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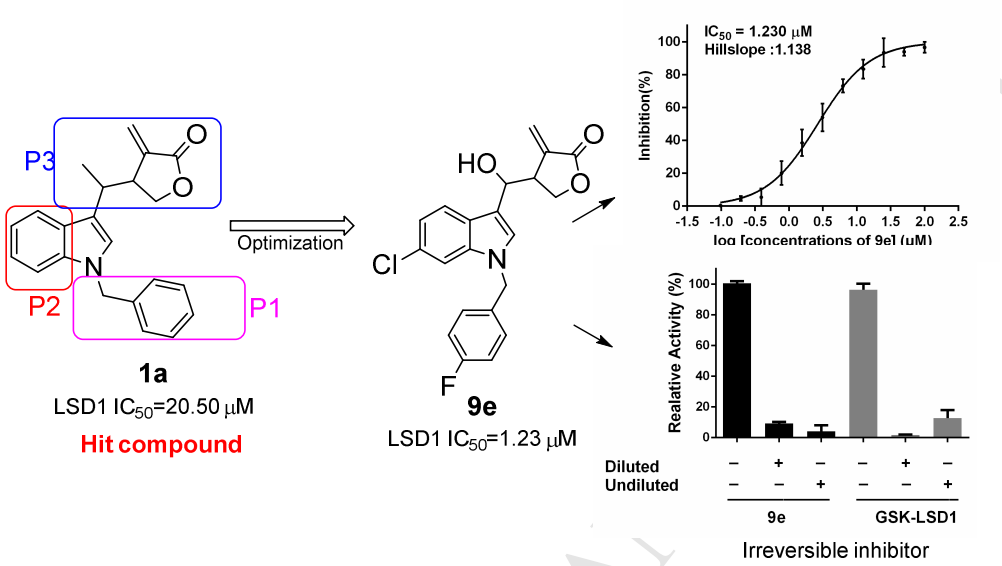

Discovery and synthesis of novel indole derivatives-containing 3-methylenedihydrofuran-2(3H)-one as irreversible LSD1 inhibitors

Hong-Min Liu [a], Feng-Zhi Suo [a], Xiao-Bo Li [a], Ying-Hua You [a], Chun-Tao Lv [a], Chen-Xing Zheng [a], Guo-Chen Zhang [a], Yue-Jiao Liu [a], Wen-Ting Kang [a], Yi-Chao Zheng [a,b,#], Hai-Wei Xu [a,#]

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Abstract: Lysine-specific demethylase 1 (LSD1), demethylase against mono- and di -methylated histone3 lysine 4 , has emerged as a promising target in oncology. More specifically, it has been demonstrated as a key promoter in acute myeloid leukemia (AML), and several LSD1 inhibitors have already entered into clinical trials for the treatment of AML. In this paper, a series of new indole derivatives were designed and synthesized based on a lead compound obtained by a high-throughput screening with our in-house compound library. Among the synthetic compounds, **9e** was characterized as a potent LSD1 inhibitor with an IC_{50} of 1.230 μM and can inhibit the proliferation of THP-1 cells effectively. And most importantly, this is the first irreversible LSD1 inhibitor that is not derived from monoamine oxidase inhibitors. Hence, the discovery of **9e** may serve as a proof of concept work for AML treatment.

Keywords: LSD1; irreversible; inhibitor; indole; lactone

1. Introduction

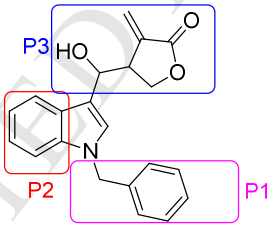
Epigenetic is the study of heritable changes in gene function that does not involve changes in DNA sequence. Example of mechanisms that produce such changes is histone modification. Histones, where multiple signaling pathways converge on, are small basic proteins in eukaryotic cell nucleus that package and order the DNA into nucleosomes, which are the fundamental units of eukaryotic chromatin [1, 2]. It can be subjected to a diverse array of post-translational modifications, such as methylation, acetylation, phosphorylation and ubiquitination, and these modifications contribute to dysregulation of target genes by modeling chromatin structure precisely [1, 3]. Among these modifications, methylated histone can be demethylated by specific erasers, such as LSD1, which is the first identified histone demethylase with flavin adenine dinucleotide (FAD) as a cofactor to demethylate mono- and di- methylation of H3K4 and H3K9 [4, 5], and it plays a vital role in several kinds of disease, such as cancer [6, 7].

As reported, LSD1 is overexpressed in a variety of tumors, such as lung cancer[8],

neuroblastoma [9], gastric cancer [10], retinoblastoma [11], breast cancer [12], and leukemia [13], and it has been considered as a potential cancer therapeutic target. Hence, many medicinal chemists tried to design and synthesize potent and selective LSD1 inhibitors. As LSD1 is a homology of monoamine oxidase (MAO) and shares similar catalytic mechanisms as MAO [14], MAO inhibitors, such as *trans*-2-phenylcyclopropylamine (TCP), were used as scaffolds for further development. Until now, tens of TCP based LSD1 inhibitors have been identified, but only six of TCP based LSD1 inhibitors have entered into clinic trials alone or in combination with other therapeutics, including ORY-1001 (Fig. 2A), ORY2001, GSK2879552 (Fig. 2A), CC-90011, IMG-7289 and INCB059872 [15, 16]. And all of them are applied for the treatment of AML as well as myelofibrosis as LSD1 is a central regulator of hematopoietic stem cell and progenitor cell [17], and it can also regulate the expression of a variety of leukemia subtype oncogenes [18-23]. Hence, extensive study on irreversible inhibitors of LSD1 with different backbone is urgently needed. Here, we reported the first irreversible LSD1 inhibitor **9e**, that is not derived from monoamine oxidase inhibitors. **9e** is the most potent one among the series of new indole derivatives we got and can inactivate LSD1 with IC_{50} value of 1.230 μM. In THP-1 cells, compound **9e** blurred cell membrane boundaries and shrank chromatin, inhibited the intracellular activity of LSD1, meanwhile, compound **9e** inhibited cell proliferation ability and colony formation dose dependently. In a nutshell, compound **9e** is a potent irreversible inhibitor of LSD1 with fully novel skeleton, which can provide a promising strategy for the development of irreversible LSD1 inhibitors for AML treatment.

2. Results and discussion

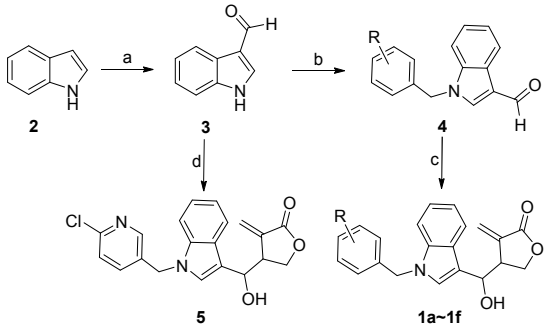
2.1. Chemistry



1a

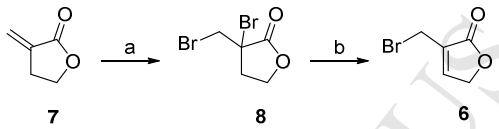
Fig.1 The structure of compound **1a**.

Recent years, our groups focused on the synthesis and bioactive research of butenolide derivatives [24, 25]. Based on our previous work on LSD1, an in-house compound library screening against LSD1 was done to discover LSD1 inhibitor. Compound **1a**, an indole derivative including butenlide moiety was obtained with IC_{50} of 20.50 μM against LSD1. With compound **1a** as a hit, series of novel indole derivatives were designed, synthesized to study the SAR by modification at the P1, P2, and P3 on compound **1a** (Fig 1).



Reagents and conditions: (a) DMF, $POCl_3$, 0°C, 98%; (b) NaH, MeCN, Benzyl chloride derivatives, r.t. 50% ~ 90%; (c) THF, Zn, NH_4Cl, 3 compound 6, r.t., 68%~85%. (d) (1) NaH, MeCN, 2-chloro-5- (chloromethyl)pyridine, r.t.; (2) THF, Zn, NH_4Cl, compound **6**, r.t. 66% for two steps.

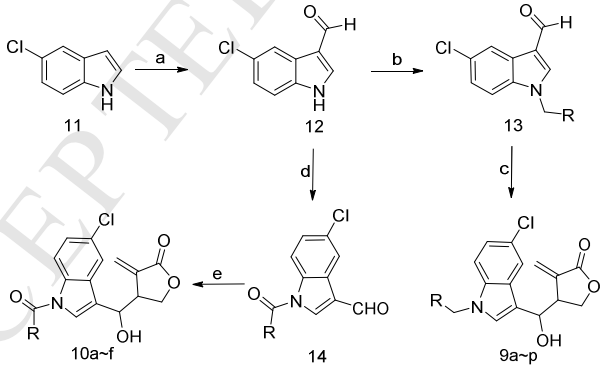
Scheme 1 Synthesis of compounds**1a~1f**.



Reagents and conditions: (a) $Me_3PhN^+Br_3$, 1,4-dioxane, r.t. 15h, 87.6%; (b) Li_2CO_3/LiBr, TBAB, MeCN, 85°C, 48.2%.

Scheme 2 Synthesis of intermediate **6.**

Firstly, compounds **1b-f** were designed to investigate the evaluation of benzyl groups of P1 at N1. Compounds **1b~1f** with substituted phenyl groups at N1 were synthesized by the formylation reaction, *N*-benzylation reaction and Blaise reaction with indole as starting material as shown in Scheme 1. The key intermediate bromolactone **6** was obtained from the commercially available tulipalin (**7)** by bromination reaction followed by regioselective elimination (Scheme 2).



ID	R	ID	R
9a		9l	
9b		9m	
9c		9n	
9d		9o	
9e		9p	
9f		10a	
9g		10b	
9h		10c	
9i		10d	

9j		**10e**	
9k		**10f**	

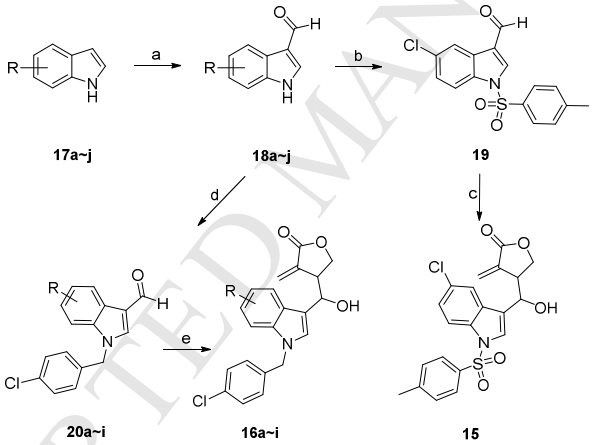
Reagents and conditions: (a) DMF, $POCl_3$, 0°C, 95%; (b) NaH, MeCN, aryl or alkyl methylene chloride, r.t. 50%~90%; (c) THF, Zn, NH_4Cl, compound **6**, r.t. 43~76%; (d) NaH, MeCN, acyl chloride, r.t. 60%~85%; (e) THF, Zn, NH_4Cl, compound **6**, r.t. 69%~80%.

Scheme 3. Synthesis of compounds **9a~9p** and compounds **10a~10f**.

Compounds **5** and **9a~9p** were designed to compare the heterocyclic methylene substitution with benzyl groups at N1. Compounds **9a~9o** were prepared by the same method to prepare compound **1** with 5-choloride indole as material(Scheme 3)**.**

In order to compare the methylene and formyl groups for P1, compounds **10a~10g** were designed and synthesized. Similarly, the *N*-acylation group was introduced at N1 on indole to yield compounds **10a~10g** (Scheme 3).

Keeping the P1 and P3, compounds **16a~16i** were designed to evaluate the contribution of various substitution such as chlorine, fluorin, and methoxyl groups at different position of indole phenyl group of P2. They were synthesized with substituted indole as raw materials by the reactions used as below (Scheme 4).



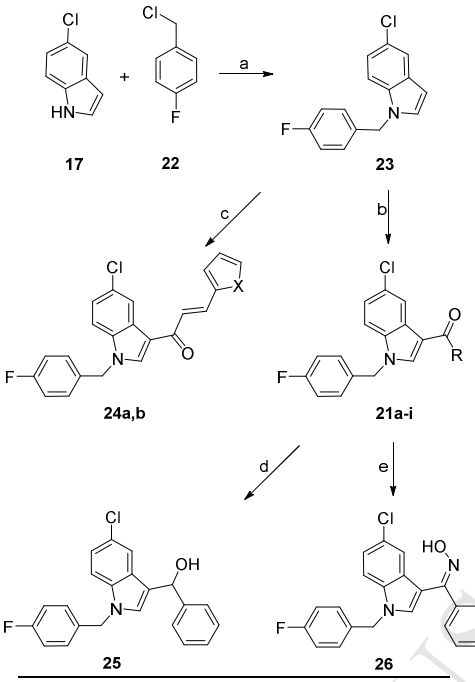
a: R= 4-OCH_3; **b:** R= 5-OCH_3; **c:** R= 6-OCH_3; **d:** R= 7-OCH_3;
e: R= 4-Cl; **f:** R= 5-Cl; **g:** R= 6-Cl; **h:** R= 7-Cl; **i:** R= 5-F;

Reagents and conditions. (a) DMF, $POCl_3$, 0°C, >90%; (b) NaH, MeCN, PTSC, r.t. 76.14%; (c) THF, Zn, NH_4Cl, compound **6**, r.t. 69%; (d) NaH, MeCN, 4-Chlorobenzyl chloride, r.t. 50%-90%; (e) THF, Zn, NH_4Cl, compound **6**, r.t. 60% ~ 83%.

Scheme 4 Synthesis of compounds **15,16a~i**.

Compound **15** was designed and synthesized to prove whether the sulfonyl group with tetrahedral pose is better than that of formyl groups with plane trigonometry to link to N1 for the LSD1 inhibition (Scheme 4).

In order to test the importance of the 4-(1-hydroxyethyl)-3-methylene dihydrofuran -2(3H)-one group at C3 (P3) on core compound **1a**, compounds **21, 24, 25** and **26,** in which the P3 was displaced with phenyl, furan, pyridinyl groups and so on, were designed and synthesized as shown in Scheme 5.



ID	R	ID	R/X
21a	CH_3	**21g**	
21b		**21h**	
21c		**21i**	
21d		**24a**	**O**
21e		**24b**	**S**
21f			

Reagents and conditions: (a) NaH, MeCN, r.t. 93.96%; (b) CH_2Cl_2, $AlCl_3$, Acyl chloride derivatives, r.t. 70%~85%; (c) (1) CH_2Cl_2, $AlCl_3$, Acetyl chloride, r.t. 78%; (2) MeOH, NaOH (aq), 2-Furfural or 2-Thenaldehyde, r.t. 68%; (d) MeOH, $NaBH_4$, r.t. 70%; (e) EtOH/Pyridine, $NH_2OH \cdot HCl$, 80°C, 90%.

Scheme 5 Synthesis of compounds **21a~i, 24a, 24b, 25** and **26**

2.2 Inhibitory Activity against LSD1 Recombinant

Table 1 LSD1 inhibition of synthetic compounds

ID	IC_{50}/μM	ID	IC_{50}/μM	ID	IC_{50}/μM
1a	20.497±1.312	**9l**	>10	**16g**	>10
1b	4.467±0.650	**9m**	>10	**16h**	>10
1c	8.959±0.952	**9n**	>10	**16i**	>10
1d	5.849±0.767	**9o**	>10	**21a**	>10
1e	9.469±0.976	**9p**	>10	**21b**	>10
1f	6.580±0.818	**10a**	>10	**21c**	>10
5	5.903±0.771	**10b**	>10	**21d**	>10
9a	>10	**10c**	>10	**21e**	>10
9b	>10	**10d**	>10	**21f**	>10
9c	>10	**10e**	>10	**21g**	>10
9d	>10	**10f**	>10	**21h**	>10
9e	1.230±0.159	**15**	1.784±0.249	**21i**	>10
9f	>10	**16a**	>10	**24a**	>10
9g	>10	**16b**	>10	**24b**	>10
9h	>10	**16c**	>10	**25**	>10

9i	8.344±0.921	**16d**	>10	**26**	>10
9j	8.410±0.925	**16e**	>10	**GSK-LSD1**	0.024±0.001
9k	>10	**16f**	>10		

The LSD1 inhibitory activities of all synthesized compounds were evaluated by the previously reported high-throughput method of our group [10]. Some of them showed inhibition against LSD1 (Table 1). Among them, compounds **1b~1f, 5, 9e, 9i, 9j** containing benzyl groups or pyridinyl methyl group at N1 of indole with IC_{50} of from 9.5μM to 1.2μM were more active than hit compound **1a**, of which **9e** with 5-chloro and N-(4-F-benzyl) indole was the best one with IC_{50} of 1.23 μM. However, compounds **9** and **10** with 5-chloro and N-aryl or alkyl methyl indole or 5-chloro and N-aryl or alkyl formyl indole showed more weak LSD1 inhibitory activity than compound **9e.** Compound **15** with 1-tosyl indole derivative, an analog of compounds **10,** showed similar LSD1 inhibition activity with IC_{50} of 1.78μM. Keeping the P3, compounds **16** with introducing different substitutions such as chlorine, fluorin, and methoxyl groups resulted in the decreasing of LSD1 inhibition of hit compound **1a**. Unfortunately, all the compounds **21**, **24, 25** and **26** without the lactone moiety P3 at C-3 position of indole compare to compound **2b** showed no inhibition against LSD1 at 10μM. Based on the above, compound **9e** was chosen for further study.

