Full Paper

Design, Synthesis, and Evaluation of 3-Aryl-4-pyrrolyl-maleimides as Glycogen Synthase Kinase-3β Inhibitors

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A series of 3-aryl-4-pyrrolyl-maleimides were designed, synthesized, and evaluated for their glycogen synthase kinase-3β (GSK-3β) inhibitory activity. Most compounds exhibited potent activity against GSK-3β. Among them, compounds **11a**, **11c**, **11h**, **11i**, and **11j** significantly reduced Aβ-induced Tau hyperphosphorylation, showing the inhibition of GSK-3β at the cellular level. Structure–activity relationships were discussed based on the experimental data obtained.

Keywords: 3-Aryl-4-pyrrolyl-maleimides / Cellular activity / Enzymatic activity / GSK-3β inhibitors

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Introduction

Glycogen synthase kinase-3 (GSK-3) is a multifunctional serine/threonine protein kinase that was first identified in the late 1970s as a consequence of its phosphorylation activity toward glycogen synthase (GS), the rate limiting enzyme of glycogen biosynthesis [1, 2]. Today, it is known that GSK-3 plays an important role in various cellular and physiological events, such as the Wnt signaling pathway [3], insulin signal transduction [4], cell survival, response to DNA damage [5], hyphosphorylation of protein Tau (one of the diagnostic features of Alzheimer's disease) [6]. From a drug discovery standpoint, inhibition of GSK-3 may provide therapy for several diseases such as cancer [7], diabetes type-2 [8], chronic inflammatory processes [9], stroke [10], bipolar disorders, and Alzheimer's disease [11]. Accordingly, searching for GSK-3 inhibitors is a very active area in both academic centers and pharmaceutical companies. GSK-3 is encoded by two isoforms in mammals, termed GSK-3α and GSK-3β, which are 98% identical within the catalytic domain, but which have shown distinct pharmacology [12, 13]. Various bisindolylmaleimides such as GF 109203X and Ro 31-8820 have been developed as potent GSK-3β inhibitors based on staurosporine, a microbial alkaloid that was identified as an early GSK-3β inhibitor (Fig. 1) [14–17]. However, most of these bisindolylmaleimides have suffered from issues including toxicity, bad solubility, and poor selectivity, especially against PKC, which make them unsuitable for the treatment of diseases such as diabetes and Alzheimer's disease [18, 19]. Recent efforts in replacing one indolyl ring of bisindolylmaleimides with other heteroaryl substituents such as imidazo[1,2-*a*]pyridinyl, 4-azaindolyl and benzofuranyl led to the emergence of some monoindolylmaleimides (Fig. 1) [20–22]. Among them, GSK-3β inhibitor 603281-31-8 has reached preclinical trials for the treatment of diabetes. These facts encouraged us to design a new series of monoindolylmaleimide analogs to find more potent and selective GSK-3β inhibitors. In this paper, we describe the synthesis and evaluation of 3-aryl-4-pyrrolyl-maleimides as potent and selective GSK-3β inhibitors. Their structure–activity relationship and *in silico* molecular modeling study in a homology GSK-3β protein are discussed as well.

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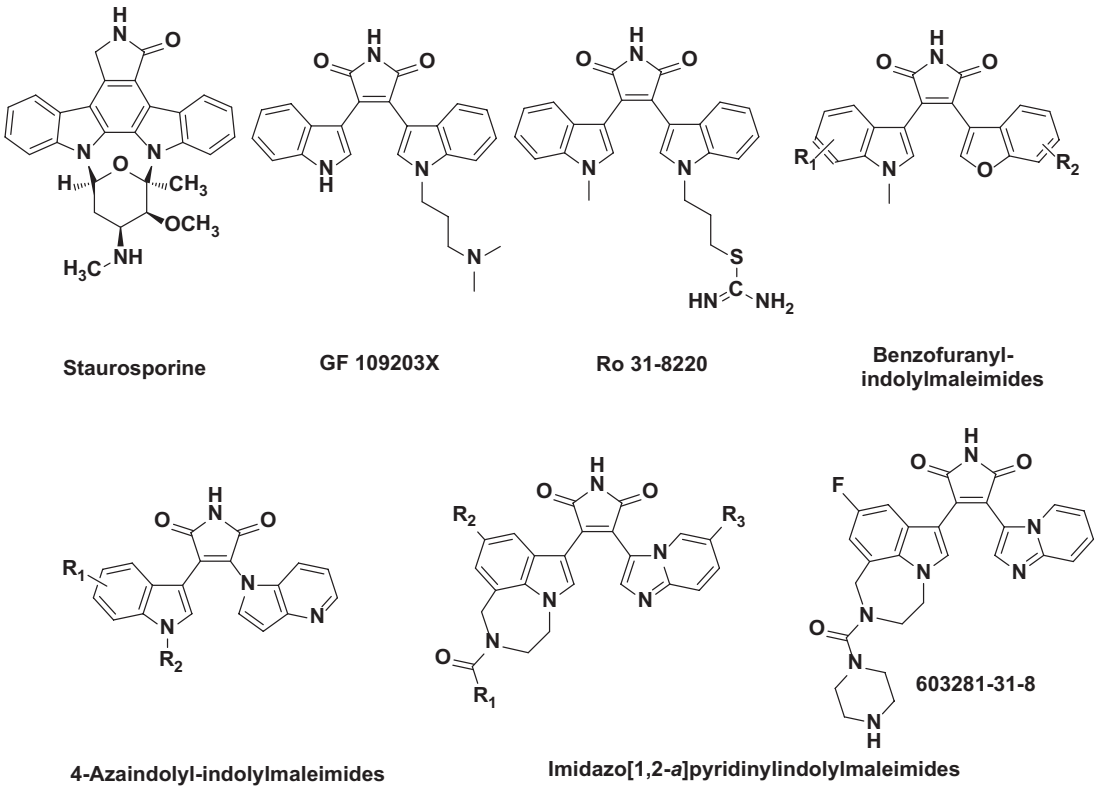


Figure 1. GSK-3β inhibitors.

Results and discussion

Chemistry

The synthetic approach to compounds **10** and **11a–k** is outlined in Scheme 1. Indole derivatives **1a–e** were reacted with oxalyl chloride in Et_2O, followed by sodium methoxide to give **2a–e**. *N*-Alkylation of **2a–e** with different alkyl halides in the presence of NaH in DMF resulted in key intermediates **3a–j**. Treatment of indole with 1,4-dibromobutane afforded **4**. Substitution reaction of **4** with morpholine by using K_2CO_3 as hydrogen bromide capture resulted in **5**, which was then treated with oxalyl chloride, followed by sodium methoxide to give another key intermediate **3k**. Condensation of glyoxilic esters **3a–k** with 2-(1*H*-pyrrol-3-yl)acetamide **9** [23, 24] in the presence of *t*-BuOK in THF afforded the target compounds **11a–k**. Similarly, compound **10** can be obtained by reaction of **2a** with **9**.

The target compounds **13a**,**b** were prepared by the condensation of ethyl pyrrole-3-glyoxalate **6** with aryl acetamides **12a**,**b**, respectively, following the same procedure described above. Similarly, compounds **15a**,**b** were obtained by condensation of glyoxilic esters **3a** with the corresponding compounds aryl acetamides **14a**,**b** (Scheme 2).

