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Total synthesis and bioactivity of the marine alkaloid pityriacitrin and some of its derivatives

Puyong Zhang [a,1], Xiaofei Sun [a,1], Bin Xu [b,c,1], Krikor Bijian [b,c], Shengbiao Wan [a], Guigen Li [d], Moulay Alaoui-Jamali [b,c,**], Tao Jiang [a,*]

[a] School of Medicine and Pharmacy, Key Laboratory of Marine Drugs, Ministry of Education, Ocean University of China, Qingdao, China
[b] Department of Medicine, Oncology and Pharmacology, Lady Davis Institute for Medical Research, McGill University, Montreal, Canada
[c] Department of Therapeutics, Lady Davis Institute for Medical Research, McGill University, Montreal, Canada
[d] Departments of Chemistry and Biochemistry, Texas Tech University, Lubbock, USA

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ABSTRACT

We report herein the chemical synthesis and biological evaluation of β-carboline alkaloid pityriacitrin and some of its new derivatives. Using tryptophan or 5-hydroxytryptophan and 5-substituted indole-3-glyoxals as the starting materials, pityriacitrin and some of its derivatives were synthesized via the acid-catalyzed Pictet–Spengler reaction and fully characterized by ^{1}H and ^{13}C NMR, mass spectroscopy and IR determinations. Biological studies revealed that pityriacitrin has a weak antiproliferative activity against a panel of breast and prostate cancer cell lines, whereas some of its derivatives exhibited stronger and potent activity, which was associated with induction of both cell apoptosis and necrosis.

1. Introduction

β-Carbolines belong to a large family of natural and synthetic indole alkaloids with different substitutions on their C-1, C-6 and C-7 positions, some of these compounds widely exist in many plants and mammals and exhibit a wide spectrum of biological activities [1–3]. Due to their unique rigid heterocyclic skeleton, many β-carbolines were able to intercalate into DNA, to inhibit cyclin-dependent protein kinases (CDKs), topoisomerases and monoamine oxidase and to bind with high affinity to benzodiazepine receptors (BzR) and 5-hydroxy serotonin receptors [4–6]. Particularly, it has been reported that the CDK inhibitory activities of β-carbolines can be improved by the introduction of appropriate substitutions at the C-1 position [7]. Even though both tryptamine and indole acid derivatives were the rich secondary metabolites in marine sponges and marine bacteria, the C-1 substituted tryptamine-derived β-carboline alkaloids were also newly found in marine creatures, such as hyrtiosulawesine [8] from the Indonesian marine sponge *H. erectus* and eudistomin U [9] from the Caribbean ascidian *Lissoclinum fragile* (Fig. 1).

Pityriacitrin **15** was originally isolated from a marine bacterium of the genus *Paracoccus* in 1999 [10]. Later on, pityriacitrin and pityriacitrin B **23** (Fig. 1) were also isolated from the yeast *Malassezia furfur* [11,12] and proven to have the novel β-carboline core structure with an indole ring attached with a carbonyl group on C-1 position. These two natural compounds were reported as a potential UV filter because of their broad UV absorption (λ_{max} 389, 315, 289, 212 nm) [11,12], which were very rare in marine β-carboline alkaloids. However until now, their further biological activities and structure–activity relationship (SAR) studies have not been investigated. Therefore, chemical synthesis of these two alkaloids in applicable quantities is necessary to study their action modes and biological implications.

In the present paper, we would like to report the first chemical synthesis of the marine alkaloid pityriacitrin, pityriacitrin B **23** and twenty-three of their new analogs, as well as their biological

* Corresponding author. Tao Jiang, School of Medicine and Pharmacy, Ocean University of China, 5 Yushan Road, Qingdao 266003, China.
** Corresponding author. Department of Medicine, Oncology and Pharmacology, Lady Davis Institute for Medical Research, McGill University, Montreal, Canada.
E-mail addresses: moulay.alaoui-jamali@mcgill.ca (M. Alaoui-Jamali), jiangtao@ouc.edu.cn (T. Jiang).
[1] These authors contributed equally to this study.