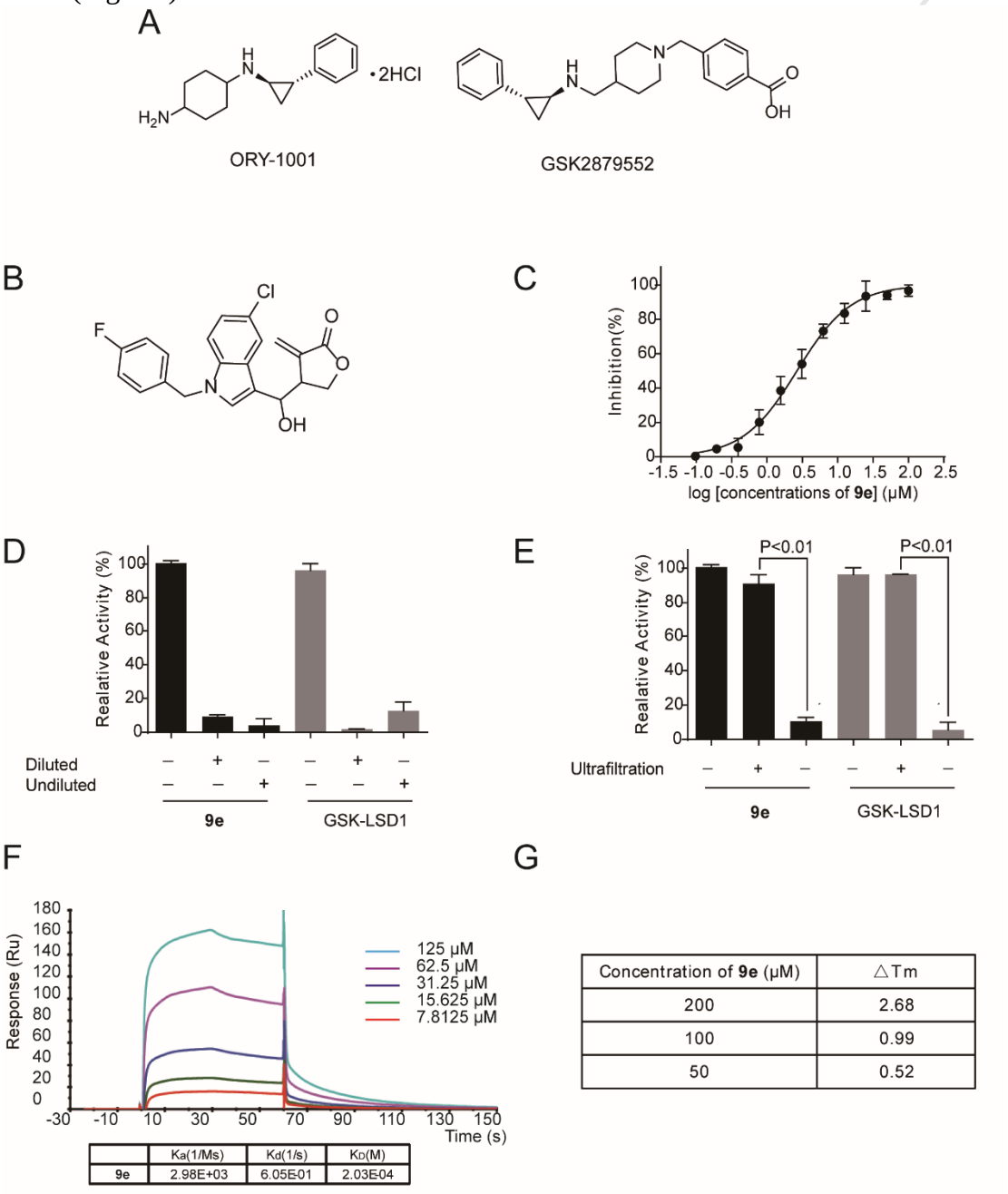
The SAR suggests that the benzyl groups at N1 and the 4-(1-hydroxyethyl)-3-methylenedihydrofuran -2(3H)-one group at C3 play important roles for their LSD1 inhibition of title compounds. The 4-(1-hydroxyethyl)-3-methylenedihydrofuran -2(3H)-one group at C3 could not be replaced by other groups. The compounds with benzyl groups or benzenesulfonyl group at N1 showed better LSD1 inhibition than that of benzoyl and heterocyclic formyl substitution derivatives, which tell us that the phenyl groups and the tetrahedral configuration of the link atom to N1 from phenyl groups are crucial for their activities.

2.3 Compound 9e can directly bind and irreversibly inactivate LSD1

To further understand the binding model of **9e** to LSD1 (Fig. 2B), several biochemical assay and biophysical experiment were performed. As shown in Figure 2C, compound **9e** inactivated LSD1 with IC_{50} = 1.230 μM (Hillslope = 1.138) , while GSK LSD1 was used as a positive control with IC_{50} = 21.840 nM (Hillslope = 1.211) (Fig. 4A). To characterize the binding model of compound **9e** to LSD1, including the binding reversibility of compound **9e** to LSD1, a dilution assay was performed (Fig. 2D). We found that not only can the irreversible positive compound GSK-LSD1 inactivate LSD1 even after being diluted by 80 folds, compound **9e** can also. This result indicates that compound **9e** may be an irreversible inhibitor. In order to further confirm the inhibition behavior of compound **9e**, we used the ultrafiltration assay, which also showed that compound **9e** may inhibit LSD1 in an irreversible manner (Fig. 2E).

To further confirm the direct interaction between compound **9e** and LSD1, surface plasmon resonance (SPR) was applied. As shown in Figure 2F, compound **9e** introduced a high signal value for the binding curve which indicated that this inhibitor can directly interact with LSD1 strongly with K_D of 2.03E-04 M. And positive control GSK-LSD1 got a similar affinity with K_D of 4.25E-04 M (Fig. 4B). For the further test of the target engagement of compound **9e** to LSD1, protein thermal shift assay, which has been extensively used in drug screening [26-28], was performed. The

results showed that Tm, which is the temperature at protein half denatured to show the protein thermal stabilization, in sample groups (containing LSD1 and compound **9e**) was higher than the reference group (containing LSD1 and DMSO) in a concentration dependent manner (Fig. 2G). As we know, a higher Tm represents a more stable protein, indicating an interaction between protein and compound. So, the result implied that compound **9e** can bind well to LSD1. When performing the same assay on GSK-LSD1, it also showed a concentration dependent manner to increase Tm value (Fig. 4C).



	Ka(1/Ms)	Kd(1/s)	KD(M)
9e	2.98E+03	6.05E-01	2.03E-04

Concentration of **9e** (μM)	△Tm
200	2.68
100	0.99
50	0.52

Fig. 2 Properties of compound **9e** against LSD1 recombinant. (A) Representative inhibitors for LSD1 that have entered into clinical studies; (B) Structure of compound **9e**; (C) LSD1 inactivation curve of compound **9e**; (D) Dilution assay of compound **9e** to LSD1 activity, GSK-LSD1 was used as a control; (E) Ultrafiltration assay of compound **9e** to LSD1 activity, GSK-LSD1 was used as a control; (F) SPR sensorgrams of the interaction between LSD1 and compound **9e** with indicated

concentrations; (G) ΔTm caused by different concentrations of compound **9e** with protein thermal shift assay, $\Delta Tm = Tm_{sample} - Tm_{reference}$. Data are the mean ± SD. $P < 0.01$ was considered statistically significant. All experiments were carried out at three times.

2.3 Evaluation of biological activity at cellular level

LSD1 inhibitors were reported to be effective against AML cell proliferation [13, 19, 21]. Moreover, overexpression of LSD1 is highly relevant to the poor prognosis of human mixed lineage leukemia (MLL) translocated AML [29-31]. Therefore, THP-1, which is an MLL-AF9 translocate AML cell line, is used to evaluate LSD1 inhibitory activity of compound **9e** at cellular level. First, THP-1 cells were treated with compound **9e** to test the anti-proliferation activity. Compound **9e** inhibited the proliferation of THP-1 cells apparently after 5 days incubation in a dose dependent manner with IC_{50} as 1.204 μM (Hillslope = 1.383). Next, colony formation assay indicated that compound **9e** and GSK-LSD1 can both suppress tumor colony formation *in vitro* even at lower concentration (Fig. 3A, Fig. 4D). The photographs implied that compared to the control group, cell populations in **9e** and GSK-LSD1 treated groups were smaller in a concentration dependent manner. In addition, hematoxylin and eosin staining (HE staining) was applied to monitor the cellular morphology change. After 3 days incubation of compound **9e** at indicated concentrations, significant morphological changes were observed, including blurred cell membrane boundaries and chromatin shrinkage with dose dependently (Fig. 3B). GSK-LSD1 also got a similar result in Figure 4E.

As reported, abrogation of LSD1 lead to differentiation of AML cells [13, 21], hence, we further focused on CD86, a type of myeloid differentiation marker whose expression level can be considered as a surrogate cellular biomarker of LSD1 [32, 33]. Flow cytometry assay was performed to compare the expression levels of CD86. The results showed that compound **9e** can significantly increase the expression level of CD86 in a concentration dependent manner in THP-1 cells (Fig. 3C). Moreover, 4 μM compound 9e performed similar potency to induce the expression of CD86 as GSK-LSD1 (Fig. 4F). So, this indicates compound **9e** may inactivate LSD1 in THP-1 cells and regulate myeloid differentiation by modulating the expression of CD86.

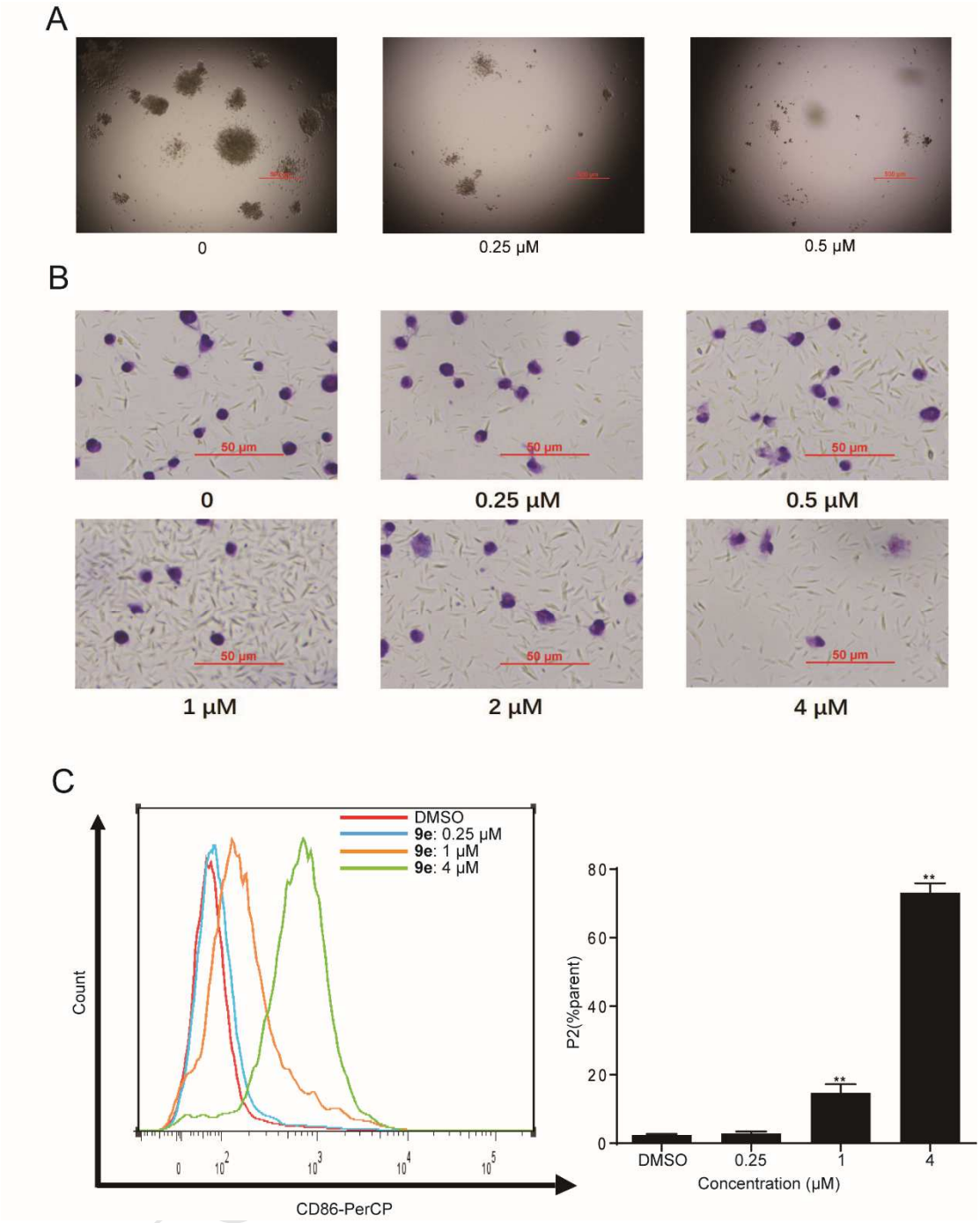
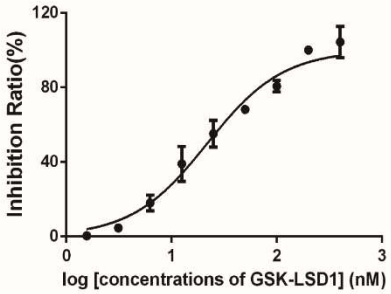
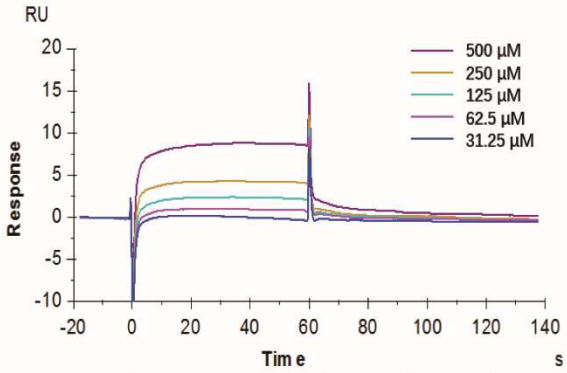


Fig. 3 Inhibitory effect of compound **9e** against LSD1 in THP-1 cells. (A) Colony formation assay for THP-1 cells treated with compound **9e** for 2 weeks; (B) HE staining of THP-1 cells treated with compound **9e** for 3 days; (C) CD86 expression in THP-1 cells treated with compound **9e** for 3 days. Data are mean ± SD. **p<0.01 was considered statistically highly significant. All experiments were carried out at least three times.