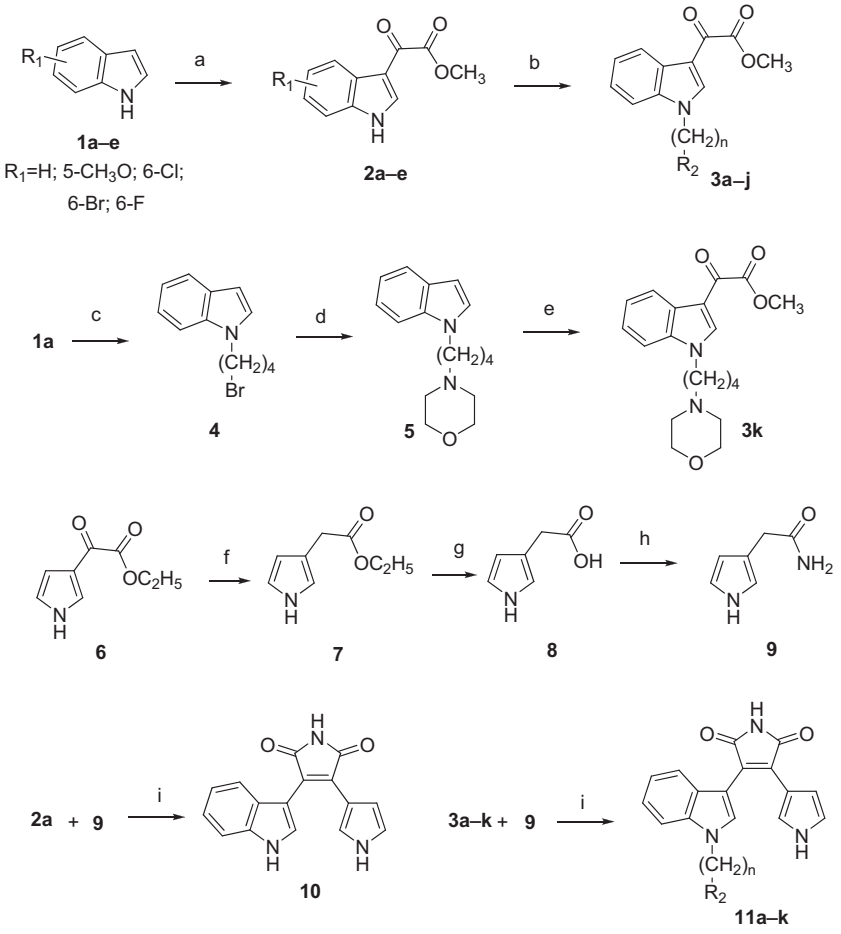
Biological activity and molecular modeling

Enzymatic activity

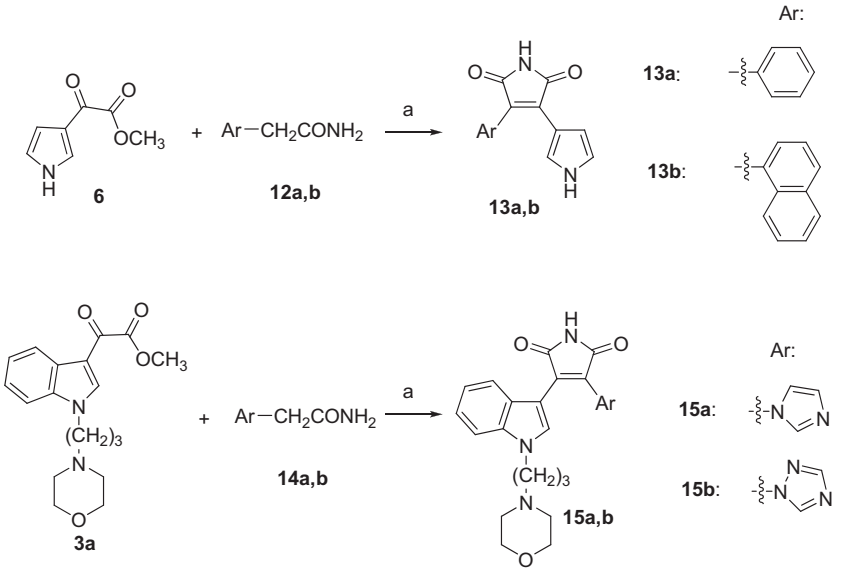
The GSK-3β inhibitory potency of all the target compounds was examined. In addition, the assays of inhibitory activity toward PKCE, IKK2, Aurora A, MEK1, and ERK1 were also conducted to determine the selectivity of the tested compounds. Staurosporine, a well-known kinase inhibitor was used as the reference compound. The results are summarized in Tables 1 and 2.

As shown in Table 1, most compounds displayed potent inhibitory activity against GSK-3β. Different aryl substituents in the maleimide ring could affect the inhibitory potency greatly. Replacement of an indole ring in **10** (IC_{50} = 1.92 μM) with a naphthyl afforded **13b** (IC_{50} = 11.86 μM), leading to a sixfold loss of potency for GSK-3β. Substitution of pyrrole in **11a** (IC_{50} = 0.10 μM) with other five-member-aryls including imidazole (**15a**, IC_{50} = 2.04 μM) and 1,3,4-triazole (**15b**, IC_{50} = 11.6 μM) resulted in a 20- and 116-fold loss of potency, respectively.

Comparing the inhibitory activity of compound **10** with **11a**, **11c**, and **11i**, it appeared that introduction of suitable hydrophilic side chains such as morpholinopropyl, imidazol-1-ylpropyl, and hydroxypropyl at the ^{1}N-position of

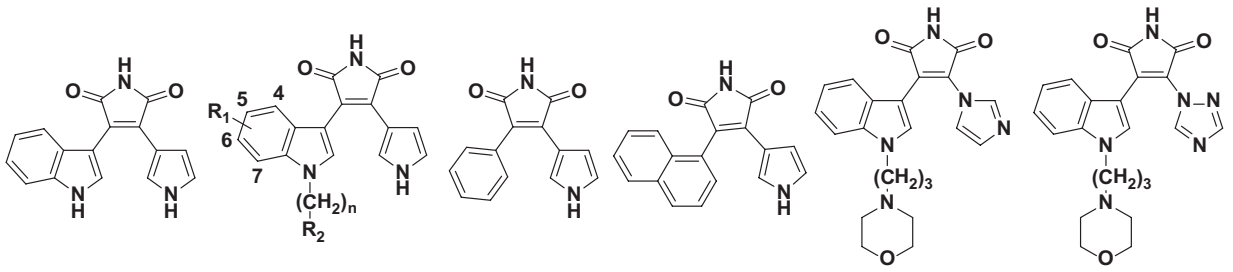


Scheme 1. Synthetic route to compounds **11a–i**. Reagents and conditions: (a) i: $(COCl)_2$, Et_2O; ii: CH_3ONa, CH_3OH. (b) NaH, DMF, $X(CH_2)_nR_2$; (c) NaH, DMF, $Br(CH_2)_4Br$; (d) K_2CO_3, DMF, morpholine; (e) i: HCl, $(COCl)_2$, Et_2O; ii: CH_3ONa, CH_3OH; (f) 10% Pd/C, $NaHPO_2$, 1,4-dioxane/H_2O; (g) i: NaOH, CH_3OH/H_2O, ii: concentrated HCl. (h) DCC, NH_3, 1,4-dioxane; i: i *t*-BuOK, THF; ii: concentrated HCl.



Scheme 2. Synthetic route to compounds **13a**,**b**, and **15a**,**b**. Reagents and conditions: (a) i: *t*-BuOK, THF; ii: concentrated HCl.

Table 1. GSK-3β inhibitory activity of the target compounds.



