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Synthesis and structure based optimization of 2-(4-phenoxybenzoyl)-5-hydroxyindole as a novel CaMKII inhibitor

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ABSTRACT

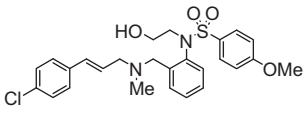
Based on 2-(4-phenoxybenzoyl)-5-hydroxyindole (**2**), a novel structural class of CaMKII inhibitors were synthesized and further optimized. The strong acidity of the hydroxyl group and the lipophilic group at the 4 and 6-positions were found to be necessary for strong CaMKII inhibition. Compound **25** was identified as a promising compound with 50-fold more potent inhibitory activity for CaMKII than **2**. Compound **25** also showed high selectivity for CaMKII over off-target kinases.

1. Introduction

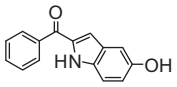
Calcium (Ca^{2+}) is an important intracellular messenger, controlling a diverse range of cellular processes, such as apoptosis, ion channel and cell cycle regulation, and cellular response to oxidative stress.[1,2] A rise in intracellular Ca^{2+} concentration leads to binding of Ca^{2+} ions to calmodulin (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 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] Upon binding 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 activated 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 proliferation and cytotoxic activity of T cells.[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 reported Ca^{2+}/CaM antagonists[18] and CaM non-competitive inhibitors.[19] Based on these reports, we considered CaMKII to be a good target for 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** (Fig. 1), was recently published.[20] In particular, we showed that a hydroxyl group in the indole moiety is important for CaMKII inhibition. Here we report in detail the structure–activity relationship (SAR) of this series compounds.



KN-93



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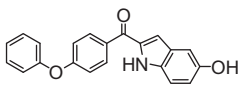

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Figure 1. Structures of KN-93, **1** and **2**.

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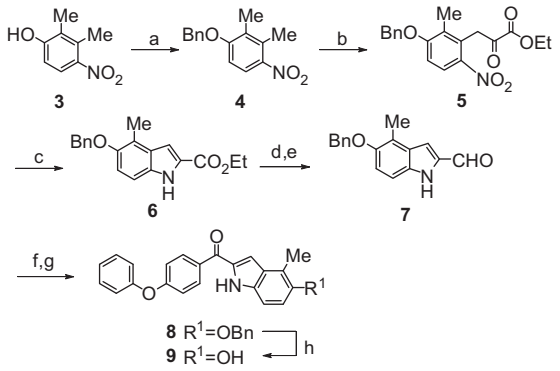
2. Chemistry

The synthetic routes to the indole compound are shown in Schemes 1–3. Benzylation of the commercially available **3**, followed by reaction with diethyl oxalate in the presence of potassium ethoxide and reduction of the nitro group with iron under acidic conditions provided **6**. Reduction of the ester group of **6** with $LiAlH_4$, followed by oxidation of the formed hydroxyl group with MnO_2 afforded the aldehyde **7**. The aldehyde **7** was reacted with 4-lithium diphenylether, which was generated by halogen–lithium exchange reaction using *n*-BuLi. Oxidation of the formed hydroxyl group, followed by deprotection of the benzyl group with BBr_3 gave the desired **9** (Scheme 1).

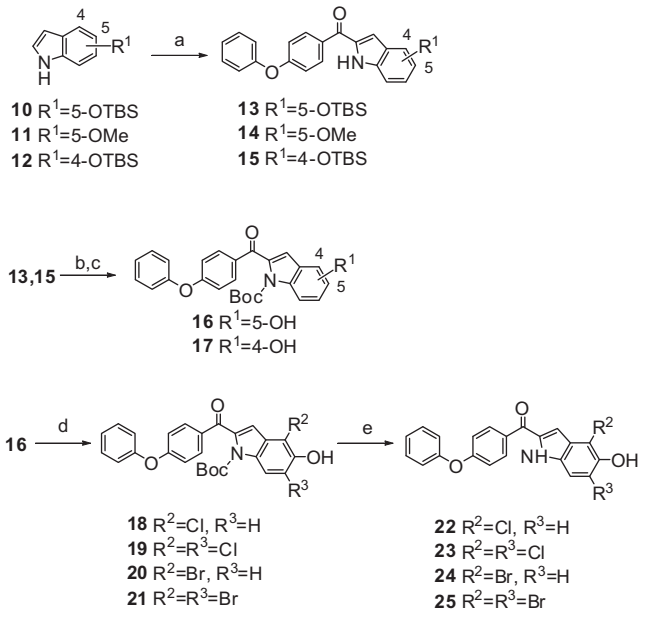
The other indole derivatives **2**, **14**, **22–25**, **32, 34–41** were prepared from the commercially available indoles **10–11** or from the reported compound **12**.[21] According to the reported method,[22] protection of NH group in **10–12** using carbon dioxide, followed by treatment with *tert*-butyllithium afforded 2-lithium carbanion. Nucleophilic addition of the carbanion to 4-phenoxybenzaldehyde, followed by oxidation of the hydroxyl group with MnO_2 produced the 2-acylindole derivatives **13–15**. Protection of NH group in **13** and **15** with di-*tert*-butyl dicarbonate, followed by deprotection of TBS group afforded compounds **16–17**. Halogenation of **16** with *N*-bromosuccinimide or *N*-chlorosuccinimide, followed by deprotection of the Boc group with trifluoroacetic acid gave **22–25** (Scheme 2). Reaction of the hydroxyindole derivative **16** with ethyl bromoacetate or ethyl 2-bromo-2-methylpropanoate, followed by deprotection of the Boc group and hydrolysis of the ester afforded **32** and **34**. Alkylation of **16–17** with 1-bromo-2-chloroethane or 1-bromo-3-chloropropane, followed by nucleophilic addition of the corresponding amines provided various amine derivatives (**35–40**). In the case of **41**, compound **16** was reacted with *N*-(2-bromoethyl)phthalimide, and the phthalimide moiety, as a protective group, was removed by methylamine (Scheme 3).

3. Results and discussion

Although compound **2** inhibited CaMKII with an IC_{50} value of 0.61 μM, this inhibitory activity was not considered high enough. In our last paper, a 4-phenoxybenzoyl group was identified as the best substituent at the 2-position of the indole in **2**, while a hydroxyl group at the 5-position was considered as important for CaMKII inhibitory activity. To improve compound **2** inhibitory activity for CaMKII, we designed further structural modifications, in particular, modification of the substituent around the hydroxyl group.

