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ORIGINAL RESEARCH

Design, synthesis and biological evaluation of novel 1,5-disubstituted isatin derivatives as antitumor agents

Huijun Zhuo[1] · Zhen Zhang[1] · Yang Liu[2] · Jingya Zhang[1] · Guisen Zhao[1]

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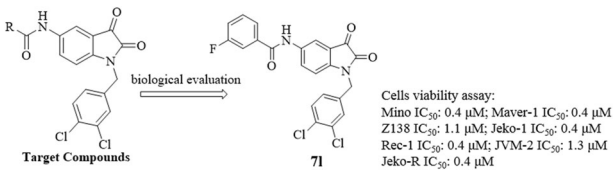
Abstract

Isatin (1*H*-indole-2,3-dione) was reported to possess anticancer activities through its effect on tumor proliferation, apoptosis, and metastasis in vitro and in vivo. Here, we described the synthesis of a novel series of 1,5-disubstituted isatin derivatives with 2-indolinone scaffold as antitumor agents. Most of the synthesized compounds revealed potent antiproliferative effects in mantle cell lymphoma (MCL) cell lines, among which **7l** possessed promising activities with IC_{50} values ranging from 0.4 to 1.3 μM. Following flow cytometric analysis, compound **7l** efficiently arrested the cell cycle at G2/M phase, and induced apoptosis. Thus, this study shows promise in therapeutics of 1,5-disubstituted isatin derivatives in MCL and provides novel potential and efficient antitumor agents.

Graphical Abstract



Keywords Apoptosis · Isatin derivatives · Mantle cell lymphoma · Antitumor

Introduction

Apoptosis, an evolutionary highly conserved form of programmed cell death, refers to the orderly death of cells controlled by genes in order to maintain a stable internal environment [1, 2]. Apoptosis can be triggered by extrinsic and intrinsic death receptors, and the intrinsic pathway is closely regulated by the B-cell lymphoma 2 (Bcl-2) family of intracellular proteins [3–6]. Escaping apoptotic cell death machinery is a hallmark of cancer [7, 8]. Mutagenic inactivation of apoptotic proteins and overexpression of anti-apoptotic proteins have been found in a variety of cancers, which are the cause of cell proliferation [5, 7, 9].

Mantle cell lymphoma (MCL) is a highly aggressive form of non-Hodgkin-lymphoma (NHL) with a median survival of ~3–5 years and accounts for 6–8% of NHL [10–12]. In spite of the low incidence rate, MCL is considered to be incurable in clinic due to poor prognosis and limited survival [13]. Increased expression of antiapoptotic proteins BCL-2, MCL-1, and Bcl-xL, and loss of proapoptotic proteins BIM is common in MCL [14–16]. Accumulating evidence suggests that the pathogenetic processes of MCL is related to impaired apoptosis regulation [14, 17–19]. Targeting antiapoptotic molecules to induce apoptosis is emerging as a promising therapeutic strategy in MCL.

Isatin (1*H*-indole-2,3-dione, Fig. 1) and its derivatives can participate in a variety of biological activities, which

✉ Guisen Zhao
guisenzhao@sdu.edu.cn

[1] Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Cheeloo College of Medicine, Shandong University, Jinan, 250012 Shandong, PR China

[2] Department of Lymphoma and Myeloma, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

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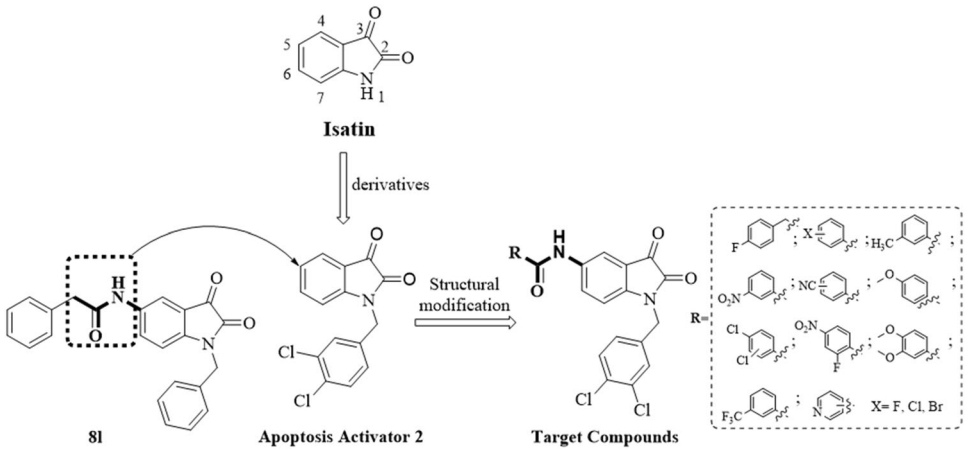
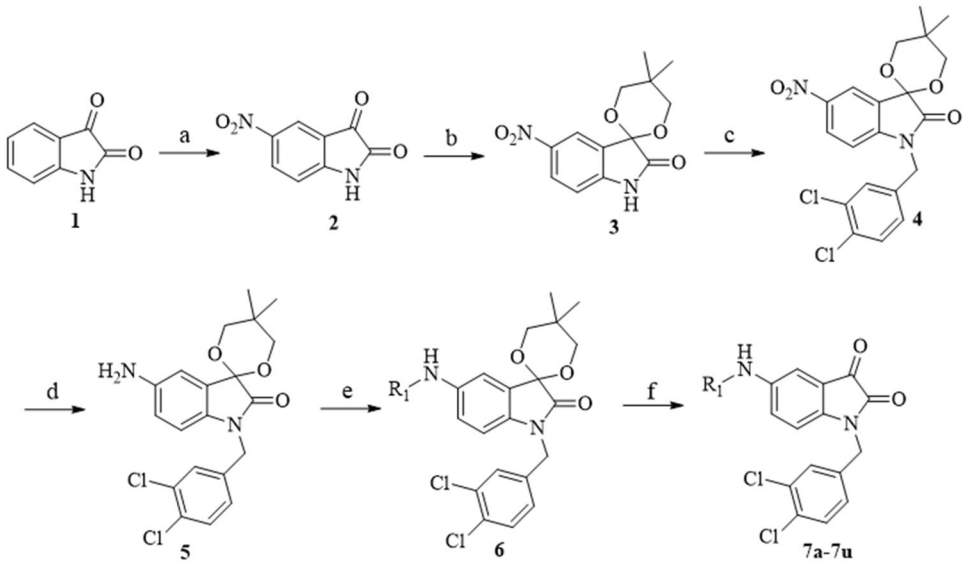


Fig. 1 Design of the target compounds



Scheme 1 Reagents and conditions: **a** fuming HNO_3, concentrated H_2SO_4, 0 °C 1 h; **b** 2,2-dimethyl-1,3-propanediol, p-toluenesulfonic acid, cyclohexane, 85 °C, 16 h; **c** 1,2-dichloro-4-(chloromethyl) benzene, K_2CO_3, DMF, 85 °C, 1.5 h; **d** H_2, 10% Pd/C, ethyl acetate, r.t., 12 h; **e** substituted aromatic formic acid, HBTU, DIEPA, DMF, r.t., 12 h; **f** acetic acid, concentrated hydrochloric acid, r.t., 12 h

have demonstrated anticancer [20, 21], antiviral [22], antibacterial [23], anti-HIV [24] efficacy, etc. Compound **apoptosis activator 2** (**AA2**, Fig. 1), a benzyl substituted isatin derivative, strongly induces caspase-3 activation, poly(ADP-ribose) polymerase cleavage, and DNA fragmentation, leading to the destruction of cells with IC_{50} of 4–9 μM [25, 26]. Notably, compound **8l** (Fig. 1) is another isatin analog with favorable in vitro antiproliferation activity that was reported in our previous article [27]. In this paper, we used 1*H*-indole-2,3-dione as the scaffold, modified the substituents at N-1 and C-5 with 3,4-dichlorobenzyl group and different substituted side chain respectively to design the novel 1,5-disubstituted isatin derivatives (Fig. 1). The biological effects of the designed compounds were investigated for their antiproliferative activity against MCL cells, as well as efficacy in cell apoptosis and cell cycle.