10 **11a–k** **13a** **13b** **15a** **15b**

Compds.	R_1	R_2	*n*	IC_{50} (μM) ± SE[a]
Staurosporine				0.028 ± 0.0[b]
10				1.92 ± 0.29
11a	H	Morpholino	3	0.10 ± 0.005
11b	H	Morpholino	2	6.45 ± 0.25
11c	H	Imidazol-1-yl	3	0.21 ± 0.01
11d	H	Piperidin-1-yl	3	1.29 ± 0.08
11e	5-OCH_3	Morpholino	3	0.57 ± 0.032
11f	6-Cl	Morpholino	3	0.36 ± 0.023
11g	6-Br	Morpholino	3	1.01 ± 0.02
11h	6-F	Morpholino	3	0.16 ± 0.009
11i	H	Hydroxyl	3	0.15 ± 0.01
11j	H	H	1	0.26 ± 0.02
11k	H	Morpholino	4	0.53 ± 0.024
13a				4.24 ± 0.17
13b				11.86 ± 1.07
15a				2.04 ± 0.15
15b				11.6 ± 0.54

[a] SE, standard error mean.
[b] Lit. [25]. IC_{50} = 0.056 ± 0.0069 μM.

the indole ring gave an obvious enhancement of GSK-3β inhibitory activity. Compound **11a** (IC_{50} = 0.1 μM) with a morpholinopropyl on the indole ring was about 19-fold more potent than **10** (IC_{50} = 1.92 μM). Replacement of the terminal morpholine group in **11a** with a hydroxy group (**11c**, IC_{50} = 0.15 μM) and imidazol-1-yl (**11i**, IC_{50} = 0.21 μM) led to a slight drop in potency. However, replacement of the morpholine group with piperidin-1-yl (**11d**, 1.29 μM) resulted in 13-fold loss in potency. Changing the length of the alkyl linker could affect the ability of the molecule to interact with GSK-3β thereby influencing the potency of the compounds. Compounds **11a** (IC_{50} = 0.10 μM) with a three methylene linker showed better inhibitory activities than **11b** (IC_{50} = 0.53 μM) with a four methylene linker and compound **11c** (IC_{50} = 6.45 μM) with a two methylene linker.

Table 2. The selectivity to tested kinases of target compounds **11a** and **11i**.[a]

Kinase assay	IC_{50} (μM) ± SE[b]		
	Staurosporine	**11a**	**11i**
GSK-3β	0.028 ± 0.0	0.10 ± 0.005	0.15 ± 0.01
PKCE	0.0015 ± 0.0001	>40	>40
IKK2	1.41 ± 0.26	>40	>40
Aurora A	0.018 ± 0.002	>40	>40
MEK1	0.67 ± 0.035	>40	>40
ERK1	1.31 ± 0.035	>40	>40

[a] ">40" means <50% inhibition at 40 μM of compound.
[b] SE, standard error mean.

Different substituents on the indole ring could affect the GSK-3β inhibitory activity (**11a**, **11e–h**). Fluorine at 6-position of the indole ring (**11h**) led to a slight drop in potency toward GSK-3β as compared to **11a**, while bromine at 6-position (**11g**) showed a 10-fold loss of activity.

As reported in the literature [14], staurosporine was found to be a potent and nonselective kinase inhibitor. Although staurosporine potently inhibits GSK-3β (IC_{50} = 0.028 μM), it also potently inhibits many other kinases (e.g., PKCE, IKK2, Aurora A, MEK1, and ERK1). The data of inhibitory activity

against PKCE, IKK2, Aurora A, MEK1, and ERK1 showed that compounds **11a** and **11i** displayed high selectivity for GSK-3β over other tested kinases (see Table 2).

Cellular activity

Among the multiple cellular processes in which GSK-3β has been implicated, the ability to hyperphosphorylate the Tau protein and induce neurofibrillary tangle was intensively studied. Therefore, the cell-based assay examining Tau phosphorylation at Serine 396 represents a direct functional assay to measure the cellular activity of GSK-3β inhibitors. Compounds **11a**, **11c**, **11e**, **11f**, **11h**, **11i**, **11j**, and **11k** were tested for the ability to reduce Tau phosphorylation at Ser 396 in human neuroblastoma SH-SY5Y cells. LiCl is a known inhibitor of GSK-3β and reduces Tau phosphorylation at Ser 396 in SH-SY5Y cells [26], which was used as a reference compound in this assay. As shown in Fig. 2, compounds **11a**, **11c**, **11h**, **11i**, and **11j** significantly reduced Aβ-induced Tau hyperphosphorylation, showing the inhibition of GSK-3β at the cell level, while **11e**, **11f**, and **11k** have no significant cellular activity.

Molecular modeling

To explore the possible binding mode of a GSK-3β inhibitor a molecular modeling study of the optimum compound **11a** was performed using the Tripos FlexiDock program [27, 28], based on the published GSK-3β crystal structure (1Q3D) [14]. As it can be seen in Fig. 3, there are some important interactions between ligand and GSK-3β. The maleimide portion of **11a** makes key hydrogen bonding contacts with residues Asp-133 and Val-135 backbone carbonyl and amide hydrogen, respectively. The oxygen atom of morpholine forms another hydrogen bond with Lys-183.

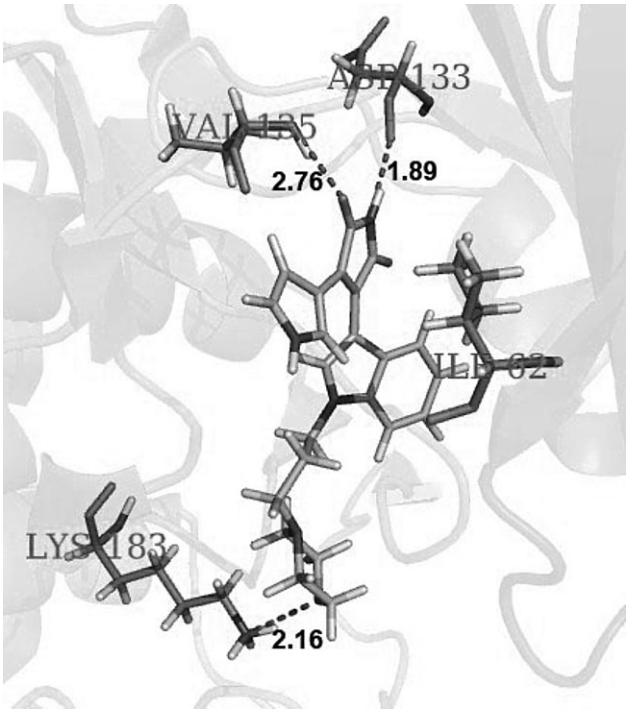


Figure 3. Docking of **11a** to the GSK-3β crystal structure.

Conclusion

In summary, a series of 3-aryl-4-indolyl-maleimides were designed, synthesized, and tested for their biological activity, and most of them showed potent activity against GSK-3β with **11a** being the most potent compound. Among them, compounds **11a** and **11i** exhibited high selectivity against PKCE, IKK2, Aurora A, MEK1, and ERK1. Further cell-based functional assay revealed that selected compounds **11a**, **11c**, **11h**, **11i**, and **11j** could significantly reduce Aβ-induced Tau hyperphosphorylation by inhibiting GSK-3β. The preliminary structure–activity relationships and molecular modeling study provided further insight into interactions between the enzyme and its ligand. The results provide valuable information for the design of GSK-3β inhibitors.

