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Benzo[*e*]isoindole-1,3-diones as Potential Inhibitors of Glycogen Synthase Kinase-3 (GSK-3). Synthesis, Kinase Inhibitory Activity, Zebrafish Phenotype, and Modeling of Binding Mode

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Benzo[*e*]isoindole-1,3-dione derivatives were synthesized, and the effects on GSK-3β activity and zebrafish embryo growth were evaluated. A series of derivatives show obvious inhibitory activity against GSK-3β. The most potent inhibitor, 7,8-dimethoxy-5-methylbenzo[*e*]isoindole-1,3-dione (**8a**), shows nanomolar IC_{50} and obvious phenotype on zebrafish embryo growth associated with the inhibition of GSK-3β at low micromolar concentration. The interaction mode between **8a** and GSK-3β was characterized by computational modeling.

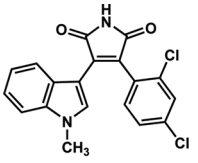
Introduction

Glycogen synthase kinase-3β (GSK-3β[a]) is a ubiquitous serine/threonine protein kinase that is involved in a broad range of biological processes, such as Wnt and Hedgehog signaling pathways, stem-cell renewal, cell-division cycle, differentiation, apoptosis, circadian rhythm, transcription, and insulin action.[1−4] Dysregulation of GSK-3β has been associated with many diseases, such as diabetes,[5−7] Alzheimer's disease,[8−10] Down syndrome,[11] bipolar disorder,[12] and colorectal cancer.[13,14] Thus, the development of GSK-3β inhibitors has been regarded as a potential therapeutic approach for these related diseases.[15−17]

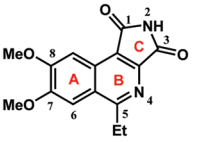
In connection with our development of a chemical genetic approach to analyzing biological systems by using zebrafish assay,[18,19] we identified compound **10** (3F8, Chart 1) from a public library, and the molecule could inhibit the eye and forebrain formation of zebrafish in embryos,[20] resembling a typical Wnt overexpression phenotype.[21] Cell reporter assay, chemical informatics analysis, and in vitro kinase experiment revealed that **10** is a selective GSK-3β inhibitor, which is more potent than **9** (SB-216763),[22] a commonly used GSK-3 inhibitor.[22,23]

Stimulated by the high potency and selectivity of **10**, as well as its unique scaffold as GSK-3 inhibitors, we have initiated a synthetic program exploring efficient strategies to synthesize **10**-related analogues from which we could identify potential GSK-3 inhibitors with improved therapeutic profiles. In this paper we report that benzo[*e*]isoindole-1,3-dione is a potential framework of **10** derivatives for the development of GSK-3 inhibitors.

Chart 1



9 (SB-216763)



5-ethyl-7,8-dimethoxy-1*H*-pyrrolo[3,4-*c*]isoquinoline-1,3(2*H*)-dione **10** (3F8)

Results and Discussion

Synthesis of the Library. Our study began with the study of the interaction of **10** with its binding site. To this end, we first conducted computational analysis,[20] and our results suggested that maleimide moiety of **10** might interact with the ATP binding site of GSK-3β, and the N-4 and C-5 positions were solvent-exposed, indicating the less important role of this region to the binding affinity. Thus, our first strategy to modify the **10** is based on the replacement of the nitrogen (N-4) on the isoquinoline with a carbon, affording an entirely new type of scaffold benzo[*e*]isoindole-1,3-dione, a scaffold that is relatively easy to be constructed and diversified.

Synthetically, benzo[*e*]isoindole-1,3-dione with a substituent at C-4 position (type I) could be expected to be made via our recent developed $Zn(OTf)_2$-catalyzed tandem annulation reaction from 1-isocyano-4-nitrobenzene and allenic esters **1a**−**d** in a one-pot fashion (Scheme 1).[24] In the event, when we mixed allenic esters **1a**−**d** and 1-isocyano-4-nitrobenzene with $Zn(OTf)_2$, followed by an oxidative photolysis in the presence of I_2, the expected intermediates **B** were obtained. Upon treatment of **B** with NH_3 in methanol, we got the desired products **2a**−**d** in satisfactory yields (Table 1).

On the basis of the chemistry developed above, 18 additional type I benzo[*e*]isoindole-1,3-diones were made, and their structures are listed in Table 2.

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[a] Abbreviations: GSK-3β, glycogen synthase kinase-3β; ATP, adenosine triphosphate; DDQ, 2,3-dichloro-5,6-dicyanobenzoquinone; DMF, dimethylformamide; PDC, pyridinium dichromate; CDK2, cyclin-dependent kinase 2; CDK4, cyclin-dependent kinase 4; dpf, days postfertilization; ELISA, enzyme-linked immunosorbent assay; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; DTT, dithiothreitol; DMSO, dimethyl sulfoxide; FLK-1, fetal liver kinase-1; EGFP, enhanced green fluorescent protein; HPLC, high performance liquid chromatography.

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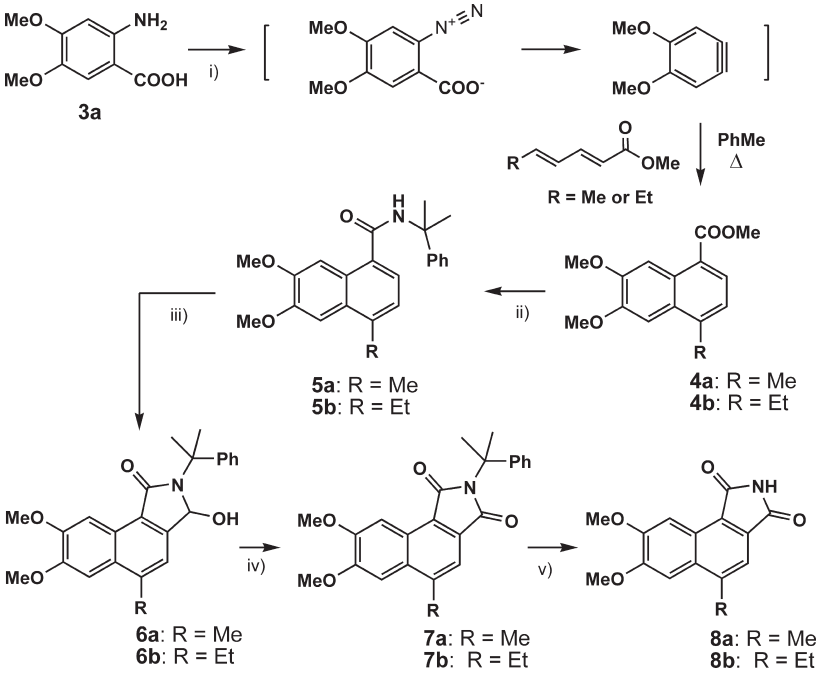
Scheme 1. Syntheses of Compounds **8a** and **8b** (Type II)[a]



[a] 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 **4a** of 11%, **4b** yield of 10%; (ii) (a) LiOH, $MeOH/H_2O$ = 3:1, 40 °C; (b) $SOCl_2$, reflux; (c) cumylamine, DCM, 0 °C to room temp, total yield for **5a** of 73%, **5b** yield of 78%; (iii) *t*-BuLi, TMEDA, THF, −78 °C, then DMF, −78 °C to room temp, **6a** yield of 88% brsm, **6b** yield of 91% brsm; (iv) PDC, DMF, room temp, **7a** yield of 85%, **7b** yield of 88%; (v) TFA, 50 °C, **8a** yield of 90%, **8b** yield of 92%.

Though the developed chemistry could quickly access the C-4 or both C-4 and C-5 substituted benzo[*e*]isoindole-1,3-diones, this methodology could not apply to the synthesis of substrates with substitution only at the C-5 position but not at the C-4 position. We therefore started to explore a different approach to synthesize the benzo[*e*]isoindole-1,3-diones with a substituent at C-5 (type II). After substantial screening of different synthetic strategies, we used the modified Snieckus' approach to construct the type II scaffold of benzo[*e*]isoindole-1,3-diones,[25] and its synthetic transformations are illustrated in Scheme 1.

