Bioorganic & Medicinal Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of novel 2,3-indolinedione derivatives against mantle cell lymphoma

Shengping Yu[a,1], Yang Liu[b,1], Zhen Zhang[a], Jingya Zhang[a], Guisen Zhao[a,*]

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

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

ARTICLE INFO

Keywords:
2,3-Indolinedione
Mantle cell lymphoma
Derivatives
Biological activities

ABSTRACT

2,3-Indolinedione derivatives have been identified as a novel class of promising agents for cancer treatment. In this study, eighteen 2,3-indolinedione derivatives were designed and synthesized, and their anticancer activities against mantle cell lymphoma (MCL) cells were evaluated. Most of them exhibited significant antiproliferative activity against the tested cell lines, and compound **K5** was the most potent (MCL cellular IC_{50} = 0.4–0.7 μM). Further, compound **K5** could induce cell apoptosis and cell cycle arrest in G2/M phase. Additionally, the results of drug-likeness analysis demonstrated that these novel 2,3-indolinedione derivatives could have potential as novel treatment strategies for MCL.

1. Introduction

Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma with poor prognosis.[1] Unlike other non-Hodgkin's lymphomas, MCL is insidious, and most patients are in clinical stage III or stage IV at the time of diagnosis and often relapse after first-line treatment.[2] Median overall survival for MCL patients is approximately 3–4 years.[3] The pathogenesis of MCL is complex and involves molecular changes at all levels, including cell cycle mechanisms (CDKN2C/RB1/TP53/BMI1), cellular survival signaling pathways (FAF1/BCL2L11/CDK4/MDM2), and DNA damage response pathways (ATM/SP100/SP140/TP53).[4,5] Currently, drugs used as targeted therapy for MCL include proteasome inhibitors,[6] BTK inhibitors,[7] PI3K/AKT/mTOR pathway inhibitors,[8,9] CDK inhibitors,[10] HDAC inhibitors,[11] targeted death proteins[12] and so on. In spite of the diverse therapeutic strategies available for MCL treatment, chemo-resistance and increasing mortality rate remain challenging. This finding emphasizes that more effective treatment plans for MCL are urgently needed.

Isatin (2,3-indolinedione, Fig. 1) is an endogenous active component in mammalian tissue and body fluids that has led to a series of derivatives that display a wide range of biological properties including antiproliferative, anticonvulsant, antifungal, anti-mycobacterial, anti-protozoal, anti-HIV and anti-inflammatory activities.[13–19] In the last several years, new isatin-based analogs have exerted significant activities against different human malignancies.[20–24]

It was reported that compound **1** (Fig. 1), a 2,3-indolinedione derivative bearing an amide moiety, could predominantly inhibit human myeloid leukemia HL-60, U-937 and human lymphoid leukemia MOLT-3 cells.[25] The previous work of our laboratory once indicated that compound **2** (Fig. 1), another 2,3-indolinedione analog with an incorporated amide group, effectively inhibited the proliferation of tumor cells.[26] Taking the above into consideration, herein, we retained the 2,3-indolinedione amide functionality and concentrated on chemical modifications of substituents on the N-1 and C-5 positions, which led to the discovery of new 2,3-indolinedione derivatives as shown in Fig. 1. All of them were evaluated with respect to antiproliferative activity against MCL cells. Moreover, cell apoptosis and cell cycle were assessed. Last, we predicted their drug-likeness with the SwissADME web tool.

2. Results and discussion

2.1. Chemistry

The syntheses of these 2,3-indolinedione derivatives are depicted in Scheme 1. Commercially available isatin **(1)** was nitrified to obtain 5-nitroisatin **(2)**. Intermediate **(3)** was prepared by treating intermediate **(2)** with 2,2-dimethylpropane-1,3-diol, with catalysis by *p*-toluenesulfonic acid. The intermediate **(4)** was obtained from reaction of intermediate **(3)** and 1,2-dichloro-4-(chloromethyl)benzene under

* Corresponding author.
E-mail address: guisenzhao@sdu.edu.cn (G. Zhao).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bmc.2019.06.009
Received 5 April 2019; Received in revised form 3 June 2019; Accepted 5 June 2019

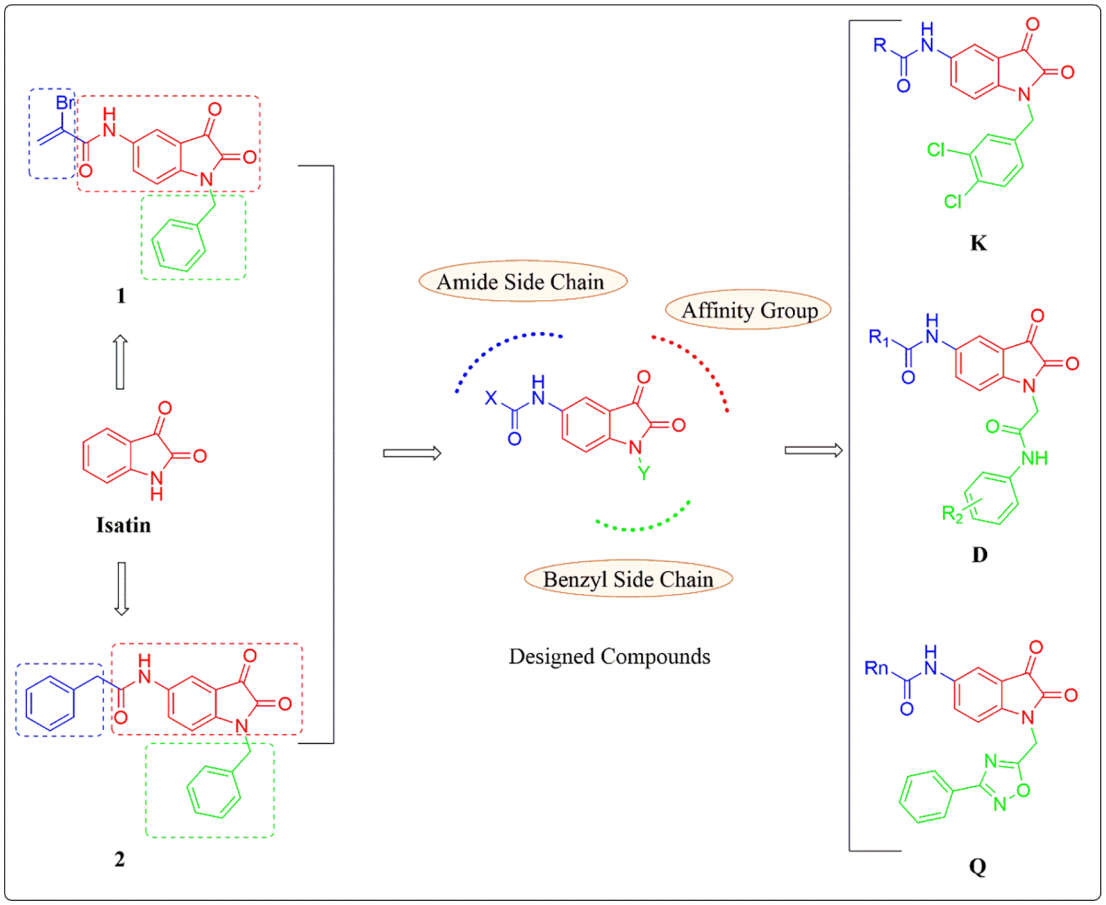


Fig. 1. Design of the target compounds.

alkaline conditions via electrophilic substitution. With Pd/C (10%, w/w) as the catalyst, the nitro group in intermediate **(4)** was converted to an amino group by hydrogenation to prepare intermediate **(5)**. The resulting compound was allowed to react with acyl chloride in the presence of anhydrous potassium carbonate to obtain acylated products **(6a-6h)**, followed by deprotection under acidic conditions to achieve the target compound **K**.