Experimental

Chemistry

Melting points were determined with a BÜCHI Melting Point B-450 apparatus (Büchi Labortechnik, Flawil, Switzerland). The ^{1}H NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Bruker Avance DMX 500 at 500 MHz (chemical shifts are expressed as δ values relative to TMS as internal standard). ESI (positive) was recorded on an Esquire-LC-00075 spectrometer. Element analyses were performed on an Eager 300 instrument. All reactions were

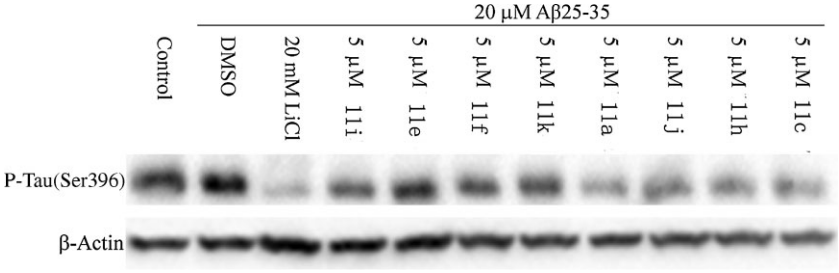


Figure 2. Effects of GSK-3β inhibitors on Tau phosphorylation (Ser396) in SH-SY5Y cells.

monitored by thin-layer chromatography (TLC). All reagents were obtained from commercial sources and used without further purification unless stated. Et_2O and THF were distilled from sodium-benzophenone. DMF was distilled from calcium hydride.

*Methyl 2-(1H-indol-3-yl)-2-oxoacetate **2a***

Oxalyl chloride (3.40 g, 26 mmol) in Et_2O (5 mL) was added dropwise to a solution of indole (3.01 g, 26 mmol) in Et_2O (30 mL) at 0–5°C. The reaction mixture kept stirring under the same conditions for 1 h, and a 20 wt% solution of CH_3ONa in MeOH (14.05 g, 52 mmol) was added dropwise at −30 to −20°C. After addition, the mixture was stirred for 30 min at room temperature, poured into cold water (100 mL). The product was isolated with filtration, washed with dichloromethane, and dried to afford 2.24 g (86.3%) **2a** as a light yellow solid, mp: 208–210°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.48 (brs, 1H), 8.46 (d, J = 3.5 Hz, 1H), 8.16 (d, J = 7.0 Hz, 1H), 7.55 (d, J = 7.0 Hz, 1H), 7.32–7.26 (m, 2H), 3.90 (s, 3H).

*Methyl 2-(5-methoxy-1H-indol-3-yl)-2-oxoacetate (**2b**)*

According to the procedure used to prepare **2a**, reaction of 5-methoxyindole with oxalyl chloride followed by CH_3ONa provided **2b** in 71.0% yield as a light yellow solid, mp: 221–223°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.34 (brs, 1H), 8.37 (d, J = 3.0 Hz, 1H), 7.66 (d, J = 2.0 Hz, 1H), 7.45 (d, J = 9.0 Hz, 1H), 6.93 (dd, J = 9.0, 2.0 Hz, 1H), 3.89 (s, 3H), 3.81 (s, 3H).

*Methyl 2-(6-chloro-1H-indol-3-yl)-2-oxoacetate (**2c**)*

According to the procedure used to prepare **2a**, reaction of 6-chloroindole with oxalyl chloride followed by CH_3ONa provided **2c** in 62.8% yield as a light yellow solid, mp: 246–248°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.52 (brs, 1H), 8.51 (d, J = 3.5 Hz, 1H), 8.15 (d, J = 8.5 Hz, 1H), 7.62 (d, J = 2.0 Hz, 1H), 7.31 (dd, J = 8.5, 2.0 Hz, 1H), 3.90 (s, 3H).

*Methyl 2-(6-bromo-1H-indol-3-yl)-2-oxoacetate (**2d**)*

According to the procedure used to prepare **2a**, reaction of 6-bromoindole with oxalyl chloride followed by CH_3ONa provided **2d** in 58.9% yield as a light yellow solid, mp: 207–209°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.50 (brs, 1H), 8.50 (s, 1H), 8.10 (d, J = 8.5 Hz, 1H), 7.75 (d, J = 2.0 Hz, 1H), 7.43 (dd, J = 8.5, 2.0 Hz, 1H), 3.90 (s, 3H).

*Methyl 2-(6-fluoro-1H-indol-3-yl)-2-oxoacetate (**2e**)*

According to the procedure used to prepare **2a**, reaction of 6-fluoroindole with oxalyl chloride followed by CH_3ONa provided **2e** in 60.3% yield as a light yellow solid, mp: 182–184°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.48 (brs, 1H), 8.48 (s, 1H), 8.15 (dd, J = 8.5, 5.5 Hz, 1H), 7.36 (dd, J = 9.5, 2.0 Hz, 1H), 7.15 (td, J = 9.5, 2.0 Hz, 1H), 3.90 (s, 3H).

*Methyl 2-(1-(3-morpholinopropyl)-1H-indol-3-yl)-2-oxoacetate (**3a**)*

Seventy percent NaH (0.51 g, 14.8 mmol) was added portionwise to a solution of **2a** (3.0 g, 14.8 mmol) in DMF (30 mL) at 0–5°C. The reaction mixture was warmed to room temperature and stirred for 30 min. After that, 4-(3-chloropropyl)morpholine (1.14 g, 19.2 mmol) was added. Then the mixture was heated to 55–60°C and reacted for 12 h. After cooling, the mixture was poured into cold water (300 mL) and extracted with ethyl acetate (3 × 100 mL). The organic phase was combined, washed with brine (3 × 300 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using ethyl acetate/methanol (50:1 v/v) as eluent to afford 3.30 g (67.5%) **3a** as a light yellow solid, mp: 103–104°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.47–8.43 (m, 2H), 7.46–7.41 (m, 1H), 7.38–7.32 (m, 2H), 4.32 (t, J = 6.5 Hz, 2H), 3.95 (s, 3H), 3.78–3.72 (m, 4H), 2.44–2.38 (m, 4H), 2.28 (t, J = 6.5 Hz, 2H), 2.08–2.02 (m, 2H). ESI-MS: m/z $[M+H]^+$ 331. Anal. calcd. for $C_{18}H_{22}N_2O_4$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.27; H, 6.68; N, 8.58.

*Methyl 2-(1-(2-morpholinoethyl)-1H-indol-3-yl)-2-oxoacetate (**3b**)*

According to the procedure used to prepare **3a**, reaction of **2a** with 4-(2-chloroethyl)morpholine provided **3b** in 52.4% yield as a light yellow solid, mp: 110–112°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.51 (s, 1H), 8.49–8.44 (m, 1H), 7.43–7.39 (m, 1H), 7.38–7.35 (m, 2H), 4.29 (t, J = 6.5 Hz, 2H), 3.97 (s, 3H), 3.75–3.69 (m, 4H), 2.81 (t, J = 6.5 Hz, 2H), 2.54–2.47 (m, 4H). ESI-MS: m/z $[M+H]^+$ 317. Anal. calcd. for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.86. Found: C, 64.71; H, 6.68; N, 8.68.

*Methyl 2-(1-(3-(1H-imidazol-1-yl)propyl)-1H-indol-3-yl)-2-oxoacetate (**3c**)*

According to the procedure used to prepare **3a**, reaction of **2a** with 1-(3-chloropropyl)-1*H*-imidazole provided **3c** in 52.8% yield as a light yellow solid, mp: 72–73°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.51–8.46 (m, 1H), 8.36 (s, 1H), 7.53 (s, 1H), 7.42–7.34 (m, 2H), 7.30–7.27 (m, 1H), 7.17 (s, 1H), 6.96 (s, 1H), 4.20 (t, J = 7.0 Hz, 2H), 4.00 (t, J = 7.0 Hz, 2H), 3.98 (s, 3H), 2.48–2.40 (m, 2H). ESI-MS: m/z $[M+H]^+$ 312. Anal. calcd. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.59; H, 4.88; N, 13.68.