The commercially available 2-amino-4,5-dimethoxybenzoic acid **3a** was treated with isoamylnitrite and Cl_3CCOOH to afford dimethoxybenzenediazonium-2-carboxylic acid (see Experimental Section), which was used to generate dimethoxybenzyne in situ and then underwent Diels−Alder reaction with the corresponding dienes, followed by aromatization in the presence of DDQ to produce naphthoates **4a** and **4b**. These products were further hydrolyzed and treated with sulfurous dichloride and cumylamine sequentially to give the *N*-cumylamides **5a** and **5b**, respectively. After treatment with *t*-BuLi, the formed phenyllithium was reacted with DMF to afford the corresponding naphthalimidines **6a** and **6b**. Compounds **6a** and **6b** were then subjected to the PDC oxidation, followed by TFA treatment to afford the final products **8a** and **8b**, respectively, in good yield.

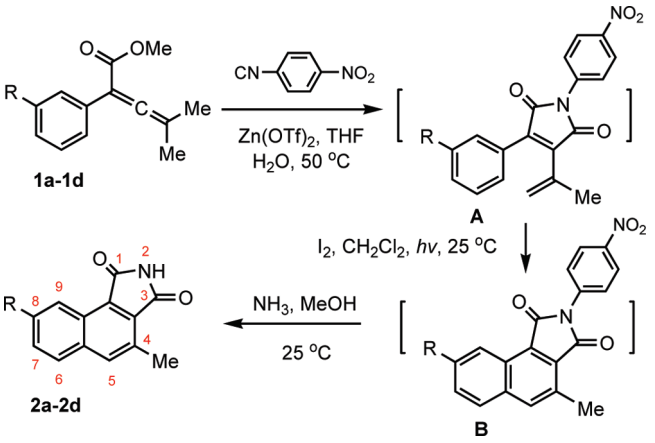
GSK-3*β* Kinase Inhibitory Activity. Synthesized compounds were tested for their inhibitory activity in vitro against GSK-3*β* using ELISA assay (see Experimental section). As shown in Figure 1, all compounds have significant inhibitory activity against GSK-3*β* at 1 *μ*M; most compounds have more than 50% inhibitory activity against GSK-3*β* at the concentration tested. In particular, compounds **8a** and **8b**, bearing 7,8-dimethoxy group on the aromatic ring, show potent GSK-3*β* inhibitory activity, comparable to the well-known GSK-3*β* inhibitor **9** (Figures 1 and 2). Removing one or two methoxy groups (**2c**,**d**) results in a loss of potency, suggesting favorable interactions of the methoxy groups at the 7 and 8 positions with its target.

Compounds with a larger substituent, such as carboxylic ester, at position 8 (**2a**,**e**−**h** vs **2c**) generally have lower inhibitory activity, and a bulkier substituent at the C-4 position results in a larger loss in potency. Substitution of carboxylic ester at position 7 also results in a loss in potency, but which is less sensitive to the substitution at the C-4 position (**2i**−**p**). Compounds with carboxylic ester at position 6 (**2q**−**v**) have a wide range of potency, having higher potency with the more rigid and bulkier alkyl group at the C-4 position (**2t**,**u**).

IC_{50} values of the most potent compounds **2u**, **8a**, and **8b** were further measured while using **9** as the control (Figure 2). The data confirmed that our compounds have comparable inhibitory activity against GSK-3*β* as that of the well-known GSK-3*β* inhibitor **9** in the in vitro kinase assay. Moreover, we tested **2u**, **8a**, and **8b** against the closely related kinase CDK2/cyclinA and the complex CDK4/cyclin D1;[26,27] **9** was used as the control (see Supporting Information). **8a** and **8b** show similar selectivity profile as that of **9** (Table 3), whereas **2u** had relatively lower selectivity against GSK-3*β*, suggesting that the substituents on the 7 or 8 position has a role in the GSK-3*β* selectivity. (The rationalization of the selectivity is shown in Figure 2S of Supporting Information.)

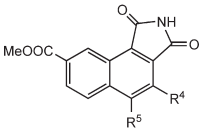
Mechanism of GSK-3*β* Inhibition. To further investigate the mechanism of GSK-3*β* inhibition of type II scaffold, kinase assays were tested with compound **8a** in varied ATP concentrations. As shown in Figure 3, the experimental data were perfectly fitted to an equation for simple competitive inhibition with respect to ATP, with an apparent K_i value of

Table 1. Syntheses of Benzo[*e*]isoindole-1,3-dione **2a**−**d** (Type I)

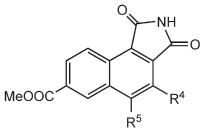


entry	R	product	yield (%)
1	COOMe	**2a**	34
2	Cl	**2b**	49
3	OMe	**2c**	44
4	H	**2d**	42

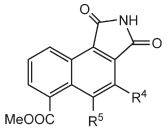
Table 2. Synthesized Type I Benzo[*e*]isoindole-1,3-diones **2e**−**v**



2e: R^4 = Et, R^5 = H
2f: R^4 = *i*Pr, R^5 = H
2g: R^4 = *i*Bu, R^5 = H
2h: R^4 = Me, R^5 = Et



2i: R^4 = Et, R^5 = H
2j: R^4 = *i*Pr, R^5 = H
2k: R^4 = *i*Bu, R^5 = H
2l: R^4 = Me, R^5 = Me
2m: R^4 = $(CH_2)_2OBn$, R^5 = H
2n: R^4 = $CH_2CH(Me)OBn$, R^5 = H
2o: R^4 = CH_2CH_2OH, R^5 = H
2p: R^4 = $CH_2CH(Me)OH$, R^5 = H



2q: R^4 = Me, R^5 = Me
2r: R^4 = Et, R^5 = H
2s: R^4 = Pr, R^5 = H
2t: R^4 = *i*Pr, R^5 = H
2u: R^4 = *i*Bu, R^5 = H
2v: R^4 = Me, R^5 = H

259 nM (Figure 3), consistent with the determined IC_{50} of **8a**. This result indicates that **8a** and its analogues indeed act as ATP competitive inhibitors of GSK-3β.

Molecular Modeling. To rationalize the structure−activity relationships of these compounds, the binding modes of the most potent inhibitors **8a** and **8b** were modeled using docking simulations. Compounds **8a** and **8b** were docked into the ATP binding site of GSK-3β,[28] and the binding modes of lowest energy were analyzed. As shown in Figure 4, compounds **8a** and **8b** fit the ATP pocket of GSK-3β well.

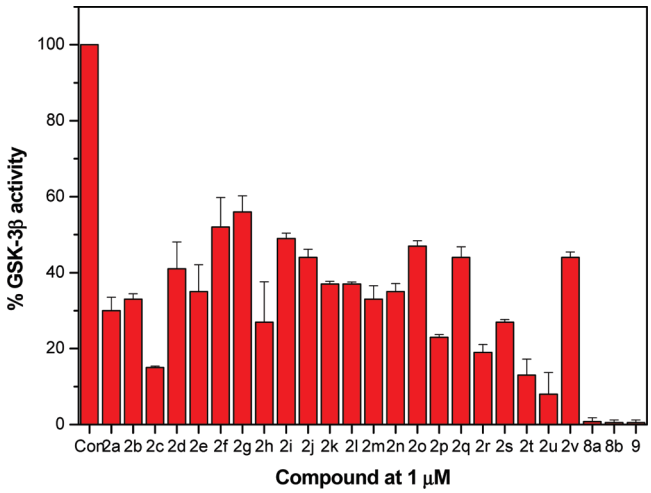


Figure 1. Inhibition of GSK-3β by benzo[*e*]isoindole-1,3-dione derivatives at 1 μM. DMSO (con) was used as blank control, and **9** was included as a positive control. Bars indicate standard deviation.