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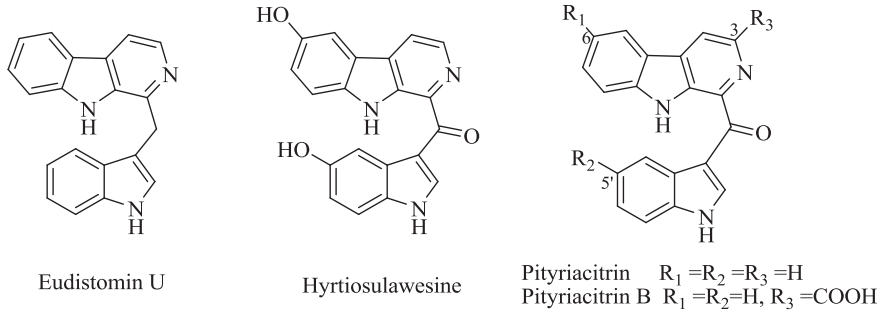


Fig. 1. The structure of the natural alkaloids.

activities on cancer cells. This paper is focused on elucidating the preliminary structure–activity relationships through the compounds **15**–**25** and their biological activities against MDA-231, MCF-7 and PC3 cells, and subsequently, inspired by lead structural information to design and synthesize their derivatives **30**–**42** as outlined in Scheme 3. Pleasantly, it has been found that these newly synthesized compounds showed greater *in vitro* inhibitory activity than previous compounds **15**–**25**.

2. Results and discussion

2.1. Chemistry

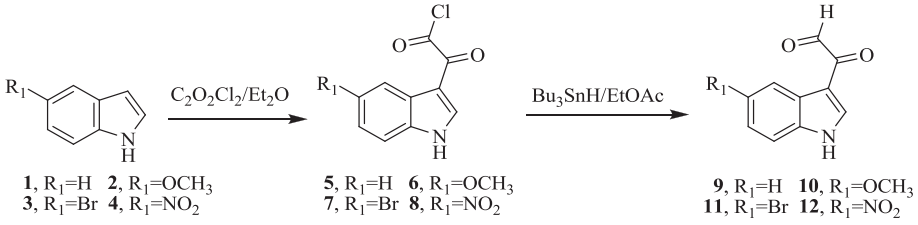
Amongst various methods available for the synthesis of β-carboline alkaloids, the most extensively used methods were the Pictet–Spengler and Bischler–Napieralski condensations. In nearly one century the Pictet–Spengler reaction has remained as one of the most efficient methods for the formation of the ring system via C–C bond formation using tryptophan as the starting material [13,14]. Although several groups have systematically studied the mechanistic aspects of the Pictet–Spengler reaction [13–15], this method still needs further understanding and modifications by finding new reaction conditions so as to achieve high synthetic efficiency. The classical method to prepare β-carboline alkaloids through Pictet–Spengler reaction was a two-step method, and involved the acid-catalyzed condensation of an aliphatic amine (attached to a sufficiently reactive aromatic nucleus) with aldehydes. Specifically, an imine was formed at the first step, which can be activated by acids. At the second step, endo cyclization occurred between a carbon nucleophile of a sufficiently reactive aromatic moiety and the activated iminium ion, resulting in N-heterocyclic ring formation through a new C–C bond, that led to tetrahydro-β-carboline. Subsequent hydrogenation converted tetrahydro-β-carboline to β-carboline [13–17]. Very interestingly, in our synthesis, the treatment of L-tryptophan with indole-3-glyoxal **9** [18–20] under the acidic conditions did not produce the expected tetrahydro-β-carboline. Surprisingly, the reaction directly resulted in a dehydrogenated β-carboline product pityriacitrin **15**. This new synthesis is outlined in Scheme 2.

The synthesis was started by reacting the commercially available indole and oxalyl chloride in anhydrous ether at 0–5 °C for 1 h. Indole-3-glyoxyloyl chlorides **5**–**8** were prepared in excellent yields consistent with literature reports [21–23]. Indole-3-glyoxyloyl chloride **5** was then treated with tributyltin in anhydrous ethyl acetate at the room temperature for 2 h, indole-3-glyoxal **9** being formed in 67% yield. The compounds **10**–**12** were synthesized by using the similar procedure to give excellent yields (65–66%). The reaction routes are outlined in Scheme 1.