Results and discussion

Chemistry

The series of 1,5-disubstituted isatin derivatives were synthesized according to Scheme 1. Commercially available isatin (**1**) was treated with fuming nitric acid under ice bath conditions to give the nitrated indoledione (**2**). The carbonyl-protection of intermediate (**3**) was generated by 2,2-dimethyl-1,3-propanediol and p-toluenesulfonic acid as catalyst in cyclohexane. Then, deprotonation at N-1 under alkaline conditions, followed by the addition of the 1,2-dichloro-4-(chloromethyl)benzene to give the intermediate (**4**). Subsequently, the nitro group was reduced under mild conditions with Palladium on carbon (Pd/C) under hydrogen atmosphere to corresponding intermediate (**5**). Acylation of aniline (**5**) was performed using standard amide

coupling conditions with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and N, N-diisopropylethylamine (DIPEA) in dimethylformamide (DMF). Final deprotection with acetic acid yielded target compounds (**7a**–**7u**) in good overall yields.

Cell antiproliferation assay

This series of compounds were investigated for their effect against MCL cell lines (Mino, Maver-1, Jeko-1, Z138, Rec-1, JVM-2 and Jeko-R). Ibrutinib (**IBN**), which has received FDA-approval in MCL treatment [28], and aforementioned **AA2** served as the positive control. The results were expressed as the IC_{50} (50% maximun inhibitory concentration). As shown in Table 1, tested compounds were generally more potent than the reference **IBN** and **AA2** in most of tested cancer cell lines, specifically compounds **7c**–**7i**, **7k**, **7l**, **7n**–**7q** and **7s**–**7u** with IC_{50} values ranged from 0.4–0.8 μM, about 5 to10-fold more potent than **IBN** (IC_{50} = 3.9 μM) against IBN-sensitive Mino cells. Furthermore, compounds **7b**, **7j** and **7r** showed moderate activities (IC_{50} = 3.7 μM, 2.2 μM and 2.2 μM, respectively) against Mino cancer cell compared to **IBN**. The IC_{50} values of tested compounds ranged from 0.4 to 4.3 μM, 0.7 to 18.1 μM, and 0.4 to 6.3 μM in comparison to **IBN** (IC_{50} = 12.7 μM, 24.2 μM and 11.7 μM, respectively) among the three different IBN-resistant cell lines (Maver-1, Z138, Jeko-R). Furthermore, replacing amide side chain with the amino group (**7a**) weakened the antiproliferation activity. As for **7b**–**7u** that possess different amide chain at C-5, substituted amide side chain were benzoyl or picolinoyl moiety exert more potent antiproliferation activity than phenylacetyl. Among them, the antiproliferation activity of **7n** was stronger than that of **7c**, **7e**, **7i**–**7j** and **7m**, which can be inferred that the contribution of meta-position substituted benzoyl moiety for activity is electron donating > withdrawing groups. Besides, the antiproliferation activity of picolinoyl moiety substituted compounds (**7s**–**7u**) gradually increased (4-picolinoyl > 3-picolinoyl > 2-picolinoyl) for IBN-resistant cell lines. All compounds with dual substituents (**7o**, **7p**, **7q**, **7r**) exhibited better antiproliferative activities in IBN-sensitive cell with twofold potent than in IBN-resistant cell. Five remarkable compounds (**7g**, **7l**, **7n**, **7t**, **7u**) showed no bias between IBN-sensitive cell and IBN-resistant cell with similar IC_{50} = 0.4–1.4 μM.

Cell apoptosis and cell cycle assay

In order to elucidate the potential antitumor mechanism, the compound (**7l**) was further evaluated for cell apoptosis assay with Rec-1 and Z138 cells by an Annexin V-FITC/PI dual staining assay, for which compound **7l** was diluted to achieve four increasing drug concentrations ranging from 0.5 to 5 μM. The results showed that compound **7l** was capable of increasing apoptosis in a dose-dependent manner. (Fig. 2a). It was demonstrated that **7l** could induce an increase in the late and early cellular apoptosis, which was in accord with its antiproliferative capacity that Rec-1 was more sensitive to **7l** than Z138 (IC_{50} = 0.4 μM and 1.1 μM, respectively). The results showed a shift of the **7l**-treated cells from the normal to the apoptotic phase. Next, to address the mechanism responsible for MCL cell cycle progression, the Rec-1 and Z138 cell lines were stained and analysed by flow cytometry. As shown in Fig. 2b, **7l** significantly blocked the cell cycle at the G2/M phase in a dose-dependent manner in Rec-1 cell after 24 h treatment. Z138 cells with **7l** were also arrested at G2/M phase, preventing cell cycle progression and promoting cell death (Fig. 2c).

Experimental

General procedures

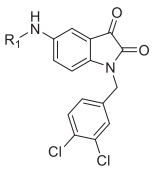
Unless otherwise stated, all starting materials, solvents, and reagents were purchased and used from commercial sources. All reagents were weighed out under ambient conditions. Flash column chromatography was performed using silica gel 60 (200–300 mesh). Thin layer chromatography (TLC) was carried out on silica gel plates and visualized by UV. Melting points using melting point apparatus (Buchi Labortechnik AG, Switzerland) were measured in open capillary tubes. Nuclear magnetic resonance spectra were recorded on Bruker Avance DRX - 400 spectrometers, at 400 MHz ^{1}H NMR frequency, 101 MHz ^{13}C NMR frequency. H and C chemical shifts are reported in ppm. Multiplicities are reported as follows: s (singlet), br (broad), d (doublet), dd (doublet of doublets), dt (doublet of triplets), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) are reported in hertz. The mass spectra (MS) were measured with LCQ FLEET (ThermoFisher, USA).

General procedure for the synthesis of 5-nitroindoline-2,3-dione (2)

To a solution of isatin **1** (200 mg, 1.36 mmol) in concentrated H_2SO_4 (3 mL) was added dropwise fuming nitric acid (124 mg, 1.77 mmol) at 0 °C. The mixture was stirred for an additional 1 h in ice bath conditions. Finally, the reaction mixture was poured into ice/water (50 mL) of crushed ice, the formed precipitate was filtered, washed several times with water and dried to afford 5-nitroisatin **2**.

Yellow solid; yield 85%; mp: 257–259 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.66 (s, 1H, NH), 8.45 (dd, J = 8.7, 2.4 Hz, 1H, Ph-H), 8.22 (d, J = 2.4 Hz, 1H, Ph-H), 7.09 (d, J = 8.7 Hz, 1H, Ph-H).

Table 1 Cell antiproliferation assay assessing the effects of novel 1,5-disubstituted isatin derivatives on MCL cell lines



Code	R_1	Cell viability assay, IC_{50} /μM						
		Mino[a]	Maver-1[b]	Jeko-1[a]	Z138[b]	Rec-1[a]	JVM-2[a]	Jeko-R[b]
IBN	---	3.9	12.7	5.9	24.2	0.5	17.0	11.7
AA2	---	2.1	3.2	0.9	32.5	1.1	10.7	5.4
7a	**H**	19.5	19.9	ND[c]	14.6	ND	ND	21.8
7b		3.7	2.0	ND	1.2	ND	ND	1.9
7c		0.8	0.4	ND	0.7	ND	ND	0.3
7d		0.8	0.4	ND	0.7	ND	ND	0.3
7e		0.8	0.4	ND	0.7	ND	ND	0.5
7f		0.4	0.5	0.4	2.4	0.4	2.2	0.5
7g		0.4	0.4	0.4	1.0	0.4	1.4	0.4
7h		0.5	0.7	0.4	4.8	0.4	2.2	0.7
7i		0.7	1.0	0.4	3.8	0.9	2.0	1.1
7j		2.2	2.5	1.0	7.4	1.8	4.1	4.1
7k		0.4	0.4	0.4	1.7	0.4	1.7	0.4
7l		0.4	0.4	0.4	1.1	0.4	1.3	0.4
7m		4.3	4.3	1.5	18.1	2.7	8.5	5.4
7n		0.4	0.4	0.4	1.4	0.4	1.4	0.4
7o		0.5	0.8	0.4	3.6	0.5	2.2	0.9
7p		0.6	0.7	0.4	4.0	0.5	2.3	1.0
7q		0.5	0.9	0.4	4.2	0.5	2.5	1.4
7r		2.2	2.7	0.5	9.8	0.9	9.2	6.3
7s		0.4	0.5	0.4	3.2	0.4	1.9	0.9
7t		0.4	0.4	0.4	1.4	0.4	1.2	0.4
7u		0.4	0.4	0.4	1.3	0.4	1.0	0.4