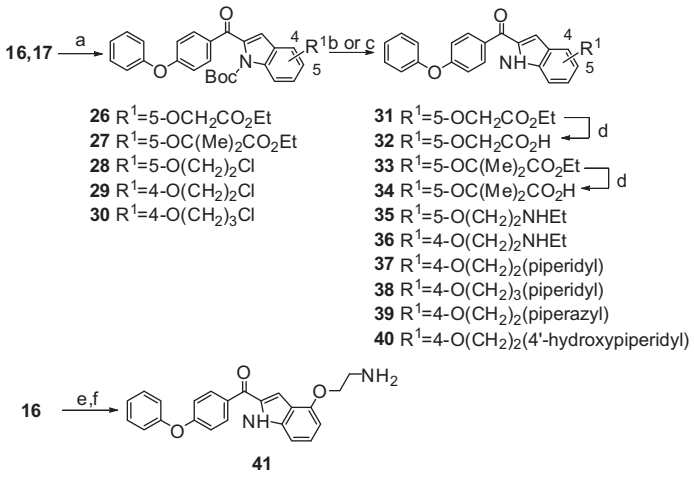


Scheme 1. Reagents and conditions: (a) BnCl, K_2CO_3, DMF, 100 °C, 2 h, 88%; (b) $(CO_2Et)_2$, KOEt, Et_2O–EtOH, rt, overnight, 36%; (c) Fe, AcOH, EtOH, reflux, 3 h, 75%; (d) $LiAlH_4$, THF, 0 °C, 1 h; (e) MnO_2, acetone, rt, overnight, 77% in two steps; (f) 4-bromodiphenylether, *n*-BuLi, THF, -20 °C, 2 h; (g) MnO_2, acetone, rt, 2 h, 38% in two steps; (h) BBr_3, $CHCl_3$, rt, 1 h, 43%.



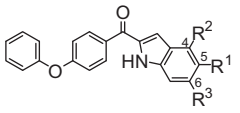
Scheme 2. Reagents and conditions: (a) (i) *n*-BuLi, CO_2(s), THF, −78 °C, 1 h; (ii) *t*-BuLi; (iii) 4-phenoxybenzaldehyde, −78 °C to rt, 2 h; (iv) MnO_2, acetone, rt, overnight, **13**:83%, **14**:56%, **15**:75%; (b) Boc_2O, THF, rt, 1 h; (c) tetrabutylammonium fluoride, THF, rt, 1 h, **16**:97%, **17**:56% in two steps; (d) *N*-chlorosuccinimide or *N*-bromosuccinimide, THF, 0 °C to rt, 1 h, **18**:33%, **19**:23%, **20**:43%, **21**:29%; (e) trifluoroacetic acid, $CHCl_3$, rt, 1 h, **22**:75%, **23**:74%, **24**:67%, **25**:98%.

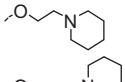
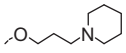
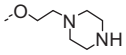
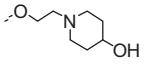
The inhibitory activity against CaMKII of the synthesized compounds is summarized in Table 1. First, we examined the effect of a substituent on the indole at the 4-position. Introduction of a methyl group, as an electron-donating group, resulted in twofold drop in the activity, while a chloro group, as an electron-withdrawing group, showed an activity comparable to that of the unsubstituted **2**, suggesting that substitution at the 4-position is tolerated. Although a methyl and chloro groups are considered to be of similar molecular size, they exert mutually opposite electronic effects. This led us to hypothesize that a hydroxyl group is related to inhibitory activity against CaMKII. Based on this hypothesis, we focused on the acidity of the hydroxyl group in the indole moiety. We first prepared the di-chloro compound **23** to increase the acidity of the hydroxyl group (calculated pK_a value of the hydroxyl group in **23** decreased over one log-fold compared to **22**). Compound **23** inhibited CaMKII with an IC_{50} value of 61 nM, which was 10-fold more potent than that of **22**. Next we replaced the chloro group in **22** with a bromo one. Surprisingly, the mono-bromo compound **24** showed potent inhibitory activity against CaMKII with an IC_{50} value of 40 nM, which was 15-fold more potent than that of the mono-chloro compound **22**. However, the calculated pK_a value of the hydroxyl group was similar. The most potent inhibition of CaMKII was seen with the di-bromo compound **25** (IC_{50} = 12 nM). As the calculated pKa values of **23** and **25** were similar (data not shown), we guessed that a strong acidic proton might be important for interaction with CaMKII protein and that lipophilic groups should be placed at the 4 and 6-positions. As reference, we prepared the methoxy **14**, as a no-acidic proton compound. Compound **14** showed complete loss of inhibitory activity against CaMKII, indicating an acidic proton is essential for strong CaMKII inhibitory activity. Next, we introduced an acidic proton via a carboxylate at the 5-position. The inhibitory activity of compound **32** was equal to that of the lead compound **2**, while that of compound **34** was moderate. These results indicated that various acidic substituents can be acceptable at the 5-position. Interestingly, the ethylaminoethoxy compound **35** also showed moderate



Scheme 3. Reagents and conditions: (a) alkyl bromide, K_2CO_3, DMF, 40 °C or 100 °C, 2 h, **26**:72%, **27**:79%, **28**:47%, **29**:83%, **30**:69%; (b) trifluoroacetic acid, $CHCl_3$, rt, 1 h, **31**:88%, **33**:95%; (c) amines, NaI, DMF, 100 °C , 6 h, **35**:26%, **36**:37%, **37**:80%, **38**:64%, **39**:69%, **40**:89%; (d) 1 M NaOH aq, THF, MeOH, rt, 2 h, **32**:67%, **34**:48%; (e) *N*-(2-bromoethyl)phthalimide, K_2CO_3, NaI, DMF, 100 °C , 3 h; (f) $MeNH_2$, MeOH, 50 °C, 1 h, 52% in two steps.