Intermediate **(7)** was obtained from intermediate **(3),** which was converted by hydrogenation. Intermediate **(7)** was reacted with acyl chloride in the presence of anhydrous potassium carbonate to obtain acylated products **(8a-8h)**. Treatment of phenyl nitrile **(9)** with hydroxylamine hydrochloride under alkaline conditions afforded intermediate **(10)**, and subsequent reaction with chloroacetyl chloride in a methanol solution provided intermediate **(11)**. Different intermediates **(12a-12b)** were reacted with bromoacetyl bromide to prepare intermediates **(13a-13b)**. Then, intermediates **(8a-8f)** reacted with intermediate **(11)** and intermediates **(8f-8h)** reacted with intermediates **(13a-13b)** by electrophilic substitution to obtain intermediates **(14a-14f)** and intermediates **(15a-15d)**, respectively. Finally, products **14** and **15** were converted to target compounds **Q** and **D** by deprotection under acidic conditions.

2.2. Cell antiproliferative activity

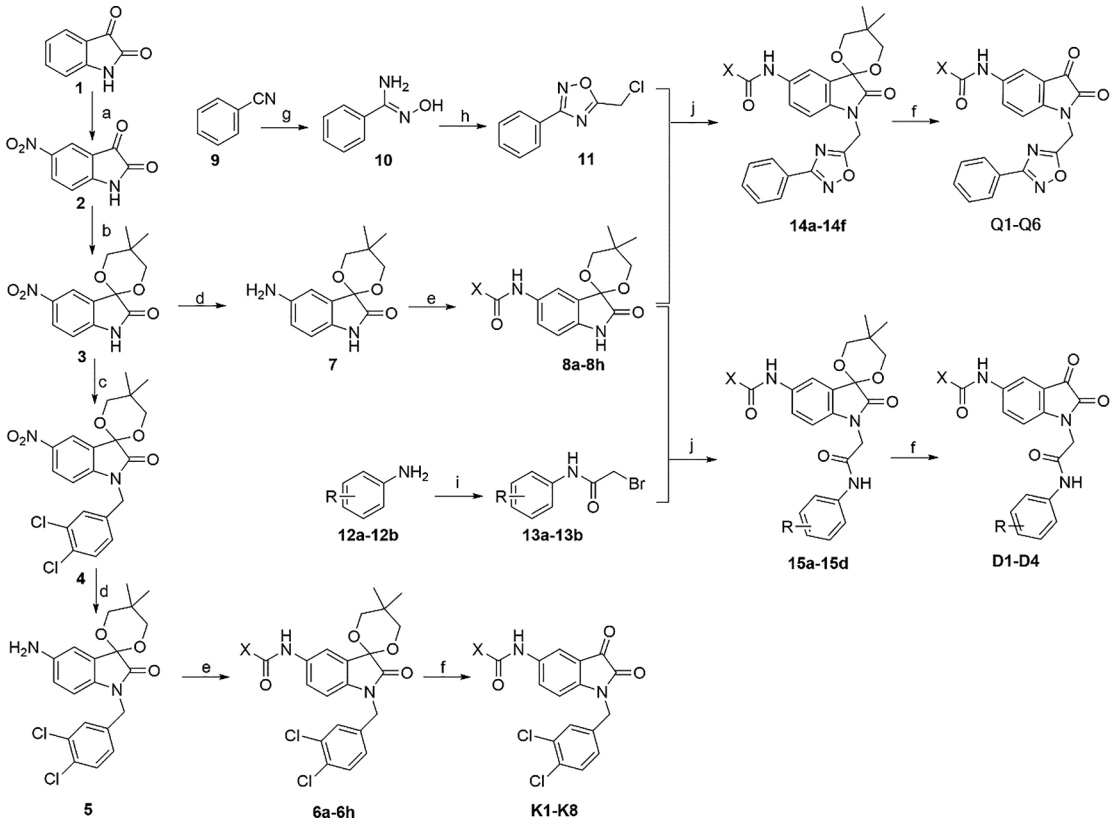
All of the newly synthesized compounds were assessed in a panel of MCL cell lines (Mino, Granta-519, Z138, Jeko-R, Maver-1) to explore the antiproliferative activities. Ibrutinib (**IBN**), which was approved by the FDA to treat MCL, served as a positive control.[27] As shown in Table 1, most of the compounds increased antiproliferative activities compared with **IBN**. Compounds with 3,4-dichlorobenzyl group and 3-phenyl-1,2,4-oxadiazol-5-methylene group at the N1 position displayed higher inhibitory potency to MCL cell lines than that with benzamide methylene moiety (**K2**, **Q6** *vs.* **D2**). Notably, series **K** and **Q** behaved better (IC_{50} = 0.5–4.9 μM) than **IBN** (IC_{50} = 20.0 μM) for Jeko-R cells, an IBN-resistant MCL cell line. **K5** was the most potent inhibitor, with IC_{50} values ranging between 0.4 and 0.7 μM against tested MCL cell lines.

2.3. Cell apoptosis and cell cycle assay

Many factors mediate apoptosis and the cell cycle to maintain a stable internal environment. Accordingly, to shed light on the underlying antitumor mechanism of compound **K5**, an apoptosis assay was performed in Rec-1 cells and Z138 cells after **K5** treatment, as well as the cell cycle assay in Rec-1 cells. Annexin V-FITC/PI staining showed that **K5** induced cell apoptosis in Rec-1 cells and Z138 cells in a dose-dependent manner, and the rate of cell apoptosis was over 90% after 24 h of treatment at a concentration of 2.5 μM (Fig. 2A–D). The cell cycle assay demonstrated that treatment with **K5** dose-dependently induced cell cycle arrest in G2/M phase after a 24-h incubation, effectively preventing cell cycle progression and promoting cell death (Fig. 2E).

2.4. Prediction of drug-likeness

To reduce time and resource consumption in drug discovery phases, molecules should be evaluated for absorption, distribution, metabolism and excretion (ADME). With the SwissADME web tool (http://www.swissadme.ch),[28] we calculated the physicochemical properties, lipophilicity, water solubility, pharmacokinetics and drug-likeness of compound **K5**. The results are summarized in Table 2: we could see that the drug-likeness of **K5** was predicted by five diverse filters (Lipinski



Scheme 1. Reagents and conditions: (a) fuming nitric acid, concentrated H_2SO_4, 0 °C, 1 h; (b) neopentyl glycol, *p*-toluenesulfonic acid, cyclohexane, 85 °C, 16 h; (c) K_2CO_3, *N,N*-dimethylformamide, 85 °C, 1.5 h; (d) Pd/C, H_2, ethyl acetate, r.t., 12 h; (e) substituted acyl chloride, K_2CO_3, ethyl acetate, 0 °C, 12 h; (f) glacial acetic acid, concentrated hydrochloric acid, r.t., 12 h; (g) hydroxylamine hydrochloride, Et_3N, CH_3OH, 60 °C, 0.5 h; (h) chloroacetyl chloride, K_2CO_3, tetrahydrofuran, 0 °C, 2 h; (i) bromoacetyl bromide, $NaHCO_3$, ethyl acetate/H_2O = 1:1, 0 °C, 1 h; (j) K_2CO_3, *N,N*-dimethylformamide, r.t., 12 h.

filter, Ghose filter, Veber filter, Egan filter and Muegge filter), and it passed all the rules of filter evaluation, which indicated that **K5** was promising for further in-depth research. From the prediction, we could ascertain that early prediction contributed to designing and synthesizing excellent compounds.

3. Conclusion

In summary, our study outlined the design, synthesis and biological evaluation of novel 2,3-indolinedione derivatives as potential agents for the treatment of MCL. Most compounds showed increased antiproliferative activity against MCL cell lines compared with **IBN**. Among these compounds, **K5** remarkably exhibited considerable efficiency in MCL cells, with IC_{50} values lower than 1 μM. Meanwhile, it induced cell apoptosis and G2/M cell cycle arrest in a dose-dependent manner. The prediction of drug-likeness proved that **K5** was worthy of thorough research. The results suggested that the 2,3-indolinedione structure did offer potential for the discovery of novel potent inhibitors for MCL.