*Methyl 2-oxo-2-(1-(3-(piperidin-1-yl)propyl)-1H-indol-3-yl)-acetate (**3d**)*

According to the procedure used to prepare **3a**, reaction of **2a** with 1-(3-chloropropyl)piperidine provided **3d** in 46.5% yield as a light yellow solid, mp: 66–67°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.47 (s, 1H), 8.20 (d, J = 7.5 Hz, 1H), 7.68 (d, J = 7.5 Hz, 1H), 7.39–7.27 (m, 2H), 4.34 (t, J = 7.0 Hz, 2H), 3.90 (s, 3H), 2.29–2.18 (m, 4H), 2.13 (t, J = 7.0 Hz, 2H), 1.96–1.90 (m, 2H), 1.55–1.41 (m, 4H), 1.40–1.30 (m, 2H). ESI-MS: m/z $[M+H]^+$ 329. Anal. calcd. for $C_{19}H_{24}N_2O_3$: C, 69.49; H, 7.37; N, 8.53. Found: C, 69.69; H, 7.68; N, 8.48.

*Methyl 2-(5-methoxy-1-(3-morpholinopropyl)-1H-indol-3-yl)-2-oxoacetate (**3e**)*

According to the procedure used to prepare **3a**, reaction of **2b** with 4-(3-chloropropyl)morpholine provided **3e** in 64.7% yield as a light yellow solid, mp: 67–68°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.38 (s, 1H), 7.95 (d, J = 2.5 Hz, 1H), 7.32 (d, J = 9.0 Hz, 1H), 6.97 (dd, J = 9.0, 2.5 Hz, 1H), 4.28 (t, J = 7.0 Hz, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 3.77–3.70 (m, 4H), 2.42–2.38 (m, 4H), 2.27 (t, J = 6.5 Hz, 2H), 2.06–2.01 (m, 2H). ESI-MS: m/z $[M+H]^+$ 361. Anal. calcd. for $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.77. Found: C, 63.49; H, 6.68; N, 7.49.

*Methyl 2-(6-chloro-1-(3-morpholinopropyl)-1H-indol-3-yl)-2-oxoacetate (**3f**)*

According to the procedure used to prepare **3a**, reaction of **2c** with 4-(3-chloropropyl)morpholine provided **3f** in 59.1% yield as a light yellow solid, mp: 115–116°C. ^{1}H NMR (500 MHz, $CDCl_3$):

δ 8.42 (s, 1H), 8.34 (d, J = 8.5 Hz, 1H), 7.49 (d, J = 1.5 Hz, 1H), 7.30 (dd, J = 8.5, 1.5 Hz, 1H), 4.28 (t, J = 6.5 Hz, 2H), 3.94 (s, 3H), 3.80–3.70 (m, 4H), 2.43–2.37 (m, 4H), 2.24 (t, J = 6.5 Hz, 2H), 2.04–2.00 (m, 2H). ESI-MS: m/z $[M+H]^+$ 365. Anal. calcd. for $C_{18}H_{21}ClN_2O_4$: C, 59.26; H, 5.80; N, 7.68. Found: C, 59.44; H, 5.78; N, 7.45.

*Methyl 2-(6-bromo-1-(3-morpholinopropyl)-1H-indol-3-yl)-2-oxoacetate (**3g**)*

According to the procedure used to prepare **3a**, reaction of **2d** with 4-(3-chloropropyl)morpholine provided **3g** in 54.9% yield as a light yellow solid, mp: 102–103°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.43 (s, 1H), 8.31 (d, J = 8.5 Hz, 1H), 7.67 (d, J = 1.5 Hz, 1H), 7.45 (dd, J = 8.5, 1.5 Hz, 1H), 4.29 (t, J = 6.5 Hz, 2H), 3.95 (s, 3H), 3.86–3.69 (m, 4H), 2.43–2.39 (m, 4H), 2.25 (t, J = 6.5 Hz, 2H), 2.05–2.01 (m, 2H). ESI-MS: m/z $[M+H]^+$ 409. Anal. calcd. for $C_{18}H_{21}BrN_2O_4$: C, 52.82; H, 5.17; N, 6.84. Found: C, 52.68; H, 5.26; N, 6.68.

*Methyl 2-(6-fluoro-1-(3-morpholinopropyl)-1H-indol-3-yl)-2-oxoacetate (**3h**)*

According to the procedure used to prepare **3a**, reaction of **2e** with 4-(3-chloropropyl)morpholine provided **3h** in 64.8% yield as a light yellow solid, mp: 116–117°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.44 (s, 1H), 8.38 (dd, J = 8.5, 5.5 Hz, 1H), 7.16 (dd, J = 9.0, 2.0 Hz, 1H), 7.13–7.06 (m, 1H), 4.27 (t, J = 6.5 Hz, 2H), 3.95 (s, 3H), 3.79–3.70 (m, 4H), 2.43–2.39 (m, 4H), 2.27 (t, J = 6.5 Hz, 2H), 2.06–2.00 (m, 2H). ESI-MS: m/z $[M+H]^+$ 349. Anal. calcd. for $C_{18}H_{21}FN_2O_4$: C, 62.06; H, 6.08; N, 8.04. Found: C, 62.18; H, 6.23; N, 8.25.

*Methyl 2-(1-(3-(tert-butyldimethylsilyloxy)propyl)-1H-indol-3-yl)-2-oxoacetate (**3i**)*

According to the procedure used to prepare **3a**, reaction of **2a** with (3-bromopropoxy) (*tert*-butyl)dimethylsilane provided **3i** in 62.5% yield as a light yellow solid, mp: 69–71°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40–8.36 (m, 1H), 8.31 (s, 1H), 7.39–7.34 (m, 1H), 7.31–7.24 (m, 2H), 4.27 (t, J = 7.0 Hz, 2H), 3.88 (s, 3H), 3.52 (t, J = 7.0 Hz, 2H), 2.06–1.92 (m, 2H), 0.87 (s, 9H), 0.01 (s, 6H). ESI-MS: m/z $[M+H]^+$ 349. Anal. calcd. for $C_{20}H_{29}NO_4Si$: C, 63.97; H, 7.78; N, 3.73. Found: C, 64.05; H, 7.86; N, 3.65.

*Methyl 2-(1-methyl-1H-indol-3-yl)-2-oxoacetate (**3j**)*

Seventy percent NaH (0.17 g, 4.9 mmol) was added portionwise to a solution of **2a** (1.0 g, 4.9 mmol) in DMF (10 mL) at 0–5°C. The reaction mixture was warmed to room temperature and stirred for 30 min. After that, iodomethane (0.85 g, 5.9 mmol) was added at 0°C and stirred for 1 h at room temperature. The mixture was then poured into cold water (100 mL) and extracted with ethyl acetate (3 × 50 mL). The organic phase was combined, washed with brine (3 × 150 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using petroleum ether/ethyl acetate (3:1 v/v) as eluent to afford 0.81 g (76.8%) **3j** as a light yellow solid, mp: 73–74°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.51–8.42 (m, 1H), 8.35 (s, 1H), 7.39–7.36 (m, 3H), 3.96 (s, 3H), 3.88 (s, 3H). ESI-MS: m/z $[M+H]^+$ 218. Anal. calcd. for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.55; H, 5.20; N, 6.59.