[a]IBN-sensitive cell lines

[b]IBN-resistance cell lines

[c]ND not detected

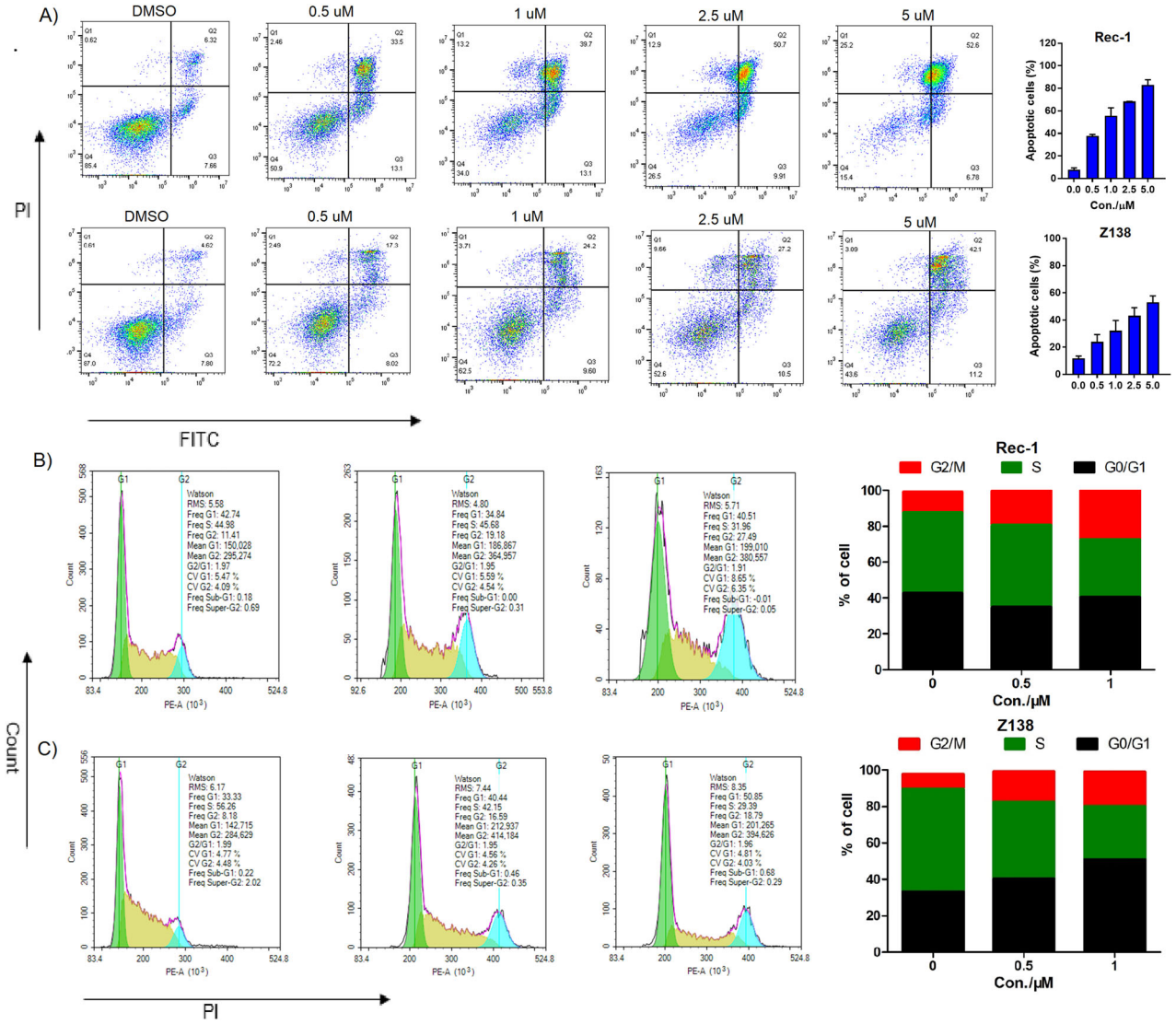


Fig. 2 Cell apoptosis and cell cycle assay with **7l** treatment in Rec-1 and Z138 cells. **a** Apoptosis assay of **7l** at the indicated concentrations in Rec-1 and Z138 cells for 24 h; **b** Cell cycle analysis of **7l** at the indicated concentrations in Rec-1 for 24 h; **c** Cell cycle analysis of **7l** at the indicated concentrations in Z138 cells for 24 h

General procedure for the synthesis of 5′,5′-dimethyl-5-nitrospiro[indoline-3,2′-[1,3]dioxan]-2-one (3)

The 2,2-dimethyl-1,3-propanediol (108 mg, 1.04 mmol) and a catalytic amount of p-toluene sulfonic acid (25 mg, 145 μmol) was added to a suspension of 5-nitroisatin **2** (200 mg, 1.04 mmol) in cyclohexane (10 mL). The resulting reaction mixture then was stirred at reflux temperature for 12 h. Upon completion, the mixture was cooled. Then, the precipitate was filtered, washed with water and dried. Purification by chromatography on silica gel (petroleum ether: ethyl acetate = 5: 1) to give the ketal of 5-nitroisatin **3**.

White solid; yield 84%; mp: 201–203 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 11.20 (s, 1H, NH), 8.28 (dd, $J = 8.7$, 2.4 Hz, 1H, Ph-H), 8.08 (d, $J = 2.4$ Hz, 1H, Ph-H), 7.04 (d, $J = 8.7$ Hz, 1H, Ph-H), 4.49 (d, $J = 11.0$ Hz, 2H, -OCH_2), 3.55 (d, $J = 11.2$ Hz, 2H, -OCH_2), 1.34 (s, 3H, C-CH_3), 0.84 (s, 3H, C-CH_3).

General procedure for the synthesis of 1-(3,4-dichlorobenzyl)-5′,5′-dimethyl-5-nitrospiro[indoline-3,2′-[1,3]dioxan]-2-one (4)

To a solution of intermediate **3** (200 mg, 0.72 mmol) and anhydrous K_2CO_3 (199 mg, 1.44 mmol) in dried DMF (2 mL) was added 1,2-dichloro-4-(chloromethyl) benzene (169 mg, 0.86 mmol). The reaction mixture was heated to 85 °C and stirred for 1.5 h. After completion of the reaction, the reaction mixture was cooled to ambient temperature and

subsequently poured into ice/water (50 mL). The precipitate was filtered, washed with water, and then recrystallized with petroleum ether and ethyl acetate to obtain the desired product **4**.

White solid; yield 80%; mp: 137–141 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 8.34 (dd, $J = 8.7$, 1.6 Hz, 1H, Ph-H), 8.14(d, $J = 2.2$ Hz, 1H, Ph-H), 7.64 (d, $J = 8.7$ Hz, 2H, aromatic), 7.31 (d, $J = 8.8$ Hz, 1H, Bn-H), 7.22 (dd, $J = 8.3$, 2.0 Hz, 1H, Bn-H), 4.98 (s, 2H, -NCH_2), 4.50 (d, $J = 11.0$ Hz, 2H, -OCH_2), 3.61 (d, $J = 10.9$ Hz, 2H, -OCH_2), 1.37 (s, 3H, C-CH_3), 0.87 (s, 3H, C-CH_3).

General procedure for the synthesis of 5-amino-1-(3,4-dichlorobenzyl)-5′,5′-dimethylspiro[indoline-3,2′-[1,3]dioxan]-2-one (5)

To a mixture of the intermediate **4** (280 mg, 640 μmol) in ethyl acetate (25 mL) was added palladium on carbon (140 mg, 10 wt %). The reaction mixture was stirred at ambient temperature for 12 h under a hydrogen atmosphere. After completion of the reaction, the mixture was filtered through diatomite powder, and the residue was washed with ethyl acetate. The filtrate was evaporated under reduced pressure to give the crude residue. Purification was achieved by recrystallized with ethyl acetate/petroleum ether.

