Supplemental Data

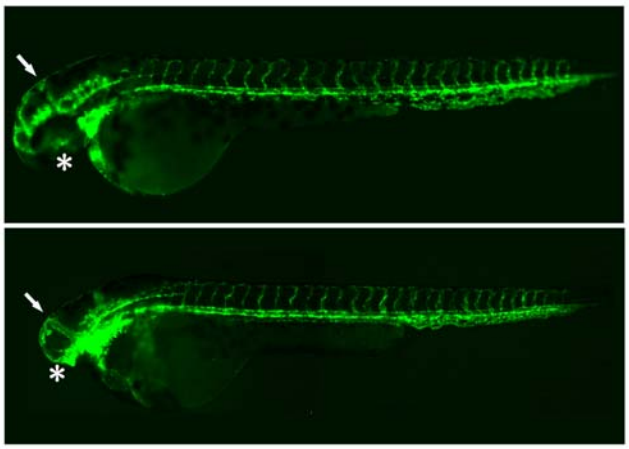


Fig S1. 3F8 does not cause abnormity outside forebrain. 2 dpf control (upper) and treated (lower) embryos are shown here. White arrows point to the metencephalic artery and medial cerebral vein. White stars (*) indicate the eye of control embryo or the forebrain residue of treated embryo. Similar observations have been obtained for liver, blood cells and pancreas (data not shown).

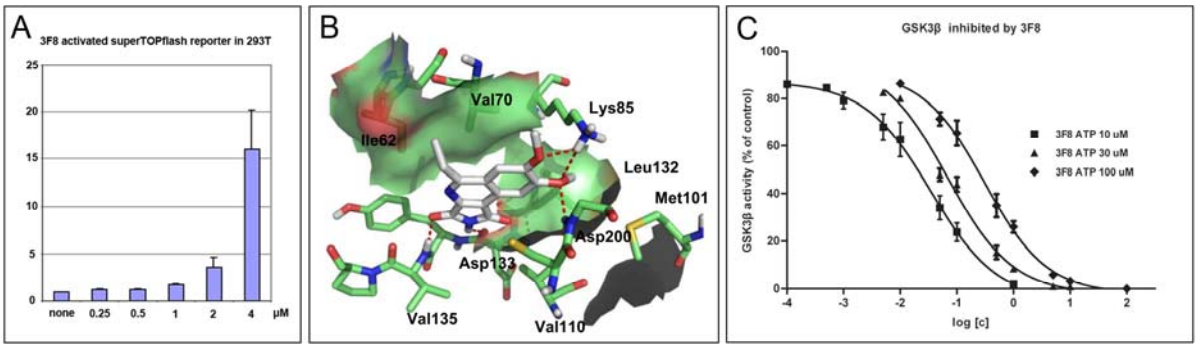



Fig S2. 3F8 activates Wnt pathway through inhibiting GSK3. A. *In vitro* β-catenin/TCF activity assay. Y-axis, folds of firefly luciferase activity induction. X-axis, 3F8 concentration (μM). Bars indicate standard deviation. B. The docking model of 3F8 in the ATP binding site of human GSK3β. The critical hydrogen bonds of 3F8 with the hinge region and Lysine85 of GSK3 are indicated by the red dashed lines. In this model the maleimide motif of 3F8 forms a pair of hydrogen bonds with the hinge region (Asp133 and Val135) and the two methoxy oxygens form another two hydrogen bonds with the positively charged Lys85. One of the methoxy group docks into the back-pocket formed by the gatekeeper residue Leu132, and the residues Met101 and Val110. In addition, the ethyl substituent of 3F8 docks to the minor hydrophobic pocket in the front of the ATP site formed by Ile62 and Val70, which enhances its affinity and selectivity against GSK3. C. GSK3 activity is inhibited by 3F8 in an ATP competitive manner. Human TAU-441 was used as substrate to measure GSK3 activity in the presence of indicated concentrations of ATP and 3F8. GSK3 activities are shown as the percentage of control reaction without 3F8. Data are mean +/- S.E.M. from two independent reactions. In the presence of 10 μM ATP, the IC50 of 3F8 is 34 nM; in the presence of 30 μM ATP, the IC50 of 3F8 is 67 nM; in the presence of 100 μM ATP, the IC50 of 3F8 is 304 nM.