A



B

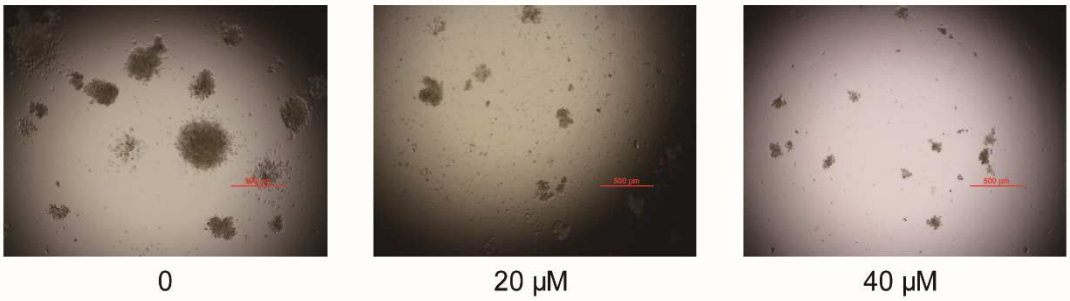


	K_a (1/Ms)	K_d (1/s)	K_D (M)
GSK-LSD1	2.60E+03	1.11E-01	4.25E-04

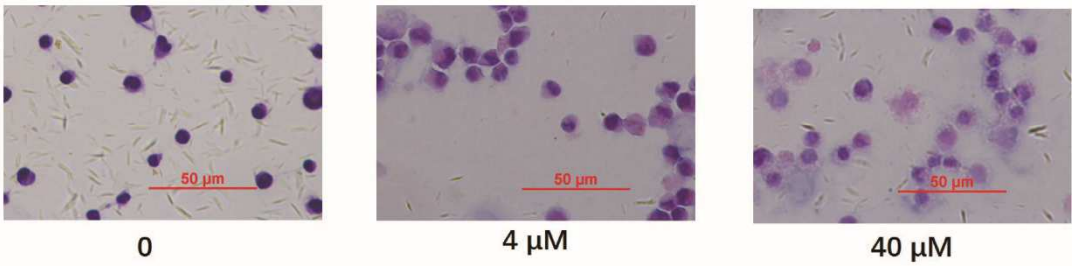
C

Concentrations of GSK-LSD1 (μM)	▵Tm
200 μM	8.37
100 μM	4.28
50 μM	2.21

D



E



F

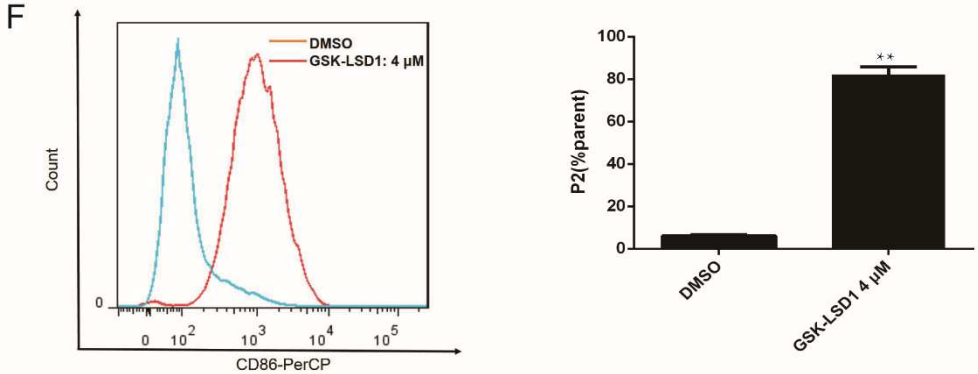


Fig. 4 Properties of GSK-LSD1 against LSD1 recombinant and LSD1 in THP-1 cells. (A) LSD1 inactivation curve of GSK-LSD1; (B) SPR sensorgrams of the interaction between LSD1 and GSK-LSD1 with indicated concentrations; (C) ▵Tm caused by different concentrations of GSK-LSD1 with protein thermal shift assay, $\Delta Tm=Tm_{sample} - Tm_{reference}$. (D) Colony formation assay for THP-1 cells treated with GSK-LSD1 for 2 weeks; (E) HE staining of THP-1 cells treated with GSK-LSD1 for 3 days; (F) CD86 expression in THP-1 cells treated with GSK-LSD1 for 3 days. Data are the mean ± SD. $P < 0.01$ was considered statistically significant. All experiments

were carried out at three times.

3. Conclusion

In summary, we have synthesized a series of novel indole derivatives based on the screening of a compound library constructed by ourselves for LSD1 activity and have identified them as a novel class of LSD1 inhibitor. Some of the indole derivatives, especially compound **9e** with 5-chloro and N-(4-F-benzyl) indole can inhibit LSD1 effectively. Dilution assay and ultrafiltration assay suggested that compound **9e** is an irreversible LSD1 inhibitor. Importantly, these indole derivatives are the first series of LSD1 targeting irreversible inhibitors that are not derived from monoamine oxidase inhibitors. Moreover, in AML cells, which with high expression of LSD1, compound **9e** significantly inhibited cell proliferation and increased the expression level of CD86 in THP-1 cells, which means compound **9e** promoted myeloid differentiation. So, our findings indicated that these indole derivatives could be served as a good starting point to design and synthesize new irreversible inhibitors targeting LSD1.

4. Experimental section

4.1 General information

Reagents and solvents were purchased from Bide Pharmatech Ltd, Aladdin, Sinopharm Chemical Reagent Co, Ltd. with purities of at least 97%. ^{1}H NMR (400 MHz)and ^{13}C NMR (100 MHz) spectra were recorded with a Bruker spectrometer. All reactions were monitored by thin-layerchromatography (TLC) on 25.4 mm × 76.2 mm silica gel plates (GF-254) and UPLC-Mass on Waters ACQUITY UPLC H-Class or Q-Tof Micro HRMS on waters. Melting points were determined on a Beijing Keyi XT4A apparatus. The silica gel used for column chromatography was 200-300 mesh or recrystallization with solvents specified in the corresponding experiments.

4.2 General Synthetic Procedure for the compounds

Synthesis of compound 3：$POCl_3$ (13.09 g, 85.36 mmol) was slowly added dropwise to DMF (10 mL) under ice bath. The mixture was stirred at ice bath for 30 minutes. Then, DMF (6.24 g, 85.36 mmol) solution of indole (5 g, 42.68 mmol) was added dropwise to the reaction system. Keeping the reaction at room temperature for 3 hours, ice water and 10% NaOH aq. were added into the reaction system to pH of 7-8 and continue to stir to a lot of white solid precipitation. The solid was recrystallized from ethyl acetate and petroleum ether to obtain compound **3** in yields of 97.6%.

General Synthesis Procedure for 1-benzyl-1H-indole-3-carbaldehydes (4) To the solution of compound **3** (1 equiv) in MeCN was added NaH (3 equiv) and the Benzyl chloride derivatives (1.5 equiv) at room temperature. The reaction mixture was stirred at room temperature for 4 h. The mixture was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, ethyl acetate/Petroleum ether) to give the desired product.

Synthesis of 4-((1-((6-chloropyridin-3-yl)methyl)- 1H-indol-3-yl)(hydroxy)methyl) -3-methylenedihydrofuran-2(3H)-one (5): To the solution of compound **3** (200 mg, 1.38 mmol) in MeCN (5 mL) was added NaH (99.19 mg, 4.13 mmol) and 2-chloro-5-(chloromethyl) pyridine (334.83 mg, 2.07 mmol) at room temperature. The reaction mixture was stirred at room temperature for 4 h. The mixture was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, ethyl

acetate/Petroleum ether) to give the desired product. It mixed with α-bromomethyl-γ-butene lactone (**6**) (78.46 mg, 443.27 μmol) and Zn powder (120.75 mg, 1.85 mmol) in THF. And then, a saturated aqueous solution of ammonium chloride was added dropwise from a constant pressure low liquid funnel. The reaction mixture was stirred at room temperature for 4 h. After suction filtration, the mixture was evaporated under reduced pressure to move THF. EtOAc (100 mL) was added, and the resulting solution was washed with brine water (3 × 50mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was purified on a silica gel column eluting with EtOAc, to give compound **5** as light yellow solid in 66.06% yield. m.p.: 55.2-55.7℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 2.1 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.38–7.30 (overlap, 2H), 7.27 –7.24(overlap, 2H), 7.22 – 7.18 (m, 1H), 7.14 (s, 1H), 6.39 (d, *J* = 2.1 Hz, 1H), 5.90 (d, *J* = 1.5 Hz, 1H), 5.32 (s, 2H), 5.04 (d, *J* = 8.0 Hz, 1H), 4.29 – 4.21 (m, 1H), 4.18 – 4.12 (m, 1H), 3.73 – 3.67 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.74, 151.23, 148.09, 137.30, 136.70, 135.36, 131.59, 126.14, 125.93, 125.32, 124.65, 123.15, 120.55, 119.86, 116.17, 109.90, 69.94, 67.95, 47.03, 44.76. HRMS (ESI) calcd. $C_{20}H_{18}ClN_2O_3$ $[M+H]^+$m/z: 369.1000/371.0971, found: 369.1002/371.0973.

Synthesis of 3-bromo-3-(bromomethyl)dihydrofuran-2(3H)-one (8): *α*-methylene -*γ*- butyrolactone **(7)** (1 g, 10.19 mmol) was added to a solution of compound **6** (4.29 g, 11.42 mmol) in dioxane (5 mL). The reaction mixture was stirred at room temperature for 2 h. After suction filtration, EtOAc (100 mL) was added, and the resulting solution was washed with brine water (3 × 50mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was purified on a silica gel column eluting with EtOAc, to give compound **8** as a red solid in 87.6% yield

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Synthesis of 3-(bromomethyl)furan-2(5H)-one (6): Compound **8** (2.65 g, 10.27mmol) was added to a solution of tetrabutylammonium bromide (TBAB) (331.24 mg, 1.03 mmol), Li_2CO_3/LiBr (1: 1, 51.37 mmol) in MeCN (30 mL). The reaction mixture was stirred at room temperature for 20h. After suction filtration, MeCN was evaporated under reduced pressure. EtOAc (100 mL) was added, and the resulting solution was washed with brine water (3 × 100mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was purified on a silica gel column eluting with EtOAc, to give compound **6** (877mg) as a red liquid in 48.22% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 –7.43(m, 1H), 4.94 – 4.82(m, 2H), 4.18–4.07(m, 2H).

Synthesis of Compounds 1a~1f with 4-((1-benzyl-1H-indol-3-yl) (hydroxy)methyl) -3-methylenedihydrofuran-2(3H)-one (1a) as a sample: Compound 6 (541.63 mg, 3.06 mmol) mixed with a solution of intermediate compound **4a** (600mg, 2.55 mmol) and Zn powder (833.62 mg, 12.75mmol) in THF. And then, a saturated aqueous solution of ammonium chloride was added dropwise from a constant pressure low liquid funnel. The reaction mixture was stirred at room temperature for 4 h. After suction filtration, THF was evaporated under reduced pressure. EtOAc (100 mL) was added, and the resulting solution was washed with brine water (3 × 50mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was purified on a silica gel column eluting with EtOAc, to give compound **1a** as a white solid in 85.8% yield. m.p.: 43.6-43.9℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.9 Hz, 1H), 7.38 – 7.29 (m, 4H), 7.27 – 7.22 (m, 1H), 7.21 – 7.16 (m, 1H), 7.16 – 7.09(m, 3H), 6.36 (d, *J* = 2.0 Hz, 1H), 5.88 (d, *J* = 1.3 Hz, 1H), 5.29 (s, 2H), 4.96 (d, *J* = 8.1 Hz, 1H), 4.24 – 4.17 (m, 1H), 4.08 (m, 1H), 3.69 – 3.62 (m, 1H); ^{13}C NMR

(100MHz, $CDCl_3$) δ 171.12, 137.04, 135.41, 128.91, 127.91, 126.90, 126.53, 126.00, 125.35, 122.60, 120.03, 119.56, 115.33, 110.30, 69.91, 68.20, 50.11, 44.64. HRMS (ESI) calcd. $C_{21}H_{20}NO_3$ $[M+H]^+$m/z: 334.1438, found: 334.1439.

Synthesis of 4-((1-(4-fluorobenzyl) -1H-indol-3-yl)(hydroxy)methyl) -3-methylene dihydrofuran-2(3H)-one (1b): Compound **4b** (600 mg, 2.37 mmol) was converted to compound 1**b** (625 mg, 75.08%) as a white solid by the same procedure as described for compound **1a**. m.p.: 265.2-266.1℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 8.3 Hz, 1H), 7.26 – 7.22(overlap, 2H), 7.21 – 7.15 (overlap, 2H), 7.10 (s, 1H), 6.90 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.37 (d, *J* = 1.9 Hz, 1H), 5.87 (d, *J* = 1.3 Hz, 1H), 5.24 (s, 2H), 5.00 (d, *J* = 8.0 Hz, 1H), 4.28 – 4.19 (m, 1H), 4.14 – 4.09 (m, 1H), 3.71 – 3.64 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.87, 137.04(d, J=42Hz), 135.32, 133.09, 132.06, 130.94, 128.67, 126.22, 126.03(d, *J*=4.6Hz) 125.40, 123.00, 120.40, 119.74, 115.87, 110.05, 69.98, 68.06, 49.08, 44.69. m.p.: HRMS (ESI) calcd. $C_{21}H_{19}FNO_3$ $[M+H]^+$m/z: 352.1343, found: 352.1344.

Synthesis of 4-(hydroxy(1-(2-(trifluoromethyl)benzyl) -1H-indol-3-yl)methyl)-3-methylenedihydrofuran-2(3H)-one (1c): Compound **1c** (580 mg, 73.04%) as a white solid was prepared from compound **4c** (600mg, 1.98mmol) by the same procedure as described for compound **1a**. m.p.: 133.9-134.7℃. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.84 – 7.80 (m, 1H), 7.77 (d, *J* = 7.7 Hz, 1H), 7.55–7.45(m, 2H), 7.41 (s, 1H), 7.21 (d, *J* = 8.0 Hz, 1H), 7.15–7.03 (overlap, 2H), 6.51 (d, *J* = 6.3 Hz, 1H), 6.07 (d, *J* = 1.0 Hz, 1H), 5.67 – 5.60 (overlap, 3H), 5.56 (s, 1H), 5.10 (t, *J*=5.5Hz, 1H), 4.37 (t, *J* = 8.7 Hz, 1H), 4.29 (dd, *J* = 9.2, 4.0 Hz, 1H), 3.67 – 3.59 (m, 1H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 171.07, 137.14, 136.69, 136.25, 133.42, 128.36, 127.92(d, *J*=5.3Hz), 126.73, 126.44, 126.16, 123.81, 122.31, 120.36, 119.76, 116.88, 110.22, 69.01, 68.56, 46.08, 44.71. HRMS (ESI) calcd. $C_{22}H_{19}F_3NO_3$ $[M+H]^+$m/z: 402.1312, found: 402.1310.

Synthesis of 4-((1-(2-fluorobenzyl)- 1H-indol-3-yl) (hydroxy)methyl) -3-methylene dihydrofuran-2(3H)-one (1d): Compound **1d** (610 mg, 73.28%) was synthesize from compound **4d** (600 mg, 2.37 mmol) as a yellow solid by the same procedure as described for compound 1a. m.p.: 54.1-54.7℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.9 Hz, 1H), 7.31 – 7.27 (overlap, 2H), 7.26–7.22 (overlap, 2H), 7.21 – 7.16 (m, 1H), 7.14-7.08 (m, 2H), 7.02–6.96 (m, 1H), 6.38 (d, *J*=1.6Hz, 1H), 5.91–5.87 (m, 1H), 5.28 (s, 2H), 5.01 (d, *J* = 8.0 Hz, 1H), 4.28 – 4.21 (m, 1H), 4.14 – 4.11 (m, 1H), 3.72 – 3.66 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.91, 145.00, 139.08(d, *J*=3Hz), 136.96, 135.38(d, *J*=54Hz), 130.21(d, *J*=6.6Hz), 128.13, 126.90, 126.33, 125.99, 124.92, 122.86, 120.65, 120.25, 119.64, 115.74, 110.13, 70.01 (d, *J*=5.3Hz), 68.10, 49.57, 44.70. HRMS (ESI) calcd. $C_{21}H_{19}FNO_3$ $[M+H]^+$m/z: 352.1343, found: 352.1344.