4. Experimental section

4.1. Chemistry

All of the chemical reagents and solvents were purchased from commercial sources and were used without further purification. Reactions were monitored using thin-layer chromatography (TLC) performed on SGF254 plates. Chromatographic separations were performed using column chromatography on silica gel (60 Å, 200–300 mesh). Melting points were determined on a Büchi capillary melting point apparatus (Buchi Labortechnik AG, Switzerland) without correction. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker Avance DRX-400 Spectrometer (Bruker, Germany) in deuterated dimethyl sulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as the internal standard. Peak multiplicities were expressed as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad singlet (br s), doublet of doublets (dd), doublet of triplets (dt), and quartet of doublets (qd). The mass spectra (MS) were measured with LCQ FLEET (ThermoFisher, USA). The purity of the compounds was determined by HPLC performed on a Shimadzu LC-20ATVP Liquid Chromatograph equipped with an SPD-M20A UV VIS Detector using a C18 column (size: 250 mm × 4.6 mm). Elution solvent: 75% methanol and 25% water. The elution rate was 1.00 mL/min, and the injection volumes were 10 μL at 25 °C and detection at 253 nm.

4.1.1. Synthesis of 5-nitroindoline-2,3-dione (2)

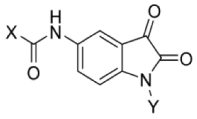
To a solution of isatin (**1**, 200 mg, 1.36 mmol) in concentrated H_2SO_4 (3 mL) was added fuming nitric acid (124 mg, 1.77 mmol) at 0 °C. The resulting reaction mixture was stirred at 0 °C for 1 h. After completion of the reaction, the mixture was slowly poured into ice-cold water (50 mL). The precipitate was filtered, washed with water (15 mL × 3), and then dried to obtain intermediate **2**.

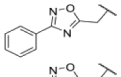
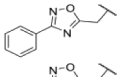
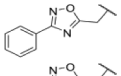
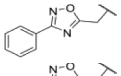
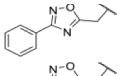
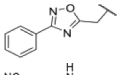
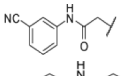
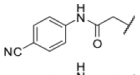
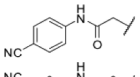
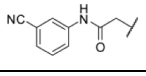
Yellow solid; yield 85%; mp: 257–259 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.66 (s, 1H), 8.45 (dd, J = 8.7, 2.4 Hz, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.09 (d, J = 8.7 Hz, 1H).

4.1.2. Synthesis of 5′,5′-dimethyl-5-nitrospiro[indoline-3,2′-[1,3]dioxan]-2-one (3)

Intermediate **2** (200 mg, 1.04 mmol), neopentyl glycol (108 mg, 1.04 mmol) and *p*-toluenesulfonic acid (25 mg, 145 μmol) were successively added and dissolved in cyclohexane (10 mL). The resulting reaction mixture was refluxed for 16 h at 85 °C. After completion of the

Table 1
Cell proliferation assay assessing the effects of novel 2,3-indolinedione derivatives on MCL cell lines.



Code	X	Y	Cell viability assay, IC_{50}/μM				
			Mino	Granta-519	Z138	Jeko-R	Maver-1
IBN	—	—	2.0	9.7	14.4	20.0	14.4
K1			0.4	1.4	0.4	0.8	0.6
K2			0.4	1.2	0.4	0.8	0.5
K3			0.4	1.1	0.4	0.5	0.5
K4			0.4	1.1	0.4	0.6	0.5
K5			0.4	0.6	0.4	0.7	0.4
K6			0.4	0.9	0.4	0.7	0.5
K7			0.4	0.8	0.4	0.5	0.5
K8			1.8	4.2	0.9	3.3	2.3
Q1			2.3	6.5	1.3	4.1	2.9
Q2			1.2	3.0	0.6	2.0	1.0
Q3			0.4	1.7	0.5	0.9	0.7
Q4			3.5	7.3	2.2	4.9	3.9
Q5			0.4	1.5	0.4	0.7	0.5
Q6			0.6	2.1	0.5	1.3	0.7
D1			7.3	[a]ND	29.6	49.4	25.3
D2			12.3	[a]ND	28.0	52.5	43.8
D3			> 60	[a]ND	> 60	> 60	> 60
D4			15.7	[a]ND	> 60	58.0	39.6

[a]ND, Not Detected.

reaction, the mixture was cooled to room temperature. Then, the precipitate was filtered, washed with water and dried. Purification by chromatography on silica gel (petroleum ether/ethyl acetate = 5/1) afforded intermediate **3**.

White solid; yield 84%; mp: 201–203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 8.28 (dd, J = 8.7, 2.4 Hz, 1H), 8.08 (d, J = 2.4 Hz, 1H), 7.04 (d, J = 8.7 Hz, 1H), 4.49 (d, J = 11.0 Hz, 2H), 3.55 (d, J = 11.2 Hz, 2H), 1.34 (s, 3H), 0.84 (s, 3H).

*4.1.3. 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 *N,N*-dimethylformamide (2 mL) was added 1,2-dichloro-4-(chloromethyl)benzene (169 mg, 0.86 mmol). The resulting reaction mixture was stirred for 1.5 h at 85 °C. The reaction mixture was cooled to room temperature and then poured into ice water (50 mL), and a precipitate was produced. The precipitate was filtered, washed with water, and then recrystallized with ethyl acetate/petroleum ether to obtain intermediate **4**.

White solid; yield 80%; mp: 137–141 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (dd, J = 8.7, 1.6 Hz, 1H), 8.14(d, J = 2.2 Hz, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.31 (d, J = 8.8 Hz, 1H), 7.22 (dd, J = 8.3, 2.0 Hz, 1H), 4.98 (s, 2H), 4.50 (d, J = 11.0 Hz, 2H), 3.61 (d, J = 10.9 Hz, 2H), 1.37 (s, 3H), 0.87 (s, 3H).