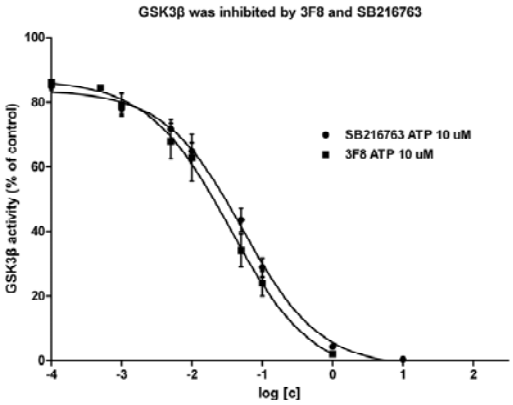



Fig S3. GSK3 activity was inhibited by both 3F8 and SB216763. In the presence of 10 μM ATP, the IC50 of SB216763 is 52 nM, while the IC50 of 3F8 is 34 nM.

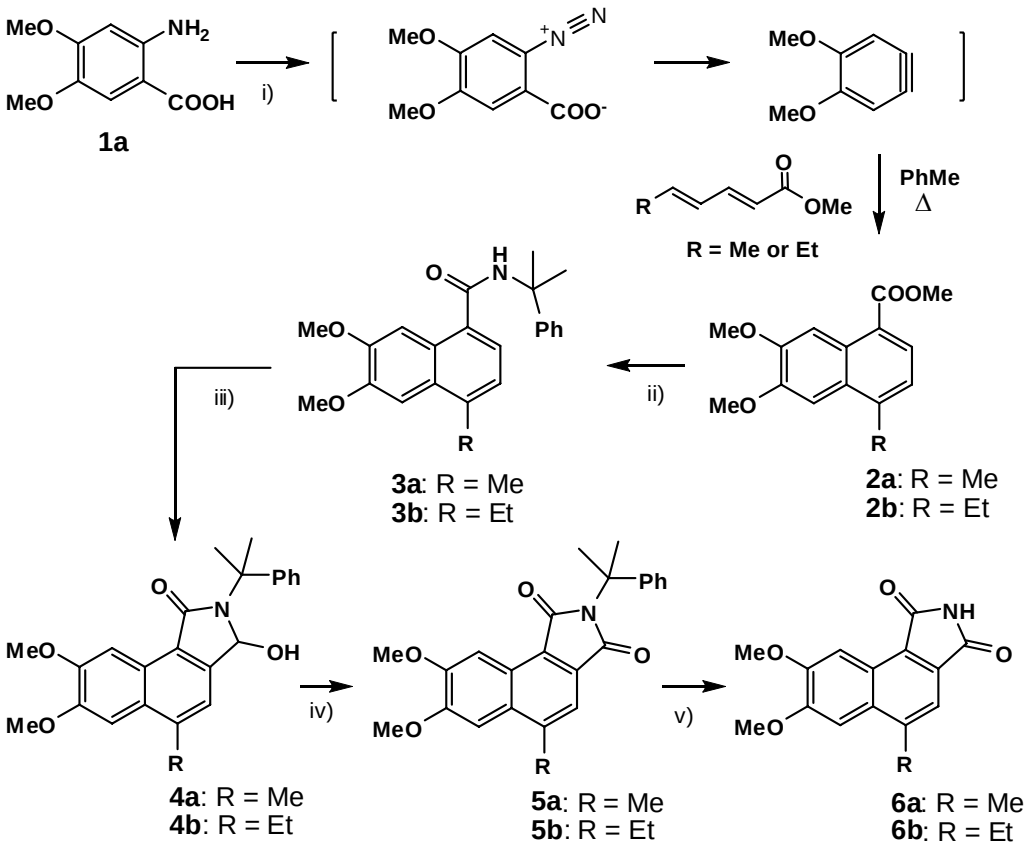



Fig S4. The synthetic route for compound **6a-6b**.Reagents and conditions: (i) (a) isoamyl nitrite, $Cl_3CCOOH$, THF, 0°C to room temp; (b) diene, toluene, 130°C; (c) DDQ, room temp, total yield for **2a**: 11%, **2b**: 10%; (ii) (a) LiOH, MeOH/$H_2O$ = 3:1, 40 °C; (b) $SOCl_2$, reflux; (c) cumyl amine, DCM, 0 °C to room temp, total yield for **3a**: 73%, **3b**: 78%; (iii) *t*-BuLi, TMEDA, THF, -78 °C, then DMF, -78 °C to room temp, **4a**: 88% brsm, **4b**: 91% brsm; (iv) PDC, DMF, room temp, **5a**: 85%, **5b**: 88%; (v) TFA, 50 °C, **6a**: 90%, **6b**: 92%.

Table S1. Comparison of 3F8 activity with four known GSK3 inhibitors

| **GSK3 inhibitors** | **$C_E$ (Effective concentration to cause no-eyes phenotype)** | **IC50** | **$C_E$ /IC50** |
|---|---|---|---|
| 3F8 | 7.5 μM | 34 nM in our assay | 221 |
| SB216763 | 20 μM | 52 nM in our assay, 34 nM on Sigma datasheet | 385,588 |
| GSK-3b Inhibitor IX | 10 μM | 5 nM on EMD datasheet | 2000 |