Table 1
Inhibitory activity of indole derivatives against CaMKII



Compd	R^1	R^2	R^3	CaMKII IC_{50} (μM)
2	OH	H	H	0.61
9	OH	Me	H	1.3
22	OH	Cl	H	0.64
23	OH	Cl	Cl	0.061
24	OH	Br	H	0.040
25	OH	Br	Br	0.012
14	OMe	H	H	>10
32	OCH_2CO_2H	H	H	0.51
34	$OC(Me)_2CO_2H$	H	H	1.5
35	$O(CH_2)_2NHEt$	H	H	1.5
36	H	$O(CH_2)_2NHEt$	H	1.1
41	H	$O(CH_2)_2NH_2$	H	2.6
37	H		H	0.82
38	H		H	0.80
39	H		H	0.27
40	H		H	0.42

n.d. = Not determined.

inhibitory activity for CaMKII (IC_{50} = 1.5 μM), suggesting that this compound inhibits CaMKII by interacting with other amino acids in CaMKII. The solubility of the above halogen-atom substituted compounds **23–25** was poor (<0.001 mg/mL at pH 7.4), indicating that further modification is necessary to identify orally available drug candidates. Generally, an amino group is useful to improve compounds water solubility. To confirm this, we carried out a preliminary examination. As the inhibitory activity of **36**, containing an ethylaminoethoxy at the 4-position, was slightly stronger than that of **35**, the 4-position was chosen for further modification. A primary amine with the same ethylene chain length as compound **41** decreased the inhibitory activity for CaMKII, while a tertiary amine as in compounds **37** and **38,** slightly improved the activity. CaMKII inhibitory activity was improved in the piperazine **39** and hydroxypiperidine **40** compared to **36**, suggesting that a bipolar group is beneficial for the activity. However, these amine compounds showed cytotoxicity in mouse bone marrow cells, forcing us to stop further examination of this series of compounds.

Table 2
Selected compounds inhibitory activity against CaMKII and their selectivity for CaMKII over other kinases

Compd	CaMKII IC_{50} (μM)	Selectivity (fold)			
		P38α	MLCK	Akt1	PKCγ
2	0.61	<1	<1	295	93
24	0.040	115	12	1500	550
25	0.012	450	63	>5000	1250

Based on the above structure–activity relationships, we focused on compounds **2**, **24** and **25**, and further examined their selectivity for CaMKII. As shown in Table 2, compound **2** exhibited no higher selectivity for CaMKII over p38α and MLCK, whereas compound **24** and **25** showed good selectivity for CaMKII over all the other selected kinases. In particular, compound **25** was considered as a promising compound considering the side-effects associated with interaction with p38α and MLCK.

4. Conclusion

Based on our previous work, we carried out an optimization study to find strong and selective CaMKII inhibitors. This optimization focused on acidity of the acid proton in the indole. Introduction of a halogen atom at the 4 and 6-positions of the indole was effective in improving both CaMKII inhibitory activity and kinase selectivity. In particular, the di-bromo compound **25** showed strong CaMKII inhibitory activity with an IC_{50} value of 12 nM as well as good selectivity for this kinase.

5. Experimental

5.1. Chemistry

Melting points (Mp) were determined on an electrothermal apparatus without correction. IR spectra were recorded on a JEOL JIR-SPX60 spectrometer as ATR. NMR spectra were recorded on a

JEOL JNM-LA300 spectrometer. Chemical shifts (δ) are given in parts per million, and 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.

5.1.1. 1-(Benzyloxy)-2,3-dimethyl-4-nitrobenzene (4)

A mixture of **3** (1.67 g, 10.0 mmol), K_2CO_3 (2.07 g, 15.0 mmol) and BnCl (1.39 g, 11.0 mmol) in DMF (17 ml) was stirred at 100 °C for 2 h. The mixture was cooled and diluted with EtOAc, then 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) to provide compound **4** (2.26 g, 88%) as a yellow solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.28 (3H, s), 2.46 (3H, s), 5.15 (2H, s), 6.80 (1H, d, J = 9.0 Hz), 7.32–7.44 (5H, m), 7.76 (1H, d, J = 9.0 Hz); MS (ESI) m/z 258 (M+1).

5.1.2. Ethyl 3-(3-(benzyloxy)-2-methyl-6-nitrophenyl)-2-oxopropanoate (5)

To a solution of **4** (1.86 g, 7.24 mmol) in Et_2O (3.7 ml) and EtOH (37 ml) was added diethyl oxalate (2.12 g, 14.5 mmol) and potassium ethoxide (670 mg, 7.96 mmol). The mixture was stirred at room temperature overnight and then quenched with saturated NH_4Cl 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 **5** (933 mg, 36%) as a yellow oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.42 (3H, t, J = 7.2 Hz), 2.26 (3H, s), 4.41 (2H, q, J = 7.2 Hz), 4.52 (2H, s), 5.18 (2H, s), 6.94 (1H, d, J = 9.2 Hz), 7.34–7.45 (5H, m), 8.01 (1H, d, J = 9.2 Hz); MS (ESI) m/z 358 (M+1).

5.1.3. Ethyl 5-(benzyloxy)-4-methyl-1H-indole-2-carboxylate (6)

A mixture of **5** (850 mg, 2.39 mmol), AcOH (287 mg, 4.78 mmol) and Fe (664 mg, 12.0 mmol) in EtOH (9.0 ml) was stirred under reflux for 3 h. The mixture was cooled and filtered through celite. The filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/hexane) to provide compound **6** (553 mg, 75%) as a white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.42 (3H, t, J = 7.2 Hz), 2.47 (3H, s), 4.40 (2H, q, J = 7.2 Hz), 5.08 (2H, s), 7.08 (1H, m), 7.18–7.48 (7H, m), 8.85 (1H, br s); MS (ESI) m/z 310 (M+1).

5.1.4. 5-(Benzyloxy)-4-methyl-1*H*-indole-2-carbaldehyde (7)

To a solution of $LiAlH_4$ (36.8 mg, 0.969 mmol) in THF (2.0 ml) at 0 °C was added a solution of **6** (100 mg, 0.323 mmol) in THF (0.5 ml). The mixture was stirred at 0 °C for 1 h and then quenched with water and 1 N NaOH solution. The mixture was filtered through celite. The filtrate was evaporated under reduced pressure. The residue was dissolved in acetone (2.0 ml) and MnO_2 (84.3 mg, 0.969 mmol) was added. The mixture was stirred at room temperature overnight and filtered through celite. The filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/hexane) to provide compound **7** (66.0 mg, 77%) as a yellow solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.50 (3H, s), 5.10 (2H, s), 7.15–7.48 (8H, m), 8.90 (1H, br s), 9.83 (1H, s); MS (ESI) m/z 266 (M+1).

5.1.5. (5-(Benzyloxy)-4-methyl-1*H*-indol-2-yl)(4-phenoxyphenyl)methanone (8)

A solution of 4-bromodiphenylether (375 mg, 1.50 mmol) in THF (1.0 ml) was cooled to −20 °C and n-BuLi in hexane (2.64 M, 0.571 ml, 1.50 mmol) was added. The mixture was stirred at −20 °C for 1 h and a solution of **7** (50.0 mg, 0.188 mmol) in THF (0.5 ml) was added. The mixture was allowed to rise to room temperature for 1 h and then quenched with saturated NH_4Cl 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 secondary alcohol intermediate. The intermediate was dissolved in acetone (1.0 ml) and MnO_2 (49.0 mg, 0.564 mmol) was added. The mixture was stirred at room temperature for 2 h and filtered through celite. The filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/hexane) to provide compound **8** (30.9 mg, 38%) as a yellow solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.48 (3H, s), 5.06 (2H, s), 7.08–7.49 (15H, m), 7.99–8.03 (2H, m), 9.14 (1H, br s); MS (ESI) m/z 434 (M+1).