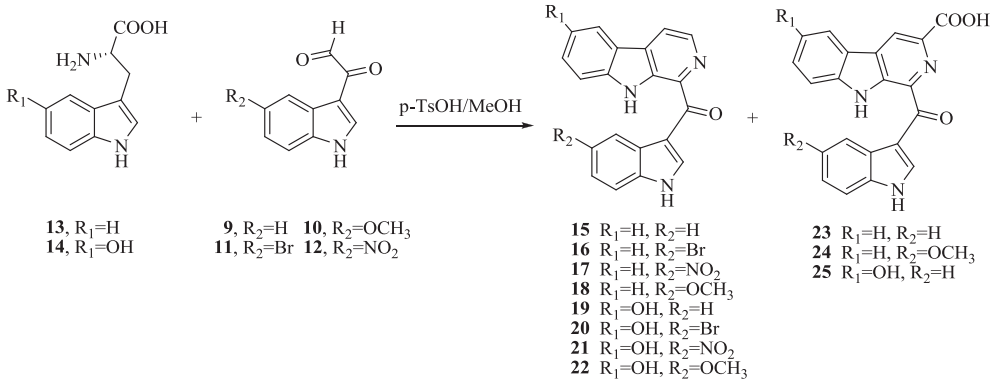
Subsequently, as shown in the Scheme 2, following the addition of indole-3-glyoxal **9** into the mixture of *p*-toluenesulfonic acid and L-tryptophan in methanol, the reaction mixture was then stirred at 50 °C for 2 h. The crude product was purified by column chromatography using a mixture of petroleum ether and acetone as the eluents to give the pityriacitrin **15** in 27% yield. Its spectral data were fully in agreement with the literature values [12] albeit the yield was lower than what we expected. Another compound, pityriacitrin B **23** was also synthesized (23% yield) and isolated by chromatography using eluents of greater polarity. Similarly, compounds **16**–**22** were prepared by using the similar procedure, and compounds **24** and **25** were also obtained from purification of the reaction mixture of compounds **18** and **19**, respectively.

As shown in Scheme 2, the modified one-pot oxidation reaction was more efficient and convenient in preparing 1-substituted β-carbolines without the need of using aromatization or decarboxylation reaction. Using this modified Pictet–Spengler reaction, we have successfully synthesized the β-carboline alkaloid pityriacitrin as well as a series of its derivatives.

The synthesis of the designed pityriacitrin derivatives with various amide substitutes on position 3 are displayed in Scheme 3. To begin the synthesis, the acid-catalyzed Pictet–Spengler cyclization of L-tryptophan **13** and **14** in the presence of indole-3-glyoxal **9** afforded the corresponding diastereoisomeric mixture 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid **26** and **27** with high yields by using 1% H_2SO_4 as the catalyst in H_2O at room temperature. In this synthesis, dehydrogenation and decarboxylation in Pictet–Spengler cyclization was not observed, which was attributed to the mild reaction conditions of lower temperature and lower acidity [7,24–27]. The final esterification of **26** and **27** with methanol in the presence of $SOCl_2$ gave corresponding methyl



Scheme 1. Synthesis of indol-3-yl-oxoacetaldehyde **9**–**12**.



Scheme 2. Synthesis of the Pityriacitrin and Its Derivatives **15**–**25**.

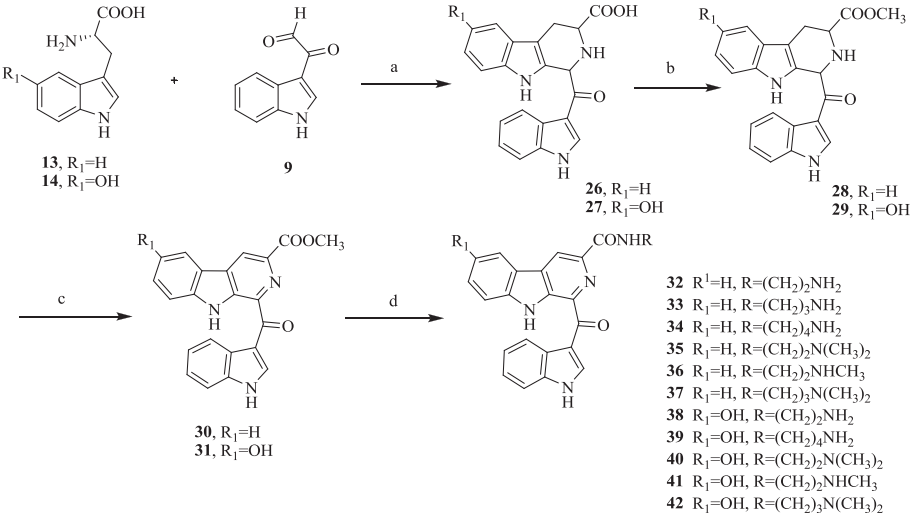
1,2,3,4-tetrahydro-β-carboline-3-carboxylate **28** and **29** with significantly higher yields (92–95%).