Light yellow solid; yield 77%; and mp: 171–174 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 7.63 (d, $J = 8.3$ Hz, 1H, Bn-H), 7.55 (d, $J = 2.0$ Hz,1H, Bn-H), 7.21 (dd, $J = 8.3$, 2.0 Hz, 1H, Bn-H), 6.76 (d, $J = 2.2$ Hz, 1H, Ph-H), 6.64 (d, $J = 8.3$ Hz, 1H, Ph-H),6.48 (dd, $J = 8.3$, 2.3 Hz, 1H, Ph-H), 4.97 (s, 2H, -NCH_2), 4.75 (s, 2H, -NH_2), 4.50 (d, $J = 10.9$ Hz, 2H -OCH_2), 3.51 (d, $J = 11.0$ Hz, 2H -OCH_2), 1.30 (s, 3H, C-CH_3), 0.84 (s, 3H, C-CH_3).

General procedure for the synthesis of intermediates (6)

HBTU (257 mg, 678 μmol), and DIEA (109 mg, 847 μmol) were added to a mixture of appropriate commercially available acid (678 μmol) in DMF (6 mL). The mixture was stirred under ice bath for about 40 min, then amine intermediate **5** (230 mg, 565 μmol) was added. The mixture was stirred at ambient temperature for overnight. TLC analysis showed that starting material was consumed. Water (100 mL) was added to the reaction, the precipitate was filtered off and washed with water. Water (100 mL) and ethyl acetate (50 mL) were added to the filtrate, the organic layer was collected, and the aqueous layer was extracted with ethyl acetate (×3). The combined organic layers were washed with brine, dried over anhydrous anhydrous magnesium sulfate, and concentrated to give the crude product which was purified by column chromatography on silica gel (petroleum ether: ethyl acetate = 5: 1) to afford the desired intermediates **6**.

General procedure for the synthesis of target compounds (7a-7u)

The appropriate intermediates **6** (300 μmol) was dissolved in acetic acid (20 mL) and treated with the concentrated hydrochloric acid (2 mL). the reaction mixture was stirred overnight at room temperature and then pour into water (100 mL). The flocculent precipitate formed after cooling was separated by filtration and purified by recrystallization in methanol to get title compounds series **7a**–**7u**.

5-Amino-1-(3,4-dichlorobenzyl)indoline-2,3-dione (7a) Black solid; yield 47%; mp: 222–224 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.72 (d, $J = 1.9$ Hz, 1H, Ph-H), 7.60 (d, $J = 8.3$ Hz, 1H, Ph-H), 7.40 (dd, $J = 8.3$, 2.0 Hz, 1H, Bn-H), 6.84–6.73 (m, 2H, aromatic), 6.66 (d, $J = 8.2$ Hz, 1H, Bn-H), 5.15 (s, 2H, -NCH_2), 4.82 (s, 2H, -NH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 184.37 (s, C=O), 158.76 (s, –NC=O), 145.98 (s, Ar–C), 140.35 (s, Ar–C), 137.68 (s, Ar–C), 131.71 (s, Ar–C), 131.18 (s, Ar–C), 130.52 (s, Ar–C), 129.85 (s, Ar–C), 128.17 (s, Ar–C), 122.58 (s, Ar–C), 118.77 (s, Ar–C), 111.97 (s, Ar–C), 109.89 (s, Ar–C), 42.19 (s, -CH_2). MS(ESI): calcd for $C_{15}H_{10}Cl_2N_2O_2$ $[M-2H]^-$ 318.01, found 318.28.

N-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-2-(4-fluorophenyl)acetamide (7b) Red solid; yield 40%; mp: 198–200 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.29 (s, 1H, -CONH), 7.88 (d, $J = 2.1$ Hz, 1H, aromatic), 7.76 (d, $J = 2.0$ Hz, 1H, aromatic), 7.66–7.58 (m, 2H, phenylacetyl), 7.44 (dd, $J = 8.3$, 2.0 Hz, 1H, aromatic), 7.35 (dd, $J = 8.6$, 5.6 Hz, 2H, aromatic), 7.14 (t, $J = 8.9$ Hz, 2H, phenylacetyl), 6.89 (d, $J = 8.5$ Hz,1H, aromatic), 4.89 (s, 2H, -NCH_2), 3.62 (s, 2H, -NH_2). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 183.34 (s, –C=O), 169.59 (s, -NHC=O),161.60 (d, $^1J = 240.8$ Hz, -C_6H_5F), 159.11 (s, -NC=O), 145.80 (s, Ar–C), 137.30 (s, Ar–C), 135.51 (s, Ar–C), 132.35 (d, $^4J = 3.1$ Hz, -C_6H_5F), 131.74 (s, Ar–C), 131.46 (d, $^3J = 8.0$ Hz, 2 C, -C_6H_5F), 131.15 (s, Ar–C), 130.57 (s, Ar–C), 129.79 (s, Ar–C), 128.38 (s, Ar–C), 128.19 (s, Ar–C), 118.43 (s, Ar–C), 115.75 (s, Ar–C), 115.49 (d, $^2J = 21.1$ Hz, 2 C, -C_6H_5F), 111.56 (s, Ar–C), 42.57 (s, -NCH_2), 42.28 (s, -$COCH_2$). MS (ESI): calcd for $C_{23}H_{15}Cl_2FN_2O_3$ $[M-H]^-$ 455.04, found 455.15.

N-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-4-fluorobenzamide (7c) Red solid; yield 33%; mp: 285–286 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): 10.36 (s, 1H, -CONH), 8.08–7.96 (m, 3H, aromatic), 7.85 (dd, $J = 8.5$, 2.1 Hz, 1H, Ph-H), 7.79 (d, $J = 1.7$ Hz, 1H, aromatic), 7.62 (d, $J = 8.3$ Hz, 1H, aromatic), 7.46 (dd, $J = 8.3$, 1.8 Hz, 1H, Bn-H), 7.38 (t, $J = 8.8$ Hz, 2H, benzoyl), 6.96 (d, $J = 8.5$ Hz, 1H, aromatic), 4.92 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz,

DMSO-*d6*): δ 183.34 (s, –C=O), 164.85 (s, –NHC=O), 164.63 (d, $^{1}J = 247.8$ Hz, -C_6H_5F), 159.18 (s, –NC=O), 146.17 (s, Ar–C), 137.32 (s, Ar–C), 135.37 (s, Ar–C), 131.76 (s, Ar–C), 131.35 (d, $^{4}J = 2.8$ Hz, -C_6H_5F), 131.17 (s, Ar–C), 130.85 (d, $^{3}J = 9.0$ Hz, 2 C, -C_6H_5F), 130.59 (s, Ar–C), 129.82 (s, Ar–C), 129.73 (s, Ar–C), 128.21 (s, Ar–C), 118.41 (s, Ar–C), 117.08 (s, Ar–C), 115.89 (d, $^{2}J =$ 21.8 Hz, 2C, -C_6H_5F), 111.44 (s, Ar–C), 42.32 (s, -NCH_2). MS (ESI): calcd for $C_{22}H_{13}Cl_2FN_2O_3$ $[M - H]^-$ 441.03, found 441.03.

4-Chloro-N-(1-(3,4-dichlorobenzyl)-2,3-dioxoindolin-5-yl) benzamide (7d) Red solid; yield 18%; mp: 247–250 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.42 (s, 1H, -CONH), 8.05–8.01 (m, 1H, aromatic), 7.98 (dd, $J = 8.6$, 1.8 Hz, 2H, Ph-H), 7.85 (dt, $J = 8.5$, 2.1 Hz, 1H, benzoyl), 7.62 (d, $J =$ 8.4 Hz, 2H), 7.48–7.28 (m, 3H, aromatic), 6.98 (dd, $J =$ 12.8, 8.5 Hz, 1H, Bn-H), 4.92 (s, 2H, –NCH_2). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 183.32 (s, –C=O), 164.85 (s, –NHC=O), 159.18 (s, -NC=O), 146.23 (s, Ar–C), 137.32 (s, Ar–C), 137.05 (s, Ar–C), 135.28 (s, Ar–C), 133.58 (s, Ar–C), 131.76 (s, Ar–C), 131.17 (s, Ar–C), 130.09 (s, 2 C, benzoyl), 129.82 (s, Ar–C), 129.13 (s, Ar–C), 129.00 (s, 2C, benzoyl), 128.22 (s, Ar–C), 127.83 (s, Ar–C), 118.40 (s, Ar–C), 117.13 (s, Ar–C), 111.44 (s, Ar–C), 42.32 (s, -CH_2). MS (ESI): calcd for $C_{22}H_{13}Cl_3N_2O_3$ $[M - H]^-$ 458.00, found 458.10.