Synthesis of 4-((1-(4-chlorobenzyl) -1H-indol-3-yl)(hydroxy)methyl) -3-methylene dihydrofuran-2(3H)-one (1e): Compound **4e** (600 mg, 2.22 mmol) was converted to compound 1**e** (620 mg, 75.77%) as a light-yellow solid by the same procedure as described for compound 1a. m.p.: 106.3-106.7℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.9 Hz, 1H), 7.30 – 7.26 (overlap, 3H), 7.26 – 7.21 (m, 1H), 7.19 – 7.14 (m, 1H), 7.09 (s, 1H), 7.04 (d, *J* = 8.4 Hz, 2H), 6.34 (d, *J* = 2.0 Hz, 1H), 5.84 (d, *J* = 1.3 Hz, 1H), 5.25 (s, 2H), 4.97 (d, *J* = 7.9 Hz, 1H), 4.24 – 4.14(m, 1H), 4.14 – 4.10 (dd, *J*

= 5.7, 3.7 Hz, 1H), 3.70 – 3.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.15, 136.85, 135.63, 135.33, 133.62, 129.04, 128.21, 126.44, 126.07, 125.33, 122.69, 120.13, 119.66, 115.57, 110.18, 69.85, 68.24, 49.47, 44.66. HRMS (ESI) calcd. $C_{21}H_{19}ClNO_3$ $[M+H]^+$m/z: 368.1048/370.1018, found: 368.1049/370.1050.

Synthesis of 4-((1-(3,4-dichlorobenzyl)-1H -indol-3-yl)(hydroxy)methyl) -3-methylene dihydrofuran-2(3H)-one (1f): Compound **4f** (300 mg, 2.22 mmol) was converted to compound 1**f** (280 mg, 68.44%) as a yellow solid by the same procedure as described for compound **1a.** m.p.: 60.9-61.3℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.25–7.21 (m, 1H), 7.19 – 7.14 (m, 1H), 7.12–7.06 (overlap, 3H), 7.05–6.97 (overlap, 2H), 6.39 (d, *J* = 2.0 Hz, 1H), 5.93 (d, *J* = 1.4 Hz, 1H), 5.27 (s, 2H), 4.99 (dd, *J* = 8.2, 2.3 Hz, 1H), 4.25–4.19 (m, 1H), 4.12 – 4.07 (m, 1H), 3.73 – 3.66 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.80, 163.59, 161.14, 136.94, 135.45, 132.59(d, *J*=3.2Hz), 128.57(d, *J*=8.2Hz), 126.20, 125.95, 125.38, 122.81, 120.23, 119.62, 115.99, 115.77, 115.42, 110.19, 70.04, 67.99, 49.51, 44.69. HRMS (ESI) calcd. $C_{21}H_{18}Cl_2NO_3$ $[M+H]^+$m/z: 402.0658, found: 402.0657.

Synthesis of 5-chloro-1H-indole-3-carbaldehyde (12): compound **11** (5.00 g, 32.98mmol) was converted to compound **12** (5.58 g, 94.72%) as a white solid by the same procedure as described for **3**.

***General procedure for* Synthesis of 5-chloro-1- methylene -1H-indole-3-carbaldehyde (13)**: Compound **13** was synthesized from compound **12** used the same reaction for the preparation of compound **4**.

Synthesis of 4-((5-chloro-1-((5-chlorothiophen-2-yl) methyl)-1H-indol-3-yl) (hydroxy)methyl) -3-methylenedihydrofuran-2(3H)-one (9a): compound **13a** (700 mg, 2.37 mmol) was converted to compound **9a** (520mg, 56.52%) as a white solid by the same procedure as described for compound 1a. m.p.: 113.2-113.8℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.69 (d, *J* = 1.9 Hz, 1H), 7.50 (d, *J* = 8.8 Hz, 1H), 7.39 (s, 1H), 7.09 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.92 (d, *J* = 3.8 Hz, 1H), 6.89 (d, *J* = 3.8 Hz, 1H), 5.95 (d, *J* = 1.1 Hz, 1H), 5.55 (d, *J* = 4.7 Hz, 1H), 5.47 (s, 2H), 5.29 (s, 1H), 4.94 (t, *J* = 5.3 Hz, 1H), 4.26 (t, *J*=8.7Hz, 1H), 4.13(dd, *J*=9.1, 4.0Hz, 1H), 3.51 – 3.38 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ170.55, 139.77, 135.58, 134.38, 128.08, 127.51, 127.24, 126.50, 126.30, 123.99, 123.19, 121.47, 119.00, 116.44, 111.79, 68.27, 68.23, 44.18, 43.98. HRMS (ESI) calcd. $C_{19}H_{15}Cl_2NO_3SNa$ $[M+Na]^+$m/z: 430.0042, found: 430.0049.

Synthesis of 4-((5-chloro-1-((2-chlorothiazol-5-yl) methyl)-1H-indol-3-yl) (hydroxy) methyl) -3-methylenedihydrofuran-2(3H)-one (9b): compound **13b** (700 mg, 2.24 mmol) was converted to compound **9b** (485mg, 52.76%) as a white solid by the same procedure as described for compound **1a**. m.p.: 135.3-135.6℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.77 (t, *J* = 5.3 Hz, 1H), 7.72 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.49 (s, 1H), 7.18 (dd, *J* = 8.7, 1.8 Hz, 1H), 6.03 (s, 1H), 5.70 – 5.61 (overlap, 3H), 5.37 (s, 1H), 5.04 (t, *J* = 5.2 Hz, 1H), 4.35 (t, *J*=8.7Hz, 1H), 4.23(dd, *J*=9.1, 3.8Hz, 1H), 3.53 (s, 1H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 170.56, 150.10, 140.12, 138.03, 135.55, 134.29, 127.91, 127.30, 124.19, 123.22, 121.65, 119.10, 116.73, 111.69, 68.27, 68.21, 43.98, 41.51. HRMS (ESI) calcd. $C_{18}H_{14}Cl_2N_2O_3SNa$ $[M+Na]^+$m/z: 430.9994, found: 431.0004.

Synthesis of 4-((1-((2-aminothiazol-5-yl)methyl)- 5-chloro-1H-indol-3-yl) (hydroxy) methyl)-3-methylenedihydrofuran-2(3H)-one (9c): compound **13c** (700mg, 2.40 mmol) was converted to compound **9c** (576mg, 61.58%) as an oily yellow liquid by the same procedure as described for compound **1a**. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.59 (d, *J*=1.9Hz, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.22 (s, 1H), 6.98 (dd, *J*=8.7, 2.0Hz, 1H), 6.78 (s, 2H), 6.17 (s, 1H), 5.92 (d, *J* = 1.3 Hz, 1H), 5.45 (d, *J* = 4.7 Hz, 1H), 5.30 (s, 1H), 4.98 (s, 2H), 4.87 – 4.81 (m, 1H), 4.18 (t, *J*=8.7Hz, 1H), 4.03 (dd, *J*=9.2, 4.0Hz, 1H), 3.42 – 3.32 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.64, 168.81, 147.90, 135.55, 134.75, 128.62, 126.92, 123.51, 121.05, 118.70, 115.43, 111.94, 103.16, 68.29, 56.63, 55.80, 45.92, 44.04.HRMS (ESI) calcd. $C_{18}H_{16}ClN_3O_3SNa$ $[M+Na]^+$m/z: 412.0493/414.0464, found: 412.0498/414.0496.

Synthesis of 4-((5-chloro-1-((3,5-dimethylisoxazol-4-yl) methyl)-1H-indol-3-yl) (hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (9d): compound **13d** (700mg, 2.41 mmol) was converted to compound **9d** (456mg, 48.71%) as a light yellow oily liquid by the same procedure as described for compound **1a**. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.77 (d, *J* = 4.8 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.32 (s, 1H), 7.16 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.04 (s, 1H), 5.59 (d, *J* = 4.7 Hz, 1H), 5.50 (s, 1H), 5.21 (s, 2H), 5.03 – 4.98 (m, 1H), 4.30 (t, *J* = 8.7 Hz, 1H), 4.18 (dd, *J* = 9.2, 4.0 Hz, 1H), 3.60 – 3.50 (m, 1H), 2.37 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.50, 166.67, 159.04, 135.76, 134.58, 127.96, 127.06, 123.83, 123.29, 121.43, 119.04, 115.92, 111.51, 110.36, 68.25(m), 55.79, 44.06, 38.11, 32.05, 29.56 (d, *J* = 4.4 Hz).HRMS (ESI) calcd. $C_{20}H_{19}ClN_2O_4Na$ $[M+Na]^+$m/z: 409.0926/411.0896, found: 409.0932/411.0912.

Synthesis of 4-((5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl) (hydroxy)methyl) -3-methylenedihydrofuran-2(3H)-one (9e): compound **13e** (700mg, 2.59 mmol) was converted to compound **9e** (720mg, 76.70%) as a white solid by the same procedure as described for compound **1a. m.p.**: 152.4-152.8℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.77(d, J=1.9Hz, 1H), 7.53 – 7.47(m, 2H), 7.27 – 7.21(m, 2H), 7.19 – 7.08(overlap, 3H), 6.04(s, 1H), 5.63(d, *J*=4.6Hz, 1H), 5.40(s, 2H), 5.03(t, *J*=5.4Hz, 1H), 4.35(t, J=8.7Hz, 1H), 4.23(dd, *J*=9.2Hz, 3.9Hz, 1H), 3.59 – 3.49(m, 1H).^{13}C NMR (100 MHz, DMSO-*d*6) δ 175.82, 166.69(d, *J*=242Hz), 140.91, 139.77, 139.38 (d, *J*=3.0Hz), 134.31(d, *J*=8.3Hz), 133.88, 132.43, 129.01, 128.52, 126.58, 124.17, 121.15, 120.65, 120.43, 117.10, 73.51(d, *J*=11.5Hz), 53.60, 49.30. HRMS (ESI) calcd. $C_{21}H_{17}ClFNO_3Na$ $[M+Na]^+$m/z: 408.0773/410.0744, found: 408.0775/140.0735.

Synthesis of 4-((5-chloro-1-(pyridin-4-ylmethyl)- 1H-indol-3-yl)(hydroxy) methyl) -3-methylenedihydrofuran-2(3H)-one (9f): compound **13f** (700mg, 2.43 mmol) was converted to compound 9**f** (643mg, 67.42%) as a white solid by the same procedure as described for compound 1a. m.p.: 64.3-64.7℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 – 8.19 (m, 2H), 7.74 (d, *J* = 1.3 Hz, 1H), 7.15 – 7.10(overlap, 2H), 7.10–7.03 (m, 1H), 6.81(d, *J*=4.9Hz, 2H), 6.32 (s, 1H), 5.81 (s, 1H), 5.25 (s, 2H), 4.98 (d, *J* = 7.5 Hz, 1H), 4.24 (t, *J*=8.8Hz, 1H), 4.18 – 4.07(m, 1H), 3.67–3.57 (m, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ 170.90, 149.80, 146.31, 135.26, 135.17, 127.58, 127.14, 126.14, 125.22, 123.26, 121.32, 119.54, 116.26, 110.96, 69.58, 68.07, 49.06, 44.72.RMS (ESI) calcd. $C_{20}H_{17}ClN_2O_3Na$ $[M+Na]^+$m/z: 391.0820/393.0790, found: 391.0825/393.0821.

Synthesis of 4-((5-chloro-1- ((5-methylpyridin-3-yl) methyl)-1H-indol-3-yl) (hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (9g): compound **13g** (700mg, 2.46 mmol) was converted to compound 9**g** (613mg, 65.13%) as an oily yellow liquid solid by the same procedure as described for compound 1a. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.31 (s, 2H), 7.76 (d, *J* = 1.8 Hz, 1H), 7.52 (d, *J* = 10.2 Hz, 2H), 7.36 (s, 1H), 7.12(dd, J=8.7, 2.0Hz, 1H), 6.03 (s, 1H), 5.62 (d, *J* = 4.6 Hz, 1H), 5.41 (s, 3H), 5.04 (t, *J* = 5.3 Hz, 1H), 4.35(t, *J*=8.7Hz, 1H) 4.24 (dd, *J*=9.1, 3.8Hz, 1H), 3.59 – 3.51 (m, 1H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.52, 149.15, 145.63, 135.70, 134.99, 134.50, 132.86(d, *J*=5Hz), 128.58, 127.23, 123.87, 123.15, 121.44, 118.98, 116.02, 111.77, 68.32, 68.13, 46.59, 44.10, 29.53(d, *J*=5Hz) 17.73. HRMS (ESI) calcd. $C_{21}H_{19}ClN_2O_3Na$ $[M+Na]^+$m/z: 405.0976/407.0947, found: 405.0981/407.0952.

Synthesis of 4-((5-chloro-1-((5-chloropyridin-2-yl) methyl)-1H-indol-3-yl) (hydroxy) methyl) -3-methylenedihydrofuran-2(3H)-one (9h): compound **13h** (700mg, 2.29 mmol) was converted to compound 9**h** (653mg, 70.59%) as a white solid by the same procedure as described for compound 1a. m.p.: 163.1-163.8℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.57 (d, *J* = 2.4 Hz, 1H), 7.87 (dd, *J*=8.4, 2.5Hz 1H), 7.78 (d, *J* = 1.9 Hz, 1H), 7.49 (s, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.11 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.06 (d, *J* = 1.2 Hz, 1H), 5.65 (d, *J* = 4.7 Hz, 1H), 5.51 (s, 2H), 5.44 (s, 1H), 5.04 (t, *J* = 5.3Hz, 1H), 4.35 (t, *J*=8.7Hz, 2H), 4.23 (dd, *J*=9.2, 3.9Hz, 1H), 3.59–3.51(m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.61, 155.74, 147.77, 136.83, 135.57, 134.78, 129.96, 128.89, 127.18, 123.88, L.123.40, 122.57, 121.43, 118.93, 116.08, 111.80, 68.28 (d, *J* = 8.5 Hz), 55.79, 50.41, 44.05.HRMS (ESI) calcd. $C_{20}H_{16}Cl_2N_2O_3Na[M+Na]^+$m/z: 425.0430, found: 425.0435.

Synthesis of 4-((5-chloro-1-((6-chloropyridin-3-yl)methyl)- 1H-indol-3-yl) (hydroxy) methyl)-3-methylenedihydrofuran-2(3H)-one (9i): compound **13i** (700mg, 2.29 mmol) was converted to compound 9**i** (596mg, 64.43%) as a white solid by the same procedure as described for compound 1a. m.p.: 172.2-172.8℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.35 (d, *J* = 1.9 Hz, 1H), 7.77 (s, 1H), 7.60 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.57–7.52 (d, overlap, 2H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.14 (dd, *J* = 8.7, 1.8 Hz, 1H), 6.03 (s, 1H), 5.63 (d, *J* = 4.5 Hz, 1H), 5.46 (s, 2H), 5.40 (s, 1H), 5.03 (t, *J* = 5.1 Hz, 1H), 4.34(t, *J*=8.6Hz, 1H), 4.22(dd, *J*=9.1, 3.7Hz, 1H), 3.59–3.49 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.54, 149.41, 148.65, 138.51, 135.67, 134.44, 133.09, 128.51, 127.27, 124.24, 124.00, 123.25, 121.54, 119.03, 116.32, 111.78, 68.28, 68.16, 45.85, 44.04.HRMS (ESI) calcd. $C_{20}H_{16}Cl_2N_2O_3Na$ $[M+Na]^+$m/z: 425.0430, found: 425.0437.

Synthesis of 4-((5-chloro-1-((4-(3-methoxypropoxy)-3- methylpyridin-2-yl) methyl)-1H-indol-3-yl)(hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (9j): compound **13j** (700mg, 1.88 mmol) was converted to compound 9**j** (425mg, 48.07%) as a white solid by the same procedure as described for compound 1a. m.p.: 71.2-71.4℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.18 (d, *J* = 5.6 Hz, 1H), 7.74 (d, *J* = 1.7 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.31 (s, 1H), 7.08 (dd, *J* = 8.7, 1.9 Hz, 1H), 6.93 (d, *J* = 5.7 Hz, 1H), 6.02 (s, 1H), 5.57 (d, *J* = 4.8 Hz, 1H), 5.46 (s, 2H), 5.43 (s, 1H), 5.01 (t, *J* = 5.3 Hz, 1H), 4.33(t, *J*=8.7Hz, 1H), 4.20(dd, *J*=9.1, 4.0Hz, 1H) 4.08(t, *J*=6.2Hz, 2H), 3.56 –3.50 (m, 1H), 3.47 (t, *J* = 6.2 Hz, 2H), 3.23 (s, 3H), 2.12 (s, 3H), 2.01 –1.91 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.62, 162.69, 154.82,

147.74, 135.52, 135.29, 128.93, 126.90, 123.51, 123.30, 121.06, 119.64, 118.67, 115.41, 111.99, 106.46, 68.34, 68.29, 65.04, 57.92, 49.80, 44.03, 28.64, 9.85. HRMS (ESI) calcd. $C_{25}H_{28}ClN_2O_5$ $[M+H]^+$m/z: 471.1681/473.1652, found: 471.1685/ 473.1655.