The maleimide motif of type II forms a pair of hydrogen bonds with the hinge region (Glu133 and Val135) of GSK-3β, similar to the binding mode of other known maleimide GSK-3β inhibitors.[29] The two methoxy oxygen atoms form another two hydrogen bonds with the positively charged Lys85. The methyl group of the methoxy at C-8 position docks to the small back cleft of GSK-3β. This binding mode explicitly explains the important role of the two methoxy groups at C-7 and C-8 positions. In addition, the 4-ethyl group of **8b** docks to the minor hydrophobic pocket formed by Ile62 and Val70 in the front of the ATP binding site of GSK-3β (Figure 4B), which contributes to its higher binding affinity compared to **8a**.

The docking results also provide a template to understand the structure−activity relationships of other compounds. Accordingly, the large carboxylic ester at the C-7 or C-8 position (compounds **2l**, **2a**) can also potentially form hydrogen bonds with Lys85; however, the favorable electrostatic interactions are compromised by the steric hindrance. On the other hand, other elements, such as chlorine or hydrogen in compounds **2b** and **2d**, only form weak or no hydrogen bond with Lys85, which accounts for their relatively weaker inhibitory activity compared to compounds **8a** and **8b**. In addition, the carboxylic ester at the C-6 position in **2v** cannot form a hydrogen bond with Lys85. As a result, no favorable electrostatic interactions exist between the substrate and the ATP site.

The docking results also explain the substituent effects at C-4 and C-5 positions. As shown in Figure 4C, to avoid the steric interactions with Lys85, a large carboxylic ester at the C-8 position moves toward the back cleft of ATP site, in turn moving the substitutions at C-4 or C-5 positions into the flat ATP site. Large substituents at C-4 or C-5 positions cause steric repulsion with the front of the ATP site; therefore, those compounds have lower potency compared to smaller ones (compounds **2e**−**g** vs **2a**). On the other hand, a large carboxylic ester on the 7 position would move toward the front of the ATP site and the less hydrophobic surface of the tricyclic scaffold is buried, resulting in a loss in potency. Meanwhile, the 4 or 5 position can tolerate relatively large substituents. However, the hydrogen bond between the carboxylic ester with Lys85 restricts the optimal interactions

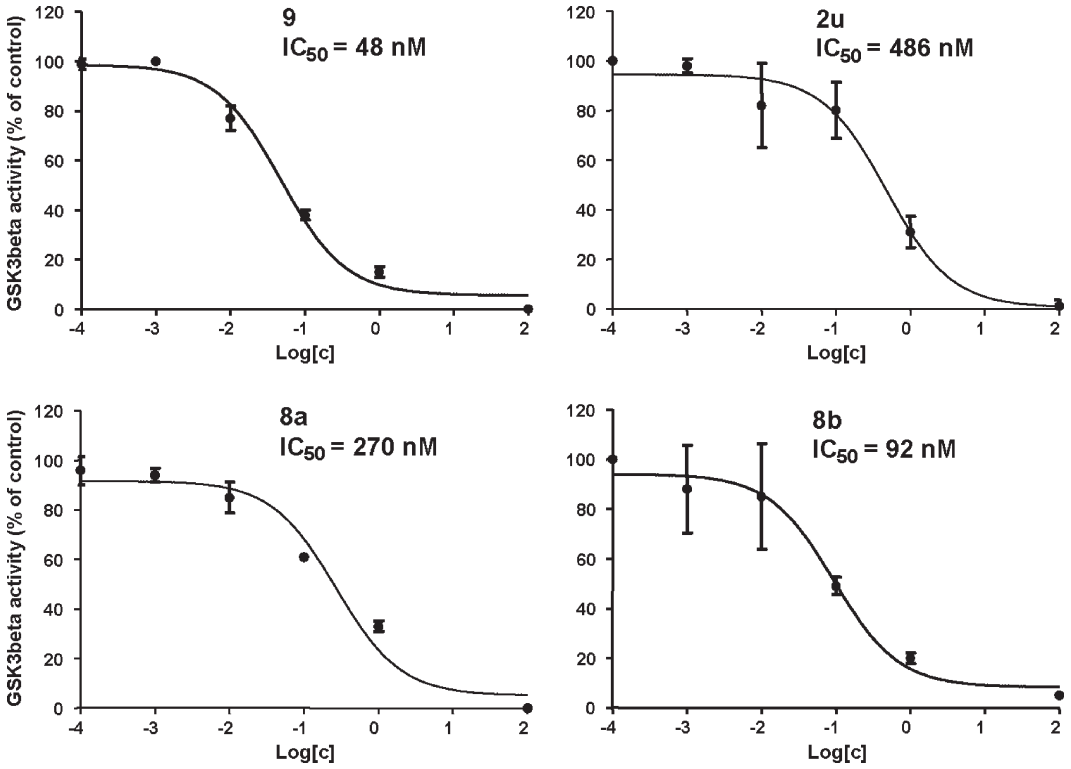


Figure 2. Dose-dependent inhibition of GSK-3β by compounds **2u**, **8a**, and **8b**. **9** was used as a control.

Table 3. Inhibitory Activity (IC_{50}) vs Human GSK-3β, CDK2, and CDK4[a]

compd	GSK-3β (μM)	CDK2 (μM)	CDK4 (μM)
9	0.048	0.269	32
8a	0.270	0.787	16
8b	0.092	ND	19
2u	0.486	ND	6

[a] ND: not determined.

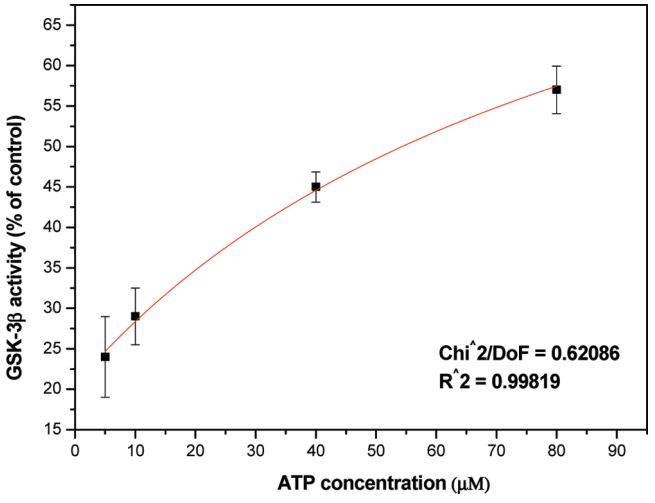


Figure 3. Plot of relative GSK-3β activity in the presence of **8a** (1 μM) as a function of ATP concentration. The data were fitted to an equation for simple competitive inhibition: GSK-3β activity (% of control) = $(K_{d\text{-ATP}} + [ATP]) \times 100/\{K_{d\text{-ATP}} + [ATP] + (K_{d\text{-ATP}}/K_{i\text{-}8a})[\mathbf{8a}]\}$, where [**8a**] = 1 μM.

between the substituents at C-4 or C-5 positions and the front region of the ATP site; the substituents just have modest contribution to the binding affinity (compounds **2i**−**n** vs **2d**). A carboxylic ester at the C-6 position has no steric and hydrogen bonding interactions with Lys85 or other residues within the ATP site. The substituents at C-4 or C-5 positions can fully fit into the front region of the ATP site. Larger substitutions have more hydrophobic interactions and higher binding affinity.

In Vivo Activity on Zebrafish Embryos. After the rationalization of the in vitro enzymatic activities of these compounds by the molecular modeling, the most potent compounds **8a** and **8b** were further tested for their in vivo activity on the zebrafish embryos. The known GSK-3β inhibitor **9** was included for comparison. GSK-3β is a negative regulator of the Wnt signal transduction pathway. Specific inhibition of GSK-3β activity leads to the activation of the Wnt pathway, which would produce an eyeless phenotype of zebrafish embryos during gastrulation.[21]

Compound **8a** at 25 μM shows obvious eyeless phenotype on the zebrefish embryos after 3 days of incubation. The known GSK-3β inhibitor LiCl shows similar eyeless phenotype, while more potent inhibitors **8b** and **9** in the in vitro assay unexpectedly show no such eyeless phenotype on the zebrafish embryos growth (Figure 5; data not shown for **8b**). This result indicates that compound **8a** has better in vivo efficacy than **8b** and **9**. Highlighting more factors besides the potency should be considered in the drug discovery process.