The key step in the synthetic sequence was the dehydrogenation of the esters **28** and **29**. As discussed earlier, several methods are available for the dehydrogenation of a 1,2,3,4-tetrahydro-β-carboline to the β-carboline skeleton, the most common ones are to use Pd/C [28], elemental sulfur [29], DDQ [30], and $Pb(OAc)_4$ [24] for this step. The dehydrogenation with DDQ in refluxing THF, with Pd/C at 200 °C or with $Pb(OAc)_4$ in glacial acetic acid gave poor yields of 10–15%. Nevertheless, the best method proved to be the dehydrogenation with sulfur in refluxing xylene. Using this method, 1-substituted β-carboline-3-carboxylate **30** and **31** were obtained with good yields of 71% and 70%, respectively. In the last step, to avoid dimerization of carboline, a large excess of alkyl diamine and milder reaction temperature was employed. This strategy seems to have worked since the amidation [31] of compound **30** and **31** with alkyl diamine afforded desired compounds **32**–**42** with high yields (80–98%) as shown in Scheme 3.

2.2. Biological evaluation

The *in vitro* inhibitory activity of compounds **15**–**25** was evaluated against the breast carcinoma cell lines MCF-7 and MDA-231, as well as the prostate carcinoma cell line PC_3, using the 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic assay. After 18 h cells were continuously treated with compounds **15**–**25**. Following this, after 96 h, cell survival was evaluated. The determined inhibitory activity (IC_{50}) of the various compounds on cell proliferation is reported in Table 1. The structure–activity relationship of relative β-carboline compounds was studied. Surprisingly, pityriacitrin **15** was found to have a poor antiproliferative activity. However, some of its derivatives exhibited a potent activity against all the three cell lines tested. In particular, 5′-methoxy substituted compound **18** and 6-hydroxy substituted compound **19** were the most potent, and the 3-carboxy substituted compound **23** and 5′-methoxy and 3-carboxy substituted compound **24** also exhibited a high antiproliferative activity which suggests that the conserved carboxyl group on the position 3 of β-carboline is critical to the antiproliferative activity. In contrast, 5′-bromo substituted compound **16** and 5′-nitro substituted compound **17**, as well as 6-hydroxy, 5′-methoxy, 5′-bromo and 5′-nitro double substituted compounds **20**, **21**, **22**, respectively, exhibited a weak antiproliferative activity. Therefore, positions 3 and 6 of β-carboline should be the most sensitive positions for antiproliferative activity and will be further structure modified. Thus the derivative structures of **30**–**42** were designed and synthesized (Scheme 3).

Although the compounds **30**–**42** were tested only against one cell line, the results of antiproliferative activity tests *in vitro* showed that all of them had stronger cytotoxic activities against



Scheme 3. Synthesis of the Pityriacitrin Amide Derivatives **32**–**42**. Reagent and conditions: (a) H_2SO_4/H_2O(1/100, v/v), room temperature; (b) $SOCl_2$/methanol, room temperature; (c) S/Xylene, reflux; (d) alkyl diamine, room temperature.

Table 1
Cytotoxicity of β-carboline derivatives **15**–**25** *in vitro*[a] (μM).