| GSK-3b Inhibitor XII, TWS119 | At 40 μM, embryos were still normal. | 30 nM on EMD datasheet | >1333 |
| GSK-3 Inhibitor XV | 1 μM | 0.6 nM on EMD datasheet | 1667 |

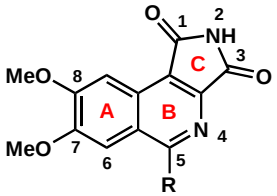
Table S2. Kinase profile of 3F8 (5 μM)

| **Kinase name** | **Inhibition (%)** |
|---|---|
| | **3F8** |
| ABL1 | 7 |
| CAMK1D (CaMKI delta) | -3 |
| CAMK2B (CaMKII beta) | 1 |
| CDK2/cyclin A | 71 |
| CHEK2 (CHK2) | -1 |
| CLK1 | 10 |
| CSNK1D (CK1 delta) | 9 |
| FLT3 | 23 |
| GSK3B (GSK3 beta) | 91 |
| KDR (VEGFR2) | 53 |
| LCK | 15 |
| MAP2K1 (MEK1) | 12 |
| MAP3K9 (MLK1) | 25 |
| MAPK1 (ERK2) | 7 |
| MAPK8 (JNK1) | 10 |
| MAPKAPK2 | 2 |
| MYLK2 (skMLCK) | 7 |
| NEK2 | 1 |
| PLK1 | 4 |
| RPS6KA1 (RSK1) | 41 |
| SGK (SGK1) | 2 |
| STK3 (MST2) | 38 |

Table S3. Data of dose-vs-timing effect of 3F8 treatment

| **Stage** | **Time (hpf)** | **Concentration (μM)** | **Number of no-eyes embryos** | **Number of total embryos** |
|---|---|---|---|---|
| Born | 0 | 2.5 | 37 | 39 |
| 2-cell | 0.75 | 3.75 | 57 | 57 |
| 1k-cell | 3 | 5 | 38 | 38 |
| High | 3.3 | 3.75 | 31 | 33 |
| Dome | 4.3 | 7.5 | 30 | 30 |
| Germ ring | 5.7 | 7.5 | 41 | 44 |
| Shield | 6 | 11.25 | 51 | 72 |
| Tail bud | 10 | 60 | 1 | 12 |
| 2s | 10.7 | 75 | 0 | 17 |