3-Chloro-*N*-(1-(3,4-dichlorobenzyl)-2,3-dioxoindolin-5-yl) benzamide (7e) Red solid; yield 14%; mp: 222–224 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.44 (s, 1H, -CONH), 8.05–7.98(m, 2H, aromatic), 7.94–7.88 (m, 1H, aromatic), 7.85 (dt, $J = 8.5$, 2.1 Hz, 1H, benzoyl), 7.79 (d, $J = 2.0$ Hz, 1H, aromatic),7.68 (ddd, $J = 8.0$, 2.0, 1.0 Hz, 2H, benzoyl), 7.65–7.55 (m, 1H, aromatic), 7.46 (dd, $J = 8.4$, 2.0 Hz, 1H, aromatic), 6.97 (d, $J = 8.5$ Hz, 1H, aromatic), 4.92 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 183.30 (s, –C=O), 164.47 (s, –NHC = O), 159.18 (s, –NC=O), 146.30 (s, Ar–C), 137.31 (s, Ar–C), 136.88 (s, Ar–C), 135.16 (s, Ar–C), 133.73 (s, Ar–C), 132.05 (s, Ar–C), 131.76 (s, Ar–C), 131.17 (s, Ar–C), 130.96 (s, Ar–C), 130.59 (s, Ar–C), 129.82 (s, Ar–C), 129.13 (s, Ar–C), 128.21 (s, Ar–C), 127.82 (s, Ar–C), 126.95 (s, Ar–C), 118.43 (s, Ar–C), 117.12 (s, Ar–C), 111.46 (s, Ar–C), 42.33 (s, -CH_2). MS (ESI): calcd for $C_{22}H_{13}Cl_3N_2O_3$ $[M - H]^-$ 457.00, found 457.12.

4-Bromo-*N*-(1-(3,4-dichlorobenzyl)-2,3-dioxoindolin-5-yl) benzamide (7f) Red solid; yield 81%; mp: 285–287 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.43 (s, 1H, -CONH), 8.03 (d, $J = 2.0$ Hz, 1H, aromatic), 7.90 (d, $J = 8.5$ Hz, 2H, benzoyl), 7.84 (dd, $J = 8.5$, 2.1 Hz, 1H, aromatic), 7.79–7.75 (m, 3H, aromatic), 7.62 (d, $J = 8.3$ Hz, 1H, aromatic), 7.47 (dd, $J = 8.3$, 1.8 Hz, 1H, aromatic), 6.96 (d, $J = 8.5$ Hz, 1H, aromatic), 4.92 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 183.31 (s, –C=O), 164.98 (s, –NHC = O), 159.18 (s, –NC=O), 146.25 (s, Ar–C), 137.32 (s, Ar–C), 135.23 (s, Ar–C), 133.97 (s, Ar–C), 131.96 (s, 2 C, benzoyl), 131.75 (s, Ar–C), 131.17 (s, Ar–C), 130.58 (s, Ar–C), 130.22 (s, 2 C, benzoyl), 129.82 (s, Ar–C), 129.76 (s, Ar–C), 128.22 (s, Ar–C), 126.02 (s, Ar–C), 118.43 (s, Ar–C), 117.10 (s, Ar–C), 111.45 (s, Ar–C), 42.32 (s, -CH_2). MS (ESI): calcd for $C_{22}H_{13}BrCl_2N_2O_3$ $[M-H + 2]^-$ 502.95, found 503.05.

4-Cyano-*N*-(1-(3,4-dichlorobenzyl)-2,3-dioxoindolin-5-yl) benzamide (7g) Red solid; yield 74%; mp: 295–297 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.58 (s, 1H, -CONH), 8.10 (d, $J = 8.3$ Hz, 2H, aromatic), 8.04 (d, $J = 7.8$ Hz, 3H, aromatic), 7.85 (dd, $J = 8.5$, 2.0 Hz, 1H, aromatic), 7.79 (d, $J = 1.5$ Hz, 1H, aromatic), 7.62 (d, $J = 8.3$ Hz, 1H, aromatic), 7.47 (dd, $J = 8.3$, 1.6 Hz, 1H, aromatic), 6.96 (t, $J =$ 8.6 Hz, 1H, aromatic), 4.92 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 183.26 (s, –C=O), 164.55 (s, –NHC = O), 159.19 (s, –NC=O), 146.43 (s, Ar–C), 138.93 (s, Ar–C), 137.30 (s, Ar–C), 135.00 (s, Ar–C), 133.01 (s, 2 C, Ar–C), 131.76 (s, Ar–C), 131.17 (s, Ar–C), 130.58 (s, Ar–C), 129.81 (s, Ar–C), 128.96 (s, 2 C, Ar–C), 128.21 (s, Ar–C), 118.76 (s, Ar–C), 118.46 (s, Ar–C), 117.12 (s, Ar–C), 114.47 (s, Ar–C), 111.50 (s, Ar–C), 42.33 (s, -NCH_2). MS (ESI): calcd for $C_{23}H_{13}C_{l2}N_3O_3$ $[M - H]^-$ 448.03, found 448.11.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-4-methoxy-benzamide (7h)** Red solid; yield 81%; mp: 276–277 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.20 (s, 1H, -CONH), 8.04 (d, $J = 1.9$ Hz, 1H, Ph-H), 7.95 (d, J = 8.8 Hz, 2H, benzoyl), 7.86 (dd, $J = 8.5$, 2.0 Hz, 1H, Ph-H), 7.79 (d, $J =$ 1.4 Hz, 1H, aromatic), 7.63 (d, $J = 8.3$ Hz, 1H, aromatic), 7.47 (dd, $J = 8.3$, 1.5 Hz, 1H, Bn-H), 7.07 (d, $J = 8.8$ Hz, 2H, benzoyl), 6.95 (d, $J = 8.5$ Hz, 1H, Bn-H), 4.92 (s, 2H, -NCH_2), 3.84 (s, 3H, -OCH_3). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 183.40 (s, –C=O), 165.33 (s, –NHC = O), 162.50 (s, Ar–C), 159.18 (s, –NC=O), 145.95 (s, Ar–C), 137.35 (s, Ar–C), 135.69 (s, Ar–C), 131.76 (s, Ar–C), 131.17 (s, Ar–C), 130.58 (s, Ar–C), 130.04 (s, 2 C, Ar–C), 129.83 (s, Ar–C), 129.64 (s, Ar–C), 128.21 (s, Ar–C), 126.92 (s, Ar–C), 118.37 (s, Ar–C), 117.02 (s, Ar–C), 114.14 (s, 2 C, Ar–C), 111.38 (s, Ar–C), 55.92 (s, -OCH_3), 42.32 (s, -NCH_2). MS (ESI): calcd for $C_{23}H_{16}Cl_2N_2O_4$ $[M - H]^-$ 453.05, found 453.14.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-3-nitroben-zamide (7i)** Red solid; yield 73%; mp: 241–243 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.69 (s, 1H, -CONH), 8.80 (s, 1H, aromatic), 8.46 (dd, $J = 8.2$, 1.2 Hz, 1H, aromatic),