Compd.	R_1	R_3	R_2	IC_{50} (μM)[b]		
				MCF-7[c]	MDA-231[c]	PC3[c]
15	H	H	H	>100	>100	55.11
16	H	H	Br	60.85	>100	>100
17	H	H	NO_2	>100	>100	38.84
18	H	H	OMe	6.94	18.82	49.89
19	OH	H	H	12.94	6.35	16.37
20	OH	H	Br	>100	>100	>100
21	OH	H	NO_2	>100	>100	>100
22	OH	H	OMe	>100	44.54	>100
23	H	COOH	H	23.45	24.78	16.9
24	H	COOH	OMe	3.4	12.09	32.3
25	OH	COOH	H	79	42	57.93

[a] Data represent the mean values of three independent determinations.
[b] Cytotoxicity as IC_{50} for each cell line, is the concentration of compound that causes a 50% growth inhibition to untreated cells using the MTT assay.
[c] Cell lines include breast carcinoma cell lines MCF-7 and MDA-231, prostate carcinoma cell line PC3.

Table 2
Cytotoxicity of β-carboline derivatives **30**–**42** *in vitro*[a] (μM).

Compd.	R_1	R_3	IC_{50} (μM)[b]MDA-231[c]
30	H	$COOCH_3$	12.6
31	OH	$COOCH_3$	3.16
32	H	$CONH(CH_2)_2NH_2$	12.6
33	H	$CONH(CH_2)_3NH_2$	2.48
34	H	$CONH(CH_2)_4NH_2$	3.04
35	H	$CONH(CH_2)_2N(CH_3)_2$	3.56
36	H	$CONH(CH_2)_2NHCH_3$	3.98
37	H	$CONH(CH_2)_3N(CH_3)_2$	6.02
38	OH	$CONH(CH_2)_2NH_2$	15.8
39	OH	$CONH(CH_2)_4NH_2$	8.7
40	OH	$CONH(CH_2)_2N(CH_3)_2$	1.33
41	OH	$CONH(CH_2)_2NHCH_3$	4.36
42	OH	$CONH(CH_2)_3N(CH_3)_2$	2.51

[a] Data represent the mean values of three independent determinations.
[b] Cytotoxicity as IC_{50} for MDA-231, is the concentration of compound that causes a 50% growth inhibition to untreated cells using the MTT assay.
[c] Breast carcinoma cell line MDA-231.

MDA-231 than compounds 15–25 (Table 2 and Table 1). Among them, compounds 30–42 represented a subseries of compounds bearing an ester or amide group on the 3-position of β-carboline core. First, ester compound 31 with a 6-hydroxy group displays high inhibition with an IC_{50} of 3.16 compared with ester compound 30. Even less potency was observed on the amide compounds 32 and 38 (IC_{50} was 12.6 and 15.8 respectively). It was intriguing, however, that amides without 6-hydroxy group of β-carboline, such as 33, 34, 35, 36, and the amides with 6-hydroxy group, such as 40, 41, and 42 displayed higher antiproliferative activity against MDA-231 with the IC50 of 1.33–4.36μM. These results indicated that the methyl carboxylate side chain or carboxamide side chains at the 3-position of β-carboline is beneficial to inhibitory activity on tumor cell line and might be due to the hydrogen bonding between the amino groups, as well as the methoxy groups at the end of the side chain and DNA bases target [31,32]. Protecting the amino group with methyl in the 3-position amide fragment led to compound 35, 36 (compared with 32) and compounds 40, 41 (compared with 38) possessing an enhanced potency against MDA-231 cell line. These results indicated that the polarity of molecular is of significant importance to the tumor cell inhibition potency.

Apoptosis assay using the MDA-231 cell line revealed that compounds **19** and **23** were able to induce apoptosis in a dose-dependent manner, and the activity was higher at the concentration of 50 μM level. Paclitaxel was used as a positive control (Table 3 and Fig. 2). As compared with 3-carboxy substituted compound **23**, 6-hydroxy substituted compound **19** exhibited a higher apoptosis activity in comparison with the control, i.e., 50 μM induced 41% apoptotic activity. Besides apoptosis, these active molecules were also able to induce necrosis but to a lesser extent than apoptosis (not shown). These results suggest that the antiproliferative activity of these pityriacitrin-derived molecules occurs, at least in part, via modulation of the apoptotic pathway.

The inhibitory activity of compound **19** was tested against various kinases *in vitro*. Briefly, human recombinant full-length kinases were incubated in kinase buffer containing ATP and substrate (Poly Glu: Tyr) for 4 h at room temperature with or without the presence of compound **19** at 10 μM final concentrations. Remaining ATP in solution was then quantified utilizing the Kinase-Glo-luminescence kit (Promega). The ability of compound **19** to inhibit the kinase activity of these kinases was evaluated (Table 4). As such, the antiproliferative and pro-apoptotic activity of compound **19** do not seem to be modulated by any of the kinases tested.