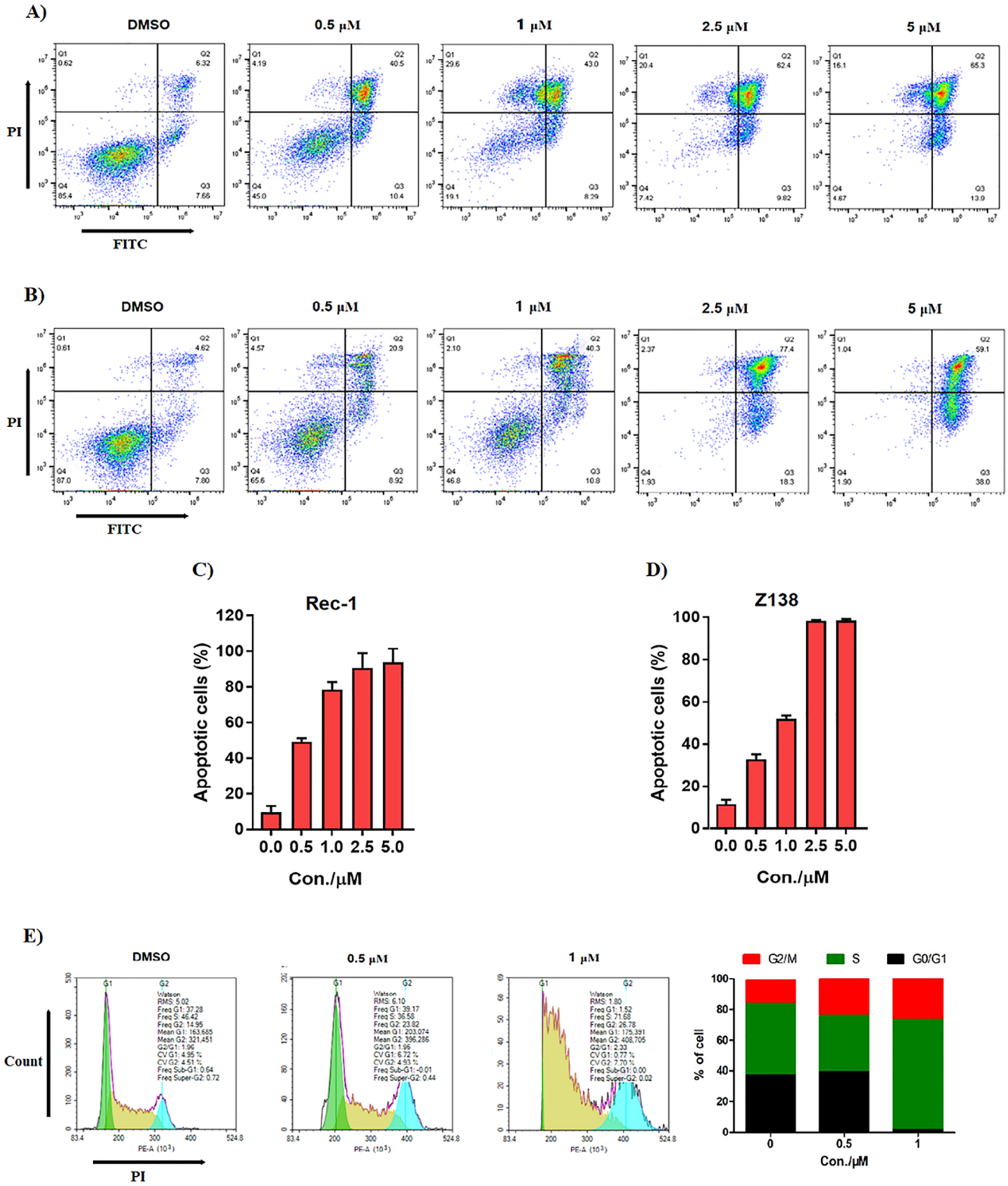


Fig 2. Cell apoptosis with **K5** treatment in Rec-1 cells and Z138 cells, and cell cycle assay with **K5** treatment in Rec-1 cells. A/C) Apoptosis assay of **K5** at the indicated concentrations in Rec-1 cells for 24 h; B/D) Apoptosis assay of **K5** at the indicated concentrations in Z138 cells for 24 h; E) Cell cycle analysis of **K5** at the indicated concentrations in Rec-1 cells for 24 h.

4.1.4. Synthesis of 5-amino-1-(3,4-dichlorobenzyl)-5′,5′-dimethylspiro [indoline-3,2′-[1,3]dioxan]-2-one (5)

To a solution of intermediate **4** (280 mg, 640 μmol) in ethyl acetate (25 mL) was added 10% Pd/C (140 mg), and the mixture was subjected to hydrogen for 12 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude material was recrystallized with ethyl acetate/petroleum ether to obtain intermediate **5**.

Light yellow solid; yield 77%; mp: 171–174 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (d, J = 8.3 Hz, 1H), 7.55 (d, J = 2.0 Hz, 1H), 7.21 (dd,

Table 2
Several important properties of compound **K5** as determined using SwissADME web tools.

Properties	**K5**
Physicochemical Properties	
Molecular weight	397.64 g/mol
Num. heavy atoms	25
Num. arom. heavy atoms	12
Fraction Csp3	0.17
Num. rotatable bonds	5
Num. H-bond acceptors	3
Num. H-bond donors	1
Molar Refractivity	100.67
TPSA[a]	66.48 Å^2
Lipophilicity	
Log P (average of iLOGP, XLOGP3, WLOGP, MLOGP, SILICOS-IT)	3.06
Water Solubility	
Log *S* (ESOL)[b]	−4.50
Solubility	1.25e-02 mg/ml; 3.13e-05 mol/l
Class	Moderately soluble
Pharmacokinetics	
GI absorption	High
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)[c]	−6.28 cm/s
Drug-likeness	
Lipinski (Pfizer) filter	Yes; 0 violation
Ghose filter	Yes
Veber (GSK) filter	Yes
Egan (Pharmacia) filter	Yes
Muegge (Bayer) filter	Yes
Bioavailability Score[d]	0.55

[a] TPSA: Topological Polar Surface Area. Molecular polar surface area (PSA) calculated as sum of tabulated surface contributions of polar fragments.[29]

[b] ESOL – Estimated SOLubility: Simple method for estimating the aqueous solubility of a compound directly from its structure.[30] Solubility Class: Log S scale: −Insuluble < −10 < Poorly < −6 < Moderately < −4 < Soluble < −2 < Very < 0 < Highly.

[c] LogKp: Permeability coefficient from the Quantitative Structure Permeability Relationships (QSPR) model based upon permeant size [molecular volume (MV) or molecular weight (MW)] and octanol/water partition coefficient (Koct).[31]

[d] Abbott Bioavailability Score: Probability that a compound will have F > 10% in the rat.[32]

J = 8.3, 2.0 Hz, 1H), 6.76 (d, *J* = 2.2 Hz, 1H), 6.64 (d, *J* = 8.3 Hz, 1H),6.48 (dd, *J* = 8.3, 2.3 Hz, 1H), 4.97 (s, 2H), 4.75 (s, 2H), 4.50 (d, *J* = 10.9 Hz, 2H),3.51 (d, *J* = 11.0 Hz, 2H), 1.30 (s, 3H), 0.84 (s, 3H).

4.1.5. Synthesis of intermediates (6a-6h)

To a solution of intermediate **5** (200 mg, 491 μmol) and anhydrous K_2CO_3 (81 mg, 589 μmol) in ethyl acetate (30 mL) was added differentially substituted acyl chloride (589 μmol) at 0 °C. The resulting solution was stirred overnight at room temperature. After completion of the reaction, the mixture was filtered. The organic phase was successively washed with water (15 mL × 3) and saturated brine (15 mL × 3) and then evaporated under reduced pressure to obtain intermediates **6a-6h**, which were directly used for the next step without further purification.

4.1.6. Synthesis of target compounds (K1-K8)

To the solvent of glacial acetic acid (20 mL) and concentrated hydrochloric acid (2 mL) was added intermediate **6** (421 μmol). The mixture was stirred overnight at room temperature and then poured into water (100 mL), and a precipitate was produced. The precipitate was filtered and purified by recrystallization in methanol to obtain compounds **K1-K8**.

*4.1.6.1. N-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)propionamide (**K1**).* Red solid; yield 81%; mp: 227–230 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 7.90 (s, 1H), 7.76 (s, 1H), 7.61 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.89 (s, 2H), 2.30 (dd, *J* = 14.9, 7.5 Hz, 2H), 1.07 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.43 (s), 172.53 (s), 159.11 (s), 145.56 (s), 137.34 (s), 135.76 (s), 131.74 (s), 131.16 (s), 130.57 (s), 129.81 (s), 128.23 (s), 128.21 (s), 118.39 (s), 115.66 (s), 111.50 (s), 42.28 (s), 29.84 (s), 10.05 (s); MS (ESI): calcd for $C_{18}H_{14}Cl_2N_2O_3$ $[M-H]^-$ 375.04, found 375.33; HPLC: t_R 5.566 min, purity 95.0%.

*4.1.6.2. N-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)butyramide (**K2**).* Red solid; yield 77%; mp: 248–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 7.91 (s, 1H), 7.76 (s, 1H), 7.61 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 4.89 (s, 2H), 2.26 (t, *J* = 7.3 Hz, 2H), 1.65–1.53 (m, 2H), 0.88 (t, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.42 (s), 171.68 (s), 159.10 (s), 145.59 (s), 137.33 (s), 135.73 (s),131.74 (s), 131.16 (s), 130.57 (s), 129.81 (s), 128.26 (s), 128.20 (s), 118.37 (s), 115.70 (s), 111.50 (s), 42.29 (s), 38.65 (s), 18.97 (s), 14.04 (s); MS (ESI): calcd for $C_{19}H_{16}Cl_2N_2O_3$ $[M-H]^-$ 389.05, found 389.07; HPLC: t_R 6.710 min, purity 92.3%.

*4.1.6.3. (E)-N-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)but-2-enamide (**K3**).* Red solid; yield 72%; mp: 257–259 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 7.97 (d, *J* = 2.1 Hz, 1H), 7.77 (d, *J* = 2.0 Hz, 1H), 7.65 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.44 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.80 (dq, *J* = 13.8, 6.9 Hz, 1H), 6.06 (dd, *J* = 15.2, 1.7 Hz, 1H), 4.89 (s, 3H), 1.86 (dd, *J* = 6.9, 1.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.38 (s), 163.97 (s), 159.10 (s), 145.73 (s), 140.78 (s), 137.32 (s), 135.69 (s), 131.75 (s), 131.16 (s), 130.58 (s), 129.81 (s), 128.38 (s), 128.20 (s), 126.06 (s), 118.43 (s), 115.80 (s), 111.56 (s), 42.30 (s), 18.01 (s); MS (ESI): calcd for $C_{19}H_{14}Cl_2N_2O_3$ $[M-H]^-$ 387.04, found 387.18; HPLC: t_R 7.598 min, purity 87.1%.