8.40 (d, $J = 7.8$ Hz, 1H, benzoyl), 8.05 (d, $J = 1.9$ Hz, 1H, aromatic), 7.86 (t, $J = 8.1$ Hz, 2H, benzoyl), 7.80 (d, $J =$ 1.6 Hz, 1H, aromatic), 7.63 (d, $J = 8.3$ Hz, 1H, aromatic), 7.48 (dd, $J = 8.3$, 1.7 Hz, 1H, aromatic), 6.99 (d, $J =$ 8.5 Hz, 1H, aromatic), 4.93 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 183.26 (s, –C=O), 163.76 (s, –NHC = O), 159.19 (s, –NC=O), 148.25 (s, Ar–C), 146.48 (s, Ar–C), 137.31 (s, Ar–C), 136.28 (s, Ar–C), 134.93 (s, Ar–C), 134.62 (s, Ar–C), 131.76 (s, Ar–C), 131.18 (s, Ar–C), 130.77 (s, Ar–C), 130.59 (s, Ar–C), 129.95 (s, Ar–C), 129.82 (s, Ar–C), 128.22 (s, Ar–C), 126.82 (s, Ar–C), 122.81 (s, Ar–C), 118.48 (s, Ar–C), 117.30 (s, Ar–C), 111.51 (s, Ar–C), 42.35 (s, -NCH_2). MS (ESI): calcd for $C_{22}H_{13}Cl_2N_3O_5$ $[M - H]^-$ 468.02, found 468.13.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-3-(trifluoromethyl)benzamide (7j)** Red solid; yield 81%; mp: 210–213 °C; ^{1}H NMR (400 MHz, DMSO-*d6*): δ 10.57 (s, 1H, -CONH), 8.29 (s, 1H, aromatic), 8.26 (d, $J = 7.9$ Hz, 1H, aromatic), 8.03 (d, $J = 1.9$ Hz, 1H, aromatic), 7.99 (d, J = 7.8 Hz, 1H, aromatic), 7.86 (dd, $J = 8.5$, 2.0 Hz, 1H, aromatic), 7.82–7.78 (m, 2H, aromatic), 7.63 (d, $J = 8.3$ Hz, 1H, aromatic), 7.48 (dd, $J = 8.3$, 1.6 Hz, 1H, aromatic), 6.98 (d, $J = 8.5$ Hz, 1H, aromatic), 4.93 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-*d6*): δ 183.28 (s, –C=O), 164.45 (s, –NHC = O), 159.19 (s, –NC=O), 146.40 (s, Ar–C), 137.31 (s, Ar–C), 135.80 (s, Ar–C), 135.05 (s, Ar–C), 132.29 (s, Ar–C), 131.76 (s, Ar–C), 131.17 (s, Ar–C), 130.59 (s, Ar–C), 130.30 (s, Ar–C), 129.82 (s, Ar–C), 129.69 (q, $^2J = 32.2$ Hz, -$\underline{C}_6H_5CF_3$), 128.77 (q, $^3J = 3.4$ Hz, -$\underline{C}_6H_5CF_3$), 128.22 (s, Ar–C), 125.78 (s, Ar–C), 124.63 (q, $^3J = 4.0$ Hz, -$\underline{C}_6H_5CF_3$), 124.43 (q, $^1J = 273.0$ Hz, -$C_6H_5\underline{C}F_3$), 118.46 (s, Ar–C), 117.28 (s, Ar–C), 111.48 (s, Ar–C), 42.34 (s, -NCH_2). MS (ESI): calcd for $C_{23}H_{13}Cl_2F_3N_2O_3$ $[M - H]^-$ 491.03, found 491.13.

3-Bromo-*N*-(1-(3,4-dichlorobenzyl)-2,3-dioxoindolin-5-yl) benzamide (7k) Red solid; yield 84%; mp: 239–240 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.45 (s, 1H, -CONH), 8.14 (s, 1H, aromatic), 8.03 (d, $J = 1.9$ Hz, 1H, aromatic), 7.95 (d, $J = 7.9$ Hz, 1H, aromatic), 7.85 (dd, $J = 8.6$, 2.0 Hz, 1H, aromatic), 7.81 (d, $J = 8.2$ Hz, 1H, aromatic), 7.79 (d, $J = 1.6$ Hz, 1H, aromatic), 7.63 (d, $J = 8.3$ Hz, 1H, aromatic), 7.52 (t, $J = 7.9$ Hz, 1H, aromatic), 7.47 (dd, $J =$ 8.3, 1.5 Hz, 1H, aromatic), 6.97 (d, $J = 8.5$ Hz, 1H, aromatic), 4.92 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 183.30 (s, –C=O), 164.39 (s, –NHC = O), 159.18 (s, –NC=O), 146.31 (s, Ar–C), 137.32 (s, Ar–C), 137.08 (s, Ar–C), 135.15 (s, Ar–C), 134.94 (s, Ar–C), 131.76 (s, Ar–C), 131.21 (s, Ar–C), 131.17 (s, Ar–C), 130.64 (s, Ar–C), 130.59 (s, Ar–C), 129.82 (s, Ar–C), 129.79 (s, Ar–C), 128.21 (s, Ar–C), 127.32 (s, Ar–C), 122.20 (s, Ar–C), 118.43 (s, Ar–C), 117.13 (s, Ar–C), 111.46 (s, Ar–C), 42.34 (s, -NCH_2). MS (ESI): calcd for $C_{22}H_{13}BrCl_2N_2O_3$ $[M - H + 2]^-$ 502.95, found 503.14.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-3-fluorobenzamide (7l)** Red solid; yield 75%; mp: 247–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.42 (s, 1H, -CONH), 8.03 (d, $J = 1.8$ Hz, 1H, aromatic), 7.85 (dd, $J = 8.5$, 1.8 Hz, 1H, aromatic), 7.82–7.75 (m, 3H, aromatic), 7.64–7.58 (m, 2H, aromatic), 7.48–7.44 (m, 2H, aromatic), 6.97 (d, $J = 8.5$ Hz, 1H, aromatic), 4.92 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 183.30 (s, –C=O), 164.56 (s, –NHC = O), 162.41 (d, $^1J = 243$ Hz, C_6H_5-F), 159.18 (s, –NC = O), 146.31 (s, Ar–C), 137.22 (d, $^3J = 6.9$ Hz, C_6H_5-F), 135.16 (s, Ar–C), 131.76 (s, Ar–C), 131.19 (s, Ar–C), 131.17 (s, Ar–C), 131.11 (s, Ar–C), 130.59 (s, Ar–C), 129.82 (s, Ar–C), 129.79 (s, Ar–C), 128.22 (s, Ar–C), 124.3(d, $^3J =$ 3.7 Hz, C_6H_5-F), 119.139 (d, $^2J = 21$ Hz, C_6H_5-F), 118.43 (s, Ar–C), 117.12 (s, Ar–C), 114.92 (d, $^2J = 22.8$ Hz, C_6H_5-F), 111.46 (s, Ar-C), 42.34 (s, -NCH_2). MS (ESI): calcd for $C_{22}H_{13}Cl_2FN_2O_3$ $[M - H]^-$ 441.03, found 441.13.

3-Cyano-*N*-(1-(3,4-dichlorobenzyl)-2,3-dioxoindolin-5-yl) benzamide (7m) Red solid; yield 76%; mp 256–257 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.53 (s, 1H, -CONH), 8.40 (s, 1H, aromatic), 8.25 (d, $J = 8.0$ Hz, 1H, aromatic), 8.09 (d, $J = 7.9$ Hz, 1H, aromatic), 8.03 (d, $J = 1.8$ Hz, 1H, aromatic), 7.85 (dd, $J = 8.5$, 2.0 Hz, 1H, aromatic), 7.79 (s, 1H, aromatic), 7.76 (d, $J = 7.8$ Hz, 1H, aromatic), 7.63 (d, J = 8.3 Hz, 1H, aromatic), 7.47 (dd, $J = 8.5$, 1.4 Hz, 1H, aromatic), 6.98 (d, $J = 8.5$ Hz, 1H, aromatic), 4.93 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 183.27 (s, –C=O), 164.10 (s, -NHC = O), 159.18 (s, –NC = O), 146.41 (s, Ar–C), 137.30 (s, Ar–C), 135.94 (s, Ar–C), 135.01 (s, Ar–C), 132.96 (s, Ar–C), 131.76 (s, Ar–C), 131.70 (s, Ar–C), 131.18 (s, Ar–C), 130.59 (s, Ar–C), 130.41 (s, Ar–C), 129.81 (s, Ar–C), 129.76 (s, Ar–C), 128.21 (s, Ar–C), 118.75 (s, Ar–C), 118.47 (s, Ar–C), 117.08 (s, Ar–C), 112.05 (s, -CN), 111.51 (s, Ar–C), 42.34 (s, -NCH_2). MS (ESI): calcd for $C_{22}H_{13}Cl_2N_3O_3$ $[M - H]^-$ 448.03, found 448.03.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-3-methylbenzamide (7n)** Red solid; yield 80%; mp: 218–221 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.32 (s, 1H, -CONH), 8.04 (d, $J = 2.0$ Hz, 1H, aromatic), 7.86 (dd, $J = 8.5$, 2.0 Hz, 1H, aromatic), 7.79–7.73 (m, 3H, aromatic), 7.62 (d, $J = 8.3$ Hz, 1H, aromatic), 7.48–7.41 (m, 3H, aromatic), 6.96 (d, $J = 8.5$ Hz, 1H, aromatic), 4.92 (s, 2H, -CH_2), 2.40 (s, 3H, -CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ 183.37 (s, –C=O), 166.08 (s, –NHC = O), 159.18 (s, –NC = O), 146.08 (s, Ar–C), 138.26 (s, Ar–C), 137.34 (s, Ar–C), 135.52 (s, Ar–C), 134.94 (s, Ar–C), 132.80 (s, Ar–C), 131.75 (s, Ar–C), 131.17 (s, Ar–C), 130.58 (s, Ar–C),