*1-(4-Bromobutyl)-1H-indole (**4**)*

Seventy percent NaH (1.77 g, 51.6 mmol) was added portionwise to a solution of indole (5.0 g, 43 mmol) in DMF (50 mL) at 0–5°C. The mixture was warmed to room temperature and stirred for 30 min. After that, it was added dropwise to a mixture of 1,4-dibromobutane (46.42 g, 215 mmol) in DMF (10 mL) at room temperature, and stirred for 12 h. Then the mixture was poured into cold water (300 mL) and extracted with ethyl acetate (3 × 100 mL). The organic phase was combined, washed with brine (3 × 300 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using petroleum ether/ethyl acetate (80:1 v/v) as eluent to afford 7.6 g (71.2%) **4** as a colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.66 (d, J = 8.0 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.25–7.22 (m, 1H), 7.14–7.11 (m, 2H), 6.53 (d, J = 3.0 Hz, 1H), 4.19 (t, J = 7.0 Hz, 2H), 3.40 (t, J = 6.5 Hz, 2H), 2.07–2.00 (m, 2H), 1.90–1.85 (m, 2H).

*4-(4-(1H-Indol-1-yl)butyl)morpholine (**5**)*

A mixture of **4** (2.0 g, 7.9 mmol), morpholine (6.9 g, 79.0 mmol), and potassium carbonate (1.9 g, 13.8 mmol) in DMF (30 mL) was stirred at 50°C for 6 h [29–37]. After cooling, the mixture was poured into cold water (200 mL) and extracted with ethyl acetate (3 × 100 mL). The organic phase was combined, washed with brine (3 × 300 mL), dried over Na_2SO_4, and concentrated in vacuum. The residue was purified by flash column chromatography on silica gel using petroleum ether/ethyl acetate/triethylamine (20:100:1 by volume) as eluent to afford 1.63 g (80.1%) **5** as a colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.65 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.24–7.21 (m, 1H), 7.14–7.11 (m, 2H), 6.51 (d, J = 3.0 Hz, 1H), 4.17 (t, J = 7.0 Hz, 2H), 3.75–3.70 (m, 4H), 2.39–2.33 (m, 6H), 1.93–1.87 (m, 2H), 1.57–1.50 (m, 2H). ESI-MS: m/z $[M+H]^+$ 259. Anal. calcd. for $C_{16}H_{22}N_2O$: C, 74.38; H, 8.58; N, 10.84. Found: C, 74.53; H, 8.64; N, 10.93.

*Methyl 2-(1-(4-morpholinobutyl)-1H-indol-3-yl)-2-oxoacetate (**3k**)*

One molar HCl in dioxane (5.5 mL, 5.5 mmol) was added to a solution of **5** (1.29 g, 5 mmol) in 30 mL CAN and 30 mL Et_2O at room temperature and stirred for 30 min. After that, oxalyl chloride (0.76 g, 6.0 mmol) in Et_2O (5 mL) was added dropwise at 0–5°C and the reaction mixture was stirred for 2 h at the same temperature. MeOH (10 mL) was added dropwise to the mixture at 0–5°C and the result solution was stirred for 2 h at room temperature, then poured into a cold aqueous $NaHCO_3$ solution and extracted with ethyl acetate (3 × 50 mL). The organic phase was combined, washed with brine (150 mL), dried over Na_2SO_4, and concentrated in vacuum. The residue was purified by flash column chromatography on silica gel using ethyl acetate/methanol (50:1 v/v) as eluent to afford 0.94 g (54.6%) **5** as a light yellow solid, mp: 84–85°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.48–8.44 (m, 1H), 8.40 (s, 1H), 7.44–7.38 (m, 1H), 7.39–7.34 (m, 2H), 4.23 (t, J = 7.5 Hz, 2H), 3.97 (s, 3H), 3.73–3.65 (m, 4H), 2.39–2.34 (m, 6H), 2.02–1.92 (m, 2H), 1.59–1.51 (m, 2H). ESI-MS: m/z $[M+H]^+$ 345. Anal. calcd. for $C_{19}H_{24}N_2O_4$: C, 66.26; H, 7.02; N, 8.13. Found: C, 66.40; H, 7.11; N, 8.32.

*3-(1H-Indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**10**)*

A solution of *t*-BuOK (134 mg, 1.2 mmol) in THF was added dropwise to a solution of **2a** (106 mg, 0.52 mmol) and **9**

(50 mg, 0.4 mmol) in THF (20 mL) at −5 to 0°C. After stirring for 2 h at at room temperature, 5 mL concentrated hydrochloric acid was added and the result mixture was stirred for 30 min at room temperature, then poured into 10% $NaHCO_3$ aqueous solution (100 mL) and extracted with ethyl acetate (3 × 50 mL). The organic phase was combined and washed with brine (3× 150 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using dichloromethane/methanol (50:1 v/v) as eluent to afford 12.4 mg (11.2%) **10** as a red solid, mp: 169–170°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.85 (brs, 1H), 11.28 (brs, 1H), 10.73 (brs, 1H), 7.66 (d, J = 3.0 Hz, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.34 (brs, 1H), 7.11 (t, J = 8.0 Hz, 1H), 6.98 (d J = 8.0 Hz, 1H), 6.91 (t, J = 8.0 Hz, 1H), 6.67 (brs, 1H), 6.08 (brs, 1H). ESI-MS: m/z $[M+H]^+$ 278. Anal. calcd. for $C_{16}H_{11}N_3O_2$: C, 69.31; H, 4.00; N, 15.15. Found: C, 69.55; H, 4.11; N,15.02.

*3-(1-(3-Morpholinopropyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11a**)*

A solution of *t*-BuOK (134 mg, 1.2 mmol) in THF was added dropwise to a solution of **3a** (172 mg, 0.52 mmol) and **9** (50 mg, 0.4 mmol) in THF (20 mL) at −5 to 0°C. After stirring for 2 h at room temperature, 5 mL concentrated hydrochloric acid was added and the result mixture was stirred for 30 min at room. The mixture was then poured into 10% $NaHCO_3$ aqueous solution (100 mL) and extracted with ethyl acetate (3 × 50 mL). The organic phase was combined and washed with brine (3 × 150 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using dichloromethane/methanol/triethylamine (90:3:1 by volume) as eluent to afford 20.7 mg (12.8%) **11a** as a red solid, mp: 133–135°C. ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6): δ 10.19 (brs, 1H), 9.47 (brs, 1H), 7.62 (s, 1H), 7.44–7.42 (m, 2H), 7.19 (t, J = 8.0 Hz, 1H) 7.09 (d, J = 8.0 Hz, 1H), 6.97 (t, J = 8.0 Hz, 1H), 6.62 (brs, 1H), 6.27 (brs, 1H), 4.32 (t, J = 7.0 Hz, 2H), 3.76–3.72 (4H, m), 2.48–2.45 (4H, m), 2.35 (t, J = 7.0 Hz, 2H), 2.07–2.05 (2H, m). ESI-MS: m/z $[M+H]^+$ 405. Anal. calcd. for $C_{23}H_{24}N_4O_3$: C, 68.30; H, 5.98; N, 13.85. Found: C, 68.53; H, 6.03; N, 14.13.