*4.1.6.4. N-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)acrylamide (**K4**).* Red solid; yield 74%; mp: 220–222 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 7.99 (d, *J* = 2.1 Hz, 1H), 7.77 (d, *J* = 2.0 Hz, 1H), 7.68 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.45 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.39 (dd, *J* = 17.0, 10.0 Hz, 1H), 6.26 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.77 (dd, *J* = 10.0, 2.1 Hz, 1H), 4.90 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.33 (s), 163.67 (s), 159.11 (s), 145.97 (s), 137.31 (s), 135.39 (s), 131.97 (s), 131.75 (s), 131.17 (s), 130.58 (s), 129.82 (s), 128.52 (s), 128.21 (s), 127.68 (s), 118.48 (s), 115.89 (s), 111.61 (s), 42.31 (s); MS (ESI): calcd for $C_{18}H_{12}Cl_2N_2O_3$ $[M-H]^-$ 373.02, found 373.11; HPLC: t_R 5.523 min, purity 88.9%.

*4.1.6.5. 2-Chloro-N-(1-(3,4-dichlorobenzyl)-2,3-dioxoindolin-5-yl) acetamide (**K5**).* Red solid; yield 83%; mp: 205–206 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 7.88 (d, *J* = 2.1 Hz, 1H), 7.77 (d, *J* = 1.9 Hz, 1H), 7.61 (dd, *J* = 8.4, 2.2 Hz, 2H), 7.45 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 4.90 (s, 2H), 4.25 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.32 (s), 165.26 (s), 159.11 (s), 146.24 (s), 137.26 (s), 134.76 (s), 131.76 (s), 131.16 (s), 130.59 (s), 129.81 (s), 128.72 (s), 128.21 (s), 118.51 (s), 116.00 (s), 111.65 (s), 43.85 (s), 42.32 (s); MS (ESI): calcd for $C_{17}H_{11}Cl_3N_2O_3$ $[M-H]^-$ 394.98, found 395.19; HPLC: t_R 5.300 min, purity 94.1%.

*4.1.6.6. N-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl) cyclopropanecarboxamide (**K6**).* Red solid; yield 75%; mp: 247–250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 7.89 (d, *J* = 2.1 Hz, 1H),

7.76 (d, J = 2.0 Hz, 1H), 7.65–7.58 (m, 2H), 7.44 (dd, J = 8.4, 2.0 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 4.89 (s, 2H), 1.76–1.67 (m, 1H), 0.80 (d, J = 6.3 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.41 (s), 172.17 (s), 159.10 (s), 145.54 (s), 137.34 (s), 135.77 (s), 131.75 (s), 131.16 (s), 130.58 (s), 129.81 (s), 128.20 (s), 128.14 (s), 118.41 (s), 115.64 (s), 111.52 (s), 42.29 (s), 14.99 (s), 7.76 (s, 2C); MS (ESI): calcd for $C_{19}H_{14}Cl_2N_2O_3$ $[M-H]^-$ 387.04, found 387.48; HPLC: t_R 7.195 min, purity 99.6%.

*4.1.6.7. N-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)benzamide (**K7**).* Red solid; yield 70%; mp: 238–240 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.05 (d, J = 2.2 Hz, 1H), 7.98–7.93 (m, 2H), 7.87 (dd, J = 8.6, 2.2 Hz, 1H), 7.79 (d, J = 1.9 Hz, 1H), 7.61 (dd, J = 11.0, 7.8 Hz, 2H), 7.54 (t, J = 7.3 Hz, 2H), 7.47 (dd, J = 8.4, 2.0 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 4.92 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.36 (s), 165.97 (s), 159.19 (s), 146.12 (s), 137.34 (s), 135.50 (s), 134.92 (s), 132.23 (s), 131.75 (s), 131.17 (s), 130.58 (s), 129.83 (s), 129.70 (s), 128.92 (s, 2C), 128.22 (s), 128.10 (s, 2C), 118.40 (s), 117.05 (s), 111.43 (s), 42.32 (s); MS (ESI): calcd for $C_{22}H_{14}Cl_2N_2O_3$ $[M-H]^-$ 423.04, found 423.42; HPLC: t_R 9.789 min, purity 97.4%.

*4.1.6.8. N-(1-(3,4-Dichlorobenzyl)-2,3-dioxoindolin-5-yl)-2-phenylacetamide (**K8**).* Red solid; yield 75%; mp: 257–259 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 7.90 (d, J = 2.1 Hz, 1H), 7.76 (d, J = 1.9 Hz, 1H), 7.62 (dd, J = 13.5, 5.2 Hz, 2H), 7.44 (dd, J = 8.3, 2.0 Hz, 1H), 7.32 (d, J = 4.4 Hz, 4H), 7.29–7.20 (m, 1H), 6.89 (d, J = 8.5 Hz, 1H), 4.89 (s, 2H), 3.62 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.35 (s), 169.69 (s), 159.11 (s),145.76 (s), 137.30 (s), 136.24 (s), 135.60 (s), 131.75 (s), 131.15 (s), 130.58 (s),129.80 (s), 129.56 (s, 2C), 128.79 (s, 2C), 128.37 (s), 128.19 (s), 127.04 (s), 118.41(s), 115.73 (s), 111.55 (s), 43.62 (s), 42.28 (s); MS (ESI): calcd for $C_{23}H_{16}Cl_2N_2O_3$ $[M-H]^-$ 437.05, found 437.22; HPLC: t_R 8.042 min, purity 97.2%.

*4.1.7. Synthesis of 5-amino-5′,5′-dimethylspiro[indoline-3,2′-[1,3]dioxan]-2-one (**7**)*

To a solution of intermediate **3** (395 mg, 1.42 mmol) in ethyl acetate (40 mL) was added 10% Pd/C (240 mg). The mixture was subjected to hydrogen for 12 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude material was recrystallized with ethyl acetate/petroleum ether to obtain intermediate **7**.

White solid; yield 81%; mp: 212–214 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 6.68 (s, 1H), 6.47 (s, 2H), 4.82 (s, 2H), 4.49 (d, J = 10.8 Hz, 2H), 3.43 (d, J = 11.0 Hz, 2H), 1.27 (s, 3H), 0.81 (s, 3H).

*4.1.8. Synthesis of intermediates (**8a-8h**)*

To a solution of intermediate **7** (240 mg, 967 μmol) and anhydrous K_2CO_3 (160 mg, 1.16 mmol) in ethyl acetate (20 mL) was added differentially substituted acyl chloride (1.16 μmol) at 0 °C. The resulting solution was stirred overnight at room temperature. After completion of the reaction, the mixture was filtered. The organic phase was successively washed with water (15 mL × 3) and saturated brine (15 mL × 3) and then evaporated under reduced pressure to obtain intermediates **8a-8h**, which were directly used for the next step without further purification.

*4.1.9. Synthesis of (E)-N′-hydroxybenzimidamide (**10**)*

Benzonitrile **9** (2 g, 19.39 mmol), hydroxylamine hydrochloride (2.7 g, 38.79 mmol) and Et_3N (3.93 g, 38.79 mmol) were successively added and dissolved in methanol (100 mL). The mixture was stirred for 30 min at 60 °C. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (50 mL). The organic phase was successively washed with water (30 mL × 3) and saturated brine (30 mL × 3), dried over $MgSO_4$ and then filtered and concentrated under reduced pressure to obtain intermediate **10** as a white solid.

*4.1.10. Synthesis of 5-(chloromethyl)-3-phenyl-1,2,4-oxadiazole (**11**)*

To a solution of intermediate **10** (100 mg, 734 μmol) and anhydrous K_2CO_3 (152 mg, 1.1 mmol) in tetrahydrofuran (15 mL) was added chloroacetyl chloride (166 mg, 1.47 mmol) at 0 °C. The mixture was stirred for 2 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (25 mL). The organic phase was successively washed with water (20 mL × 3) and saturated sodium carbonate solution (20 mL × 3), dried over Na_2SO_4 and then filtered and concentrated under reduced pressure to obtain intermediate **11** as yellow oil.