129.83 (s, Ar–C), 129.64 (s, Ar–C), 128.83 (s, Ar–C), 128.54 (s, Ar–C), 128.22 (s, Ar–C), 125.26 (s, Ar–C), 118.40 (s, Ar–C), 117.01 (, Ar–C s), 111.42 (s, Ar–C), 42.32 (s, -NCH_2), 21.43 (s, -CH_3). MS (ESI): calcd for $C_{23}H_{16}Cl_2N_2O_3$ $[M-H]^-$ 437.05, found 437.13.

3,4-Dichloro-*N*-(1-(3,4-dichlorobenzyl)-2,3-dioxoindolin-5-yl)benzamide (7o) Red solid; yield 82%; mp: 279–281 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.42 (s, 1H, -CONH), 8.14 (d, $J = 1.8$ Hz, 1H, aromatic), 7.94 (d, $J = 1.9$ Hz, 1H, aromatic), 7.86 (dd, $J = 8.4$, 1.8 Hz, 1H, aromatic), 7.77 (br, 1H, aromatic), 7.75 (br, 1H, aromatic), 7.72 (d, $J = 1.3$ Hz, 1H, aromatic), 7.55 (d, $J = 8.3$ Hz, 1H, aromatic), 7.40 (dd, $J = 8.3$, 1.5 Hz, 1H, aromatic), 6.90 (d, $J = 8.5$ Hz, 1H, aromatic), 4.85 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 183.26 (s, –C=O), 163.59 (s, –NHC = O), 159.18 (s, –NC = O), 146.40 (s, Ar–C), 137.31 (s, Ar–C), 135.21 (s, Ar–C), 135.01 (s, Ar–C), 134.99 (s, Ar–C), 131.83 (s, Ar–C), 131.76 (s, Ar–C), 131.32 (s, Ar–C), 131.17 (s, Ar–C), 130.59 (s, Ar–C), 130.01 (s, Ar–C), 129.82 (s, 2 C, Ar–C), 128.49 (s, Ar–C), 128.22 (s, Ar–C), 118.46 (s, Ar–C), 117.15 (s, Ar–C), 111.49 (s, Ar–C), 42.34 (s). MS (ESI): calcd for $C_{22}H_{12}C_{l4}N_2O_3$ $[M-H]^-$ 492.96, found 493.07.

2,4-Dichloro-*N*-(1-(3,4-dichlorobenzyl)-2,3-dioxoindolin-5-yl)benzamide (7p) Red solid; yield 83%; mp: 237–238 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.67 (s, 1H, -CONH), 7.98 (d, $J = 1.9$ Hz, 1H, Ph-H), 7.78 (d, $J = 1.4$ Hz, 2H, aromatic), 7.73 (dd, $J = 8.5$, 2.0 Hz, 1H, aromatic), 7.63 (dd, $J = 10.0$, 8.4 Hz, 2H, aromatic), 7.57 (dd, $J = 8.2$, 1.8 Hz, 1H, aromatic), 7.46 (dd, $J = 8.3$, 1.6 Hz, 1H, aromatic), 6.95 (d, $J = 8.5$ Hz, 1H, aromatic), 4.92 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 183.24 (s, –C=O), 164.49 (s, –NHC = O), 159.16 (s, –NC=O), 146.31 (s, Ar–C), 137.26 (s, Ar–C), 135.81 (s, Ar–C), 135.51 (s, Ar–C), 134.99 (s, Ar–C), 131.75 (s, Ar–C), 131.73 (s, Ar–C), 131.16 (s, Ar–C), 130.83 (s, Ar–C), 130.58 (s, Ar–C), 129.79 (s, Ar–C), 129.75 (s, Ar–C), 128.83 (s, Ar–C), 128.20 (s, Ar–C), 128.00 (s, Ar–C), 118.54 (s, Ar–C), 116.14 (s, Ar–C), 111.66 (s, Ar–C), 42.31 (s, -NCH_2). MS (ESI): calcd for $C_{22}H_{12}C_{l4}N_2O_3$ $[M-H]^-$ 492.96, found 493.18.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-2-fluoro-4-nitrobenzamide (7q)** Red solid; yield 89%; mp: 247–249 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.83 (s, 1H, -CONH), 8.30 (dd, $J = 9.6$, 1.9 Hz, 1H, aromatic), 8.21 (dd, $J = 8.6$, 1.7 Hz, 1H, aromatic), 7.99–7.94 (m, 2H, aromatic), 7.79 (d, $J = 1.1$ Hz, 1H, aromatic), 7.75 (dd, $J = 8.6$, 1.9 Hz, 1H, aromatic), 7.63 (d, $J = 8.3$ Hz, 1H, aromatic), 7.47 (dd, $J = 8.4$, 1.7 Hz, 1H, aromatic), 6.97 (d, $J = 8.5$ Hz, 1H, aromatic), 4.93 (s, 2H, -NCH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ 183.18 (s, –C=O), 161.62 (s, –NHC = O), 159.16 (s, –NC = O), 158.86 (d, $^1J = 252.9$ Hz, C_6H_5-F), 149.84 (d, $^3J = 8.8$ Hz, C_6H_5-F), 146.57 (s, Ar–C), 137.25 (s, Ar–C), 134.64 (s, Ar–C), 131.76 (s, Ar–C), 131.65 (d, $^3J = 3.3$ Hz, C_6H_5-F), 131.17 (s, Ar–C), 130.86 (d, $^2J = 15.8$ Hz, C_6H_5-F), 130.59 (s, Ar–C), 129.80 (s, Ar–C), 129.19 (s, Ar–C), 128.21 (s, Ar–C), 120.26 (d, $^4J = 3.4$ Hz, C_6H_5-F), 118.58 (s, Ar–C), 116.46 (s, Ar–C), 112.67 (d, $^2J = 27.2$ Hz, C_6H_5-F), 111.69 (s, Ar–C), 42.34 (s, -NCH_2). MS (ESI): calcd for $C_{22}H_{12}Cl_2FN_2O_5$ $[M-H]^-$ 486.01, found 486.05.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-3,4-dimethoxybenzamide (7r)** Red solid; yield 82%; mp: 276–278 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.18 (s, 1H, -CONH), 8.01 (d, $J = 2.0$ Hz, 1H, aromatic), 7.86 (dd, $J = 8.5$, 2.1 Hz, 1H, aromatic), 7.79 (d, $J = 1.7$ Hz, 1H, aromatic), 7.64–7.60 (m, 2H, aromatic), 7.52 (d, $J = 1.8$ Hz, 1H, aromatic), 7.47 (dd, $J = 8.3$, 1.8 Hz, 1H, aromatic), 7.09 (d, $J = 8.5$ Hz, 1H, aromatic), 6.95 (d, $J = 8.5$ Hz, 1H, aromatic), 4.92 (s, 2H, -NCH_2), 3.84 (s, 6H,-OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.31 (s, –C=O), 164.26 (s, –NHC = O), 158.11 (s, –NC = O), 151.17 (s, Ar–C), 147.74 (s, Ar–C), 144.91 (s, Ar–C), 136.28 (s, Ar–C), 134.53 (s, Ar–C), 130.68 (s, Ar–C), 130.09 (s, Ar–C), 129.50 (s, Ar–C), 128.76 (s, Ar–C), 128.68 (s, Ar–C), 127.14 (s, Ar–C), 125.85 (s, Ar–C), 120.42 (s, Ar–C), 117.32 (s, Ar–C), 116.08 (s, Ar–C), 110.35 (s, Ar–C), 110.32 (s, Ar–C), 110.30 (s, Ar–C), 55.08 (s, -OCH_3), 55.03 (s, -OCH_3), 41.25 (s, -NCH_2). MS (ESI): calcd for $C_{24}H_{18}Cl_2N_2O_5$ $[M-H]^-$ 483.06, found 483.16.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)picolinamide (7s)** Red solid; yield 76%; mp: 241–243 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.88 (s, 1H, -CONH), 8.75 (d, $J = 4.6$ Hz, 1H, aromatic), 8.21 (d, $J = 1.9$ Hz, 1H, aromatic), 8.16 (d, $J = 7.7$ Hz, 1H, aromatic), 8.08 (td, $J = 7.7$, 1.3 Hz, 1H, aromatic), 8.02 (dd, $J = 8.5$, 2.0 Hz, 1H, aromatic), 7.79 (br, 1H, aromatic), 7.70–7.67 (m, 1H, aromatic), 7.63 (d, $J = 8.3$ Hz, 1H, aromatic), 7.47 (dd, $J = 8.3$, 1.4 Hz, 1H, aromatic), 6.97 (d, $J = 8.6$ Hz, 1H, aromatic), 4.92 (s, 2H, -NCH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 183.33 (s, –C=O), 163.16 (s, –NHC = O), 159.17 (s, –NC=O), 150.09 (s, $\underline{C}_{pyridyl}$-C = O), 148.92 (s, Ar–C), 146.33 (s, Ar–C), 138.64 (s, Ar–C), 137.34 (s, Ar–C), 134.83 (s, Ar–C), 131.75 (s, Ar–C), 131.17 (s, Ar–C), 130.60 (s, Ar–C), 129.96 (s, Ar–C), 129.89 (s, Ar–C), 128.27 (s, Ar–C), 127.51 (s, Ar–C), 122.98 (s, Ar–C), 118.39 (s, Ar–C), 117.12 (s, Ar–C), 111.41 (s, Ar–C), 42.33 (s, -NCH_2). MS (ESI): calcd for $C_{21}H_{13}Cl_2N_3O_3$ $[M-H]^-$ 424.03, found 424.16.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)nicotinamide (7t)** Red solid; yield 49%; mp: 287–290 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.79 (s, 1H, -CONH), 9.24 (s, 1H, aromatic), 8.88 (d, $J = 6.3$ Hz, 1H, aromatic), 8.55 (d, $J = 6.7$ Hz, 1H, aromatic), 8.06 (d, $J = 1.6$ Hz, 1H, aromatic), 7.88 (d, $J = 8.5$ Hz, 1H, aromatic), 7.80–7.77 (m, 2H, aromatic), 7.63 (d, $J = 8.3$ Hz, 1H, aromatic), 7.47 (dd, $J = 8.3$, 1.6 Hz, 1H, aromatic), 6.98 (d, $J = 8.5$ Hz, 1H, aromatic), 4.93 (s, 2H, -NCH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 183.24 (s, –C=O), 162.90 (s, –NHC = O), 159.18 (s, –NC = O), 148.75 (s, Ar–C), 146.53 (s, Ar–C), 145.80 (s, Ar–C), 140.45 (, Ar–C s), 137.30 (s, Ar–C), 134.88 (s, Ar–C), 132.01 (s, Ar–C), 131.76 (s, Ar–C), 131.17 (s, Ar–C), 130.59 (s, Ar–C), 129.84 (s, Ar–C), 129.82 (s, Ar–C), 128.24 (s, Ar–C), 125.71 (s, Ar–C), 118.47 (s, Ar–C), 117.12 (s, Ar–C), 111.53 (s, Ar–C), 42.35 (s, -NCH_2). HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{13}Cl_2N_3O_3$: 426.0334, found: 426.0381.