Synthesis of 4-((5-chloro-1-(pyrimidin-2-ylmethyl)-1H-indol-3-yl) (hydroxy) methyl)-3-methylenedihydrofuran-2(3H)-one (9k): compound **13k**(700mg, 2.58 mmol) was converted to compound 9**k** (538mg, 56.47%) as an oily yellow liquid solid by the same procedure as described for compound 1a. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.25 (dd, *J*=5.6Hz, 1H), 8.02–7.85 (m, 1H), 7.83 (d, *J* = 8.1 Hz, 1H), 7.78 (d, *J* = 2.0 Hz, 1H), 7.73 – 7.67 (m, 1H), 7.55 – 7.48 (m, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.08 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.05 (d, *J* = 1.2 Hz, 1H), 5.69 (s, 2H), 5.64 (d, *J* = 4.9 Hz, 1H), 5.48(s, 1H), 5.07 (t, *J* = 5.3 Hz, 1H), 4.43 – 4.23 (m, 2H), 3.62–3.48 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 208.89, 185.24, 169.67, 159.94, 148.82, 143.08, 136.09, 134.53, 127.90 (d, *J* = 7.8 Hz), 127.26, 125.74 (d, *J* = 5.5 Hz), 123.60, 122.51, 120.02, 116.70, 113.06, 68.58, 55.63, 52.74.HRMS (ESI) calcd.$C_{19}H_{17}ClN_3O_3$ $[M+H]^+$m/z: 370.0953/372.0923, found: 370.0957/372.0933.

Synthesis of 4-((5-chloro-1-((4-methylquinazolin-2-yl)methyl) -1H-indol-3-yl) (hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (9l): compound **13l** (700mg, 2.08 mmol) was converted to compound 9**l** (589mg, 65.12%) as a yellow solid by the same procedure as described for compound 1a. m.p.: 59.8-60.4℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.74 (d, *J* = 4.9 Hz, 2H), 7.77 (d, *J* = 2.0 Hz, 1H), 7.44 (s, 1H), 7.43–7.36 (overlap, 2H), 7.09 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.05 (d, *J* = 1.4 Hz, 1H), 5.68 – 5.56 (overlap, 3H), 5.43 (s, 1H), 5.04(t, *J*=5.4Hz, 1H), 4.36 (t, *J*=8.7Hz, 1H), 4.22(dd, *J*=9.1, 4.0Hz, 1H)3.59 – 3.44 (m, 1H), 3.36 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.69, 165.70, 157.61, 135.37, 135.29, 129.33, 127.03, 123.62, 123.49, 121.21, 120.24, 118.73, 115.79, 111.75, 68.49, 68.40, 68.33, 56.63, 55.80, 51.80, 43.96, 32.06, 29.57, 29.52. MS (ESI) calcd. $C_{24}H_{20}ClN_3O_3Na$ $[M+Na]^+$m/z: 456.1085/458.1056, found: 456.1081/458.1052.

Synthesis of 4-((1-(benzo[d]oxazol-2-ylmethyl) -5-chloro-1H-indol-3-yl) (hydroxy) methyl)-3-methylenedihydrofuran-2(3H)-one (9m): compound **13m** (700mg, 2.25 mmol) was converted to compound 9**m** (396mg, 43.00%) as a yellow solid by the same procedure as described for compound 1a. m.p.: 67.2-67.4℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.62 (d, *J* = 1.8 Hz, 1H), 7.61–7.55 (m, 1H), 7.40–7.36(m, 1H), 7.33 (d, *J* = 8.8 Hz, 1H), 7.30 – 7.21 (overlap, 2H), 7.19 (s, 1H), 7.15 (dd, *J* = 8.8, 1.9 Hz, 1H), 6.29 (d, *J* = 2.0 Hz, 1H), 5.75 (d, *J* = 1.4Hz, 1H), 5.40 (s, 2H), 4.87 (d, *J* = 7.8 Hz, 1H), 4.16 (t, *J*=9.4Hz, 1H), 4.04(dd, *J*=9.6, 4.2Hz, 1H) 3.56–3.48 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ165.60, 155.25, 145.63, 135.26, 130.15, 129.76, 122.48, 121.85, 121.19, 120.64, 120.30, 119.66, 118.24, 115.11, 114.04, 110.93, 105.87, 105.63, 64.41, 62.82, 39.26, 38.65. MS (ESI) calcd. $C_{22}H_{17}ClN_2O_4Na$ $[M+Na]^+$m/z: 431.0769/433.0740, found: 431.0768/433.0743.

Synthesis of 4-((1-(benzo[d]thiazol-2-ylmethyl)-5-chloro -1H-indol-3-yl) (hydroxy) methyl)-3-methylenedihydrofuran-2(3H)-one (9n): compound **13n** (700mg, 2.14 mmol) was converted to compound 9**n** (508mg, 55.82%) as a yellow solid by the same procedure as described for compound 1a. m.p.: 71.2-71.4℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.2 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.71 (d, *J* = 1.7 Hz, 1H), 7.51–7.43 (m, 1H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 7.22 (s, 1H),

7.18 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.36(d, *J* = 1.9 Hz, 1H), 5.84 (d, *J* = 1.3 Hz, 1H), 5.61 (s, 2H), 4.96 (d, *J* =7.7 Hz, 1H), 4.29 –4.21 (m, 1H), 4.13(dd, *J*=9.6, 4.3Hz, 1H), 3.66 – 3.56 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.80, 166.88, 152.84, 135.27, 135.05, 134.96, 127.53, 127.21, 126.58, 126.53, 125.77, 125.54, 123.54, 123.23, 121.85, 119.39, 116.43, 111.18, 69.72, 68.04, 48.67, 44.56. MS (ESI) calcd. $C_{22}H_{18}ClN_2O_3S$ $[M+H]^+$m/z: 425.0721/427.0692, found: 425.0721/427.0691.

Synthesis of 4-((1-((1H-benzo[d]imidazol-2-yl)methyl) -5-chloro-1H-indol-3-yl) (hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (9o): compound **13o** (700mg, 2.26 mmol) was converted to compound 9**o** (639mg, 69.33%) as a yellow solid by the same procedure as described for compound 1a. m.p.: 58.6-59.0℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.77 (d, *J*=1.8Hz, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 7.56–7.50(overlap, 1H), 7.48(s, 1H) 7.20 – 7.11 (overlap, 3H), 6.03 (s, 1H), 5.66 – 5.62 (m, 1H), 5.60 (s, 2H), 5.44 (s, 1H), 5.03 – 4.98 (m, 1H), 4.59–4.49 (m, 1H), 4.35(t, *J*=8.7Hz, 1H), 4.20 (dd, *J*=9.1Hz, 1H), 3.56-3.48 (m, 1H). ^{13}C NMR (101 MHz, DMSO-*d*6) δ 170.66, 150.12, 135.51, 135.01, 128.67, 127.11, 123.90, 123.51, 121.43, 118.85, 116.22, 111.88, 68.68, 68.49, 68.37, 68.27, 56.63, 55.80, 43.98, 43.85, 32.06, 29.57.MS (ESI) calcd. $C_{22}H_{18}ClN_3O_3Na$ $[M+Na]^+$m/z: 430.0929/432.0899, found: 430.0927/432.0898.

Synthesis of 4-((5-chloro-1-(cyclopropylmethyl) -1H-indol-3-yl) (hydroxy) methyl)-3-methylenedihydrofuran-2(3H)-one (9p): compound **13p** (700mg, 3.00 mmol) was converted to compound 9**p** (716mg, 72.04%) as an oily yellow liquid solid by the same procedure as described for compound 1a. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 1.9 Hz, 1H), 7.30 – 7.26 (m, 1H), 7.23 (s, 1H), 7.19 (dd, *J* = 8.7, 1.9 Hz, 1H), 6.37 (d, *J* = 2.1 Hz, 1H), 5.89 (d, *J* = 1.5 Hz, 1H), 4.92 (d, *J* = 8.2 Hz, 1H), 4.26 – 4.16 (m, 1H), 4.07 (dd, *J* = 9.6, 4.4 Hz, 1H), 3.93 (dd, *J* = 6.8, 3.7 Hz, 2H), 3.67–3.58 (m, 1H), 2.16 (s, 1H), 0.68–0.58 (m, 2H), 0.38–0.33 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.86, 135.35, 135.30, 127.00, 126.73, 125.54, 125.40, 122.59, 118.94, 114.27, 111.01, 69.88, 68.06, 50.91, 44.67, 11.07, 4.18, 4.15.MS (ESI) calcd. $C_{18}H_{18}ClNO_3Na$ $[M+Na]^+$m/z: 354.0867/356.0838, found: 354.0863/356.0837.

General Procedure for 1-formyl-5-chloro-1H-indole-3-carbaldehyde (14): To the compound **12** (1 equiv) in MeCN was added NaH (3 equiv) and Acyl chloride derivatives (1.5 equiv) at room temperature. The reaction mixture was stirred at room temperature for 4 h and was then adjust with HCl (1 M) until pH 6-7. The mixture was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, ethyl acetate/Petroleum ether) to give the desired product.

Synthesis of 4-((5-chloro-1-(3-methylthiophene-2-carbonyl) -1H-indol-3-yl) (hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (10a): compound **14a** (600mg, 1.98 mmol) was converted to compound **10a** (586mg, 73.82%) as a white solid by the same procedure as described for compound **1a**. m.p.: 194.8-195.6℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.21 (d, *J* = 8.8 Hz, 1H), 8.00 – 7.89 (overlap, 2H), 7.55 (s, 1H), 7.42 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.17 (d, *J* = 5.0 Hz, 1H), 6.07 (d, *J* = 1.6 Hz, 1H), 5.84 (d, *J* = 5.1 Hz, 1H), 5.39 (d, *J* = 0.9 Hz, 1H), 5.07 (t, *J* = 5.3 Hz, 1H), 4.40 (t, *J*=8.7Hz, 1H), 4.27(dd, *J*=9.2, 3.7Hz, 1H), 3.66 – 3.48 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.46, 162.05, 143.78, 135.13, 134.23, 131.39, 131.37, 129.97, 128.22, 128.10, 126.69, 124.75, 123.58, 122.45, 119.90, 117.08, 68.37, 67.81,

43.29, 15.43.MS (ESI) calcd. $C_{20}H_{16}ClNO_4$[M+Na] $^+$m/z: 424.0381/426.0351, found: 424.0382/426.0351.

Synthesis of 4-((5-chloro-1-(furan-2-carbonyl)-1H-indol-3-yl) (hydroxy)methyl) -3-methylenedihydrofuran-2(3H)-one (10b): compound **14b** (600mg, 2.19 mmol) was converted to compound **10b** (653mg, 80.12%) as a yellow solid by the same procedure as described for compound **1a**. m.p.: 65.3-65.9℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, *J* = 8.9 Hz, 1H), 7.97 (s, 1H), 7.68(d, J=0.9Hz, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 7.42 (d, *J* = 3.5 Hz, 1H), 7.23–7.26 (m, 1H), 6.64 (dd, *J* = 3.6, 1.7 Hz, 1H), 6.34 (d, *J* = 2.1 Hz, 1H), 5.75 (d, *J* = 1.6 Hz, 1H), 4.95 (d, *J* = 7.2 Hz, 1H), 4.36 – 4.25 (m, 1H), 4.20(dd, *J*=9.6, 3.9Hz, 1H), 3.62–3.53 (m, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 170.83, 156.22, 146.94, 146.36, 135.32, 134.44, 129.84, 128.98, 125.75, 125.42, 121.86, 121.69, 119.32, 117.98, 112.70, 69.39, 68.12, 43.94, 29.20.MS (ESI) calcd. $C_{19}H_{14}ClNO_5K$ [M+K] $^+$m/z: 410.0192/412.0163, found: 410.0192/412.0161.

Synthesis of 4-((5-chloro-1-(3,5-dimethylisoxazole-4-carbonyl) -1H-indol-3-yl) (hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (10c): compound **14c** (600mg, 1.97 mmol) was converted to compound **10c** (560mg, 70.60%) as a light-yellow solid by the same procedure as described for compound 1a. m.p.: 145.8-146.2℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.28 (d, *J* = 8.8 Hz, 1H), 7.93 (d, *J* = 1.9 Hz, 1H), 7.53 (s, 1H), 7.44 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.09 (d, *J* = 1.1 Hz, 1H), 5.81 (t, *J* = 5.4 Hz, 1H), 5.57 (s, 1H), 4.99 (t, *J* = 5.9 Hz, 1H), 4.36(t, J=8.7Hz, 1H), 4.22(dd, J=9.2, 3.9Hz, 1H),, 3.67 – 3.55 (m, 1H), 2.44 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 171.29, 170.40, 160.85, 158.78, 135.32, 133.89, 130.43, 128.27, 126.38, 124.85, 123.89, 122.96, 120.03, 117.23, 111.63, 68.12, 67.71, 43.31, 12.58, 10.29. MS (ESI) calcd. $C_{20}H_{17}ClN_2O_5Na$ [M+Na] $^+$m/z: 423.0718/425.0689, found: 423.0716/425.0688.

Synthesis of 4-((5-chloro-1-cinnamoyl-1H-indol-3-yl)(hydroxy) methyl)-3-methylenedihydrofuran-2(3H)-one (10d): compound **14d** (600mg, 1.94 mmol) was converted to compound **10d** (589mg, 74.55%) as a yellow solid by the same procedure as described for compound **1a**. m.p.: 177.5-178.2℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.50 (d, *J* = 8.9 Hz, 1H), 8.37 (s, 1H), 8.00–7.53 (overlap, 3H), 7.91 (d, *J* = 2.1 Hz, 1H), 7.79 (d, *J* = 15.4 Hz, 1H), 7.54 – 7.47 (m, 3H), 7.41 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.13 (d, *J* = 1.5 Hz, 1H), 5.91 (d, *J* = 4.9 Hz, 1H), 5.57 (s, 1H), 4.99 (t, J=8.7Hz, 1H), 4.42(t, J=8.7Hz, 1H), 4.27(dd, J=9.3, 3.8Hz, 1H), 3.73 – 3.55 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.47, 164.08, 146.65, 135.25, 134.36, 134.26, 130.89, 130.21, 129.06, 128.86, 127.98, 125.44, 124.68, 124.02, 122.57, 119.69, 117.88, 117.39, 68.23, 67.79, 43.32. MS (ESI) calcd. $C_{23}H_{18}ClNO_4$ [M+Na]$^+$m/z: 430.0817/432.0787, found: 430.0815/432.0789.

Synthesis of 4-((5-chloro-1-(2-chloronicotinoyl)-1H-indol-3-yl) (hydroxy) methyl)-3- methylenedihydrofuran-2(3H)-one (10e): compound **14e** (600mg, 1.88 mmol) was converted to compound **10e** (610mg, 77.76%) as a white solid by the same procedure as described for compound **1a**. m.p.: 74.2-74.6℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.48 (dd, *J* = 4.9, 1.9 Hz, 1H), 8.17 (s, 1H), 8.09 (dd, *J* = 7.6, 1.9 Hz, 1H), 7.75 (d, *J* = 2.1 Hz, 1H), 7.49 (dd, *J* = 7.6, 4.9 Hz, 1H), 7.28 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.95 (s, 1H), 5.83 (d, *J* = 1.8 Hz, 1H), 5.59 (d, *J*=5.4Hz, 1H), 5.15 (s, 1H), 4.77(t, *J*=5.5Hz, 1H), 4.14(t, *J*=8.7Hz, 1H), 3.98 (dd, *J*=9.2, 3.7Hz, 1H), 3.39 –3.23 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.44, 164.01, 151.96, 145.88, 138.76, 134.95,

133.86, 130.42, 130.31, 128.86, 125.76, 125.38, 123.99, 123.71, 123.62, 120.20, 117.37, 68.38, 67.69, 43.07.MS (ESI) calcd.$C_{20}H_{14}Cl_2N_2O_4K[M+K]^+$m/z: 454.9962, found: 455.1000.