5.1.6. (5-Hydroxy-4-methyl-1*H*-indol-2-yl)(4-phenoxy phenyl)methanone (9)

To a solution of **8** (18.0 mg, 0.042 mmol) in $CHCl_3$ (1.0 ml) at 0 °C was added BBr_3 in CH_2Cl_2 (1.0 M, 0.5 ml, 0.500 mmol). The mixture was stirred at room temperature for 1 h and then 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) to provide compound **9** (6.0 mg, 43%) as a white solid.

Mp = 143–144 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.44 (3H, s), 4.58 (1H, s), 6.95 (1H, d, J = 8.8 Hz), 7.07–7.14 (5H, m), 7.18–7.26 (2H, m), 7.40–7.45 (2H, m), 8.01 (2H, m), 9.16 (1H, br s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 11.9, 109.6, 110.2, 113.7, 117.2, 120.1, 124.8, 128.7, 130.4, 131.5, 132.8, 134.1, 148.1, 155.2, 160.6, 184.8; IR (ATR) 3292, 1622 cm^{-1}; MS (ESI) m/z 344 (M+1); Anal. Calcd for $C_{22}H_{17}NO_3 0.75H_2O$: C, 74.04; H, 5.22; N, 3.92. Found: C, 74.09; H, 5.17; N, 3.80.

5.1.7. (5-(*tert*-Butyldimethylsilyloxy)-1*H*-indol-2-yl)(4-phenoxy phenyl)methanone (13)

According to a literature procedure, a solution of **10** (4.95 g, 20.0 mmol) in THF (100 ml) was cooled to −78 °C and n-BuLi in hexane (2.64 M, 9.09 ml, 24.0 mmol) was added. The mixture was stirred at −78 °C for 1 h and then excess of carbon dioxide (dry ice, 10 g) was added. The solution was allowed to rise to room temperature for 1 h. 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 dissolved in THF (100 ml) and the solution was cooled to −78 °C. t-BuLi in hexane (1.57 M, 14.0 ml, 22.0 mmol) was added and stirred at −78 °C for 1 h, and then a solution of 4-phenoxybenzaldehyde (4.36 g, 22.0 mmol) in THF (5.0 ml) was added at −78 °C. The solution was allowed to rise to room temperature for 1 h and then quenched with saturated NH_4Cl 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 secondary alcohol intermediate. The intermediate was dissolved in acetone (100 ml) and MnO_2 (7.00 g, 60.0 mmol) was added. The mixture was stirred at room temperature overnight and filtered through celite. The filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc/hexane) to provide compound **13** (7.40 g, 83%) as a white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.18 (6H, s), 1.01 (9H, s), 6.91–7.13 (6H, m), 7.19–7.45 (5H, m), 7.98–8.03 (2H, m), 9.24 (1H, br s); MS (ESI) *m*/*z* 444 (M+1).

5.1.8. (5-Methoxy-1*H*-indol-2-yl)(4-phenoxyphenyl) methanone (14)

Compound **14** was prepared from **11** using the procedure for **13** with 56% as a white solid.

Mp = 155–156 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.86 (3H, s), 7.03–7.14 (7H, m), 7.20–7.26 (1H, m), 7.37–7.45 (3H, m), 8.00 (2H, m), 9.30 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 55.7, 102.7, 111.7, 113.1, 117.4, 118.2, 120.1, 124.5, 128.0, 130.1, 131.5, 132.4, 132.8, 134.8, 154.8, 155.6, 161.5, 185.6; IR (ATR) 3280, 1618 cm^{-1}; MS (ESI) *m*/*z* 344 (M+1); Anal. Calcd for $C_{22}H_{17}NO_3$: C, 76.95; H, 4.99; N, 4.08. Found: C, 77.00; H, 4.93; N, 4.10.

5.1.9. (4-(*tert*-Butyldimethylsilyloxy)-1*H*-indol-2-yl)(4-phenoxy phenyl)methanone (15)

Compound **15** was prepared from **12** using the procedure for **13** with 75% as a yellow solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.21 (6H, s), 0.98 (9H, s), 6.50 (1H, m), 6.96 (1H, s), 7.09–7.29 (7H, m), 7.46–7.51 (2H, m), 7.95 (2H, m), 11.99 (1H, br s); MS (ESI) *m*/*z* 444 (M+1).

5.1.10. *tert*-Butyl 5-hydroxy-2-(4-phenoxybenzoyl)-1*H*-indole-1-carboxylate (16)

To a solution of **13** (6.40 g, 14.4 mmol) in CH_2Cl_2 (72 ml) was added Boc_2O (3.46 g, 15.8 mmol) and DMAP (2.10 g, 17.3 mmol). The mixture was stirred at room temperature for 1 h and diluted with $CHCl_3$, then washed with water and brine. The organic fraction was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was dissolved in THF (72 ml) and TBAF in THF (1.0 M, 14.4 ml, 14.4 mmol) was added. The mixture was stirred at room temperature for 1 h and diluted with EtOAc, and then 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) to provide compound **16** (6.02 g, 97%) as a white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (9H, s), 4.90 (1H, s), 6.77 (1H, s), 6.94–7.10 (6H, m), 7.18–7.44 (3H, m), 7.87–7.92 (2H, m), 8.05 (1H, m); MS (ESI) *m*/*z* 430 (M+1).

5.1.11. *tert*-Butyl 4-hydroxy-2-(4-phenoxybenzoyl)-1*H*-indole-1-carboxylate (17)

Compound **17** was prepared from **15** using the procedure for **16** with 56% as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.34 (9H, s), 6.67 (1H, m), 7.09–7.29 (7H, m), 7.44–7.49 (3H, m), 7.88 (2H, m), 10.13 (1H, br s); MS (ESI) *m*/*z* 430 (M+1).