Conclusion

We have synthesized two types of benzo[*e*]isoindole-1,3-dione derivatives as potential GSK-3β inhibitors based on the scaffold of **10**. The structure−activity relationships of these compounds are analyzed using molecular modeling, which elucidates the critical aspects of the inhibitors with higher inhibitory activity. The zebrafish embryo assay shows that compound **8a** has desirable in vivo efficacy and possible better pharmacokinetic properties, like better cell permeability.

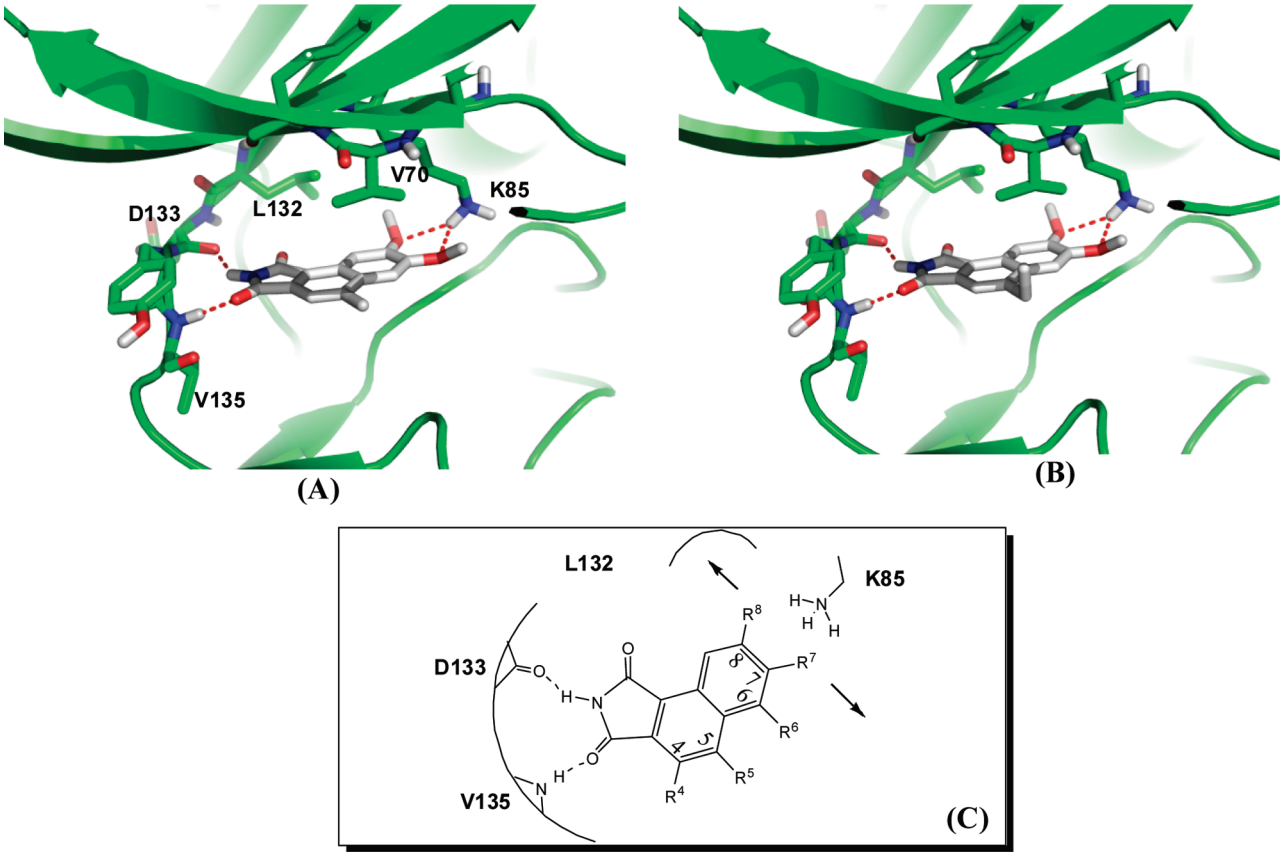


Figure 4. Docked binding modes of compounds **8a** (A) and **8b** (B) in the ATP binding site of GSK-3β. The small molecules and the critical interacting residues of GSK-3β are represented by sticks. Hydrogen bonds are shown as red dash lines. (C) Schematic representation of the binding mode of the inhibitors in the ATP binding site of GSK-3β. The arrows indicate the moving directions of the substituents compared to compound **8a**.

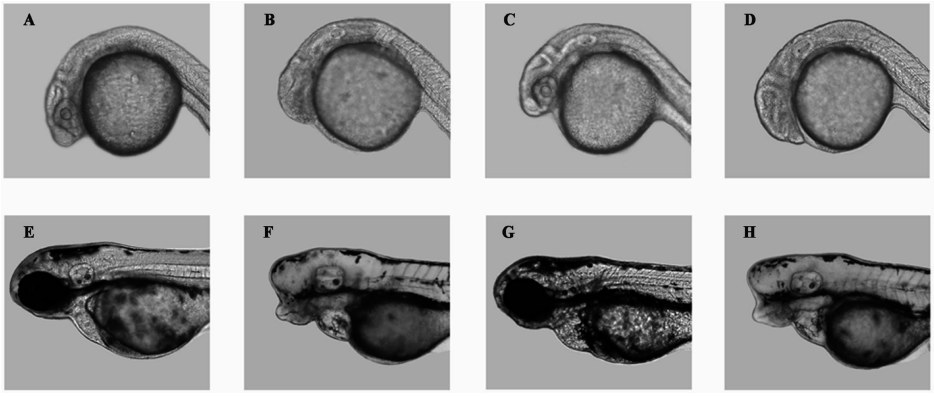


Figure 5. Effects on the zebrafish embryos by LiCl, **9**, and compound **8a**. (A, E) Heads of 1 and 3 dpf control embryos. (B, F) Heads of 1 and 3 dpf embryos treated with 0.3 M LiCl. This compound causes eyeless phenotype of zebrafish embryos. (C, G) Heads of 1 and 3 dpf embryos treated with 25 mM **9**. This compound shows no obvious effect on zebrafish embryos. (D, H) Heads of 1 and 3 dpf embryos treated with 25 mM compound **8a**. This compound causes eyeless phenotype of zebrafish embryos.

Additional studies with compound **8a** and its derivatives will be reported in due course.

Experimental Section

General Information. All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. All the chemicals were purchased commercially and used without further purification. Anhydrous THF and diethyl ether were distilled from sodium benzophenone, and dichloromethane was distilled from calcium hydride. The boiling point of petroleum ether is between 60 and 90 °C. Yields refer to chromatographic yields, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Tsingdao silica gel plates (60F-254) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate and heat as developing agents. Tsingdao silica gel (60, particle size 0.040−0.063 mm) was used for flash column chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 (^{1}H, 300 MHz; ^{13}C, 75.5 MHz) or Bruker Avance 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer. The following abbreviations were

used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad. Mass spectrometric data were obtained using a ZAB-HS mass spectrometer. Melting points were measured by X-6 micromelting point detector. Purities (>95%) of test compounds (**2a**−**v**, **8a**,**b**) were established by HPLC. HPLC experiments were performed on a Agilent 1200 series LC system equipped with a quaternary pump (G1311A), a vacuum degasser (G1322A), a diode array detector (G1315D), and a manual injector (G1328B), using a Zorbax SB-C18 (4.6 mm × 250 mm, 5 μm) column. The mobile phase was constituted of H_2O and CH_3CN. A gradient from 30% to 90% of CH_3CN over 20 min and then from 90% to 100% of CH_3CN over 5 min was used at a flow rate of 1.00 mL/min. The signal was collected under UV detection at 254 nm.