Synthesis of 4-((5-chloro-1-(cyclopropanecarbonyl)-1H-indol-3-yl) (hydroxy) methyl) -3- methylenedihydrofuran-2(3H)-one (10f): compound **14f** (600mg, 2.42 mmol) was converted to compound 10f (581mg, 69.36%) as a light-yellow solid by the same procedure as described for compound 1a.mp: 75.3-75.9℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.34 (d, *J* = 8.9 Hz, 1H), 8.16 (s, 1H), 7.90 (d, *J* = 2.0 Hz, 1H), 7.36 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.11 (d, *J* = 1.4 Hz, 1H), 5.91 (d, *J* = 5.0 Hz, 1H), 5.49 (s, 1H), 5.05 (t, *J* = 5.5 Hz, 1H), 4.42 (t, *J*=8.7Hz, 1H), 4.27(dd, *J*=9.2, 3.8Hz, 1H)3.69–3.49 (m, 1H), 2.80 –2.64 (m, 1H), 1.23 (s, 2H), 0.91 – 0.75 (m, 2H). MS (ESI) calcd.$C_{18}H_{16}ClNO_4K[M+K]^+$ m/z: 384.0399/386.0370, found: 384.0400/386.0372.

General Procedure for synthesis of substitued-1H-indole-3-carbaldehydes (18a-i)*:* compounds **18a-i** was prepared from compounds **17a-i** by the same procedure as described for compound **3**.

Synthesis of 5-chloro-1-tosyl-1H-indole-3-carbaldehyde (19): To the solution of compound **18f** (200mg, 1.11 mmol) in MeCN(5mL) was added NaH (80.17mg, 3.34 mmol) and paratoluensulfonyl chloride (318.44mg, 1.67 mmol) at room temperature. The reaction mixture was stirred at room temperature for 4 h. The mixture was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, ethyl acetate/Petroleum ether) to give the product compound **19** (283mg, 76.14%).

synthesis of 4-((5-chloro-1-tosyl-1H-indol-3-yl)(hydroxy)methyl) -3-methylene dihydrofuran-2(3H)-one (15): compound **19** (600mg, 2.42 mmol) was converted to compound 1**5**(581mg, 69.36%) as a white solid by the same procedure as described for compound **1a**. m.p.: 185.3-185.7℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.97 (d, *J* = 8.9 Hz, 1H), 7.93 – 7.85(overlap, 3H), 7.66 (s, 1H), 7.40 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.15 – 7.11 (m, 1H), 7.11 – 7.08 (m, 1H), 5.98 (d, *J* = 1.6 Hz, 1H), 5.86 (d, *J* = 5.1 Hz, 1H), 5.06 – 4.99 (m, 2H), 4.34 (t, *J*=8.7Hz, 1H), 4.21(dd, *J*=9.2, 3.7Hz, 1H), 3.80 (s, 3H), 3.55–3.44 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.40, 163.93, 135.03, 133.17, 129.89, 129.11, 128.02, 127.99, 125.52, 124.85, 123.53, 123.28, 120.29, 115.05, 114.87, 68.32, 67.71, 55.90, 43.00. MS (ESI) calcd. $C_{21}H_{18}ClNO_5SK$ [M+K]+m/z: 470.0226/472.0196, found: 470.0227/472.0194.

General Procedure for synthesis of substituted-1-(4-chlorobenzyl)- 1H-indole -3-carbaldehydes (20a-i): To the solution of compounds **18a-i** (1 equiv) in MeCN was added NaH (3 equiv) and 4-Chlorobenzyl chloride (1.5 equiv) at room temperature. The reaction mixture was stirred at room temperature for 4 h. The mixture was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, ethyl acetate/Petroleum ether) to give the desired product.

Synthesis of 4-((1-(4-chlorobenzyl)-4-methoxy-1H-indol-3-yl) (hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (16a): compound **20a** (200mg, 667.21 μmol) was converted to compound 1**6a** (165mg, 62.16%) as a white solid by the same procedure as described for compound **1a**. m.p.: 134.5-135.1℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.38 (d, *J*=8.4Hz, 2H), 7.26 (s, 1H), 7.19 (s, 1H), 7.17 (s, 1H), 7.04 – 6.97 (overlap, 2H), 6.57 – 6.47 (t, *J*=1.0Hz, 1H), 5.94 (s, 1H), 5.39 (d, *J*=5.1Hz, 1H), 5.36 (s, 2H), 5.29 (t, *J* = 4.9 Hz, 1H), 4.94 (s, 1H), 4.42(t, *J*=8.5Hz, 1H), 4.31(dd, *J*=9.0, 3.5Hz, 1H), 3.84 (s, 3H), 3.55 – 3.44 (m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.89, 153.72, 137.60, 137.31, 135.04, 131.86, 128.82, 128.42, 125.88, 122.67, 122.31, 116.77, 115.61, 103.65, 99.72, 69.10, 68.78, 55.16, 48.38, 44.77.HRMS (ESI) calcd. $C_{22}H_{20}ClNO_4Na$ $[M+Na]^+$m/z: 420.0973/422.0944, found: 420.0977/422.0935.

Synthesis of 4-((1-(4-chlorobenzyl)-5-methoxy- 1H-indol-3-yl)(hydroxy) methyl) -3-methylenedihydrofuran-2(3H)-one (16b): compound **20b** (200mg, 667.21 μmol) was converted to compound **16b** (186mg, 70.07%) as a yellow solid by the same procedure as described for compound 1a. m.p.: 62.4-62.6℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.36 (s, 2H), 7.34(s, 1H), 7.27 (d, *J* = 8.9 Hz, 1H), 7.21 – 7.10 (overlap, 3H), 6.74 (dd, *J*=8.9, 2.3Hz, 1H), 6.05 (d, *J* = 1.2 Hz, 1H), 5.59 (d, *J* = 4.3 Hz, 1H), 5.46 (s, 1H), 5.33 (s, 2H), 5.02 (t, *J*=5.4Hz, 1H), 4.35 (t, *J* = 8.7 Hz, 1H), 4.24 (dd, *J*=9.2, 4.0Hz, 1H), 3.75 (s, 3H).^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.66, 153.36, 137.39, 135.78, 131.84, 131.23, 128.73, 128.40, 127.57, 126.55, 123.28, 115.60, 111.44, 110.89, 101.54, 68.46, 68.19, 55.38, 48.39, 43.97.HRMS (ESI) calcd. $C_{22}H_{20}ClNO_4Na$ $[M+Na]^+$m/z: 420.0973/422.0944, found: 420.0976/422.0940.

Synthesis of 4-((1-(4-chlorobenzyl)-6-methoxy-1H-indol -3-yl)(hydroxy)methyl) -3-methylenedihydrofuran-2(3H)-one (16c): compound **20c** (200mg, 667.21 μmol) was converted to compound **16c** (189mg, 71.20%) as a deep yellow solid by the same procedure as described for compound 1a. m.p.: 59.5-60.3℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J* = 8.7 Hz, 1H), 7.28 (s, 1H), 7.26 (s, 1H), 7.02 (s, 1H), 7.00 (s, 1H), 6.95 (s, 1H), 6.80 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.68 (d, *J* = 2.1 Hz, 1H), 6.34 (d, *J* = 2.2 Hz, 1H), 5.88 (d, *J* = 1.3 Hz, 1H), 5.18 (s, 2H), 4.92 (d, *J* = 7.5 Hz, 1H), 4.23 – 4.15 (m, 1H), 4.12– 4.07 (m, 1H), 3.79 (s, 3H), 3.68– 3.57 (m, 1H).HRMS (ESI) calcd. $C_{22}H_{20}ClNO_4Na$ $[M+Na]^+$m/z: 420.0973/422.0944, found: 420.0978/422.0950.

Synthesis of 4-((1-(4-chlorobenzyl)-7-methoxy-1H -indol-3-yl)(hydroxy) methyl) -3-methylenedihydrofuran-2(3H)-one (16d): compound **20d** (200mg, 667.21 μmol) was converted to compound 1**6d** (181mg, 68.18%) as a yellow solid by the same procedure as described for compound **1a**. m p : 70.1-70.6℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.32 (d, *J*=8.4Hz, 2H), 7.28 (s, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 5.1 Hz, 2H), 6.92 (t, *J* = 7.9 Hz, 1H), 6.64 (d, *J* = 7.7 Hz, 1H), 6.00 (d, *J* = 1.4 Hz, 1H), 5.51 (d, *J* = 6.6 Hz, 2H), 5.34 (s, 1H), 4.99 (d, *J* = 5.9 Hz, 1H), 4.34 (t, *J*=8.7Hz, 1H), 4.24(dd, *J*=9.2, 3.8Hz, 1H), 3.77 (s, 3H), 3.54 – 3.50 (m, 1H).^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.80, 146.99, 138.81, 135.35, 131.55, 128.49, 128.33, 128.23, 127.97, 125.34, 123.47, 119.94, 116.22, 112.24, 103.02, 68.42, 68.23, 55.22, 50.97, 43.99. HRMS (ESI) calcd. $C_{22}H_{20}ClNO_4Na$ $[M+Na]^+$m/z: 420.0973/422.0944, found: 420.0974/422.0945.

Synthesis of 4-((4-chloro-1-(4-chlorobenzyl)- 1H-indol-3-yl) (hydroxy) methyl)-3-methylenedihydrofuran-2(3H)-one (16e): compound **20e**(200mg, 657.53 μmol) was converted to compound 1**6e** (190mg, 71.83%) as a white solid by the same

procedure as described for compound **1a**. ^{1}H NMR (400 MHz, $CDCl_3$, this molecule appears as conformers in a 1: 2 ratio) δ 7.34 –7.27(overlap, 2H), 7.26 –7.07(overlap, 4H), 7.06 – 6.98(overlap, 2H), 6.35(d, *J*=2.6Hz, 0.34H), 6.29(d, *J*=2.1Hz, 0.62H), 5.78 (d, *J* = 4.5 Hz, 0.32H), 5.68 (d, *J* = 6.4 Hz, 0.62H), 5.62 (d, *J* = 2.2 Hz, 0.34H), 5.44 (d, *J* = 1.1 Hz, 0.63H), 5.26 (s, 2H), 4.56(dd, *J*=9.4, 4.0Hz, 0.35H), 4.40 (t, *J*=8.9Hz, 0.65H), 4.31 (dd, *J* = 9.4, 4.0 Hz, 0.64H), 4.25 (t, *J* = 8.9 Hz, 0.32H), 3.84 – 3.74 (m, 0.33H), 3.71 – 3.60 (m, 0.65H). HRMS (ESI) calcd. $C_{21}H_{17}Cl_2NO_3Na$ $[M+Na]^+$m/z: 424.0478, found: 424.0485.

Synthesis of 4-((5-chloro-1-(4-chlorobenzyl)- 1H-indol-3-yl) (hydroxy) methyl) -3-methylenedihydrofuran-2(3H)-one (16f): compound **20f** (200mg, 657.53 μmol) was converted to compound 1**6f** (220mg, 83.17%) as a light-yellow solid by the same procedure as described for compound 1a. m.p.: 144.8-145.2℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.72 (s, 1H), 7.30 (s, 1H), 7.27 (s, 1H), 7.20 – 7.14 (overlap, 2H), 7.14 – 7.07 (m, 1H), 7.02 (s, 1H), 6.99 (s, 1H), 6.38 (d, *J* = 2.1 Hz, 1H), 5.88 (d, *J* = 1.5 Hz, 1H), 5.24 (s, 2H), 4.94(d, *J*=8.7Hz, 1H), 4.26 – 4.19(m, 1H), 4.10(dd, *J*=9.6, 4.2Hz, 1H), 3.67–3.58 (m, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 170.63, 135.29, 135.21, 134.92, 133.99, 129.19, 128.08, 127.52, 126.94, 126.11, 125.45, 123.18, 119.24, 115.10, 111.24, 69.88, 67.87, 49.77, 44.64. HRMS (ESI) calcd. $C_{21}H_{17}Cl_2NO_3Na$ $[M+Na]^+$m/z: 424.0478, found: 424.0480.

Synthesis of 4-((6-chloro-1-(4-chlorobenzyl)-1H-indol-3-yl)(hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (16g): compound **20g** (200mg, 657.53 μmol) was converted to compound 1**6g** (196mg, 74.10%) as a white solid by the same procedure as described for compound 1a. m.p.: 150.6-150.8℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* =8.5 Hz, 1H), 7.29 (s, 1H), 7.26(d, *J*=2.6Hz, 1H), 7.24 (d, *J* = 1.6 Hz, 1H), 7.10 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.06 (s, 1H), 7.01 (s, 1H), 6.99 (s, 1H), 6.32 (d, *J* = 2.0 Hz, 1H), 5.81 (d, *J* = 1.4 Hz, 1H), 5.19 (s, 2H), 4.94 (d, *J* = 7.8 Hz, 1H), 4.23–4.16 (m, 1H), 4.13 – 4.04 (m, 1H), 3.64–3.54 (m, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 170.85, 137.25, 135.14, 134.92, 133.89, 129.16, 128.79, 128.10, 126.91, 125.38, 124.54, 120.90, 120.61, 115.75, 110.08, 69.75, 67.98, 49.53, 44.69. HRMS (ESI) calcd. $C_{21}H_{17}Cl_2NO_3Na$ $[M+Na]^+$m/z: 424.0478, found: 424.0480.

Synthesis of 4-((7-chloro-1-(4-chlorobenzyl)-1H-indol-3 -yl)(hydroxy)methyl)-3-methylenedihydrofuran-2(3H)-one (16h): compound **20h** (200mg, 657.53 μmol) was converted to compound 1**6h** (161mg, 60.87%) as a white solid by the same procedure as described for compound 1a. m.p.: 131.2-131.5℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 7.9 Hz, 1H), 7.28–7.25 (overlap, 2H), 7.24(s, 1H), 7.18 (d, *J* = 7.5 Hz, 1H), 7.07 (s, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 6.95 (s, 1H), 6.93 (s, 1H), 6.34 (d, *J* = 2.1 Hz, 1H), 5.81 (d, *J* = 1.5 Hz, 1H), 5.76–5.62 (m, 2H), 4.98 (d, *J* = 7.8 Hz, 1H), 4.22(t, *J*=8.7Hz, 1H), 4.10 (dd, *J*=9.6, 4.2Hz, 1H).^{13}C NMR (100 MHz, DMSO-*d*6) δ 165.52, 131.77, 129.85, 128.20, 126.90, 123.82, 123.76, 123.74, 122.35, 120.23, 119.40, 115.82, 113.12, 111.90, 110.76, 64.38, 62.72, 46.05, 39.36. HRMS (ESI) calcd. $C_{21}H_{17}Cl_2NO_3Na$ $[M+Na]^+$m/z: 424.0478, found: 420.0485.

Synthesis of 4-((1-(4-chlorobenzyl)-5-fluoro-1H- indol-3-yl)(hydroxy)methyl) -3-methylene dihydrofuran-2(3H)-one (16i): compound **20i** (600mg, 695.12 μmol) was converted to compound **16i** (213mg, 79.42%) as an oily yellow liquid by the same procedure as described for compound 1a. m.p.: 67.3-67.7℃. ^{1}H NMR (400

MHz, DMSO-*d*6) δ 7.51 (s, 1H), 7.47 (dd, *J* = 10.1, 2.5 Hz, 1H), 7.41 (dd, *J* = 9.0, 4.5 Hz, 1H), 7.37(d, *J*=8.4Hz, 2H), 7.19(d, *J*=8.4Hz, 2H), 7.00 – 6.92(m, 1H), 6.06 (d, *J* = 1.3 Hz, 1H), 5.66 (d, *J* = 4.6 Hz, 1H), 5.46 (s, 1H), 5.40 (s, 2H), 5.03 (t, *J*=5.4Hz, 1H), 4.35 (t, *J*=8.7Hz, 1H), 4.26(dd, *J*=9.2, 3.9Hz, 1H), 3.62 – 3.56(m, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ 170.65, 156.90 (d, *J*=230Hz), 137.02, 135.61, 132.71, 132.00, 128.89, 128.78, 128.46, 126.38 (d, *J* = 10 Hz), 123.41, 116.07 (d, *J* = 4 Hz), 111.26 (d, *J* = 10 Hz), 109.59 (d, *J* = 26 Hz), 104.46 (d, *J* = 23 Hz), 68.38, 68.15, 48.49, 44.03.HRMS (ESI) calcd. $C_{21}H_{17}ClFNO_3Na$ $[M+Na]^+$m/z: 408.0773/410.0744, found: 408.0776/410.0750.