5.1.12. *tert*-Butyl 4-chloro-5-hydroxy-2-(4-phenoxybenzoyl)-1*H*-indole-1-carboxylate (18)

To an ice cooled solution of **16** (70.0 mg, 0.163 mmol) in THF (1.2 ml) and *i*-PrOH (0.3 ml) was added *N*-chlorosuccinimide (17.4 mg, 0.130 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 **18** (25.0 mg, 33%) as a white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.40 (9H, s), 5.50 (1H, s), 6.88 (1H, s), 7.01–7.26 (6H, m), 7.39–7.45 (2H, m), 7.88–7.93 (2H, m), 8.02 (1H, m); MS (ESI) *m*/*z* 464 (M+1).

5.1.13. *tert*-Butyl 4,6-dichloro-5-hydroxy-2-(4-phenoxy benzoyl)-1*H*-indole-1-carboxylate (19)

To a solution of **16** (100 mg, 0.233 mmol) in THF (1.6 ml) and *i*-PrOH (0.4 ml) was added *N*-chlorosuccinimide (78.0 mg, 0.583 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 **19** (23.0 mg, 23%) as a white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (9H, s), 5.82 (1H, s), 6.87 (1H, s), 7.01–7.11 (4H, m), 7.20–7.26 (1H, m), 7.38–7.45 (2H, m), 7.88–7.92 (2H, m), 8.21 (1H, s); MS (ESI) *m*/*z* 498 (M+1).

5.1.14. *tert*-Butyl 4-bromo-5-hydroxy-2-(4-phenoxybenzoyl)-1*H*-indole-1-carboxylate (20) and *tert*-Butyl 4,6-dibromo-5-hydroxy-2-(4-phenoxybenzoyl)-1*H*-indole-1-carboxylate (21)

To an ice cooled solution of **16** (4.30 g, 10.0 mmol) in THF (50 ml) was added *N*-bromosuccinimide (1.82 g, 10.2 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 **20** (2.37 g, 43%) as a white solid and compound **21** (1.72 g, 29%) as a white solid.

Compound **20**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (9H, s), 5.44 (1H, s), 6.83 (1H, s), 7.01–7.26 (6H, m), 7.42–7.46 (2H, m), 7.88–7.93 (2H, m), 8.04 (1H, m); MS (ESI) *m*/*z* 510 (M+1).

Compound **21**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.38 (9H, s), 5.83 (1H, s), 6.82 (1H, s), 7.01–7.11 (4H, m), 7.20–7.45 (3H, m), 7.86–7.91 (2H, m), 8.41 (1H, m); MS (ESI) *m*/*z* 587 (M+1).

5.1.15. (4-Chloro-5-hydroxy-1*H*-indol-2-yl)(4-phenoxy phenyl)methanone (22)

To a solution of **18** (17.0 mg, 0.037 mmol) in $CHCl_3$ (1.0 ml) was added trifluoroacetic acid (1.0 ml). 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 **22** (10.0 mg, 75%) as a white solid.

Mp = 173–174 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.39 (1H, s), 7.15–7.18 (6H, m), 7.20–7.33 (2H, m), 7.40–7.46 (2H, m), 8.02 (2H, m), 9.33 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 109.1, 111.9, 117.0, 117.4, 120.3, 124.7, 126.7, 130.1, 131.5, 131.9, 132.6, 135.2, 146.1, 155.4, 161.9, 185.4; IR (ATR) 3282, 1624 cm^{-1}; MS (ESI) *m*/*z* 364 (M+1); Anal. Calcd for $C_{21}H_{14}ClNO_30{\cdot}3H_2O$: C, 68.32; H, 3.99; N, 3.79. Found: C, 68.66; H, 4.38; N, 3.77.

5.1.16. (4,6-Dichloro-5-hydroxy-1*H*-indol-2-yl)(4-phenoxy phenyl)methanone (23)

Compound **23** was prepared from **19** using the procedure for **22** with 74% as a white solid.

Mp = 206–207 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.69 (1H, s), 7.08–7.15 (5H, m), 7.20–7.26 (1H, m), 7.40–7.46 (3H, m), 8.01 (2H, m), 9.30 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 108.2, 112.1, 117.3, 120.1, 123.1, 124.8, 125.5, 130.4, 131.6, 131.8, 132.0, 135.5, 143.0, 155.0, 161.0, 184.6; IR (ATR) 3334, 1618 cm^{-1}; MS (ESI) *m*/*z* 398 (M+1); Anal. Calcd for $C_{21}H_{13}Cl_2NO_3$: C, 63.34; H, 3.29; N, 3.52. Found: C, 63.02; H, 3.36; N, 3.60.

5.1.17. (4-Bromo-5-hydroxy-1*H*-indol-2-yl)(4-phenoxy phenyl)methanone (24)

Compound **24** was prepared from **20** using the procedure for **22** with 67% as a yellow solid.

Mp = 192–193 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.38 (1H, s), 7.04 (1H, m), 7.10–7.15 (5H, m), 7.20–7.26 (1H, m), 7.33–7.46 (3H, m), 8.02 (2H, m), 9.36 (1H, br s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 99.1, 109.7, 112.8, 117.3, 117.5, 120.1, 124.8, 128.4, 130.4, 131.5, 132.3, 132.5, 134.8, 148.2, 155.1, 160.9, 184.8; IR (ATR) 3280, 1612 cm^{-1}; MS (ESI) *m*/*z* 408 (M+1); Anal. Calcd for $C_{21}H_{14}BrNO_3$: C, 61.78; H, 3.46; N, 3.43. Found: C, 61.72; H, 3.55; N, 3.54.

5.1.18. (4,6-Dibromo-5-hydroxy-1*H*-indol-2-yl)(4-phenoxy phenyl)methanone (25)

Compound **25** was prepared from **21** using the procedure for **22** with 98% as a yellow solid.

Mp = 205–206 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.85 (1H, s), 7.12–7.28 (5H, m), 7.47 (2H, m), 7.66 (1H, s), 7.98 (2H, m), 9.47 (1H, s), 12.20 (1H, br s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 103.5, 109.9, 113.0, 115.7, 117.3, 120.2, 124.8, 128.0, 130.4, 131.6, 132.0, 132.7, 135.4, 144.5, 155.0, 161.0, 184.8; IR (ATR) 3317, 1610 cm^{-1}; MS (ESI) *m*/*z* 488 (M+1); Anal. Calcd for $C_{21}H_{13}Br_2NO_3$: C, 51.78; H, 2.69; N, 2.88. Found: C, 52.07; H, 2.79; N, 2.99.