3. Conclusion

In summary, we have developed the synthesis of the natural β-carboline alkaloid pityriacitrin and its novel derivatives using the modified Pictet–Spengler reaction. Our preliminary structure–activity relationship (SAR) studies suggested that the electron-donating substitutions (OH, OCH_3) on the position 5′ or 6 as well as the carboxyl group on the position 3 of pityriacitrin were able to confer biological activity to the pityriacitrin molecule, whereas the double substitutions at the position 5′ and 6 were not able to induce the bioactivity. We conclude that the active pityriacitrin derivatives are promising bioactive compounds deserving further biological and molecular studies.

4. Experimental Section

4.1. Materials and methods

General. The starting materials and reagents, purchased from commercial suppliers, were used without further purification. All reactions were monitored by thin-layer chromatography (TLC), on aluminium sheets (Silica gel 60-F_{254}, E. Merck). Compounds were visualized by UV light. Column chromatography was carried out using silica gel (200–300 mesh). All reaction solvents were dried prior to use according to standard procedures. All primary reagents were commercially available. Silica gel chromatography solvents were of analytical grade. Melting points were recorded on a micro melting point apparatus MP-500D and were uncorrected. IR spectra were recorded using KBr pellets for solid samples on a Nicolet Nexus 470 FT-IR. NMR spectra were recorded on a Jeol JNM-ECP spectrometer at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR with TMS as the internal standard. Chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), brs (broad singlet), etc. Mass spectra were recorded using a Q-TOF Ultima™ Global by chemical ionization.

4.2. General procedure for compounds (*9*–*12*)

To a stirred suspension mixture of appropriated indole-3-glyoxyloyl chloride **5**–**8** (20 mmol) in anhydrous ethyl acetate (30 mL) at 0–5 °C under N_2, was added dropwise a solution of trialkyltin hydride (20 mmol) in anhydrous ethyl acetate (20 mL). After 10 min, the cooling bath was removed, and the mixture was stirred at room temperature for 2 h. The resulting suspension

Table 3
Apoptosis induction by the active pityriacitrin derivatives **19** and **23** using the MDA-231 cell line.

Control	concentration	Apoptosis,%
Taxol	10 nM	11.93
	50 nM	17.23
	10 μM	3.95
19	20 μM	11.16
	50 μM	41.00
	10 μM	2.86
23	20 μM	4.89
	50 μM	17.68

mixture was diluted with 80 mL of hexane, and the solid material **9**–**12** was collected by filtration and washed well with hexane.

*4.2.1. Indole-3-glyoxal (**9**)*

Yield, 67%; pale yellow powder; TOF MS ES-: *m*/*z* 172.0 $[M-H]^-$. ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 8.26 (s, 1H), 7.45 (dd, $J = 1.4$, 6.8 Hz, 1H), 7.24–7.21 (m, 2H), 5.4 (s, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 190.5, 136.7, 135.4, 126.2, 123.2, 122.1, 121.5, 112.7, 111.6, 95.9.

Table 4
Inhibitory kinase activity of compound **19** [10 μM] against various kinases.

Kinase	Aurora B	EGFR	MEK1	FAK	Src
% Inhibition	10	9	11	11	8

*4.2.2. 5-Methoxy-indole-3-glyoxal (**10**)*

Yield, 66%; deep yellow powder; TOF MS ES-: *m*/*z* 202.1 $[M-H]^-$. ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 8.20 (s, 1H), 7.79 (d, $J = 2.3$ Hz, 1H), 7.33 (d, $J = 8.7$ Hz, 1H), 6.87 (dd, $J = 2.3$, 8.7 Hz, 1H), 5.39 (s, 3H), 3.83 (s, 3 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 190.5, 156.5, 135.5, 131.5, 127.1, 113.2, 112.3, 103.2, 95.8, 54.7.

*4.2.3. 5-Bromo-indole-3-glyoxal (**11**)*

Compound **11** was obtained as yellow powder in 65% yield; TOF MS ES-: *m*/*z* 250.1 $[M-H]^-$.