General Procedure for the Synthesis of Maleimides 2a−n, 2q−v. To a solution of allenic esters **1a**−**m** (0.5 mmol) in THF (8 mL) and H_2O (0.8 mL) were added 1-isocyano-4-nitrobenzene (67 mg, 0.45 mmol) and $Zn(OTf)_2$ (4.9 mg, 0.0135 mmol), and the mixture was stirred at 50 °C for 24 h. After the mixture was cooled to room temperature, the solvent was removed under vacuum, and the residue was filtered off through a silica gel pad. The filtrate was concentrated under vacuum, and the residue was used in the next step without further purification.

To a solution of the crude product made above in dichloromethane (100 mL) was added I_2 (572 mg, 2.25 mmol), and the reaction mixture was then irradiated under a 300 W high-pressure mercury lamp for 4 h at 25 °C. The reaction mixture was quenched with a saturated solution of $Na_2S_2O_3$ (10 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic layer was first dried over anhydrous Na_2SO_4 and then concentrated under vacuum to give the crude product, which was eventually treated at 25 °C with a saturated solution of NH_3 in methanol (10 mL) for 12 h. The reaction was worked up by removal of the solvent under vacuum, and the residue was purified by a flash chromatography on silica gel to give the target molecules **2a**−**n**, **2q**−**v**.

Syntheses of Methyl 4-Methyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-8-carboxylate (2a) and Methyl 4-Methyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[e]isoindole-6-carboxylate (2v). Compounds **2a** and **2v** were made from **1a** as a pair of regioisomers in 59% total yield by the standard procedure. The formed reaction mixture was purified by flash chromatography on silica gel (hexane/ethyl acetate = 25/1, then hexane/THF = 25/1) to give **2a** (41 mg) as a solid in 34% yield and **2v** (30 mg) as a solid in 25% yield for three steps.

Compound 2a. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.59 (s, 1H), 8.23 (dd, J_1 = 8.7 Hz, J_2 = 1.6 Hz, 1H), 7.96 (s, 1H), 7.92 (d, J = 8.7 Hz, 1H), 7.55 (brs, 1H), 4.03 (s, 3H), 2.84 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.6, 168.5, 166.7, 138.7, 135.8, 135.4, 130.7, 130.4, 129.7, 128.7, 128.2, 127.7, 126.4, 52.7, 18.3. HRMS (EI): calcd for $C_{15}H_{11}NO_4$ (M^+) 269.0688; found 269.0686. Mp: 286.8 °C.

Compound 2v. ^{1}H NMR (500 MHz, DMSO): δ 11.33 (brs, 1H), 9.04 (d, J = 8.3 Hz, 1H), 8.81 (s, 1H), 8.23 (d, J = 7.1 Hz, 1H), 7.78 (t, J = 7.8 Hz, 1H), 3.96 (s, 3H), 2.72 (s, 3H). ^{13}C NMR (125 MHz, DMSO): δ 170.1, 169.8, 166.8, 133.5, 133.1, 132.5, 131.9, 129.8, 128.6, 127.9, 127.4, 127.1, 126.7, 52.5, 17.9. HRMS (EI): calcd for $C_{15}H_{11}NO_4$ (M^+) 269.0688; found 269.0687. Mp: 255.0 °C.

8-Chloro-4-methyl-1*H*-benzo[*e*]isoindole-1,3(2*H*)-dione (2b). Compound **2b** was prepared from **1b** by the standard procedure and was purified by a flash chromatography on silica gel (first eluted with hexane/ethyl acetate = 50/1, then 5/1) to give **2b** (54 mg) as a solid in 49% yield for three steps. ^{1}H NMR (500 MHz, DMSO): δ 11.32 (s, 1H), 8.70 (d, J = 2.0 Hz, 1H), 8.15 (s, 1H), 8.07 (d, J = 8.9 Hz, 1H), 7.70 (dd, J_1 = 8.9 Hz, J_2 = 2.0 Hz, 1H), 2.69 (s, 3H). ^{13}C NMR (125 MHz, DMSO): δ 170.0, 169.9, 135.4, 134.4, 133.4, 132.6, 130.6, 130.3, 129.1, 126.9, 126.4, 122.4, 17.4. HRMS (EI): calcd for $C_{13}H_8ClNO_2$ (M^+) 245.0244; found 245.0248. This compound decomposed when the temperature was above 270.0 °C.

8-Methoxy-4-methyl-1*H*-benzo[*e*]isoindole-1,3(2*H*)-dione (2c). Compound **2c** was prepared from **1c** by the standard procedure and purified by a flash chromatography on silica gel (dichloromethane/THF = 400/1) to give **2c** (48 mg) as a solid in 44% yield for three steps. ^{1}H NMR (500 MHz, DMSO): δ 11.15 (brs, 1H), 8.07 (s, 1H), 8.00 (s, 1H), 7.91 (d, J = 9.1 Hz, 1H), 7.32 (d, J = 9.1 Hz, 1H), 3.91 (s, 3H), 2.65 (s, 3H). ^{13}C NMR (125 MHz, DMSO): δ 170.6, 170.2, 159.2, 135.2, 132.0, 129.8, 129.6, 129.3, 127.6, 125.9, 121.4, 101.6, 55.2, 17.0. HRMS (EI): calcd for $C_{14}H_{11}NO_3$ (M^+) 241.0739; found 241.0728. Mp: 222.9 °C.

4-Methyl-1*H*-benzo[*e*]isoindole-1,3(2*H*)-dione (2d). Compound **2d** was prepared from **1d** by the standard procedure and was washed with methanol to give **2d** (40 mg) as a solid in 42% yield for three steps. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (d, J = 8.1 Hz, 1H), 7.93 (s, 1H), 7.87 (d, J = 7.4 Hz, 1H), 7.68−7.62 (m, 2H), 7.49 (brs, 1H), 2.81 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.2, 137.1, 136.0, 132.9, 130.0, 129.1, 128.8, 128.4, 128.0, 127.3, 125.1, 18.2. HRMS (EI): calcd for $C_{17}H_9NO_2$ (M^+) 211.0633; found 211.0631. Mp: 229.4 °C.

Syntheses of Methyl 4-Ethyl-1,3-dioxo-2,3-dihydro-1*H*-benzo-[*e*]isoindole-8-carboxylate (2e), Methyl 4,5-Dimethyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-6-carboxylate (2q), and Methyl 4-Ethyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-6-carboxylate (2r). Compounds **2e**, **2q**, and **2r** were made from **1e** as a mixture of regioisomers in 54% total yield by the standard procedure and were first purified by flash chromatography on silica gel (hexane/ethyl acetate = 20/1) to give **2r** (27 mg) as a solid in 21% yield for three steps, together with a mixture of **2e** and **2q**, which were further purified by a flash chromatography on silica gel (dichloromethane/THF = 400/1) to give **2e** (25 mg) as a solid in 20% yield and to give **2q** (17 mg) as a solid in 13% yield for three steps.

Compound 2e. ^{1}H NMR (500 MHz, DMSO): δ 11.35 (brs, 1H), 9.40 (s, 1H), 8.21 (s, 1H), 8.15 (d, J = 8.7 Hz, 1H), 8.11 (dd, J_1 = 8.7 Hz, J_2 = 1.7 Hz, 1H), 3.95 (s, 3H), 3.16 (q, J = 7.5 Hz, 2H), 1.30 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75.5 MHz, DMSO): δ 170.0, 169.8, 165.9, 140.9, 138.0, 133.7, 130.1, 129.3, 129.1, 128.9, 127.3, 126.3, 125.2, 52.6, 24.2, 14.8. HRMS (EI): calcd for $C_{16}H_{13}NO_4$ (M^+) 283.0845; found 283.0847. Mp: 242.4 °C.