Synthesis of 5-chloro-1-(4-fluorobenzyl)-1H-indole (23): To the solution of 5-chloroindole (**17)** (200mg, 1.11 mmol) in MeCN(3mL) was added NaH (80.17mg, 3.34 mmol). The resulting mixture was stirred at room temperature for 1h, then a solution of 4-fluorobenzylchloride (**22)**（241.49mg, 1.67 mmol）in MeCN(2mL) added dropwise to the system. The reaction mixture was stirred at room temperature for 4 h. The mixture was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, ethyl acetate/Petroleum ether=12: 1) to give the product **23**(300mg, 93.63%).

Synthesis of (E)-1-(5-chloro-1- (4-fluorobenzyl)- 1H-indol-3-yl)-3- (furan-2-yl) prop-2-en-1-one (24a): To the mixture of $AlCl_3$ (205.36mg, 1.54mmol) in CH_2Cl_2(5mL) was added acetyl chloride (120.90mg, 1.54 mmol). The resulting mixture was stirred at room temperature for 1h, then a solution of compound **23** （200mg, 770.09 μmol）in CH_2Cl_2(1mL) added dropwise. The reaction mixture was stirred at room temperature for 12 h. CH_2Cl_2 was evaporated in vacuo. The mixture was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, ethyl acetate/Petroleum ether=1: 4) to give the intermediate compound (180mg, 77.46%). It (100mg, 331.41 μmol) mixed with a solution of 2-furfural(47.76mg, 497.11μmol) in EtOH (2mL), NaOH(aq)(30μl) at room temperature. After workup, the resulting mixture was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by flash chromatography on silica gel to give the product **24a**(85mg, 67.53%). m.p.: 107.3-106.8℃. ^{1}H NMR (400 MHz, Acetone-*d*6) δ8.67 (s, 1H), 8.50 (d, *J* = 2.0 Hz, 1H), 7.70 (dd, *J* = 13.0, 2.3 Hz, 1H), 7.56 (d, *J* = 15.3 Hz, 1H), 7.51 (d, *J* = 8.7 Hz, 1H), 7.47–7.35 (overlap, 3H), 7.24 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.17–7.04 (m, 2H), 6.86 (d, *J* = 3.4 Hz, 1H), 6.60 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.60 (s, 2H).^{13}C NMR (100 MHz, Acetone-*d*6) δ183.80, 163.29(d, *J*= 243Hz), 152.92, 145.64, 138.15, 136.51, 133.72(d, *J*=3Hz), 130.33 (d, *J* = 8 Hz), 129.23, 128.75, 128.11, 124.41, 122.80, 122.19, 118.14, 116.46(d, *J*=22Hz), 115.83, 113.46, 113.17, 50.75.HRMS (ESI) calcd. $C_{22}H_{15}ClFNO_2Na$ $[M+Na]^+$m/z: 402.0668/404.0638, found: 402.0672/406.0640.

Synthesis of (E)-1-(5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl) -3-(thiophen-2-yl) prop-2-en-1-one (24b): Compound **23** was converted to compound **24b** as a yellow solid by the same procedure as described for compound **24a**. m.p.: 109.6-111.7℃. ^{1}H NMR (400 MHz, Acetone-*d*6) δ 8.72 (s, 1H), 8.49 (d, *J* = 2.0 Hz, 1H), 7.90 (d, *J* = 15.3 Hz, 1H), 7.59 (d, *J* = 5.1 Hz, 1H), 7.55–7.47 (overlap, 2H), 7.46–7.36 (overlap, 3H), 7.25 (dd, *J*=8.7, 2.1Hz, 1H), 7.19–7.06 (overlap, 3H), 5.60 (s, 2H).^{13}C NMR

(100 MHz, Acetone-*d*6)δ183.74, 163.27(d, *J*=243Hz), 141.48, 138.27, 136.52, 134.17, 133.76 (d, *J*=3Hz), 132.22, 130.21 (d, *J* = 8 Hz), 129.25, 129.18(d, *J*=4Hz), 128.74, 124.40, 123.56, 122.79, 118.02, 116.56, 116.34, 113.19, 50.72. HRMS (ESI) calcd. $C_{22}H_{16}ClFNOS$ $[M+H]^+$m/z: 396.0620/398.0590, found: 396.0628/398.0485.

Synthesis of 1-(5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl)-1-ones (21a~21i) with compound 21a as sample: To the solution of $AlCl_3$ (205.36mg) in CH_2Cl_2 was added acetyl chloride compounds (120.90mg, 1.54 mmol). The resulting mixture was stirred at room temperature for 1h, then a solution of compound **23**（200mg, 770.09 μmol）in CH_2Cl_2 was added dropwise. The reaction mixture was stirred at room temperature for 12 h. CH_2Cl_2 was evaporated in vacuo. The mixture was extracted with ethyl acetate(3×50mL), washed with brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel) to give the product compound **21a**. m.p.: 118.2-120.1℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.63 (s, 1H), 8.18 (s, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 7.37 (dd, *J* = 8.6, 5.5 Hz, 2H), 7.26 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.17 (t, *J* = 8.9 Hz, 2H), 5.50 (s, 2H), 2.47 (s, 3H)；^{13}C NMR (100 MHz, DMSO-*d*6) δ 192.33, 161.63(d, *J*=242Hz), 138.63, 134.91, 132.97, 129.46, 129.38, 126.99, 122.97, 120.63, 115.62, 115.41, 112.78, 49.08, 27.22. HRMS (ESI) calcd. $C_{17}H_{13}ClFNONa$ $[M+Na]^+$m/z: 324.0562/326.0532, found: 324.0564/326.0622.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl)(cyclopropyl)methanone (21b): Compound **23** was converted to compound **21b** as a yellow solid by the same procedure as described for compound **21a**. m.p.: 95.5-96.8℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.81 (s, 1H), 8.18 (d, *J* = 2.1 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.42–7.33 (overlap, 2H), 7.25 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.21–7.13 (overlap, 2H), 5.51 (s, 2H), 2.70–2.60 (m, 1H), 1.02–0.95 (m, 2H), 0.94–0.86 (m, 2H)；^{13}C NMR (100 MHz, DMSO-*d*6) δ 194.01, 161.64(d, *J*=242Hz), 138.07, 134.91, 132.96, 129.44 (d, *J* = 8 Hz), 126.94 (d, *J* = 4 Hz), 123.02, 120.77, 115.98, 115.66, 115.45, 112.74, 49.14, 17.36, 9.58. HRMS (ESI) calcd. $C_{19}H_{15}ClFNONa$ $[M+Na]^+$m/z: 350.0718/352.0689, found: 350.0726/352.0718.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl)(furan-2-yl)methanone (21c): Compound **23** was converted to compound **21c** as a yellow solid by the same procedure as described for compound **21a**. m.p, 119.8-121.2℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ8.88 (s, 1H), 8.32 (d, *J* = 2.0 Hz, 1H), 8.05–8.03(overlap, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.44 (d, *J* = 3.5 Hz, 1H), 7.43–7.37 (overlap, 2H), 7.31 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.22–7.14 (overlap, 2H), 6.79 (m, 1H), 5.61 (s, 2H)；^{13}C NMR (100 MHz, DMSO-*d*6) δ175.02, 161.62(d, *J*=242Hz), 152.76, 146.58, 138.59, 134.56, 132.91(d, *J*=3Hz), 129.41(d, *J*=8Hz), 128.30, 127.25, 123.34, 120.89, 116.91, 115.64, 115.43, 112.86(d, 19Hz), 112.32, 49.18. HRMS (ESI) calcd. $C_{20}H_{14}ClFNO_2$ $[M+H]^+$m/z: 354.0692/356.0662, found: 354.0698/356.0677.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl)(3-methyl-2,5-dihydrothiophen -2-yl)methanone (21d): Compound **23** was converted to compound **21d** as a white solid by the same procedure as described for compound **21a**. m.p.: 123.5-125.1℃. ^{1}H NMR (400 MHz, Acetone-*d*6) δ8.37 (d, *J* = 2.0 Hz, 1H), 8.32 (s, 1H), 7.62 (d, *J* = 5.0 Hz, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 7.44 -7.38 (overlap, 2H), 7.28 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.16-7.10 (m, 2H), 7.08 (d, *J* = 5.0 Hz, 1H), 5.63

(s, 2H), 2.49 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 181.77, 161.63(d, *J*=243Hz), 142.14, 138.68, 134.93 (d, *J* = 16 Hz), 132.92, 131.90, 129.52 (d, *J* = 8 Hz), 123.42, 120.74, 115.64, 115.43, 112.99, 49.03, 15.81. HRMS (ESI) calcd. $C_{17}H_{16}ClFNOS$ $[M+H]^+$m/z: 384.0620/386.0590, found: 384.0625/386.0672.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl)(phenyl)methanone (21e): Compound **23** was converted to compound **21e** as a light-yellow solid by the same procedure as described for compound **21a**. m.p.: 104.8-106.5℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ8.38 (s, 1H), 8.27 (d, *J* = 1.7 Hz, 1H), 7.83 (d, *J* = 7.4 Hz, 2H), 7.70–7.50 (overlap, 4H), 7.41–7.34 (overlap, 2H), 7.31 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.20–7.10 (overlap, 2H), 5.55 (s, 2H); ^{13}C NMR (100 MHz, DMSO-*d*6)δ189.43, 161.61(d, *J*=242Hz), 139.91, 134.95, 132.89(d, *J* = 3Hz), 131.39, 129.42(d, *J*=8Hz), 129.23, 128.47 (d, *J*=6Hz), 128.17, 127.28, 123.36, 120.90, 115.59, 115.38, 113.86, 112.95, 49.12. HRMS (ESI) calcd. $C_{22}H_{16}ClFNO$ $[M+H]^+$m/z: 364.0899/366.0869, found: 364.0906/366.0825.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl) (4-fluorophenyl) methanone (21f): Compound **23** was converted to compound **21f** as a yellow solid by the same procedure as described for compound **21a**. m.p.: 107.4-109.8℃. ^{1}H NMR (400 MHz, DMSO-*d*6)δ8.40 (s, 1H), 8.26 (d, *J* = 2.1 Hz, 1H), 8.03 -7.97 (m, 1H), 7.95–7.88 (overlap, 2H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.44–7.36 (overlap, 4H), 7.20–7.12 (overlap, 2H), 5.55 (s, 2H).^{13}C NMR (100 MHz, DMSO-*d*6)δ187.98, 165.77(d, *J*=111Hz), 161.07(d, *J*=136Hz), 160.39, 139.95, 136.39 (d, *J*=3Hz), 134.93, 132.86 (d, *J*=3Hz), 132.06(d, 9Hz), 131.15(d, *J*=9Hz), 129.44(d, *J*=8Hz), 128.14, 127.29, 123.40, 120.84, 115.58(d, *J*=2Hz), 115.36(d, *J*=2Hz), 113.35(d, *J*=68Hz), 49.15. HRMS (ESI) calcd. $C_{22}H_{15}ClF_2NO$ $[M+H]^+$m/z: 382.0805/384.0775, found: 382.0809/384.0662.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl)(p-tolyl)methanone (21g): Compound **23** was converted to compound **21g** as a light-yellow solid by the same procedure as described for compound **21a**. m.p.: 126.0-129.1℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.34 (s, 1H), 8.24 (d, *J* = 2.1 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.39- 7.32 (overlap, 4H), 7.28 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.18–7.10 (overlap, 2H), 5.53 (s, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, DMSO-*d*6)δ189.14, 161.59(d, *J*=242Hz), 141.49, 139.64, 137.20, 134.89, 129.44 (d, *J* = 8 Hz), 129.03, 128.59, 128.19, 127.15, 123.28, 120.86, 115.60, 115.38, 113.91, 112.93, 49.08, 21.03. HRMS (ESI) calcd. $C_{23}H_{18}ClFNO$ $[M+H]^+$m/z: 378.1055/380.1026, found: 378.1066/380.1255.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl)(pyridin-4-yl)methanone (21h): Compound **23** was converted to compound **21h** as a white solid by the same procedure as described for compound **21a. m.p.**: 142.1-144.7℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.81 (d, *J* = 1.6 Hz, 2H), 8.80(d, *J*=1.5Hz) 8.45 (s, 1H), 8.26 (d, *J* = 2.1Hz, 1H), 7.72 (d, *J* = 1.6 Hz, 1H), 7.71 (d, *J* = 1.6 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.42- 7.36 (overlap, 2H), 7.34(dd, *J* = 8.8, 2.2 Hz, 1H), 7.22–7.11 (overlap, 2H), 5.55 (s, 2H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 188.02, 161.63(d, *J*=242Hz), 150.24, 146.34, 140.98, 135.06, 132.69(d, *J*=3Hz), 129.50(d, *J*=8Hz), 127.73(d, *J*=8Hz), 123.71, 122.13, 120.79, 115.50(d, *J*=21Hz), 113.30 (d, *J* = 16 Hz), 49.25. HRMS (ESI) calcd. $C_{21}H_{15}ClFN_2O$ $[M+H]^+$m/z: 365.0851/367.0822, found: 365.0854/367.0844.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl) (2-chloropyridin-4-yl) methanone (21i): Compound **23** was converted to compound **21i** as a sandy beige solid by the same procedure as described for compound **21a**. m.p.: 124.6-127.0℃. ^{1}H NMR (400 MHz, Acetone-*d*6) δ8.58 (d, *J* = 5.0 Hz, 1H), 8.39 (d, *J* = 2.1 Hz, 1H), 8.37 (s, 1H), 7.75 (s, 1H), 7.72 (dd, *J* = 5.0, 1.3 Hz, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 7.44–7.36 (overlap, 2H), 7.32 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.14–7.07 (overlap, 2H), 5.62 (s, 2H); ^{13}C NMR(100 MHz, Acetone-*d*6)δ187.26, 163.30 (d, *J*=243Hz), 152.43, 151.37, 151.29, 141.32, 136.57, 133.43(d, *J*= 9Hz), 130.27 (d, *J* = 9 Hz), 129.43, 129.24, 124.94, 123.61, 122.38 (d, *J* = 10 Hz), 116.55, 116.33, 115.00, 113.64, 50.89. HRMS (ESI) calcd. $C_{21}H_{13}Cl_2FN_2ONa$ $[M+Na]^+$m/z: 421.0281, found: 421.0288.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl)(phenyl)methanol (25): To a stirred solution of compound **21f** (500mg, 1.37 mmol) in anhydrous MeOH (4mL) and THF (1mL) was added dropwise $NaBH_4$ in MeOH (1mL) and NaOH (1%, 1mL) at room temperature. The reaction mixture was stirred at room temperature for 2-5 h. After work up, the resulting mixture was extracted with ethyl acetate(3×50mL), washed with brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by flash chromatography on silica gel (ethyl acetate/Petroleum ether=1: 8) to give the product **25** (85mg, 67.53%). m.p.: 96.5-97.9℃. ^{1}H NMR (400 MHz, Acetone-*d*6) δ7.55 (d, *J* = 2.0 Hz, 1H), 7.52 (s, 1H), 7.51 (s, 1H), 7.37–7.28 (overlap, 3H), 7.27 (s, 1H), 7.25–7.18 (overlap, 3H), 7.08–7.06 (m, 1H), 7.06 - 7.02 (overlap, 2H), 6.09 (d, *J* = 4.4 Hz, 1H), 5.37 (s, 2H), 4.69 (t, *J* = 5.9 Hz, 1H). HRMS (ESI) calcd. $C_{22}H_{17}ClFNONa$ $[M+Na]^+$m/z: 388.0875/390.0845, found: 388.0881/390.1012.