*4.2.4. 5-Nitro-indole-3-glyoxal (**12**)*

Compound **12** was obtained as pale yellow powder in 65% yield; TOF MS ES-: *m*/*z* 217.0 $[M-H]^-$.

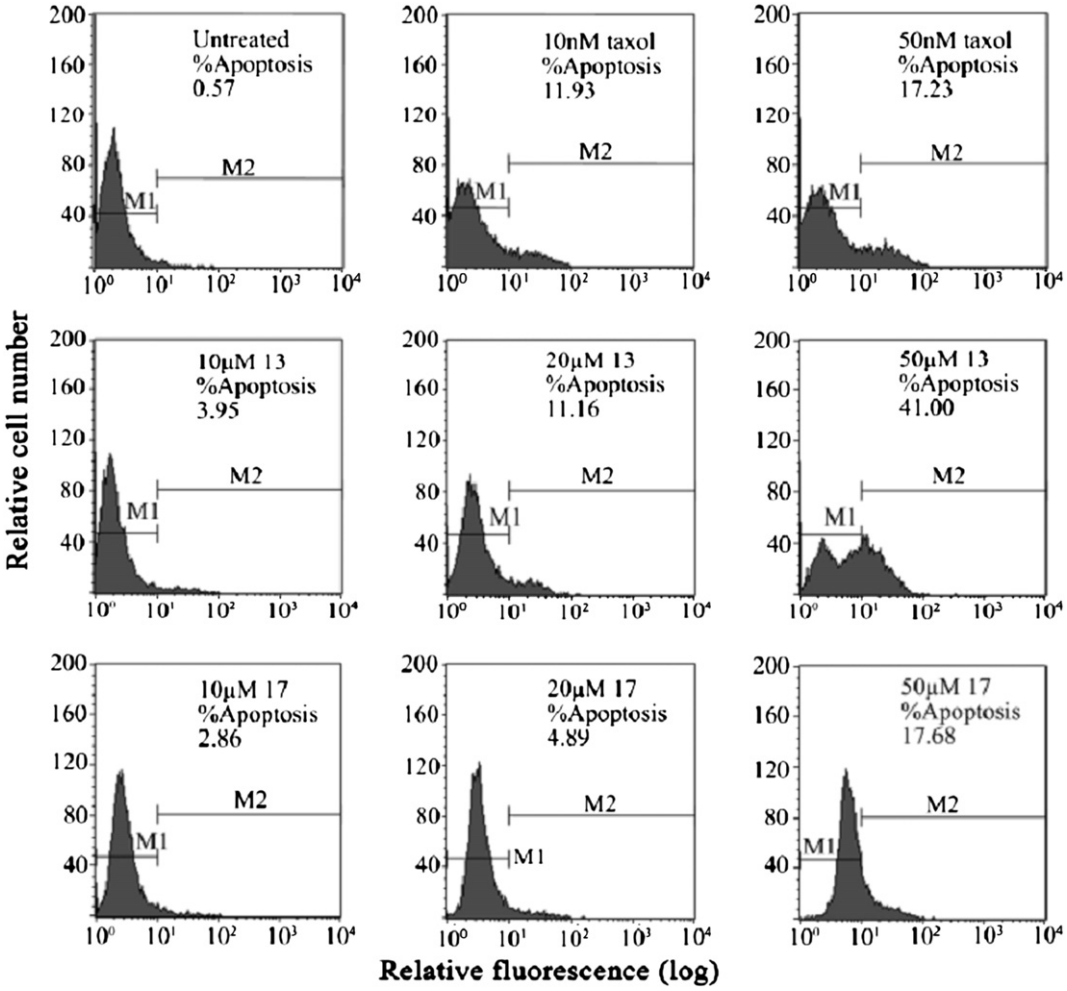


Fig. 2. Effect of compounds **19** and **23** on the induction of apoptosis in MDA-231 cell line using 7-AAD/Annexin V assay. Taxol (Paclitaxel) is used as a positive control. At least 10000 events were examined for each condition.

4.3. General procedure for compounds (**15**–**25**)

To a stirred solution of L-tryptophan **13** or 5-Hydroxy-L-tryptophan **14** (2.6 mmol) and *p*-toluenesulfonic acid (2.6 mmol) in absolute methanol (30 mL), was added appropriated indole-3-glyoxal **9**–**12** (2 mmol). The resulting solution was stirred at 50 °C for 2 h. TLC showed that the reaction was completed. The reaction mixture was poured into water and the precipitate was filtered and purified by flash chromatography on silica gel (PE/acetone) to afford the desired compounds **15**–**25**.