5.1.19. *tert*-Butyl 5-(2-ethoxy-2-oxoethoxy)-2-(4-phenoxy benzoyl)-1*H*-indole-1-carboxylate (26)

A mixture of **16** (50.0 mg, 0.116 mmol), K_2CO_3 (32.0 mg, 0.232 mmol) and ethyl bromoacetate (39.0 mg, 0.232 mmol) in DMF (1.0 ml) was stirred at 40 °C for 2 h. The mixture was cooled and diluted with EtOAc, then 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) to provide compound **26** (43.0 mg, 72%) as a colorless oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (3H, t, *J* = 7.2 Hz), 1.39 (9H, s), 4.28 (2H, q, *J* = 7.2 Hz), 4.68 (2H, s), 6.82 (1H, s), 6.99–7.24 (7H, m), 7.37–7.44 (2H, m), 7.87–7.91 (2H, m), 8.12 (1H, m); MS (ESI) *m*/*z* 516 (M+1).

5.1.20. *tert*-Butyl 5-(1-ethoxy-2-methyl-1-oxopropan-2-yloxy)-2-(4-phenoxybenzoyl)-1*H*-indole-1-carboxylate (27)

Compound **27** was prepared from **16** and ethyl 2-bromoisobutyrate using the procedure for **26** with 79% as a colorless oil.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.18 (3H, t, *J* = 7.2 Hz), 1.32 (9H, s), 1.52 (6H, s), 4.17 (2H, q, *J* = 7.2 Hz), 7.01–7.27 (8H, m), 7.46 (2H, m), 7.86–7.96 (3H, m); MS (ESI) *m*/*z* 544 (M+1).

5.1.21. *tert*-Butyl 5-(2-chloroethoxy)-2-(4-phenoxybenzoyl)-1*H*-indole-1-carboxylate (28)

A mixture of **16** (50.0 mg, 0.116 mmol), K_2CO_3 (32.0 mg, 0.580 mmol) and 1-bromo-2-chloroethane (49.9 mg, 0.348 mmol) in DMF (0.58 ml) was stirred at 100 °C for 2 h. The mixture was cooled and diluted with EtOAc, then 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) to provide compound **28** (27.0 mg, 47%) as a colorless oil.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.32 (9H, s), 3.97 (2H, m), 4.37 (2H, m), 7.03–7.28 (8H, m), 7.47 (2H, m), 7.87 (2H, m), 8.02 (1H, m); MS (ESI) *m*/*z* 492 (M+1).

5.1.22. *tert*-Butyl 4-(2-chloroethoxy)-2-(4-phenoxybenzoyl)-1*H*-indole-1-carboxylate (29)

Compound **29** was prepared from **17** using the procedure for **28** with 83% as a colorless oil.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.35 (9H, s), 3.99 (2H, m), 4.41 (2H, m), 6.90 (1H, m), 7.00 (1H, m), 7.08–7.16 (4H, m), 7.28 (1H, m), 7.41–7.47 (3H, m), 7.66 (1H, m), 7.88 (2H, m); MS (ESI) *m*/*z* 492 (M+1).

5.1.23. *tert*-Butyl 4-(3-chloropropoxy)-2-(4-phenoxybenzoyl)-1*H*-indole-1-carboxylate (30)

Compound **30** was prepared from **17** and 1-bromo-3-chloropropane using the procedure for **28** with 69% as a colorless oil.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.34 (9H, s), 2.17–2.26 (2H, m), 3.85 (2H, m), 4.23 (2H, m), 6.87 (1H, m), 7.08–7.15 (5H, m), 7.25 (1H, m), 7.37–7.50 (3H, m), 7.63 (1H, m), 7.88 (2H, m); MS (ESI) *m*/*z* 506 (M+1).

5.1.24. Ethyl 2-(2-(4-phenoxybenzoyl)-1*H*-indol-5-yloxy)acetate (31)

To a solution of **26** (40.0 mg, 0.078 mmol) in $CHCl_3$ (0.5 ml) was added trifluoroacetic acid (1.0 ml). 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 **31** (28.0 mg, 88%) as a yellow solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.20 (3H, t, *J* = 7.2 Hz), 4.16 (2H, q, *J* = 7.2 Hz), 4.74 (2H, s), 6.99–7.28 (8H, m), 7.39–7.50 (3H, m), 7.98 (2H, m), 11.85 (1H, s); MS (ESI) *m*/*z* 416 (M+1).

5.1.25. 2-(2-(4-Phenoxybenzoyl)-1*H*-indol-5-yloxy)acetic acid (32)

To a solution of **31** (28.0 mg, 0.0674 mmol) in THF (0.5 ml) and MeOH (0.5 ml) was added 2 N NaOH solution (0.078 ml, 0.156 mmol). The mixture was stirred at room temperature for 2 h and the solvent was evaporated under reduced pressure. The residue was dissolved in water (1.0 ml) and neutralized with 2 N HCl solution. The resulting solid was filtered and dried to afford compound **32** (20.0 mg, 67%) as a yellow solid.

Mp = 166–167 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 4.62 (2H, s), 6.98–7.18 (7H, m), 7.23–7.28 (1H, m), 7.39 (1H, d, *J* = 8.8 Hz), 7.45–7.50 (3H, m), 7.97 (2H, m), 11.83 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 65.1, 103.6, 111.2, 113.7, 117.2, 117.6, 120.1, 124.8, 127.2, 130.4, 131.5, 132.6, 133.6, 134.7, 152.5, 155.1, 161.7, 170.5, 184.9; IR (ATR) 3288, 1747, 1622 cm^{-1}; MS (ESI) *m*/*z* 388 (M+1); Anal. Calcd for $C_{23}H_{17}NO_5 0.25H_2O$: C, 70.49; H, 4.50; N, 3.57. Found: C, 70.42; H, 4.43; N, 3.63.

5.1.26. Ethyl 2-methyl-2-(2-(4-phenoxybenzoyl)-1*H*-indol-5-yloxy)propanoate (33)

Compound **33** was prepared from **27** using the procedure for **31** with 95% as a white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.18 (3H, t, *J* = 7.2 Hz), 1.48 (6H, s), 4.16 (2H, q, *J* = 7.2 Hz), 4.74 (2H, s), 6.92 (1H, m), 7.08–7.50 (10H, m), 7.97 (2H, m), 11.87 (1H, s); MS (ESI) *m*/*z* 444 (M+1).

5.1.27. 2-Methyl-2-(2-(4-phenoxybenzoyl)-1*H*-indol-5-yloxy)propanoic acid (34)

Compound **34** was prepared from **33** using the procedure for **32** with 48% as a light brown amorphous.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.47 (6H, s), 6.94–7.50 (11H, m), 7.97 (2H, m), 11.85 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 25.1, 79.0, 111.0, 111.3, 113.2, 117.2, 120.0, 121.2, 124.7, 127.1, 130.4, 131.5, 132.5, 134.2, 134.9, 149.2, 155.1, 160.7, 175.2, 184.9; IR (ATR) 3340, 1718, 1618 cm^{-1}; MS (ESI) *m*/*z* 416 (M+1); Anal. Calcd for $C_{25}H_{21}NO_5 0.75H_2O$: C, 70.00; H, 5.29; N, 3.27. Found: C, 69.80; H, 5.02; N, 3.33.