*3-(1-(2-Morpholinoethyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11b**)*

According to the procedure used to prepare **11a**, reaction of **3b** with **9** provided **11b** in 14.5% yield as a red solid, mp: 212–214°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.26 (brs, 1H), 10.75 (brs, 1H), 7.74 (s, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.37 (brs, 1H) 7.16 (t, J = 8.5 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 6.93 (t, J = 8.0 Hz, 1H), 6.27 (brs, 1H), 6.10 (brs, 1H), 4.38 (t, J = 7.0 Hz, 2H), 3.56–3.52 (m, 4H), 2.71 (t, J = 7.0 Hz, 2H), 2.47–2.43 (m, 4H). ESI-MS: m/z $[M+H]^+$ 391. Anal. calcd. for $C_{22}H_{22}N_4O_3$: C, 67.68; H, 5.68; N, 14.35. Found: C, 67.51; H, 5.82; N, 14.19.

*3-(1-(3-(1H-Imidazol-1-yl)propyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11c**)*

According to the procedure used to prepare **11a**, reaction of **3c** with **9** provided **11c** in 11.2% yield as a red solid, 234–236°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.22 (brs, 1H), 10.79 (brs, 1H), 7.75–7.68 (m, 2H), 7.48 (d, J = 7.5 Hz, 1H), 7.37 (s, 1H), 7.28 (brs, 1H), 7.18 (t, J = 7.5 Hz, 1H), 6.90–6.70 (3H, m), 6.67 (brs, 1H), 6.07 (brs, 1H), 4.26 (t, J = 7.0 Hz, 2H), 4.03 (t, J = 7.0 Hz, 2H), 2.35–2.30 (2H, m), ESI-MS: m/z $[M+H]^+$ 386. Anal. calcd. for $C_{22}H_{19}N_5O_2$: C, 68.56; H, 4.97; N, 18.17. Found: C, 68.41; H, 4.83; N, 18.33.

*3-(1-(3-(Piperidin-1-yl)propyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11d**)*

According to the procedure used to prepare **11a**, reaction of **3d** with **9** provided **11d** in 10.6% yield as a red solid, mp: 235–237°C. ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6): δ 11.20 (brs, 1H), 10.75 (brs, 1H), 7.72 (s, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.36 (brs, 1H), 7.17 (t, J = 8.0 Hz, 1H), 6.95–6.98 (m, 2H), 6.67 (brs, 1H), 6.07 (brs, 1H), 4.33 (t, J = 7.0 Hz, 2H), 2.39–2.34 (m, 6H), 2.09–2.07 (m, 2H), 1.62–1.59 (4H, m), 1.42–140 (m, 2H). ESI-MS: m/z $[M+H]^+$ 403. Anal. calcd. for $C_{24}H_{26}N_4O_2$: C, 71.62; H, 6.51; N, 13.92. Found: C, 71.49; H, 6.71; N, 13.86.

*3-(5-Methoxy-1-(3-morpholinopropyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11e**)*

According to the procedure used to prepare **11a**, reaction of **3e** with **9** provided **11e** in 15.6% yield as a red solid, mp: 186–188°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.44 (brs, 1H), 7.72 (s, 1H), 7.42 (brs, 1H), 7.37 (brs, 1H), 7.30 (d, J = 8.5 Hz, 1H), 6.85 (dd, J = 8.5, 2.0 Hz, 1H), 6.72 (d, J = 2.0 Hz, 1H), 6.44 (brs, 1H), 6.38 (brs, 1H), 4.29 (t, J = 7.0 Hz, 2H), 3.77–3.72 (m, 4H), 3.50 (s, 3H), 2.47–2.35 (m, 6H), 2.09–2.06 (2H, m). ESI-MS: m/z $[M+H]^+$ 433. Anal. calcd. for $C_{24}H_{26}N_4O_4$: C, 66.34; H, 6.03; N, 12.89. Found: C, 66.42; H, 6.09; N, 12.62.

*3-(6-Chloro-1-(3-morpholinopropyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11f**)*

According to the procedure used to prepare **11a**, reaction of **3f** with **9** provided **11f** in 18.5% yield as a red solid, mp: 102–104°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.55 (brs, 1H), 7.81 (brs, 1H), 7.65 (s, 1H), 7.51 (brs, 1H), 7.45 (s, 1H), 6.98–6.92 (m, 2H), 6.66 (brs, 1H), 6.25 (brs, 1H), 4.27 (t, J = 7.0 Hz, 2H), 3.80–3.72 (m, 4H), 2.48–2.40 (m, 4H), 2.31 (t, J = 7.0 Hz, 2H), 2.07–2.04 (m, 2H). ESI-MS: m/z $[M+H]^+$ 439. Anal. calcd. for $C_{23}H_{23}ClN_4O_3$: C, 62.94; H, 5.28; N, 12.77. Found: C, 66.71; H, 5.35; N, 12.62.

*3-(6-Bromo-1-(3-morpholinopropyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11g**)*

According to the procedure used to prepare **11a**, reaction of **3g** with **9** provided **11g** in 13.8% yield as a red solid, mp: 83–85°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.47 (brs, 1H), 7.69–7.65 (m, 2H), 7.53 (s, 1H), 7.40 (brs, 1H), 7.01 (d, J = 8.5 Hz, 1H), 6.93 (d, J = 8.5 Hz, 1H), 6.68 (brs, 1H), 6.27 (brs, 1H), 4.29 (t, J = 7.0 Hz, 2H), 3.83–3.75 (m, 4H), 2.49–2.43 (m, 4H), 2.31 (t, J = 7.0 Hz, 2H), 2.08–2.03 (m, 2H). ESI-MS: m/z $[M+H]^+$ 483. Anal. calcd. for $C_{23}H_{23}BrN_4O_3$: C, 57.15; H, 4.80; N, 11.59. Found: C, 57.29; H, 4.89; N, 11.78.

*3-(6-Fluoro-1-(3-morpholinopropyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11h**)*

According to the procedure used to prepare **11a**, reaction of **3h** with **9** provided **11h** in 14.9% yield as a red solid, mp: 149–150°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.49 (brs, 1H), 7.67 (s, 1H), 7.58 (brs, 1H), 7.53 (brs, 1H), 7.13 (dd, J = 9.0, 2.5 Hz, 1H), 7.01–6.98 (m, 1H), 6.76 (td, J = 9.0, 2.5 Hz, 1H), 6.67 (brs, 1H), 6.27 (brs, 1H), 4.27 (t, J = 7.0 Hz, 2H), 3.80–3.74 (m, 4H), 2.48–2.42 (m, 4H), 2.34 (t, J = 7.0 Hz, 2H), 2.08–2.04 (m, 2H). ESI-MS: m/z $[M+H]^+$ 423.

Anal. calcd. for $C_{23}H_{23}FN_4O_3$: C, 65.39; H, 5.49; N, 13.26. Found: C, 65.58; H, 5.56; N, 13.03.

*3-(1-(3-Hydroxypropyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11i**)*

According to the procedure used to prepare **11a**, reaction of **3i** with **9** provided **11i** in 15.6% yield as a red solid, mp: 160–162°C. ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6): δ 10.23 (brs, 1H), 9.79 (brs, 1H), 7.33 (s, 1H), 7.14–7.12 (m, 2H), 6.87 (t, J = 8.0 Hz, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.64 (t, J = 8.0 Hz, 1H), 6.30 (brs, 1H), 5.97 (brs, 1H), 4.05–4.02 (m, 3H), 3.29–3.27 (m, 2H), 1.78–1.76 (m, 2H). ESI-MS: m/z $[M+H]^+$ 336. Anal. calcd. for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11; N, 12.53. Found: C, 68.26; H, 5.18; N, 12.70.