***N*-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)isonicotinamide (7u)** Red solid; yield 82%; mp: 270–273 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.01 (s, 1H, -CONH), 8.98 (d, $J = 5.9$ Hz, 2H, pyridly), 8.22 (d, $J = 5.8$ Hz, 2H, pyridly), 8.08 (d, $J = 1.4$ Hz, 1H, aromatic), 7.91 (dd, $J = 8.5$, 1.6 Hz, 1H, aromatic), 7.79 (s, 1H, aromatic), 7.62 (d, $J =$ 8.3 Hz, 1H, aromatic), 7.47 (dd, $J = 8.2$, 0.7 Hz, 1H, aromatic), 7.00 (d, $J = 8.5$ Hz, 1H, aromatic), 4.93 (s, 2H, -NCH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 183.19 (s, –C=O), 163.14 (s, -NHC = O), 159.19 (s, –NC=O), 147.11 (s, 2 C, pyridly), 146.71 (s, Ar–C), 145.68 (s, Ar–C), 137.28 (s, Ar–C), 134.62 (s, Ar–C), 131.76 (s, Ar–C), 131.17 (s, Ar–C), 130.59 (s, Ar–C), 129.98 (s, Ar–C), 129.83 (s, Ar–C), 128.23 (s, Ar–C), 123.95 (s, 2 C, pyridly), 118.49 (s, Ar–C), 117.26 (s, Ar–C), 111.55 (s, Ar–C), 42.35 (s, -NCH_2). MS (ESI): calcd for $C_{21}H_{13}C_{l2}N_3O_3$ $[M - H]^-$ 424.03, found 424.12.

Cell antiproliferation assay

Cell culture

Experimental cells (MCL cell lines) were purchased from Amercian Type Culture Collection (ATCC, USA) and cultured in RPMI - 1640 (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco) at 37 °C with humidified atomosphere containing 5% CO_2 in a culture incubator. When 70% of the plate had been covered, cells were incubated, harvested and used for proliferation and cycle analysis.

Cell viability assay

The in vitro inhibitory effects of the compounds were measured on a panel of MCL cell lines using the CellTiter-Glo Luminecent cell viability assay kit (Promega, USA). Briefly, the different kinds of cells were seeded independently in a 96-well microtiter plate with containing 1×10^4 cells/well. The cells were exposed to different concentrations (0.93–60 μmol/L) of compounds and incubated for 72 h at 37 °C. After treatment, CellTiter-Glo (30 μL) solution was added to each well. The absorbance of the samples was determined at a wavelength of 570 nm with HTX Multi-Mode Microplate Reader (BioTek, USA). Each independent experiment was run three times and IC_{50} values were calculated with Prism 6.0 Software (GraphPad, USA).

Flow cytometry assay (cell cycle and apoptosis)

Cells were plated on six-well culture plates at a density of 2×10^5 cells/well and were treated with the indicated concentrations (0.5, 1, 2.5, 5 μM) of **7l** for 24 h after they adherence. After culture, cells were centrifuged (1500 rpm, 5 min) and washed twice in cold phosphate-buffered saline (PBS) before exposure to staining buffer. To the cell suspension were stained with 2 μL of FITC-Annexin V and 2 μL of PI, according to the manufacturer's protocol. After being gently mixed, the flow tube should be incubated for 15 min at room temperature in the dark. After addition of 200 μL of binding buffer, apoptosis detection was performed by flow cytometry using a Novocyte Flow Cytometer (ACEA Biosciences, USA). Data were analyzed using Flowjo software. For cell cycle assay, the treated cells were harvested by centrifugation and washed twice with PBS, then fixed with ice-cold 70% ethanol overnight at 4 °C and collected again. The fixed cells were then washed twice with PBS and stained with 100 μL of PI (100 μg/mL) in the presence of RNaseA (0.5 mg) at room temperature, then they were analyzed by flow cytometry. The results were analyzed by Novoexpress software.

Conclusion

In summary, we designed and synthesized a novel series of 1,5-disubstituted isatin derivatives. According to the biological assay of compounds against MCL cell lines, most compounds showed decreased proliferation ability compared with **IBN** and **AA2**, among which compound **7l** had demonstrated potent efficacy with IC_{50} values from 0.4 up to 1.1 μM in MCL cell lines. Moreover, in mechanistic studies, the representative compound **7l** also induced significant cell cycle arrest in the G2/M phase and increased cell apoptosis. Collectively, these structurally promise in discovery of new anticancer therapeutics by inducing MCL cells apoptosis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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