*4.1.11. Synthesis of intermediates (**14a-14f**)*

To a solution of intermediate **8a-8f** (532 μmol) in dried *N,N*-dimethylformamide (5 mL) was added anhydrous K_2CO_3 (147 mg, 1.06 mmol). The mixture was stirred for 30 min at room temperature. Intermediate **11** (124 mg, 639 μmol) was then added to the mixture. The mixture was stirred for 12 h at room temperature. The reaction mixture was poured into ice water (100 mL) and a precipitate was produced. The precipitate was filtered, washed with water, and dried to obtain intermediates **14a-14f**.

*4.1.12. Synthesis of target compounds (**Q1-Q6**)*

Glacial acetic acid (30 mL) and concentrated hydrochloric acid (4 mL) were added to intermediate **14** (421 μmol). The mixture was stirred overnight at room temperature and then poured into distilled water (100 mL), and a precipitate was produced. The precipitate was filtered and purified by column chromatography with dichloromethane/methanol (20:1) to obtain compounds **Q1-Q6**.

*4.1.12.1. N-(2,3-Dioxo-1-((3-phenyl-1,2,4-oxadiazol-5-yl)methyl)indolin-5-yl)cyclopropanecarboxamide (**Q1**).* Red solid; yield 61%; mp: 216–219 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.31 (s, 1H), 7.88 (d, J = 2.1 Hz, 1H), 7.62 (dd, J = 8.5, 2.2 Hz, 1H), 7.42 (d, J = 7.0 Hz, 2H), 7.34 (t, J = 7.3 Hz, 2H), 7.28 (t, J = 7.2 Hz, 1H), 6.92 (d, J = 8.5 Hz, 1H), 4.88 (s, 2H), 1.71 (s, 1H), 0.80 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.70 (s), 175.25 (s), 172.33 (s), 168.25 (s), 158.69 (s), 145.33 (s), 136.23 (s), 132.26 (s), 129.79 (s, 2C), 128.68 (s), 127.54 (s, 2C), 126.15 (s), 118.16 (s), 115.67 (s), 111.89 (s), 36.22 (s), 14.99 (s), 7.80 (s, 2C); MS (ESI): calcd for $C_{21}H_{16}N_4O_4$ $[M-H]^-$ 387.12, found 387.72; HPLC: t_R 4.209 min, purity 97.9%.

*4.1.12.2. N-(2,3-Dioxo-1-((3-phenyl-1,2,4-oxadiazol-5-yl)methyl)indolin-5-yl)propionamide (**Q2**).* Red solid; yield 55%; mp: 207–209 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.01–7.91 (m, 3H), 7.76 (dd, J = 8.6, 2.2 Hz, 1H), 7.61–7.52 (m, 3H), 7.26 (d, J = 8.6 Hz, 1H), 5.38 (s, 2H), 2.32 (q, J = 7.5 Hz, 2H), 1.08 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.71 (s), 175.26 (s), 172.64 (s), 168.25 (s), 158.69 (s), 145.36 (s), 136.18 (s), 132.26 (s), 129.80 (s, 2C), 128.77 (s), 127.54 (s, 2C), 126.15 (s), 118.16 (s), 115.70 (s), 111.87 (s), 36.22 (s), 29.86 (s), 10.03 (s); MS (ESI): calcd for $C_{20}H_{16}N_4O_4$ $[M-H]^-$ 375.12, found 375.78; HPLC: t_R 4.008 min, purity 98.5%.

*4.1.12.3. N-(2,3-Dioxo-1-((3-phenyl-1,2,4-oxadiazol-5-yl)methyl)indolin-5-yl)-2-phenylacetamide (**Q3**).* Red solid; yield 66%; mp: 189–192 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.37 (s, 1H), 7.99–7.91 (m, 3H), 7.77 (dd, J = 8.6, 2.2 Hz, 1H), 7.63–7.52 (m, 3H), 7.33 (d, J = 4.4 Hz, 4H), 7.27 (d, J = 8.7 Hz, 2H), 5.38 (s, 2H), 3.63 (d, J = 7.9 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.62 (s), 175.24 (s), 169.78 (s), 168.24 (s), 158.68 (s), 145.60 (s), 136.17 (s), 135.93 (s), 132.27 (s), 129.80 (s, 2C), 129.57 (s, 2C), 128.92 (s), 128.81 (s, 2C), 127.54 (s, 2C), 127.08 (s), 126.15 (s), 118.21 (s), 115.80 (s), 111.94 (s), 43.63 (s), 36.21 (s); MS (ESI): calcd for $C_{25}H_{18}N_4O_4$ $[M-H]^-$ 437.13, found 437.21; HPLC: t_R 5.342 min, purity 96.7%.

*4.1.12.4. N-(2,3-Dioxo-1-((3-phenyl-1,2,4-oxadiazol-5-yl)methyl)indolin-5-yl)benzamide (**Q4**)*. Red solid; yield 67%; mp: 264–266 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H), 8.10 (s, 1H), 8.06–7.90 (m, 5H), 7.66–7.49 (m, 6H), 7.34 (d, J = 8.5 Hz, 1H), 5.42 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.65 (s), 175.26 (s), 168.27 (s), 166.06 (s), 158.76 (s), 145.92 (s), 135.93 (s), 134.86 (s), 132.27 (s), 130.29 (s), 129.80 (s, 2C), 128.92 (s, 2C), 128.89 (s), 128.16 (s, 2C), 127.55 (s, 2C), 126.16 (s), 118.15 (s), 117.16 (s), 111.80 (s), 36.26 (s); MS (ESI): calcd for $C_{24}H_{16}N_4O_4$ $[M-H]^-$ 423.12, found 423.28; HPLC: t_R 5.233 min, purity 85.5%.

*4.1.12.5. (E)-N-(2,3-Dioxo-1-((3-phenyl-1,2,4-oxadiazol-5-yl)methyl) indolin-5-yl)but-2-enamide (**Q5**)*. Red solid; yield 72%; mp: 200–203 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H), 8.02 (d, J = 1.8 Hz, 1H), 7.97 (d, J = 6.7 Hz, 2H), 7.81 (dd, J = 8.6, 2.0 Hz, 1H), 7.65–7.52 (m, 3H), 7.28 (d, J = 8.6 Hz, 1H), 6.82 (dq, J = 13.9, 6.8 Hz, 1H), 6.10 (d, J = 15.2 Hz, 1H), 5.39 (s, 2H), 1.87 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.67 (s), 175.24 (s), 168.27 (s), 164.10 (s), 158.68 (s), 145.54 (s), 140.81 (s), 136.15 (s), 132.25 (s), 129.79 (s, 2C), 128.97 (s), 127.55 (s, 2C), 126.17 (s), 126.10 (s), 118.20 (s), 115.89 (s), 111.92 (s), 36.24 (s), 28.56 (s); MS (ESI): calcd for $C_{21}H_{16}N_4O_4$ $[M-H]^-$ 387.12, found 387.29; HPLC: t_R 4.416 min, purity 97.0%.

*4.1.12.6. N-(2,3-Dioxo-1-((3-phenyl-1,2,4-oxadiazol-5-yl)methyl)indolin-5-yl)butyramide (**Q6**)*. Red solid; yield 78%; mp: 205–208 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H), 8.03–7.91 (m, 3H), 7.76 (dd, J = 8.6, 2.2 Hz, 1H), 7.64–7.54 (m, 3H), 7.26 (d, J = 8.6 Hz, 1H), 5.38 (s, 2H), 2.28 (t, J = 7.3 Hz, 2H), 1.67–1.54 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.70 (s), 175.25 (s), 171.82 (s), 168.25 (s), 158.70 (s), 145.39 (s), 136.14 (s), 132.26 (s), 129.80 (s, 2C), 128.80 (s), 127.54 (s, 2C), 126.15 (s), 118.15 (s), 115.73 (s), 111.86 (s), 38.65 (s), 36.21 (s), 18.97 (s), 14.06 (s); MS (ESI): calcd for $C_{21}H_{18}N_4O_4$ $[M-H]^-$ 389.13, found 389.42; HPLC: t_R 4.614 min, purity 98.7%.

