preferable as R^2 substituents. In addition, the results of **14** and **21** suggest that the planarity of R^2 substituent is important for CaMKII inhibition. On the other hand, regard to the position of the hydroxyl group in the indole moiety (B), compound **15** with the hydroxyl group at 6th position resulted in fourfold drop in activity, and replacement with a hydrogen atom also gave decreased activity (12-fold). These findings confirmed that the hydroxyl group in the indole moiety is important for CaMKII inhibition, and that its placement in the 5th position is better.

Second, we focused on the effects of a substitution in the indole moiety (R^3), and the results are shown in Table 2. Substitution with an electron-withdrawing group, such as a benzensulfonyl group (**27**), showed in diminished inhibitory activity. Among the substituted alkyl compounds (**28–31**), the methyl (**28**), 2-hydroxyethyl (**29**), and carboxymethyl (**31**) showed a 2–3-fold loss of activity, leading us to speculate that the acidic proton at the *N*(1)-position is important for CaMKII inhibition. However, IC_{50} value of the ethoxycarbonylmethyl (**30**) was almost equivalent to that of **14**, suggesting that further optimization studies are necessary to find the best substituent.

Finally, in order to clarify the importance of the hydroxyl group at the 5th position, we modified the indole ring to azaindole. Both **34** and **35** exhibited decreased or diminished activity compared to the indole **14**. We therefore considered two possibilities to explain these results. One is that the lipophilic ring is better for the activity, and the other is that the pyridone form is generally stable in **34** and **35**. Further optimization work to confirm these two ideas is still ongoing and will be presented in a future paper.

In summary, we disclosed in this report a novel class of CaMKII inhibitors. An extensive SAR study based on hit compound **2** obtained by HTS identified the optimal substituents, which are the hydroxyl group as R^1 and the *para*-phenoxy group as R^2. Actually, compound **14** showed the most potent inhibitory activity against CaMKII enzyme with IC_{50} value of 0.61 μM, and the inhibitory activity was stronger than that of **1**, a known CaMKII inhibitor.

References and notes

1. Bootman, M. D.; Collins, T. J.; Peppiatt, C. M.; Prothero, L. S.; MacKenzie, L.; De Smet, P.; Travers, M.; Tovey, S. C.; Seo, J. T.; Berridge, M. J.; Ciccolini, F.; Lipp, P. *Semin. Cell Dev. Biol.* **2001**, *12*, 3.
2. Howe, C. J.; Lahair, M. M.; Mccubrey, J. A.; Franklin, R. A. *J. Biol. Chem.* **2004**, *279*, 44573.
3. Hook, S. S. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 471.
4. Braun, A. P.; Schulman, H. *Ann. Rev. Physiol.* **1995**, *57*, 417.
5. Hanson, P. I.; Schulman, H. *Annu. Rev. Biochem.* **1992**, *12*, 559.
6. Colbran, R. J.; Soderling, T. R. *Curr. Top. Cell. Regul.* **1990**, *279*, 181.
7. Gaertner, T. R.; Kolodziej, S. J.; Wang, D.; Kobayashi, R.; Koomen, J. M.; Stoops, J. K.; Waxham, M. N. *J. Biol. Chem.* **2004**, *279*, 12484.
8. Wayman, G. A.; Lee, Y. S.; Tokumitsu, H.; Silva, A.; Soderling, T. R. *Neuron* **2008**, *59*, 914.
9. Ang, E. S.; Zhang, P.; Steer, J. H.; Tan, J. W.; Yip, K.; Zheng, M. H.; Joyce, D. A.; Xu, J. *J. Cell. Physiol.* **2007**, *212*, 787.
10. Lin, M. Y.; Zal, T.; Ch'en, I. L.; Gascoigne, N. R. J.; Hedrick, S. M. *J. Immunol.* **2005**, *174*, 5583.
11. Sumi, M.; Kiuchi, K.; Ishikawa, T.; Ishii, A.; Hagiwara, M.; Nagatsu, T.; Hidaka, H. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 968.
12. Ishida, A.; Kameshita, I.; Okuno, S.; Kitani, T.; Fujisawa, H. *Biochem. Biophys. Res. Commun.* **1995**, *212*, 806.
13. Colomer, J.; Schmitt, A. A.; Toone, E. J.; Means, A. R. *Biochemistry* **2010**, *49*, 4244.
14. Asano, S.; Komiya, M.; Koike, N.; Koga, E.; Nakatani, S.; Isobe, Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6696.
15. Igarashi, S.; Isaka, M.; Inami, H.; Hara, H.; Kamitoku, H. JP 07145147.
16. Katritzky, A. R.; Akutagawa, K. *Tetrahedron Lett.* **1985**, *26*, 5935.
17. Frydman, B.; Reil, S. J.; Boned, J.; Rapoport, H. *J. Org. Chem.* **1968**, *33*, 3762.
18. Expression and purification of CaMKII: CaMKII was purified according to the method of Brickey et al. (*Biochem. Biophys. Res. Commun.* **1990**, *173*, 578) with some modifications. cDNA encoding CaMKII was sub-cloned into the transfer vector pFastBac1. Bacmids were generated in DH10Bac cells, and CaMKII recombinant baculovirus stocks were prepared according to the protocol of Bac-to-Bac Baculovirus Expression System (invitrogen, USA). Sf9 cells were maintained in Sf900 II medium and infected with CaMKII recombinant baculovirus stocks. The cells were harvested 3 days post-infection and CaMKII was affinity purified using Calmodulin Sepharose 4B (GE healthcare).
19. CaMKII kinase assay: Autocamtide-2 and CaM were purchased from Millipore, and γ-^{33}P ATP was obtained from GE Healthcare. ATP was purchased from Sigma–Aldrich. The test substances and purified CaMKII were added to the assay buffer containing 25 mM Tris–HCl, pH 7.5, 2 mM dithiothreitol, 10 mM $MgCl_2$, 0.1% CHAPS, and 1 mM $CaCl_2$. Autocamtide-2 was diluted to a final concentration of 10 μM with assay buffer containing varying concentrations of CaM (500 μM, 100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, and 3.125 μM), and ATP (final concentration 10 μM and 350–1500 cpm/pmol γ-^{33}P ATP) was added to the diluted test substances and the whole was incubated for 20 min at 30 °C. After incubation, the reaction was terminated by transfer of a 16 μL aliquot onto the appropriate area of a P30 Filtermat (PerkinElmer, USA), and the labeled substrate was captured by a negatively charged filtermat. Phosphorylation was linear with respect to time under these conditions. The filtermat was washed three times, each for 5 min with 75 mM phosphoric acid, dried, and then spotted by MicroScintO (PerkinElmer, USA). The radioactivity was counted in TopCount NXT (PerkinElmer, USA). CPM counts were calculated for transfer of a phosphate group per minute per 1 mg CaMKII.
20. Compound **14**: Mp = 162–163 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.73 (1H, s), 6.91–7.24 (8H, m), 7.34–7.45 (3H, m), 8.01 (2H, m), 9.16 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 106.1, 111.5, 113.1, 117.4, 120.2, 124.6, 128.3, 130.1, 131.5, 132.3, 133.0, 135.1, 150.2, 155.6, 161.6, 185.8; IR (ATR) 3300, 1618 cm^{-1}; MS (ESI) *m*/*z* 330 (M+1); Anal. Calcd for $C_{21}H_{15}NO_3 \cdot 0.2H_2O$: C, 75.75; H, 4.66; N, 4.21. Found: C, 75.96; H, 4.55; N, 4.27.