5.1.28. (5-(2-(Ethylamino)ethoxy)-1*H*-indol-2-yl)(4-phenoxy phenyl)methanone (35)

A mixture of **28** (23.0 mg, 0.0468 mmol), $EtNH_2$ in water (12 M, 0.039 ml, 0.468 mmol) and NaI (7.01 mg, 0.0468 mmol) in DMF

(0.23 ml) was stirred at 100 °C for 6 h. The mixture was cooled and diluted with EtOAc, then 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 (MeOH/$CHCl_3$) to provide compound **35** (4.89 mg, 26%) as a light brown amorphous.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.02 (3H, t, J = 7.2 Hz), 2.58 (2H, q, J = 7.2), 2.86 (2H, t, J = 5.7 Hz), 3.99 (2H, t, J = 5.7 Hz), 6.96–7.27 (8H, m), 7.36–7.50 (3H, m), 7.96 (2H, m), 11.81 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 15.2, 43.5, 48.2, 67.8, 103.4, 111.1, 113.6, 117.2, 117.8, 120.0, 124.7, 127.3, 130.4, 131.5, 132.6, 133.4, 134.6, 153.3, 155.2, 160.6, 184.9; IR (ATR) 3288, 1620 cm^{-1}; MS (ESI) m/z 401 (M+1); Anal. Calcd for $C_{25}H_{24}N_2O_3$: C, 70.98; H, 6.04; N, 7.00. Found: C, 74.71; H, 6.20; N, 6.98.

5.1.29. (4-(2-(Ethylamino)ethoxy)-1*H*-indol-2-yl)(4-phenoxyphenyl)methanone (36)

Compound **36** was prepared from **29** using the procedure for **35** with 37% as a yellow solid.

Mp = 143–144 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.01 (3H, t, J = 7.2 Hz), 2.59 (2H, q, J = 7.2), 2.93 (2H, t, J = 5.7 Hz), 4.13 (2H, t, J = 5.7 Hz), 6.55 (1H, m), 7.05–7.28 (8H, m), 7.48 (2H, m), 7.97 (2H, m), 11.97 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 15.2, 43.5, 48.0, 67.6, 100.3, 105.4, 109.0, 117.3, 118.6, 120.0, 124.7, 126.9, 130.4, 131.4, 132.6, 133.2, 139.3, 153.7, 155.1, 160.6, 184.7; IR (ATR) 1606 cm^{-1}; MS (ESI) m/z 401 (M+1); Anal. Calcd for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 75.00; H, 6.09; N, 7.10.

5.1.30. (4-Phenoxyphenyl)(4-(2-(piperidin-1-yl)ethoxy)-1*H*-indol-2-yl)methanone (37)

Compound **37** was prepared from **29** and piperidine using the procedure for **35** with 80% as a yellow amorphous.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.30–1.51 (6H, m), 2.44–2.49 (4H, m), 2.71 (2H, m), 4.17 (2H, m), 6.56 (1H, m), 6.99–7.27 (8H, m), 7.47 (2H, m), 7.95 (2H, m), 11.97 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 23.9, 25.6, 54.4, 57.3, 65.9, 100.6, 105.5, 108.8, 117.2, 118.6, 120.0, 124.7, 126.9, 130.3, 131.3, 132.6, 133.2, 139.3, 153.6, 155.1, 160.6, 184.8; IR (ATR) 3300, 1614 cm^{-1}; MS (ESI) m/z 441 (M+1); Anal. Calcd for $C_{28}H_{28}N_2O_3 0{\cdot}4H_2O$: C, 75.11; H, 6.48; N, 6.26. Found: C, 75.21; H, 6.41; N, 6.31.

5.1.31. (4-Phenoxyphenyl)(4-(3-(piperidin-1-yl)propoxy)-1*H*-indol-2-yl)methanone (38)

Compound **38** was prepared from **30** and piperidine using the procedure for **35** with 64% as a yellow solid.

Mp = 131–132 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.30–1.49 (6H, m), 1.88 (2H, m), 2.28–2.43 (6H, m), 4.10 (2H, t, J = 6.4 Hz), 6.53 (1H, m), 7.00–7.29 (8H, m), 7.47 (2H, m), 7.95 (2H, m), 11.96 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 24.1, 25.6, 26.3, 54.1, 55.2, 66.0, 100.4, 105.4, 108.7, 117.2, 118.6, 120.0, 124.7, 126.9, 130.3, 131.4, 132.6, 133.2, 139.3, 153.7, 155.1, 160.6, 184.7; IR (ATR) 3286, 1604 cm^{-1}; MS (ESI) m/z 455 (M+1); Anal. Calcd for $C_{29}H_{30}N_2O_3$: C, 76.63; H, 6.65; N, 6.16. Found: C, 76.53; H, 6.71; N, 6.21.

5.1.32. (4-Phenoxyphenyl)(4-(2-(piperazin-1-yl)ethoxy)-1*H*-indol-2-yl)methanone (39)

Compound **39** was prepared from **29** and piperadine using the procedure for **35** with 69% as a yellow amorphous.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.41–2.54 (4H, m), 2.69–2.76 (6H, m), 4.19 (2H, t, J = 6.4 Hz), 6.56 (1H, m), 7.00–7.29 (8H, m), 7.47 (2H, m), 7.95 (2H, m), 11.96 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 45.6, 54.6, 57.3, 65.7, 100.6, 105.5, 108.7, 117.3, 118.6, 120.0, 124.7, 126.9, 130.4, 131.3, 132.6, 133.2, 139.3, 153.5, 155.1, 160.6, 184.7; IR (ATR) 3317, 1608 cm^{-1}; MS (ESI) m/z 442 (M+1); Anal. Calcd for $C_{27}H_{27}N_3O_3 1{\cdot}5H_2O$: C, 69.21; H, 6.45; N, 8.97. Found: C, 69.20; H, 6.28; N, 9.13.

5.1.33. (4-(2-(4-Hydroxypiperidin-1-yl)ethoxy)-1*H*-indol-2-yl)(4-phenoxyphenyl)methanone (40)

Compound **40** was prepared from **29** and 4-hydroxypiperidine using the procedure for **35** with 89% as a yellow solid.