*4.1.13. Synthesis of intermediates (**13a-13b**)*

To differentially substituted aniline **12** (5 mmol) and $NaHCO_3$ (1.26 g, 15 mmol) in ethyl acetate/water (10 mL/10 mL) was added bromoacetyl bromide (2.02 g, 10 mmol) at 0 °C. The mixture was stirred for 1 h at 0 °C. After completion of the reaction, the mixture was dissolved in ethyl acetate/water (1:1, 100 mL). The aqueous phase was washed with ethyl acetate (30 mL × 2). The organic phase was then washed with saturated brine (30 mL × 1) and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was recrystallized with ethyl acetate to obtain intermediates **13a-13b**.

*4.1.13.1. 2-Bromo-N-(4-cyanophenyl)acetamide (**13a**)*. White solid; yield 84%; mp: 123–125 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.84 (s, 1H), 7.81 (d, J = 8.8 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H), 4.09 (s, 2H).

*4.1.13.2. 2-Bromo-N-(3-cyanophenyl)acetamide (**13b**)*. White solid; yield 87%; mp: 153–155 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.75 (s, 1H), 8.07 (s, 1H), 7.81–7.78 (m, 1H), 7.57–7.56 (m, 2H), 4.08 (s, 2H).

*4.1.14. General synthesis of intermediates (**15a-15d**)*

To a solution of intermediate **8f-8h** (1.03 mmol) in dried *N,N*-dimethylformamide (20 mL) was added anhydrous K_2CO_3 (1.55 mmol). The mixture was stirred for 30 min at room temperature. Then, the intermediates **13a-13b** (1.24 mmol) were added to the mixture. The mixture was stirred overnight at room temperature and poured into ice water (100 mL), and a precipitate was produced. The precipitate was filtered, washed with water, and dried and purified by column chromatography with petroleum ether/ethyl acetate (5:1) to obtain intermediates **15a-15d**.

*4.1.15. Synthesis of target compounds (**D1-D4**)*

Glacial acetic acid (15 mL) and concentrated hydrochloric acid (3 mL) were added to intermediate **15** (354.22 μmol). The mixture was stirred overnight at room temperature and then poured into ice water (100 mL), and a precipitate was produced. The precipitate was filtered, washed with water, and dried. The crude material was recrystallized with methanol to obtain compounds **D1-D4**.

*4.1.15.1. N-(1-(2-((3-Cyanophenyl)amino)-2-oxoethyl)-2,3-dioxoindolin-5-yl)acrylamide (**D1**)*. Red solid; yield 75%; mp: 283–285 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.57 (s, 1H), 10.34 (s, 1H), 8.03 (d, J = 4.3 Hz, 2H), 7.80 (s, 2H), 7.57 (s, 2H), 7.16 (d, J = 7.6 Hz, 1H), 6.40 (m, 6.44–6.37, 1H), 6.28 (d, J = 16.7 Hz, 1H), 5.79 (d, J = 10.3 Hz, 1H), 4.59 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.39 (s), 166.10 (s), 163.73 (s), 159.04 (s), 146.84 (s), 139.57 (s), 135.52 (s), 131.95 (s), 130.86 (s), 129.24 (s), 127.89 (s), 127.77 (s), 124.61 (s), 122.75 (s), 118.99 (s), 117.94 (s), 115.82 (s), 112.14 (s), 111.90 (s), 43.71 (s); MS (ESI): calcd for $C_{20}H_{14}N_4O_4$ $[M-H]^-$ 373.10, found 373.22; HPLC: t_R 2.943 min, purity 98.8%.

*4.1.15.2. N-(1-(2-((4-Cyanophenyl)amino)-2-oxoethyl)-2,3-dioxoindolin-5-yl)butyramide (**D2**)*. Red solid; yield 77%; mp: 290–293 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 10.06 (s, 1H), 7.94 (d, J = 1.5 Hz, 1H), 7.81 (d, J = 8.7 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.71 (dd, J = 8.6, 1.6 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 4.61 (s, 2H), 2.28 (t, J = 7.3 Hz, 2H), 1.68–1.55 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.47 (s), 171.76 (s), 166.25 (s), 159.03 (s), 146.51 (s), 143.02 (s), 135.87 (s), 133.85 (s, 2C), 129.01 (s), 119.99 (s, 2C), 119.38 (s), 117.79 (s), 115.60 (s), 111.76 (s), 106.05 (s), 43.76 (s), 38.65 (s), 18.96 (s), 14.07 (s); MS (ESI): calcd for $C_{21}H_{18}N_4O_4$ $[M-H]^-$ 389.13, found 389.30; HPLC: t_R 3.146 min, purity 95.2%.

*4.1.15.3. 2-(5-Acetamido-2,3-dioxoindolin-1-yl)-N-(4-cyanophenyl) acetamide (**D3**)*. Red solid; yield 73%; mp: 282–285 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 10.10 (s, 1H), 7.91 (d, J = 2.0 Hz, 1H), 7.80 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 8.8 Hz, 2H), 7.69 (dd, J = 8.5, 2.1 Hz, 1H), 7.12 (d, J = 8.5 Hz, 1H), 4.60 (s, 2H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.46 (s), 168.91 (s), 166.26 (s), 159.02 (s), 146.53 (s), 143.01 (s), 135.87 (s), 133.85 (s, 2C), 128.94 (s), 120.00 (s, 2C), 119.38 (s), 117.80 (s), 115.53 (s), 111.78 (s), 106.06 (s), 43.78 (s), 24.32 (s); MS (ESI): calcd for $C_{19}H_{14}N_4O_4$ $[M-H]^-$ 361.10, found 361.22; HPLC: t_R 2.790 min, purity 98.1%.

*4.1.15.4. N-(1-(2-((3-Cyanophenyl)amino)-2-oxoethyl)-2,3-dioxoindolin-5-yl)butyramide (**D4**)*. Red solid; yield 77%; mp: 243–245 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 10.04 (s, 1H), 8.03 (s, 1H), 7.94 (d, J = 2.0 Hz, 1H), 7.83–7.76 (m, 1H), 7.71 (dd, J = 8.5, 2.0 Hz, 1H), 7.56 (dd, J = 3.6, 2.0 Hz, 2H), 7.12 (d, J = 8.5 Hz, 1H), 4.58 (s, 2H), 2.28 (t, J = 7.3 Hz, 2H), 1.70–1.54 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.48 (s), 171.75 (s), 166.11 (s), 159.05 (s), 146.46 (s), 139.57 (s), 135.87 (s), 130.85 (s), 128.98 (s), 127.88 (s), 124.61 (s), 122.75 (s), 118.99 (s), 117.85 (s), 115.59 (s), 112.13 (s), 111.75 (s), 43.69 (s), 38.66 (s), 18.96 (s), 14.07 (s); MS (ESI): calcd for $C_{21}H_{18}N_4O_4$ $[M-H]^-$ 389.13, found 389.30; HPLC: t_R 3.170 min, purity 98.0%.

4.2. Cell proliferation activity

The cell proliferation assay was evaluated on a panel of MCL cell lines using the CellTiter-Glo Luminescent cell viability assay kit (Promega) following the manufacturer's protocol. MCL cell lines were purchased from American Type Culture Collection (ATCC, USA) and cultured in RPMI-1640 (Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco), 10,000 units/mL penicillin, and 10 mg/mL streptomycin at 37 °C in a humidified incubator with 5% CO_2. In short, the cells were plated in 96-well plates at a density of 10,000 cells/well. After

attachment of the cells to the bottom, compounds to be tested were dissolved to concentrations of 0.93 μM to 60 μM in DMSO and then added to the plates with no more than a 1‰ final percentage of DMSO. The treatment lasted for 72 h, and then CellTiter-Glo (30 μL) was added. The absorbance at 570 nm was measured using an ELX800 Microplate Reader (BioTek, USA) three times for each plate, and IC_{50} values were calculated by Prism 6.0 Software (GraphPad Software, USA).

4.3. Cell apoptosis assay

Apoptosis was quantified by the Annexin V/Propidium Iodide (PI)-binding assay. Cells were seeded in 6-well plates at a density of 200,000 cells/well, with 0.5 μM, 1 μM, 2.5 μM and 5 μM of **K5** for 24 h. The cells were then incubated in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h. Treated cells were washed twice with cold phosphate buffered saline (PBS) and then resuspended in 100 μL of binding buffer, to which 2 μL of Annexin V-FITC and 2 μL of PI were added. The samples were gently vortexed and incubated for 15 min at room temperature in the dark. After addition of 200 μL of binding buffer, samples were immediately analyzed by flow cytometry using a Novocyte Flow Cytometer (ACEA Biosciences, USA). The number of apoptotic cells was determined using Flowjo software.