**Chemical Synthesis Procedures**




Synthesis of methyl 6,7-dimethoxy-4-methyl-1-naphthoate **2a**: To a solution of 2-amino-4,5-dimethoxybenzoic acid (**1a**) (4.9 g, 25 mmol) and trichloroacetic acid (37.5 mg, 0.23 mmol) in THF (38 mL) was added at isoamyl nitrite (6.4 mL, 47.5 mmol) during 5 min. at 0 °C (cooled in an ice-water bath), and the mixture was allowed to warm to room temperature over a period of 1 hour, and the formed solution was stirred for an additional 1.5 h. The mixture was then cooled back to 0 °C, and the formed product was collected by filtration and washed with cold THF until the filtrate was colorless (caution: the filter cake should not be allowed to become dry). The solvent-wet benzene-diazonium-2-carboxylate was dispersed in toluene (50 mL) in a dried tube, to this solution was added methyl hexa-2,4-dienoate (30 mmol), and the reaction mixture was warmed up to 130 °C and stirred for 48 h. After cooling to room temperature, the reaction mixture was treated with DDQ (6.8 g, 30 mmol), and the resulted mixture was stirred at the room temperature for 6 h. The reaction was worked up by filtration of the mixture and the solid was washed with ethyl acetate (3 x 20 mL). The filtrate was washed with water (3 x 20 mL), brine (1 x 20 mL), and dried over anhydrous $Na_2SO_4$. The solvent was removed under vacuum, and the residue was purified by a flash chromatography on silica gel (hexane/ethyl acetate = 5/1) to give naphthoate **2a** (715 mg) in 11% yield for 3 steps. $^1$H NMR (500 MHz, $CDCl_3$): δ 8.59 (s, 1H), 8.02 (d, $J$ = 7.5 Hz, 1H), 7.20-7.18 (m, 2H), 4.05 (s, 3H), 4.01 (s, 3H), 3.96 (s, 3H), 2.66 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 168.2, 150.5, 149.1, 138.7, 128.9, 128.7, 128.1, 124.1, 123.0, 105.5, 103.1, 55.8, 55.6, 51.7, 20.2; HRMS (ESI): calcd for $C_{15}H_{16}NaO_4$ ($M + Na^+$) 283.0946; found 283.0941.

Synthesis of methyl 4-ethyl-6,7-dimethoxy-1-naphthoate **2b**: **2b** was made under the identical conditions as the preparation of **2a** by replacing the methyl hexa-2,4-dienoate with methyl

hepta-2,4-dienoate to do the Diels-Alder reaction, and the formed crude product was purified by a flash chromatography on silica gel (hexane/ethyl acetate = 5/1) to give **2b** (685 mg) in 10% yield for 3 steps. $^1$H NMR (500 MHz, $CDCl_3$): δ 8.58 (s, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.24 (s, 1H), 7.16 (d, *J* = 7.6 Hz, 1H), 4.03 (s, 3H), 3.98 (s, 3H), 3.93 (s, 3H), 3.01 (q, *J* = 7.5 Hz, 2H), 1.35 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 168.1, 150.3, 149.0, 144.4, 128.7, 128.3, 128.0, 122.8, 122.0, 105.5, 102.6, 55.6, 55.5, 51.6, 26.4, 14.1; HRMS (ESI): calcd for $C_{16}H_{18}NaO_4$ ($M + Na^+$) 297.1103; found 297.1097.