Compound 2q. ^{1}H NMR (500 MHz, DMSO): δ 11.26 (brs, 1H), 9.02 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 7.0 Hz, 1H), 7.72−7.69 (m, 1H), 3.93 (s, 3H), 2.72 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (75.5 MHz, DMSO): δ 170.3, 170.0, 140.5, 133.2, 131.6, 130.9, 130.0, 129.5, 127.2, 126.91, 126.86, 125.5, 52.9, 18.1, 14.1. HRMS (EI): calcd for $C_{16}H_{13}NO_4$ (M^+) 283.0845; found 283.0840. Mp: 240.7 °C.

Compound 2r. ^{1}H NMR (500 MHz, DMSO): δ 11.32 (brs, 1H), 9.06 (d, J = 8.5 Hz, 1H), 8.89 (s, 1H), 8.25 (d, J = 7.3 Hz, 1H), 7.80−7.77 (m, 1H), 3.97 (s, 3H), 3.15 (q, J = 7.5 Hz, 2H), 1.28 (t, J = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, DMSO): δ 170.0, 169.5, 166.7, 139.7, 133.3, 131.9, 130.9, 129.3, 128.6, 128.2, 127.4, 127.0, 126.7, 52.4, 24.4, 14.7. HRMS (EI): calcd for $C_{16}H_{13}NO_4$ (M^+) 283.0845; found 283.0848. Mp: 205.7 °C.

Syntheses of Methyl 4-Isopropyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-8-carboxylate (2f) and Methyl 4-Isopropyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-6-carboxylate (2t). Compounds **2f** and **2t** were made from **1f** as a pair of regioisomers in 50% total yield by the standard procedure and were purified by a flash chromatography on silica gel (hexane/ethyl acetate = 15/1) to give **2f** (35 mg) as a solid in 26% yield and **2t** (32 mg) as a solid in 24% yield for three steps.

Compound 2f. ^{1}H NMR (500 MHz, DMSO): δ 11.34 (s, 1H), 9.38 (s, 1H), 8.29 (s, 1H), 8.15 (d, J = 8.7 Hz, 1H), 8.07 (dd, J_1 = 8.7 Hz, J_2 = 1.7 Hz, 1H), 4.07−4.02 (m, 1H), 3.95 (s, 3H), 1.34 (d, J = 6.9 Hz, 6H). ^{13}C NMR (75.5 MHz, DMSO): δ 169.9, 169.8, 165.8, 145.7, 138.1, 130.9, 129.5, 129.2, 129.04, 128.99, 127.2, 126.3, 125.0, 52.5, 27.6, 22.8. HRMS (EI): calcd for $C_{17}H_{15}NO_4$ (M^+) 297.1001; found 297.1002. Mp: 270.9 °C.

Compound 2t. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.33 (s, 1H), 9.23 (d, J = 8.4 Hz, 1H), 8.35 (dd, J_1 = 7.4 Hz, J_2 = 1.1 Hz, 1H),

7.68 (dd, J_1 = 8.4 Hz, J_2 = 7.4 Hz, 1H), 7.60 (brs, 1H), 4.18−4.12 (m, 1H), 4.04 (s, 3H), 1.43 (d, J = 6.9 Hz, 6H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 168.9, 168.5, 167.3, 145.9, 134.9, 133.0, 129.9, 129.8, 128.8, 128.2, 127.5, 127.3, 127.0, 52.5, 28.5, 23.2. HRMS (EI): calcd for $C_{17}H_{15}NO_4$ (M^+) 297.1001; found 297.1005. Mp: 302.5 °C.

Syntheses of Methyl 4-Isobutyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-8-carboxylate (2g) and Methyl 4-Isobutyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-6-carboxylate (2u). Compounds **2g** and **2u** were made from **1g** as a pair of regioisomers in 35% total yield by standard procedure and were purified by a flash chromatography on silica gel (hexane/ethyl acetate = 20/1) to give **2g** (24 mg) as a solid in 17% yield and **2u** (25 mg) as a solid in 18% yield for three steps.

Compound 2g. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.60 (s, 1H), 8.22 (d, J = 8.7 Hz, 1H), 7.94−7.92 (m, 2H), 7.59 (brs, 1H), 4.02 (s, 3H), 3.09 (d, J = 7.1 Hz, 2H), 2.09−2.03 (m, 1H), 0.99 (d, J = 6.3 Hz, 6H). ^{13}C NMR (125 MHz, DMSO): δ 169.8, 169.6, 165.7, 138.3, 137.5, 135.1, 130.4, 129.1, 129.0, 128.7, 127.1, 126.2, 125.2, 52.3, 29.2, 22.0. HRMS (EI): calcd for $C_{18}H_{17}NO_4$ (M^+) 311.1158; found 311.1164. Mp: 200.0 °C.

Compound 2u. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.21 (d, J = 8.4 Hz, 1H), 9.10 (s, 1H), 8.33 (dd, J_1 = 7.3 Hz, J_2 = 1.2 Hz, 1H), 7.80 (brs, 1H), 7.68 (dd, J_1 = 8.4 Hz, J_2 = 7.3 Hz, 1H), 4.03 (s, 3H), 3.12 (d, J = 7.2 Hz, 2H), 2.09−2.02 (m, 1H), 0.99 (d, J = 6.7 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.0, 168.5, 167.2, 138.7, 134.4, 134.3, 132.9, 129.90, 129.86, 128.3, 127.7, 127.3, 127.1, 52.4, 40.7, 30.2, 22.3. HRMS (EI): calcd for $C_{18}H_{17}NO_4$ (M^+) 311.1158; found 311.1164. Mp: 204.1 °C.

Syntheses of Methyl 5-Ethyl-4-methyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-8-carboxylate (2h) and Methyl 1,3-Dioxo-4-propyl-2,3-dihydro-1*H*-benzo[*e*]isoindole-6-carboxylate (2s). Compounds **2h** and **2s** were made from **1h** as a pair of regioisomers in 39% total yield by the standard procedure and were purified by a flash chromatography on silica gel (hexane/ethyl acetate = 20/1) to give **2h** (21 mg) as a solid in 16% yield and **2s** (31 mg) as a solid in 23% yield for three steps.

Compound 2h. ^{1}H NMR (500 MHz, DMSO): δ 11.27 (brs, 1H), 9.44 (d, J = 1.7 Hz, 1H), 8.29 (d, J = 9.1 Hz, 1H), 8.10 (dd, J_1 = 9.1 Hz, J_2 = 1.7 Hz, 1H), 3.95 (s, 3H), 3.18 (q, J = 7.5 Hz, 2H), 2.76 (s, 3H), 1.20 (t, J = 7.5 Hz, 3H). ^{13}C NMR (75.5 MHz, DMSO): δ 170.5, 170.0, 165.8, 146.7, 135.7, 132.4, 130.3, 128.4, 127.2, 127.0, 126.6, 125.6, 125.4, 52.5, 21.3, 14.1, 13.3. HRMS (EI): calcd for $C_{17}H_{15}NO_4$ (M^+) 297.1001; found 297.0996. Mp: 300.4 °C.

Compound 2s. ^{1}H NMR (500 MHz, DMSO): δ 11.31 (brs, 1H), 9.04 (d, J = 8.3 Hz, 1H), 8.85 (s, 1H), 8.24 (d, J = 7.2 Hz, 1H), 7.78−7.75 (m, 1H), 3.96 (s, 3H), 3.09 (t, J = 7.4 Hz, 2H), 1.70−1.66 (m, 2H), 0.96 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO): δ 170.0, 169.5, 166.6, 138.1, 133.1, 131.9, 131.7, 129.4, 128.6, 128.2, 127.4, 127.0, 126.7, 52.4, 33.0, 23.4, 13.5. HRMS (EI): calcd for $C_{17}H_{15}NO_4$ (M^+) 297.1001; found 297.1002. Mp: 219.0 °C.

Syntheses of Methyl 4-Ethyl-1,3-dioxo-2,3-dihydro-1*H*-benzo-[*e*]isoindole-7-carboxylate (2i) and Methyl 4,5-Dimethyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-7-carboxylate (2l). Compounds **2i** and **2l** were made from **1i** as a pair of regioisomers in 58% total yield by the standard procedure and were purified by a flash chromatography on silica gel (dichloromethane /THF = 400/1) to give **2i** (48 mg) as a solid in 38% yield and **2l** (25 mg) as a solid in 20% yield.