4.4. Cell cycle assay

Cell cycle arrest was measured using PI staining by flow cytometry. Cells were seeded in 6-well plates at a density of 200,000 cells/well, with 0.5 μM and 1 μM of **K5** for 24 h. Then, cells were harvested, washed twice with cold PBS, and subsequently fixed in cold 70% ethanol overnight at 4 °C. Samples were washed twice with PBS, followed by further treatment with 50 mL of 100 μg/mL ribonuclease and 100 μL of 100 μg/mL PI, and finally analyzed by flow cytometry. The result was determined using the NovoExpress software.

Acknowledgement

This work was supported by the key research and development project of Shandong Province (No. 2017CXGC1401).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2019.06.009.

References

1. Cheah CY, Seymour JF, Wang ML. Mantle cell lymphoma. *J Clin Oncol.* 2016;34:1256–1269.
2. Bosch F, Lopez-Guillermo A, Campo E, et al. Mantle cell lymphoma - presenting features, response to therapy and prognostic factors. *Cancer.* 1998;82:567–575.
3. Jares P, Colomer D, Campo E. Genetic and molecular pathogenesis of mantle cell lymphoma: perspectives for new targeted therapeutics. *Nat Rev Cancer.* 2007;7:750–762.
4. Jares P, Colomer D, Campo E. Molecular pathogenesis of mantle cell lymphoma. *J Clin Invest.* 2012;122:3416–3423.
5. Royo C, Salaverria I, Hartmann EM, Rosenwald A, Campo E, Bea S. The complex landscape of genetic alterations in mantle cell lymphoma. *Semin Cancer Biol.* 2011;21:322–334.
6. Lei M, Feng HY, Wang C, et al. 3D-QSAR-aided design, synthesis, in vitro and in vivo evaluation of dipeptidyl boronic acid proteasome inhibitors and mechanism studies. *Bioorg Med Chem.* 2016;24:2576–2588.
7. Wang CY, Li S, Meng Q, et al. Novel amino acid-substituted diphenylpyrimidine derivatives as potent BTK inhibitors against B cell lymphoma cell lines. *Bioorg Med Chem.* 2018;26:4179–4186.
8. Kahl BS, Spurgeon SE, Furman RR, et al. A phase 1 study of the PI3K delta inhibitor idelalisib in patients with relapsed/refractory mantle cell lymphoma (MCL). *Blood.* 2014;123:3398–3405.
9. Papadopoulos KP, Egile C, Ruiz-Soto R, et al. Efficacy, safety, pharmacokinetics and pharmacodynamics of SAR245409 (voxtalisib, XL765), an orally administered phosphoinositide 3-kinase/mammalian target of rapamycin inhibitor: a phase 1 expansion cohort in patients with relapsed or refractory lymphoma. *Leuk Lymphoma.* 2015;56:1763–1770.
10. Seftel MD, Kuruvilla J, Kouroukis T, et al. The CDK inhibitor AT7519M in patients with relapsed or refractory chronic lymphocytic leukemia (CLL) and mantle cell lymphoma. A phase II study of the Canadian Cancer Trials Group. *Leuk Lymphoma.* 2017;58:1358–1365.
11. Cheng FD, Xia B, Sahakian E, et al. Selective inhibition of histone deacetylase 6 (HDAC6) and HDAC3 as a novel therapeutic strategy in mantle cell lymphoma (MCL). *Blood.* 2014;124:5397.
12. Goy A, Hernandez-Ilzaliturri FJ, Kahl B, Ford P, Protomastro E, Berger M. A phase I/II study of the pan Bcl-2 inhibitor obatoclax mesylate plus bortezomib for relapsed or refractory mantle cell lymphoma. *Leuk Lymphoma.* 2014;55:2761–2768.
13. Gao S, Zang J, Gao QW, et al. Design, synthesis and anti-tumor activity study of novel histone deacetylase inhibitors containing isatin-based caps and *o*-phenylenediamine-based zinc binding groups. *Bioorg Med Chem.* 2017;25:2981–2994.
14. Nikalje AP, Ansari A, Bari S, Ugale V. Synthesis, biological activity, and docking study of novel isatin coupled thiazolidin-4-one derivatives as anticonvulsants. *Arch Pharm.* 2015;348:433–445.
15. Rohini R, Reddy PM, Shanker K, Hu A, Ravinder V. Antimicrobial study of newly synthesized 6-substituted indolo[1,2-c]quinazolines. *Eur J Med Chem.* 2010;45:1200–1205.
16. Gao F, Chen ZJ, Ma L, Qiu L, Lin JG, Lu GM. Benzofuran-isatin hybrids tethered via different length alkyl linkers and their in vitro anti-mycobacterial activities. *Bioorg Med Chem.* 2019;27:2652–2656.
17. Kumar K, Liu N, Yang D, et al. Synthesis and antiprotozoal activity of mono- and bis-uracil isatin conjugates against the human pathogen Trichomonas vaginalis. *Bioorg Med Chem.* 2015;23:5190–5197.
18. Devale TL, Parikh J, Miniyar P, Sharma P, Shrivastava B, Murumkar P. Dihydropyrimidinone-isatin hybrids as novel non-nucleoside HIV-1 reverse transcriptase inhibitors. *Bioorg Chem.* 2017;70:256–266.
19. Sharma PK, Balwani S, Mathur D, et al. Synthesis and anti-inflammatory activity evaluation of novel triazolyl-isatin hybrids. *J Enzym Inhib Med Ch.* 2016;31:1520–1526.
20. Lotfy G, Said MM, EI Ashry EI SH, et al. Synthesis of new spirooxindole-pyrrolothiazole derivatives: anti-cancer activity and molecular docking. *Bioorg Med Chem.* 2017;25:1514–1523.
21. Eldehna WM, Nocentini A, Al-Rashood ST, et al. Tumor-associated carbonic anhydrase isoform IX and XII inhibitory properties of certain isatin-bearing sulfonamides endowed with in vitro antitumor activity towards colon cancer. *Bioorg Chem.* 2018;81:425–432.
22. El-Naggar M, Eldehna WM, Almahli H, et al. Novel thiazolidinone/thiazolo[3,2-a]benzimidazolone-isatin conjugates as apoptotic anti-proliferative agents towards breast cancer: one-pot synthesis and in vitro biological evaluation. *Molecules.* 2018;23:1420.
23. Farooq M, Al Marhoon ZM, Abu Taha N, Baabbad AA, Al-Wadaan MA, El-Faham A. Synthesis of novel class of N-alkyl-isatin-3-iminobenzoic acid derivatives and their biological activity in zebrafish embryos and human cancer cell lines. *Biol Pharm Bull.* 2018;41:350–359.
24. Wang JB, Yun D, Yao JL, et al. Design, synthesis and QSAR study of novel isatin analogues inspired Michael acceptor as potential anticancer compounds. *Eur J Med Chem.* 2018;144:493–503.
25. Romagnoli R, Baraldi PG, Cruz-Lopez O, Preti D, Bermejo J, Estévez F. α-Bromoacrylamido N-substituted isatin derivatives as potent inducers of apoptosis in human myeloid leukemia cells. *ChemMedChem.* 2009;4:1668–1676.
26. Li PZ, Tan YM, Liu GY, et al. Synthesis and biological evaluation of novel indoline-2,3-dione derivatives as antitumor agents. *Drug Discov Ther.* 2014;8:110–116.
27. Cameron F, Sanford M. Ibrutinib: first global approval. *Drugs.* 2014;74:263–271.
28. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.* 2017;7:42717.
29. Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *Eur J Med Chem.* 2000;43:3714–3717.
30. Delaney JS. ESOL: estimating aqueous solubility directly from molecular structure. *J Chem Inf Comput Sci.* 2004;44:1000–1005.
31. Potts RO, Guy RH. Predicting skin permeability. *Pharm Res.* 1992;9:663–669.
32. Martin YC. A bioavailability score. *J Med Chem.* 2005;48:3164–3170.