Syntheses of 4-methyl-6,7-dimethoxy-N-(2-phenylpropan-2-yl)-1-naphthamide (**3a**) and 4-ethyl-6,7-dimethoxy-N-(2-phenylpropan-2-yl)-1-naphthamide (**3b**): To a solution of **2a** or **2b** (1 mmol) in a mix-solvent of MeOH/$H_2O$ (3/1, 15 mL/5 mL) was added lithium hydroxide monohydrate (1.3 g, 30 mmol), and the mixture was stirred at 40 °C for 24 h. After cooling to room temperature, the reaction was neutralized by addition of hydrochloric acid (5%, v/v) to PH = 4, and the formed aqueous solution was first extracted with ethyl acetate (3 x 50 mL), and then washed with brine (2 x 50 mL), finally dried over anhydrous $Na_2SO_4$. The solvent was removed under vacuum, and the formed crude naphthoic acid was used in the next step without purification. To make the naphthoic acyl chloride, the acid made above was added neat sulfuryl chloride (5 mL), and formed mixture was allowed to reflux for 4 h. After the mixture was cooled down to room temperature, the excess sulfuryl chloride was removed under vacuum (water pump) to afford the crude acid chloride, which was dissolved in dichloromethane (15 mL), followed by reaction with cumyl amine (288 μL, 2 mmol) at 0 °C under $N_2$, and the mixture was stirred at the same temperature for 12 h. The reaction mixture was sequentially quenched with water (10 mL), extracted with dichloromethane (3 x 50 mL), washed with brine (1 x 50 mL) and dried over anhydrous $Na_2SO_4$. The solvent was removed under vacuum, and the residue was purified by a flash chromatography on silica gel (hexane/ethyl acetate = 8/1) to give N-cumyl naphthamide **3a** or **3b**. Compound **3a** (265 mg) was obtained in 73% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.69 (s, 1H), 7.54-7.53 (m, 2H), 7.41 (d, *J* = 7.2 Hz, 1H), 7.38-7.34 (m, 2H), 7.27-7.24 (m, 1H), 7.20 (s, 1H), 7.16 (d, *J* = 7.3 Hz, 1H), 6.30 (s, 1H), 4.02 (s, 3H), 3.92 (s, 3H), 2.65 (s, 3H), 1.87 (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 169.3, 149.9, 149.6, 146.9, 135.3, 132.2, 128.8, 128.4, 126.7, 126.4, 124.8, 124.0, 122.6, 105.1, 103.1, 56.4, 55.8, 55.7, 29.3, 19.9; HRMS (ESI): calcd for $C_{23}H_{26}NO_3$ ($M + H^+$) 364.1913; found 364.1907; Compound **3b** (294 mg) was obtained in 78% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 7.68 (s, 1H), 7.54-7.52 (m, 2H), 7.43 (d, *J* = 7.3 Hz, 1H), 7.37-7.34 (m, 2H), 7.28-7.24 (m, 2H), 7.17 (d, *J* = 7.3 Hz, 1H), 6.36 (s, 1H), 4.02 (s, 3H), 3.92 (s, 3H), 3.04 (q, *J* = 7.5 Hz, 2H), 1.86 (s, 6H), 1.38 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 169.3, 149.7, 149.5, 146.9, 141.2, 132.1, 128.3, 127.9, 126.6, 124.8, 122.7, 122.1, 105.1, 102.8, 56.3, 55.7, 55.6, 29.3, 26.2, 14.4; HRMS (ESI): calcd for $C_{24}H_{28}NO_3$ ($M + H^+$) 378.2069; found 378.2064.

Syntheses of 3-hydroxy-5-methyl-7,8-dimethoxy-2-(2-phenylpropan-2-yl)-2,3-dihydro-1*H*-benzo[*e*] isoindol-1-one (**4a**) and 3-hydroxy-5-ethyl-7,8-dimethoxy-2-(2-phenylpropan-2-yl) -2,3-dihydro-1*H*-benzo[*e*] isoindol-1-one (**4b**): To a solution of **3a** or **3b** (0.5 mmol) and TMEDA (241 μL, 1.6 mmol) in THF (25 mL) was added *t*-BuLi (1.5 mol/L, 1.1 mL, 1.6 mmol) at -78 °C in a dropwise manner, and the mixture was stirred at the same temperature for 4 h. After addition of DMF (193 μL, 2.5 mmol) to the above solution, the reaction mixture was gradually warmed up to room temperature and stirred for an additional 2 h. The reaction was quenched with a saturated solution of ammonium chloride, and mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic

layer was washed with brine (3 x 15 mL), and dried over anhydrous $Na_2SO_4$. The solvent was removed under vacuum and the residue was purified by a flash chromatography on silica gel (hexane/ethyl acetate = 8/1) to give the naphthalimidine **4a** or **4b**. Compound **4a** (155 mg) was obtained in 88% yield based on recovery of starting material. $^1$H NMR (500 MHz, $CDCl_3$): δ 8.44 (s, 1H), 7.44-7.43 (m, 2H), 7.34-7.31 (m, 3H), 7.23 (t, *J* = 7.3 Hz, 1H), 7.17 (s, 1H), 6.13 (d, *J* = 10.8 Hz, 1H), 4.00 (s, 3H), 3.98 (s, 3H), 2.69 (s, 3H), 2.45 (d, *J* = 10.8 Hz, 1H), 2.01 (s, 3H), 1.95 (s, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 169.2, 151.1, 149.9, 147.6, 141.6, 138.5, 129.2, 128.4, 126.5, 125.2, 125.0, 123.1, 118.8, 103.6, 103.3, 81.8, 59.1, 56.2, 55.8, 29.3, 28.6, 20.5; HRMS (ESI): calcd for $C_{24}H_{26}NO_4$ (M + $H^+$) 392.1862; found 392.1856; Compound **4b** (166 mg) was obtained in 91% yield based on recovery of starting material. $^1$H NMR (500 MHz, $CDCl_3$): δ 8.46 (s, 1H), 7.45-7.43 (m, 2H), 7.35 (s, 1H), 7.34-7.31 (m, 2H), 7.25 (s, 1H), 7.22 (t, *J* = 7.3 Hz, 1H), 6.14 (d, *J* = 10.7 Hz, 1H), 4.00 (s, 3H), 3.98 (s, 3H), 3.11-3.05 (m, 2H), 2.54 (d, *J* = 10.7 Hz, 1H), 2.01 (s, 3H), 1.95 (s, 3H), 1.40 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 169.2, 151.0, 149.9, 147.7, 144.5, 141.8, 128.42, 128.38, 126.4, 125.5, 125.0, 123.0, 116.9, 103.7, 103.0, 81.9, 59.0, 56.1, 55.8, 29.3, 28.5, 26.8, 14.4; HRMS (ESI): calcd for $C_{25}H_{28}NO_4$ (M + $H^+$) 406.2018; found 406.2013.

Syntheses of 7,8-dimethoxy-5-methyl-2-(2-phenylpropan-2-yl)-1*H*-benzo[*e*]isoindole-1,3(2*H*)-dione (**5a**) and 5-ethyl-7,8-dimethoxy-2-(2-phenylpropan-2-yl)-1*H*-benzo[*e*] isoindole-1,3 (2*H*)-dione (**5b**): To a solution of compound **4a** or **4b** (0.2 mmol) in DMF (10 mL) under $N_2$ was added pyridinium dichromate (151 mg, 0.4 mmol), and the mixture was stirred at room temperature for 6 h. The reaction was quenched by addition of water (10 mL), and the mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were first washed with water (3 x 20 mL), and then with brine (2 x 10 mL), and finally dried over anhydrous $Na_2SO_4$. The solvent was removed under vacuum and the residue was purified by a flash chromatography on silica gel (hexane/ethyl acetate = 10/1) to give the N-cumyl maleimide **5a** and **5b**. Compound **5a** (66 mg) was obtained in 85% yield. $^1$H NMR (500 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.52 (s, 1H), 7.42-7.40 (m, 2H), 7.34-7.31 (m, 2H), 7.25-7.22 (m, 2H), 4.05 (s, 3H), 4.03 (s, 3H), 2.74 (s, 3H), 2.08 (s, 6H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 171.2, 170.2, 152.1, 151.3, 147.4, 140.2, 132.3, 129.4, 128.4, 126.6, 124.5, 124.4, 123.7, 117.9, 103.8, 103.5, 61.4, 56.2, 55.9, 29.7, 29.5, 20.6; HRMS (ESI): calcd for $C_{24}H_{24}NO_4$ (M + $H^+$) 390.1705; found 390.1700; Compound **5b** (71 mg) was obtained in 88% yield; $^1$H NMR (500 MHz, $CDCl_3$): δ 8.26 (s, 1H), 7.55 (s, 1H), 7.42-7.41 (m, 2H), 7.34-7.31 (m, 3H), 7.23 (t, *J* = 7.3 Hz, 1H), 4.04 (s, 3H), 4.03 (s, 3H), 3.12 (q, *J* = 7.5 Hz, 2H), 2.08 (s, 6H), 1.42 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (125 MHz, $CDCl_3$): δ 171.2, 170.3, 152.0, 151.3, 147.4, 146.1, 131.5, 129.5, 128.4, 126.6, 124.7, 124.5, 123.4, 116.0, 103.9, 103.1, 61.3, 56.2, 55.8, 29.5, 26.8, 14.2; HRMS (ESI): calcd for $C_{25}H_{26}NO_4$ (M + $H^+$) 404.1862; found 404.1856.