Compound 2i. ^{1}H NMR (500 MHz, DMSO): δ 11.35 (brs, 1H), 8.85 (d, J = 8.8 Hz, 1H), 8.72 (s, 1H), 8.38 (s, 1H), 8.15 (dd, J_1 = 8.8 Hz, J_2 = 1.6 Hz, 1H), 3.95 (s, 3H), 3.16 (q, J = 7.5 Hz, 2H), 1.30 (t, J = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, DMSO): δ 169.9, 169.7, 165.7, 139.2, 135.4, 135.3, 131.1, 130.5, 129.0, 127.9, 127.8, 127.1, 124.4. 52.3, 23.8, 14.6. HRMS (EI): calcd for $C_{16}H_{13}NO_4$ (M^+) 283.0845; found 283.0854. Mp: 194.2 °C.

Compound 2l. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.04 (d, J = 8.9 Hz, 1H), 8.91 (s, 1H), 8.22 (d, J = 8.9 Hz, 1H), 7.44 (brs, 1H), 4.02 (s, 3H), 2.86 (s, 3H), 2.81 (s, 3H). ^{13}C NMR (125 MHz, THF): δ 170.8, 170.2, 166.9, 143.4, 135.6, 132.7, 132.5, 130.6, 129.8, 127.8, 127.5, 127.0, 126.4, 52.4, 15.0, 14.3. HRMS (EI): calcd for $C_{16}H_{13}NO_4$ (M^+) 283.0845; found 283.0856. Mp: 297.0 °C.

Synthesis of Methyl 4-Isopropyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-7-carboxylate (2j). Compound **2j** was made from **1j** by the standard procedure and the crude product was purified by a flash chromatography on silica gel (hexane/ethyl acetate = 15/1) to give **2j** (59 mg) as a solid in 44% yield for three steps. ^{1}H NMR (500 MHz, DMSO): δ 11.33 (brs, 1H), 8.82 (d, J = 8.8 Hz, 1H), 8.74 (s, 1H), 8.48 (s, 1H), 8.11 (d, J = 8.8 Hz, 1H), 4.07−4.02 (m, 1H), 3.94 (s, 3H), 1.34 (d, J = 6.9 Hz, 6H). ^{13}C NMR (75.5 MHz, DMSO): δ 170.0, 169.9, 165.9, 144.3, 135.7, 132.9, 130.9, 130.6, 129.0, 127.9, 127.8, 127.2, 124.5, 52.5, 27.5, 23.0. HRMS (EI): calcd for $C_{17}H_{15}NO_4$ (M^+) 297.1001; found 297.1005. Mp: 289.2 °C.

Synthesis of Methyl 4-Isobutyl-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-7-carboxylate (2k). Compound **2k** was made from **1k** by the standard procedure and the crude product was purified by a flash chromatography on silica gel (dichloromethane/THF = 400/1) to give **2k** (53 mg) as a solid in 38% yield for three steps. ^{1}H NMR (500 MHz, DMSO): δ 11.35 (brs, 1H), 8.86 (d, J = 8.8 Hz, 1H), 8.73 (s, 1H), 8.33 (s, 1H), 8.15 (dd, J_1 = 8.8 Hz, J_2 = 1.6 Hz, 1H), 3.94 (s, 3H), 3.02 (d, J = 7.1 Hz, 2H), 2.03−1.97 (m, 1H), 0.92 (d, J = 6.7 Hz, 6H). ^{13}C NMR (75.5 MHz, DMSO): δ 170.1, 169.9, 165.9, 137.2, 136.9, 135.2, 131.7, 130.7, 129.1, 128.1, 127.9, 127.3, 124.6, 52.6, 29.4, 22.2. HRMS (EI): calcd for $C_{18}H_{17}NO_4$ (M^+) 311.1158; found 311.1165. Mp: 285.6 °C.

Synthesis of Methyl 4-(2-(Benzyloxy)ethyl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-7-carboxylate (2m). Compound **2m** was made from **1l** by the standard procedure and the crude product was purified by a flash chromatography on silica gel (dichloromethane/THF = 400/1) to give **2m** (54 mg) as a solid in 31% yield for three steps. ^{1}H NMR (500 MHz, $CDCl_3$): 8.95 (d, J = 8.8 Hz, 1H), 8.61 (s, 1H), 8.22 (dd, J_1 = 8.8 Hz, J_2 = 1.5 Hz, 1H), 8.12 (s, 1H), 7.73 (brs, 1H), 7.26−7.21 (m, 5H), 4.53 (s, 2H), 4.02 (s, 3H), 3.87 (t, J = 6.2 Hz, 2H), 3.52 (t, J = 6.2 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.54, 168.48, 166.4, 138.2, 137.8, 135.9, 135.1, 131.3, 130.8, 130.3, 129.1, 128.32, 128.29, 128.1, 127.7, 127.6, 125.2, 72.9, 69.4, 52.5, 31.8. HRMS (EI): calcd for $C_{23}H_{19}NO_5$ (M^+) 389.1263; found 389.1268. Mp: 197.3 °C.

Synthesis of Methyl 4-(2-(Benzyloxy)propyl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-7-carboxylate (2n). Compound **2n** was made from **1m** by the standard procedure and the crude product was purified by a flash chromatography on silica gel (dichloromethane/THF = 400/1) to give **2n** (60 mg) as a solid in 33% yield for three steps. ^{1}H NMR (500 MHz, $CDCl_3$): 8.96 (d, J = 8.8 Hz, 1H), 8.61 (s, 1H), 8.25 (dd, J_1 = 8.8 Hz, J_2 = 1.5 Hz, 1H), 8.08 (s, 1H), 7.41 (brs, 1H), 7.13 (t, J = 7.3 Hz, 1H), 7.08−7.05 (m, 2H), 7.00 (d, J = 7.3 Hz, 2H), 4.56 (d, J = 12.1 Hz, 1H), 4.33 (d, J = 12.1 Hz, 1H), 4.03 (s, 3H), 3.92−3.88 (m, 1H), 3.43−3.40 (m, 1H), 3.34−3.29 (m, 1H), 1.33 (d, J = 6.1 Hz, 3H). ^{13}C NMR (75.5 MHz, DMSO): δ 170.0, 169.9, 165.8, 138.8, 137.9, 135.0, 134.4, 131.6, 130.6, 129.0, 128.2, 127.8, 127.7, 127.34, 127.30, 127.0, 124.5, 74.1, 69.4, 52.5, 37.9, 19.6. HRMS (EI): calcd for $C_{24}H_{21}NO_5$ (M^+) 403.1420; found 403.1420. Mp: 233.9 °C.

Synthesis of Methyl 4-(2-Hydroxyethyl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-7-carboxylate (2o). To a solution of compound **2m** (45.0 mg, 0.12 mmol) in methanol (0.5 mL) and THF (0.5 mL) was added Pd/C (10% palladium on activated carbon, 20.0 mg), and the mixture was stirred 25 °C under a balloon pressure of H_2 for 16 times. The mixture was filtered off through a funnel and washed with methanol/THF (1/1, 30 mL). The filtrate was concentrated under vacuum, and the residue was purified by a flash chromatography on silica gel (dichloromethane/methanol = 80/1) to give **2o** (30.5 mg) as a solid in 85%

yield. ^{1}H NMR (500 MHz, DMSO): δ 11.33 (brs, 1H), 8.79 (d, J = 8.8 Hz, 1H), 8.65 (s, 1H), 8.30 (s, 1H), 8.11 (d, J = 8.8 Hz, 1H), 4.80 (s, 1H), 3.92 (s, 3H), 3.74−3.73 (m, 2H), 3.28 (t, J = 6.6 Hz, 2H). ^{13}C NMR (75.5 MHz, DMSO): δ 170.3, 170.2, 166.2, 137.7, 135.4, 135.2, 131.9, 130.9, 129.4, 128.4, 128.0, 127.6, 124.7, 61.3, 52.9, 34.5. HRMS (EI): calcd for $C_{16}H_{13}NO_5$ (M^+) 299.0794; found 299.0791. Mp: 241.0 °C.