Synthesis of (5-chloro-1-(4-fluorobenzyl)-1H-indol-3-yl)(phenyl)methanone oxime (26): Hydroxylamine hydrochloride (954 mg, 13.74 mmol) was dissolved in EtOH/pyridine(1: 1, 5mL). The reaction mixture was stirred at room temperature for 30 min. Compound **21f** (500mg, 1.37mmol) was added to the reaction solution at room temperature. After workup, insoluble material was removed by diatomite. The resulting mixture was extracted with ethyl acetate(3×50mL), washed with brine, dried (Na_2SO_4), and concentrated. The crude residue was purified by flash chromatography on silica gel (acetone/Petroleum ether=1: 10) to give the product **26** (467mg, 89.70%). m.p.: 179.6-181.5℃. ^{1}H NMR (400 MHz, DMSO-*d*6) δ11.43(s, 1H), 8.19 (s, 1H), 7.55 (d, *J* = 8.7 Hz, 1H), 7.48–7.39 (overlap, 5H), 7.34–7.29 (overlap, 2H), 7.21–7.14 (overlap, 2H), 7.12 (dd, *J* = 8.8, 2.1 Hz, 1H), 6.72 (d, *J* = 2.0 Hz, 1H), 5.52 (s, 2H); ^{13}C NMR (100 MHz, DMSO-*d*6)δ 161.51(d, *J*= 242Hz), 149.71, 137.51, 134.61, 133.75, 133.65 (d, *J*=3Hz), 129.25(d, *J* =8Hz), 128.73, 128.26, 128.10, 127.73, 124.33, 121.44, 120.42, 115.55, 112.20, 106.32, 48.61. HRMS (ESI) calcd. $C_{22}H_{17}ClFN_2O$ $[M+H]^+$m/z: 379.1008/381.0978, found: 379.1011/381.1022.

4.3 Inhibit evaluation of compounds for LSD1

The activity of LSD1 was measured according to our previous publication [10]. Compounds were incubated with the recombinant LSD1 and H3K4me2. Hydrogen peroxide that produced during the demethylation of LSD1 will be quantified by Amplex Red to indicate the inhibition ability of compounds against LSD1. The data is analyzed by GraphPad prism 6.0.

4.4 Dilution assay

Inhibitors with a concentration of 20 folds of IC50 were incubated with recombinant LSD1. Then the mixture was diluted 80 folds to test the activity. If compounds inhibit

the activity of LSD1 in a reversible manner, LSD1 activity will recovery after dilution. The data is analyzed by GraphPad prism6.0.

4.5 Ultrafiltration assay

Inhibitors with a concentration of 20 folds of IC50 were incubated with recombinant LSD1. Then the mixture was added to a 1K cut-off ultrafiltration tube, and then subjected to 10000 rpm, 10 min centrifuge. The lower chamber solution is then taken to test whether it can inhibit the activity of LSD1. If compounds inhibit the activity of LSD1 in an irreversible manner, the compounds will remain firmly bound to the protein and will still be in the upper chamber of the ultrafiltration tube. Then the lower layer solution will not affect the LSD1 activity. The data is analyzed by GraphPad prism6.0.

4.6 Surface plasmon resonance (SPR) experiment

Studies of binding kinetics are performed on a Biacore S200 (GE Healthcare, USA). LSD1 is aimed for an immobilization level of approximately 8000 RU with a CM5 sensor chip, and the running buffer is PBS-P (0.2 M phosphate buffer, 0.027 M KCl, 1.37 M NaCl, 0.5% Surfactant P20, PH=7.4). The direct binding assay is tested with buffer and sample in 2% DMSO. Then compounds with a twofold dilution series are injected for 60s and dissociated for 300s at a flow rate of 30 μL/min. The data is analyzed by Biacore S200 Evaluation Software 1.0.

4.7 Protein Thermal Shift assay

SYPRO Orange was used as the dye to indicate the folding and unfolding status of LSD1. LSD1 recombinant, compound, dye, and HEPES buffer were mixed as the sample group, while the reference group was the mixture of LSD1 recombinant, DMSO, dye and HEPES buffer. Melting temperature (Tm) of sample group and reference group is obtained by gradually increasing the temperature to unfold the protein and measuring the fluorescence at each point. The fluorescence is monitored by real-time PCR instrument (Thermo Fisher, USA) and analyzed by Protein Thermal Shift Software 1.3 (Thermo Fisher, USA).

4.8 Cell cultures

The human acute myeloid leukemia cell THP-1 was obtained from Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Cells are cultured in RPMI-1640 medium plus 10% fetal bovine serum (FBS) and maintained in an incubator at 37ºC and 5% CO_2.

4.9 Cell proliferation assay

4000 cells are seeded in a 96-well plate and then incubated for 5 days in standard medium in the presence of compounds with different concentration. After the incubation period, cells are quantified with MTT (Solarbio, China). The data is analyzed by GraphPad prism6.0.

4.10 Hematoxylin and Eosin stain

THP-1 cells are seeded in 6-well plate with different concentration of compounds then incubated for 3 days in standard medium. After the incubation period, medium contain cells are centrifuged to gather the cells. Resuspend the cells by PBS and apply them evenly to low-absorbance glass. After followed steps contain staining, dehydration, cleaning with xylene and covered by coverslips upon gum (Solarbio, China), slides are photographed by light microscope (Leica, German)

4.11 Colony formation assays

Add 1 mL methylcellulose-based medium in a six-well plate, add 500 cells in every well with different concentration of compounds, then pipet the medium up and down gently to mix them thoroughly. Place the plate in an incubator at 37°C and 5% CO_2 for 2 weeks. Pictures were taken by the light microscope (Leica, German).

4.12 Flow Cytometry Assay

Cells were seeded in six-well plate, treated by **9e** at 0, 0.25 μM, 1 μM, 4 μM for 3 days before analysis. PerCP-conjugated mouse anti-human CD86 monoclonal antibody (Abcam, USA) is used for staining 40 min. Samples were acquired using BD Calibur flow cytometer (BD, USA) according to the manufacturer's instructions. Then results were analyzed with FlowJo V7.6 software (TreeStar, USA).

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References

[1] P. Cheung, C.D. Allis, P. Sassone-Corsi, Signaling to chromatin through histone modifications, Cell, 103 (2000) 263-271.

[2] B.D. Strahl, C.D. Allis, The language of covalent histone modifications, Nature, 403 (2000) 41-45.

[3] R. Karlic, H.R. Chung, J. Lasserre, K. Vlahovicek, M. Vingron, Histone modification levels are predictive for gene expression, Proceedings of the National Academy of Sciences of the United States of America, 107 (2010) 2926-2931.

[4] J. Huang, R. Sengupta, A.B. Espejo, M.G. Lee, J.A. Dorsey, M. Richter, S. Opravil, R. Shiekhattar, M.T. Bedford, T. Jenuwein, S.L. Berger, p53 is regulated by the lysine demethylase LSD1, Nature, 449 (2007) 105-108.

[5] H. Kontaki, I. Talianidis, Lysine methylation regulates E2F1-induced cell death, Molecular cell, 39 (2010) 152-160.

[6] Y. Shi, F. Lan, C. Matson, P. Mulligan, J.R. Whetstine, P.A. Cole, R.A. Casero, Y. Shi, Histone demethylation mediated by the nuclear amine oxidase homolog LSD1, Cell, 119 (2004) 941-953.

[7] E. Metzger, M. Wissmann, N. Yin, J.M. Muller, R. Schneider, A.H. Peters, T. Gunther, R. Buettner, R. Schule, LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription, Nature, 437 (2005) 436-439.

[8] C.A. Stewart, L.A. Byers, Altering the Course of Small Cell Lung Cancer: Targeting Cancer Stem Cells via LSD1 Inhibition, Cancer cell, 28 (2015) 4-6.

[9] J.H. Schulte, S. Lim, A. Schramm, N. Friedrichs, J. Koster, R. Versteeg, I. Ora, K. Pajtler, L. Klein-Hitpass, S. Kuhfittig-Kulle, E. Metzger, R. Schule, A. Eggert, R. Buettner, J. Kirfel, Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy, Cancer research, 69 (2009) 2065-2071.

[10] Y.C. Zheng, Y.C. Duan, J.L. Ma, R.M. Xu, X. Zi, W.L. Lv, M.M. Wang, X.W. Ye, S. Zhu, D. Mobley, Y.Y. Zhu, J.W. Wang, J.F. Li, Z.R. Wang, W. Zhao, H.M. Liu, Triazole-dithiocarbamate based selective lysine specific demethylase 1 (LSD1) inactivators inhibit gastric cancer cell growth, invasion, and migration, Journal of medicinal chemistry, 56 (2013) 8543-8560.

[11] A. Yokoyama, S. Takezawa, R. Schule, H. Kitagawa, S. Kato, Transrepressive function of TLX requires the histone demethylase LSD1, Molecular and cellular biology, 28 (2008) 3995-4003.

[12] Y. Wang, H. Zhang, Y. Chen, Y. Sun, F. Yang, W. Yu, J. Liang, L. Sun, X. Yang, L. Shi, R. Li, Y. Li, Y. Zhang, Q. Li, X. Yi, Y. Shang, LSD1 is a subunit of the NuRD complex and targets the metastasis

programs in breast cancer, Cell, 138 (2009) 660-672.
[13] T. Schenk, W.C. Chen, S. Gollner, L. Howell, L. Jin, K. Hebestreit, H.U. Klein, A.C. Popescu, A. Burnett, K. Mills, R.A. Casero, Jr., L. Marton, P. Woster, M.D. Minden, M. Dugas, J.C. Wang, J.E. Dick, C. Muller-Tidow, K. Petrie, A. Zelent, Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-trans-retinoic acid differentiation pathway in acute myeloid leukemia, Nature medicine, 18 (2012) 605-611.
[14] J.C. Culhane, D. Wang, P.M. Yen, P.A. Cole, Comparative analysis of small molecules and histone substrate analogues as LSD1 lysine demethylase inhibitors, Journal of the American Chemical Society, 132 (2010) 3164-3176.
[15] Y.C. Zheng, B. Yu, Z.S. Chen, Y. Liu, H.M. Liu, TCPs: privileged scaffolds for identifying potent LSD1 inhibitors for cancer therapy, Epigenomics, 8 (2016) 651-666.
[16] T. Maes, C. Mascaro, A. Ortega, S. Lunardi, F. Ciceri, T.C. Somervaille, C. Buesa, KDM1 histone lysine demethylases as targets for treatments of oncological and neurodegenerative disease, Epigenomics, 7 (2015) 609-626.
[17] A. Sprussel, J.H. Schulte, S. Weber, M. Necke, K. Handschke, T. Thor, K.W. Pajtler, A. Schramm, K. Konig, L. Diehl, P. Mestdagh, J. Vandesompele, F. Speleman, H. Jastrow, L.C. Heukamp, R. Schule, U. Duhrsen, R. Buettner, A. Eggert, J.R. Gothert, Lysine-specific demethylase 1 restricts hematopoietic progenitor proliferation and is essential for terminal differentiation, Leukemia, 26 (2012) 2039-2051.
[18] S.Y. Dent, J. Chandra, The lasting influence of LSD1 in the blood, eLife, 2 (2013) e00963.
[19] W. Fiskus, S. Sharma, B. Shah, B.P. Portier, S.G.T. Devaraj, K. Liu, S.P. Iyer, D. Bearss, K.N. Bhalla, Highly effective combination of LSD1 (KDM1A) antagonist and pan-histone deacetylase inhibitor against human AML cells, Leukemia, 31 (2017) 1658.
[20] D.B. Sykes, Y.S. Kfoury, F.E. Mercier, M.J. Wawer, J.M. Law, M.K. Haynes, T.A. Lewis, A. Schajnovitz, E. Jain, D. Lee, H. Meyer, K.A. Pierce, N.J. Tolliday, A. Waller, S.J. Ferrara, A.L. Eheim, D. Stoeckigt, K.L. Maxcy, J.M. Cobert, J. Bachand, B.A. Szekely, S. Mukherjee, L.A. Sklar, J.D. Kotz, C.B. Clish, R.I. Sadreyev, P.A. Clemons, A. Janzer, S.L. Schreiber, D.T. Scadden, Inhibition of Dihydroorotate Dehydrogenase Overcomes Differentiation Blockade in Acute Myeloid Leukemia, Cell, 167 (2016) 171-186.e115.
[21] Y. Ishikawa, K. Gamo, M. Yabuki, S. Takagi, K. Toyoshima, K. Nakayama, A. Nakayama, M. Morimoto, H. Miyashita, R. Dairiki, Y. Hikichi, N. Tomita, D. Tomita, S. Imamura, M. Iwatani, Y. Kamada, S. Matsumoto, R. Hara, T. Nomura, K. Tsuchida, K. Nakamura, A Novel LSD1 Inhibitor T-3775440 Disrupts GFI1B-Containing Complex Leading to Transdifferentiation and Impaired Growth of AML Cells, Mol. Cancer Ther., 16 (2017) 273-284.
[22] D.P. Mould, A.E. McGonagle, D.H. Wiseman, E.L. Williams, A.M. Jordan, Reversible inhibitors of LSD1 as therapeutic agents in acute myeloid leukemia: clinical significance and progress to date, Med. Res. Rev., 35 (2015) 586-618.
[23] N. Sugino, M. Kawahara, A novel LSD1 inhibitor NCD38 ameliorates MDS-related leukemia with complex karyotype by attenuating leukemia programs via activating super-enhancers, 31 (2017) 2303-2314.
[24] H.W. Xu, L.J. Zhao, H.F. Liu, D. Zhao, J. Luo, X.P. Xie, W.S. Liu, J.X. Zheng, G.F. Dai, H.M. Liu, L.H. Liu, Y.B. Liang, Synthesis and anti-BVDV activity of novel delta-sultones in vitro: implications for HCV therapies, Bioorganic & medicinal chemistry letters, 24 (2014) 2388-2391.
[25] G. Dai, H. Xu, Z. Jiang, H. Liu, Y. Wang, Z. Kuicheng, W. Zhenwei, W. Li, F. Wu, L. Mengjiao, Uses of c15-substituted andrographolide derivatives in the preparation of anti-hepatitis b virus

medicament, in, Google Patents, 2014.

[26] M.D. Cummings, M.A. Farnum, M.I. Nelen, Universal screening methods and applications of ThermoFluor, Journal of biomolecular screening, 11 (2006) 854-863.

[27] M.K. Groftehauge, N.R. Hajizadeh, M.J. Swann, E. Pohl, Protein-ligand interactions investigated by thermal shift assays (TSA) and dual polarization interferometry (DPI), Acta crystallographica. Section D, Biological crystallography, 71 (2015) 36-44.

[28] F. Forneris, R. Orru, D. Bonivento, L.R. Chiarelli, A. Mattevi, ThermoFAD, a Thermofluor-adapted flavin ad hoc detection system for protein folding and ligand binding, The FEBS journal, 276 (2009) 2833-2840.

[29] Z.K. Zhao, H.F. Yu, D.R. Wang, P. Dong, L. Chen, W.G. Wu, W.J. Ding, Y.B. Liu, Overexpression of lysine specific demethylase 1 predicts worse prognosis in primary hepatocellular carcinoma patients, World journal of gastroenterology, 18 (2012) 6651-6656.

[30] W. Fiskus, S. Sharma, B. Shah, B.P. Portier, S.G. Devaraj, K. Liu, S.P. Iyer, D. Bearss, K.N. Bhalla, Highly effective combination of LSD1 (KDM1A) antagonist and pan-histone deacetylase inhibitor against human AML cells, Leukemia, 28 (2014) 2155-2164.

[31] W.J. Harris, X. Huang, J.T. Lynch, G.J. Spencer, J.R. Hitchin, Y. Li, F. Ciceri, J.G. Blaser, B.F. Greystoke, A.M. Jordan, C.J. Miller, D.J. Ogilvie, T.C. Somervaille, The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells, Cancer cell, 21 (2012) 473-487.

[32] J.T. Lynch, M.J. Cockerill, J.R. Hitchin, D.H. Wiseman, T.C. Somervaille, CD86 expression as a surrogate cellular biomarker for pharmacological inhibition of the histone demethylase lysine-specific demethylase 1, Analytical biochemistry, 442 (2013) 104-106.

[33] J. Fang, H. Ying, T. Mao, Y. Fang, Y. Lu, H. Wang, I. Zang, Z. Wang, Y. Lin, M. Zhao, X. Luo, Z. Wang, Y. Zhang, C. Zhang, W. Xiao, Y. Wang, W. Tan, Z. Chen, C. Lu, P. Atadja, E. Li, K. Zhao, J. Liu, J. Gu, Upregulation of CD11b and CD86 through LSD1 inhibition promotes myeloid differentiation and suppresses cell proliferation in human monocytic leukemia cells, Oncotarget, 8 (2017) 85085-85101.

- Compound **9e** was designed and synthesized based on a lead compound obtained from our in-house compound library.
- Compound **9e** inhibits LSD1 (IC50 = 1.230 μM) and shows good anti-proliferation activity in THP-1 cells.
- Compound **9e** can significantly increase the expression of CD86 and induce differentiation of THP-1 cells.
- Compound **9e** is the first irreversible LSD1 inhibitor that is not derived from monoamine oxidase inhibitors.