Syntheses of 7,8-dimethoxy-5-methyl-1*H*-benzo[*e*]isoindole-1,3(2*H*)-dione (**6a**) and 5-ethyl-7,8-dimethoxy-1*H*-benzo[*e*]isoindole-1,3(2*H*)-dione (**6b**): After treatment of compound **5a** or **5b** (0.1 mmol) with TFA (15 mL) at 50 °C for 10 h, the excess of TFA was removed under vacuum, and The residue was purified by a flash chromatography on silica gel (hexane/ethyl acetate = 10/1, then 1/1) to give maleimide **6a** or **6b**. Compound **6a** (24 mg) was obtained in 90% yield. $^1$H NMR (500 MHz, DMSO): δ 11.03 (brs, 1H), 8.12 (s, 1H), 7.54 (s, 1H), 7.37 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 2.74 (s, 3H); $^{13}$C NMR (125 MHz, DMSO): δ 171.0, 169.8, 151.8, 150.9, 140.7, 131.6, 129.4, 123.9, 123.7, 117.3, 104.1, 102.5, 55.6, 55.5, 20.0; HRMS (ESI): calcd for $C_{15}H_{14}NO_4$ (M + $H^+$)

272.0923; found 272.0917; Compound **6b** was obtained (26 mg) in 92% yield. $^{1}$H NMR (500 MHz, DMSO): δ 11.04 (brs, 1H), 8.14 (s, 1H), 7.52 (s, 1H), 7.43 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H), 3.16 (q, *J* = 7.5 Hz, 2H), 1.34 (t, *J* = 7.5 Hz, 3H); $^{13}$C NMR (125 MHz, DMSO): δ 171.0, 169.8, 151.8, 151.0, 146.4, 130.8, 129.5, 124.0, 123.8, 115.5, 103.6, 102.6, 55.6, 55.5, 25.9, 14.1; HRMS (ESI): calcd for $C_{16}H_{16}NO_4$ ($M + H^+$) 286.1079; found 286.1074.

**6a-6b inhibition activity assay**

6a-6b inhibition activity against GSK3 was monitored by ELISA. Recombinant human GSK3β (Calbiochem) was mixed with human Tau-441 (Millipore), 2 μL of 10 x kinase buffer (200 mM Tris, PH = 7.4, 100 mM $MgCl_2$, 5 mM DTT), 2 μL of 100 μM ATP, the chemicals (6a and 6b) and water to a final volume of 20 μL. Control reactions were set up, including reactions with DMSO or without ATP. The mixtures were incubated at 30 □ for 45 min. The amount of phosphorylated Tau-441 was measured by a human Tau [pS396] immunoassay kit (Invitrogen), following the procedure demonstrated in the instruction book. The absorbance of each well at 450 nm was read by a Bio-Rad Model 680 Microplate Reader (Bio-Rad Laboratories).