*3-(1-Methyl-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11j**)*

According to the procedure used to prepare **10**, reaction of **3j** with **9** provided **11j** in 25.2% yield as a red solid, mp: 179–181°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.18 (brs, 1H), 10.75 (brs, 1H), 7.69 (s, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.36 (brs, 1H), 7.17 (t, J = 8.0 Hz, 1H), 6.98–6.95 (m, 2H), 6.68 (brs, 1H), 6.08 (brs, 1H), 3.89 (s, 3H). ESI-MS: m/z $[M+H]^+$ 292. Anal. calcd. for $C_{19}H_{17}N_3O_3$: C, 70.09; H, 4.50; N, 14.42. Found: C, 70.26; H, 4.48; N, 14.61.

*3-(1-(4-Morpholinobutyl)-1H-indol-3-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**11k**)*

According to the procedure used to prepare **11a**, reaction of **3k** with **9** provided **11k** in 12.6% yield as a red solid, m.p. 135–137°C. ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6): δ 9.90 (brs, 1H), 9.27 (brs, 1H), 7.49 (s, 1H) 7.38 (brs, 1H), 7.19 (d, J = 8.0 Hz, 1H), 7.08 (t, J = 8.0 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 6.87 (t, J = 8.0 Hz, 1H), 6.52 (brs, 1H), 6.16 (brs, 1H), 4.12 (t, J = 7.0 Hz, 2H), 3.59–3.50 (m, 4H), 2.35–2.20 (m, 6H), 1.85–1.82 (m, 2H), 1.49–1.45 (m, 2H). ESI-MS: m/z $[M+H]^+$ 419. Anal. calcd. for $C_{24}H_{26}N_4O_3$: C, 68.88; H, 6.26; N, 13.39. Found: C, 68.65; H, 6.29; N, 13.55.

*3-Phenyl-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**13a**)*

According to the procedure used to prepare **10**, reaction of **6** with **12a** provided **13a** in 30.6% yield as an orange solid, m.p. 50°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.35 (brs, 1H), 10.87 (brs, 1H), 7.52 (brs, 1H), 7.47–7.42 (5H, m), 6.99 (brs, 1H), 6.73 (brs, 1H). ESI-MS: m/z $[M+H]^+$ 239. Anal. calcd. for $C_{14}H_{10}N_2O_2$: C, 70.58; H, 4.23; N, 11.76. Found: C, 70.83; H, 4.28; N, 11.64.

*3-(Naphthalen-1-yl)-4-(1H-pyrrol-3-yl)-1H-pyrrole-2,5-dione (**13b**)*

According to the procedure used to prepare **10**, reaction of **6** with **12b** provided **13b** in 24.8% yield as an orange solid, m.p. 239–241°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.60 (brs, 1H), 10.26 (brs, 1H), 7.94–7.92 (m, 2H), 7.70 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.51–7.48 (m, 3H), 7.41 (t, J = 8.0 Hz, 1H), 6.46 (brs, 1H), 5.72 (brs, 1H). ESI-MS: m/z $[M+H]^+$ 289. Anal. calcd. for $C_{18}H_{12}N_2O_2$: C, 74.99; H, 4.20; N, 9.72. Found: C, 74.76; H, 4.31; N, 9.91.

*3-(1H-Imidazol-1-yl)-4-(1-(3-morpholinopropyl)-1H-indol-3-yl)-1H-pyrrole-2,5-dione (**15a**)*

According to the procedure used to prepare **11a**, reaction of **6** with **14a** provided **13b** in 10.6% yield as an orange solid, m.p. 222–224°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.88 (s, 1H), 7.76 (brs, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.25–7.21 (m, 2H), 7.15 (s, 1H), 6.98 (t, J = 8.0 Hz, 1H), 6.35 (d, J = 8.0 Hz, 1H), 4.35 (t, J = 7.0 Hz, 2H), 3.82–3.75 (4H, m), 2.48–2.40 (4H, m), 2.34 (t, J = 7.0 Hz, 2H), 2.09–2.01 (m, 2H). ESI-MS: m/z $[M+H]^+$ 406. Anal. calcd. for $C_{22}H_{23}N_5O_3$: C, 65.17; H, 5.72; N, 17.27. Found: C, 65.28; H, 5.81; N, 17.16.

*3-(1-(3-Morpholinopropyl)-1H-indol-3-yl)-4-(1H-1,2,4-triazol-1-yl)-1H-pyrrole-2,5-dione (**15b**)*

According to the procedure used to prepare **11a**, reaction of **6** with **14a** provided **15b** in 9.6% yield as an orange solid, m.p. 94–96°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.45 (brs, 1H), 8.88 (s, 1H), 8.26 (s, 1H), 8.22 (s, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.18 (t, J = 8.0 Hz, 1H), 6.86 (t, J = 8.0 Hz, 1H), 6.12 (d, J = 8.0 Hz, 1H), 4.36 (t, J = 6.5 Hz, 2H), 3.62–3.56 (m, 4H), 2.37–2.28 (m, 4H), 2.21 (t, J = 6.5 Hz, 2H), 1.96–1.90 (m, 2H). ESI-MS: m/z $[M+H]^+$ 407. Anal. calcd. for $C_{21}H_{22}N_6O_3$: C, 62.06; H, 5.46; N, 20.68. Found: C, 62.21; H, 5.31; N, 20.55.

Pharmacology

GSK-3β purification and activity assay

The GSK-3β cDNA was obtained from UniGene (3667B03) and inserted into the pGEX-KG vector. The recombinant GST-GSK-3β protein was expressed in *Escherichia coli* strain BL21-CodonPlus (DE3), purified by GSTrap affinity chromatography, and cleaved by thrombin. The GSK-3β kinase assay was carried out with the Invitrogen Z'-LYTETM Kinase Assay kit (Ser/Thr 9 Peptide substrate), with a final enzyme concentration of 50 nM. All reactions were carried out in triplicate. IC_{50} values (concentration at which 50% of enzyme inhibition is shown) were derived from a non-linear regression model (curvefit) based on a sigmoidal dose response curve (variable slope) and computed using Graghpad Prism version 5.02, Graphpad Software. Data were expressed as mean ± SEM.

Cell culture and Western blot

SH-SY5Y human neuroblastoma cells were obtained from ATCC (The American Type Culture Collection). Cells were cultured in 1:1 DMEM/Ham's F12 containing 10% v/v fetal bovine serum (HyClone), 1% penicillin, and 1% streptomycin at a humidified atmosphere with 5% CO_2. The medium was changed every 2 days. For experiments, cells were grown in 12-well plates until ~80% confluence, serum-deprived for 12 h, incubated with GSK-3β inhibitors for 1 h and $A\beta_{25-35}$ (amyloid beta peptide 25–35, Sigma) for another 6 h. Cells were rinsed twice with ice-cold PBS and lysed with 1× SDS loading buffer. Samples were electrophoresed on 10% SDS–polyacrylamide gels, and transferred to PVDF membranes. The membranes were blocked for 1 h with 5% w/v milk, incubated with rabbit anti-Tau [pS396] phosophospecific antibody (Abcam) for 2 h and the anti-rabbit secondary antibody for 1 h. Antigen–antibody complexes were detected by the ECL Kit.

PKCE, IKK2, Aurora A, MEK1, and ERK1 assays

The recombinant PKCE and IKK2 were expressed in the Bac-to-Bac baculovirus system, and recombinant Auroa A, MEK1, and ERK1 were expressed in the *E. coli* system. All these kinase assays were carried out by using the Invitrogen Z'-LYTETM Kinase Assay kits.

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