Mp = 174–175 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.34–1.38 (2H, m), 1.61–1.73 (2H, m), 2.11–2.17 (2H, m), 2.71–2.81 (4H, m), 3.41 (1H, m), 4.15–4.19 (2H, m), 4.52 (1H, m), 6.56 (1H, m), 6.99–7.50 (8H, m), 7.45–7.50 (2H, m), 7.94–7.97 (2H, m), 11.96 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 34.5, 51.4, 56.5, 66.0, 66.2, 100.6, 105.5, 108.7, 117.3, 118.6, 120.0, 124.7, 126.9, 130.4, 131.4, 132.6, 133.2, 139.3, 153.5, 155.1, 160.6, 184.7; IR (ATR) 1616 cm^{-1}; MS (ESI) m/z 457 (M+1); Anal. Calcd for $C_{28}H_{28}N_2O_4{\cdot}2H_2O$: C, 73.09; H, 6.22; N, 6.09. Found: C, 73.25; H, 6.20; N, 6.20.

5.1.34. (4-(2-Aminoethoxy)-1*H*-indol-2-yl)(4-phenoxyphenyl)methanone (41)

A mixture of **16** (100 mg, 0.233 mmol), K_2CO_3 (64.0 mg, 0.932 mmol) and *N*-(2-bromoethyl)phthalimide (237 mg, 0.932 mmol) in DMF (1.2 ml) was stirred at 100 °C for 3 h. The mixture was cooled and diluted with EtOAc, then washed with water and brine. The organic fraction was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was dissolved in MeOH (1.0 ml), and 40% $MeNH_2$ solution (1.0 ml) was added. The mixture was stirred at 50 °C for 1 h and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (MeOH/$CHCl_3$) to provide compound **41** (43.0 mg, 52%) as a brown oil.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.46 (2H, m), 2.88 (2H, m), 3.98 (2H, m), 6.46 (1H, m), 6.99–7.23 (8H, m), 7.43 (2H, m), 7.93 (2H, m); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 41.0, 70.2, 100.2, 105.4, 109.0, 117.3, 118.6, 120.1, 124.7, 126.9, 130.4, 131.4, 132.6, 133.2, 139.3, 153.8, 155.1, 160.6, 184.7; IR (ATR) 3300, 1616 cm^{-1}; MS (ESI) m/z 373 (M+1); Anal. Calcd for $C_{23}H_{20}N_2O_3 0.75H_2O$: C, 71.58; H, 5.62; N, 7.26. Found: C, 71.65; H, 5.56; N, 7.10.

5.2. Biology

5.2.1. CaMKII activity assay

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).

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 1 μM with assay buffer containing CaM (0.5 μM) and ATP (final concentration 0.1 μM and 350–1500 cpm/pmol γ-^{33}P ATP) were added to the diluted test substances and the whole was incubated for 90 min at 24 °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 one mg CaMKII.

5.2.2. P38α activity assay

P38α/SAPK2a active (p38) was purchased from Millipore. Myelin basic protein (MBP) was purchased from Alexis biochemicals. γ-^{33}P ATP was obtained from GE Healthcare. ATP was purchased from Sigma–Aldrich.

The test substances were added to the assay buffer containing 0.5 μg/mL p38, 25 mM Tris–HCl, pH 7.5, 2 mM dithiothreitol, 10 mM $MgCl_2$, 0.1% CHAPS. The substrate MBP was diluted to a final concentration of 50 μg/mL with assay buffer containing ATP (final concentration 0.1 μM and 350–1500 cpm/pmol γ-^{33}P ATP) was added to the diluted test substances and the whole was incubated for 90 min at room temperature. After incubation, the reaction was terminated by spotting the mixture of reaction solution onto the appropriate area of a P30 Filtermat (PerkinElmer, USA), and the labeled substrate was captured by a negatively charged filtermat. 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).

5.2.3. MLCK activity assay

MLCK active (MLCK), ZIPtide and calmodulin (CaM) were purchased from Millipore. γ-^{33}P ATP was obtained from GE Healthcare. ATP was purchased from Sigma–Aldrich.

The test substances were added to the assay buffer containing 0.5 μg/mL MLCK, 20 mM Tris–HCl, pH 7.5, 2 mM dithiothreitol, 10 mM MgAc, 0.1% CHAPS and 500 μM $CaCl_2$. The substrate ZIPtide was diluted to a final concentration of 75 μM with assay buffer containing ATP (final concentration 0.1 μM and 350–1500 cpm/pmol γ-^{33}P ATP) and 0.125 μg/mL CaM. Then prepared test substances were added to the substrate solution and the whole was incubated for 90 min at room temperature. After incubation, the reaction was terminated by spotting the mixture of reaction solution onto the appropriate area of a P30 Filtermat (PerkinElmer, USA), and the labeled substrate was captured by a negatively charged filtermat. 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).

5.2.4. Akt1 activity assay

Akt1 and Crosstide were purchased from Millipore. γ-^{33}P ATP was obtained from GE Healthcare. ATP was purchased from Sigma–Aldrich.

The test substances and Akt1 were added to the assay buffer containing 25 mM Tris–HCl, pH 7.5, 2 mM dithiothreitol, 15 mM $MgCl_2$, 0.1% CHAPS. The substrate Crosstide was diluted to a final concentration of 2.5 μM with assay buffer containing ATP (final concentration 0.1 μM and 350–1500 cpm/pmol γ-^{33}P ATP) was added to the diluted test substances and the whole was incubated for 90 min at room temperature. After incubation, the reaction was terminated by transferring 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. 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).

5.2.5. PKCγ activity assay

PKCγ active (PKCγ) was purchased from Millipore. Histone typeIII-SS from calf thymus (Histone) and ATP were purchased from Sigma–Aldrich. γ-^{33}P ATP was obtained from GE Healthcare. Polylysine YSi SPA Scintillation Beads (SPA beads) were purchased from Amersham Biosciences.

The test substances were added to the assay buffer containing 0.25 μg/mL PKCγ, 20 mM Tris–HCl, pH 7.5, 2.5 mM dithiothreitol, 7.5 mM $MgCl_2$, 750 μM $CaCl_2$. The substrate Histone was diluted to a final concentration of 25 μg/mL with assay buffer containing ATP (final concentration 0.1 μM and 350–1500 cpm/pmol γ-^{33}P ATP). Then prepared test substances were added to the substrate solution and the whole was incubated for 90 min at room temperature. After incubation, SPA beads were added to assay plate and stay still standing for over 30 min. at room temperature. The radioactivity was counted in 1450 MicroBeta TRILUX (PerkinElmer, USA).

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Reference and notes

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