Synthesis of Methyl 4-(2-Hydroxypropyl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*e*]isoindole-7-carboxylate (2p). According to the same procedure for the synthesis of compound **2o**, **2p** was made from **2n** as a solid in 75% yield. ^{1}H NMR (500 MHz, DMSO): δ 11.34 (brs, 1H), 8.87 (d, J = 8.8 Hz, 1H), 8.72 (s, 1H), 8.35 (s, 1H), 8.17 (d, J = 8.8 Hz, 1H), 4.60 (d, J = 4.5 Hz, 1H), 4.01 (s, 1H), 3.95 (s, 3H), 3.50−3.12 (m, 2H), 1.12 (d, J = 5.9 Hz, 3H). ^{13}C NMR (75.5 MHz, DMSO): δ 170.1, 170.0, 165.9, 137.9, 135.2, 135.1, 131.8, 130.7, 129.1, 128.1, 127.7, 127.3, 124.5, 66.4, 52.6, 23.5. HRMS (EI): calcd for $C_{17}H_{15}NO_5$ (M^+) 313.0950; found 313.0949. Mp: 257.5 °C.

Synthesis of Naphthoate 4a. To a solution of 2-amino-4,5-dimethoxybenzoic acid (**3a**) (4.9 g, 25 mmol) and trichloroacetic acid (37.5 mg, 0.23 mmol) in THF (38 mL) was added 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 h. 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, and to this solution was added methyl hexa-2,4-dienoate (30 mmol). 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 resulting mixture was stirred at room temperature for 6 h. The reaction was worked up by filtration of the mixture, and the solid was washed with ethyl acetate (3 × 20 mL). The filtrate was washed with water (3 × 20 mL) and brine (1 × 20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum, and the residue was purified by flash chromatography on silica gel (hexane/ethyl acetate = 5/1) to give naphthoate **4a** (715 mg) as a solid in 11% yield for three 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 Naphthoate 4b. 4b was made under the identical conditions as the preparation of **4a** by replacing the methyl hexa-2,4-dienoate with methyl hepta-2,4-dienoate for 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 **4b** (685 mg) as a solid in 10% yield for three 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 (5a) and 4-Ethyl-6,7-dimethoxy-*N*-(2-phenylpropan-2-yl)-1-naphthamide (5b). To a solution of **4a** or **4b** (1 mmol) in a mixed 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 the mixture was cooled to room temperature, the reaction was quenched by addition of hydrochloric acid (5%, v/v) to pH 4. The formed aqueous solution was first extracted with ethyl acetate (3 × 50 mL) and then washed with brine (2 × 50 mL) and 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 to neat sulfurous dichloride (5 mL), and the formed mixture was allowed to reflux for 4 h. After the mixture was cooled to room temperature, the excess sulfurous dichloride was removed under vacuum (water pump) to afford the crude acid chloride, which was dissolved in dichloromethane (15 mL), followed by reaction with cumylamine (288 μL, 2 mmol) at 0 °C under N_2. The mixture was stirred at room temperature for 12 h. The reaction mixture was sequentially quenched with water (10 mL), extracted with dichloromethane (3 × 50 mL), washed with brine (1 × 50 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum, and the residue was purified by flash chromatography on silica gel (hexane/ethyl acetate = 8/1) to give *N*-cumylnaphthamide **5a** or **5b**.

Compound 5a. Compound **5a** (265 mg) was obtained as a solid 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 5b. Compound **5b** (294 mg) was obtained as a solid 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 (6a) and 3-Hydroxy-5-ethyl-7,8-dimethoxy-2-(2-phenylpropan-2-yl)-2,3-dihydro-1*H*-benzo[*e*]isoindol-1-one (6b). To a solution of **5a** or **5b** (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 the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with brine (3 × 15 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by flash chromatography on silica gel (hexane/ethyl acetate = 8/1) to give the naphthalimidine **6a** or **6b**.

Compound 6a. Compound **6a** (155 mg) was obtained as a solid 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 6b. Compound **6b** (166 mg) was obtained as a solid 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 (7a) and 5-Ethyl-7,8-dimethoxy-2-(2-phenylpropan-2-yl)-1*H*-benzo[*e*]isoindole-1,3(2*H*)-dione (7b). To a solution of compound **6a** or **6b** (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 × 20 mL). The combined organic layers were first washed with water (3 × 20 mL) and then with brine (2 × 10 mL) and finally dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by flash chromatography on silica gel (hexane/ethyl acetate = 10/1) to give the *N*-cumylmaleimides **7a** and **7b**.

Compound 7a. Compound **7a** (66 mg) was obtained as a solid 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. Mp: 201.4 °C.

Compound 7b. Compound **7b** (71 mg) was obtained as a solid 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 (8a) and 5-Ethyl-7,8-dimethoxy-1*H*-benzo[*e*]isoindole-1,3(2*H*)-dione (8b). After treatment of compound **7a** or **7b** (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 **8a** or **8b**.

Compound 8a. Compound **8a** (24 mg) was obtained as a solid 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. Mp: 249.3 °C.

Compound 8b. Compound **8b** was obtained (26 mg) as a solid 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. Mp: 224.4 °C.

Computational Method. Protein coordinates for docking were obtained from the crystal structure of GSK-3β in complex with indirubin (PDB entry 1UV5).[28] The indirubin binding site was used to dock **8a** and **8b**. Waters and indirubin were removed from the PDB file, and the polar hydrogen atoms were added to the amino acid residues before the docking study. Docking was performed using AutoDock, version 4.0.[30] The illustrated structure was made using PyMOL.[31]

GSK3β Inhibitory Activity. Single Dose (1 μM) Activity. GSK3β activity was monitored by ELISA. Recombinant human GSK3β (500 ng, Calbiochem) was mixed with 1 μg of human τ-441 (Millipore), 2 μL of 10× kinase buffer (200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, 5 mM DTT), 2 μL of 100 μM ATP, the indicated amount of chemicals, 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 °C for 45 min. The amount of phosphorylated τ-441 was measured by a human τ [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).

Dose-Dependent Curve. GSK3β activity was monitored by ELISA. Recombinant human GSK3β (60 ng, Invitrogen) was mixed with 400 ng of human τ-441 (Millipore), 2 μL of 10× kinase buffer (200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, 5 mM DTT), 2 μL of 100 μM ATP, the indicated amount of chemicals, 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 °C for 45 min. The amount of phosphorylated τ-441 was measured by a human τ [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). The activity curves were drawn by Graphpad Prism 5 (GraphPad Software).

ATP Competitive Assay. GSK3β activity was monitored by ELISA. Recombinant human GSK3β (60 ng, Invitrogen) was mixed with 400 ng of human τ-441 (Millipore), 2 μL of 10× kinase buffer (200 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, 5 mM DTT), 0.4 μL of 50 μM compound **8a**, the indicated amount of ATP, 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 °C for 45 min. The amount of phosphorylated τ-441 was measured by a human τ [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). The activity curves were drawn by Graphpad Prism 5 (GraphPad Software).

In Vivo Activity on Zebrafish Embryos. FLK-1 promoter EGFP transgenic zebrafish (FLK-1:EGFP) was used in this study. Embryos were raised under standard conditions and staged according to description by Kimmel et al.[32] Live embryos were placed into 96-well plates, six embryos per well with 200 μL of Holfreter's buffer. Compounds were added to the embryos at corresponding concentration at the dome stage. For treatment with compounds except LiCl, embryos were allowed to grow in chemical compound solution up to 7 days. Embryos were exposed to 0.3 M LiCl for 10 min and allowed to grow in Holfreter's buffer up to 7 days.

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Supporting Information Available: Synthetic procedures, spectroscopic data for the compounds, purities of test compounds, and details of biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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