4.3.1. (1H-indol-3-yl)-(9H-pyrido[3,4-b]indol-1-yl)-met-hanone (**15**)

Yield, 27%; pale yellow powder; mp 229–231 °C; ^{1}H NMR (CD_3CN, 600 MHz) δ(ppm): 10.98 (brs, 1H), 10.15 (brs, 1H), 9.40 (d, J = 3.3 Hz, 1H), 8.60–8.59 (m, 1H), 8.58 (d, J = 4.7 Hz, 1H), 8.27 (dd, J = 0.8 Hz and 4.7 Hz, 1H), 8.26 (dd, J = 0.8 Hz and 7.7 Hz, 1H), 7.77 (d, J = 8.5 Hz, 1H), 7.63–7.62 (m, 1H), 7.56–7.55 (m, 1H), 7.33–7.32 (m, 3H); ^{13}C NMR (CD_3CN, 151 MHz) δ(ppm): 189.4, 142.4, 139.5, 138.7, 138.4, 137.1, 136.9, 132.1, 129.9, 128.5, 124.2, 123.3, 123.1, 121.7, 121.3, 118.9, 118.3, 115.8, 113.4, 112.9; IR (KBr, cm^{-1}): 3412, 3225, 1597, 1555, 1443, 1232, 1137, 736; HRMS: m/z calcd. for $C_{20}H_{14}N_3O^+$, 312.1137; found: 312.1132.

4.3.2. (5-Bromo-1H-indol-3-yl)-(9H-pyrido[3,4-b]indol-1-yl)-methanone (**16**)

Yield, 27%; pale yellow powder; mp 266–268 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.33 (s, 1H), 12.06 (s, 1H), 9.36 (d, J = 2.7 Hz, 1H), 8.78 (d, J = 1.9 Hz, 1H), 8.60 (d, J = 5.0 Hz, 1H), 8.44 (d, J = 4.6 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 7.87 (d, J = 8.2 Hz, 1H), 7.63–7.62 (m, 1H), 7.59 (d, J = 8.2 Hz, 1H), 7.45–7.44 (m, 1H), 7.33–7.32 (m,1H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.2, 141.6, 138.7, 137.9, 137.1, 135.1, 134.7, 130.9, 129.1, 128.8, 125.4, 123.9, 121.7, 120.0, 119.9, 118.2, 114.9, 114.4, 113.7, 113.0; IR (KBr, cm^{-1}): 3408, 3152, 1603, 1513, 1431, 1213, 1136, 743, 718, 669; HRMS: m/z calcd. for $C_{20}H_{13}N_3OBr^+$, 390.0242; found: 390.0252.

4.3.3. (5-Nitro-1H-indol-3-yl)-(9H-pyrido[3,4-b]indol-1-yl)-methanone (**17**)

Yield, 25%; pale yellow powder; mp 324–326 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.72 (brs, 1H), 12.15 (s, 1H), 9.53–9.52 (m, 2H), 8.62 (d, J = 5.0 Hz, 1H), 8.48 (d, J = 4.6 Hz, 1H), 8.35 (d, J = 8.7 Hz, 1H), 8.20 (dd, J = 2.3 Hz and 8.7 Hz, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.79 (d, J = 8.7 Hz, 1H), 7.63–7.62 (m, 1H), 7.34–7.33 (m, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.2, 142.9, 141.8, 140.8, 139.2, 137.4, 137.1, 135.2, 131.1, 128.9, 126.7, 121.8, 120.1, 120.0, 118.6, 118.3, 118.2, 115.5, 113.1, 113.1; IR (KBr, cm^{-1}): 3416, 3152, 1606, 1523, 1456, 1424, 1333, 1147, 739; HRMS: m/z calcd. for $C_{20}H_{13}N_4O_3^+$, 357.0988; found: 357.0978.

4.3.4. (5-Methoxy-1H-indol-3-yl)-(9H-pyrido[3,4-b]indol-1-yl)-methanone (**18**)

Yield, 27%; pale yellow powder; mp 214–216 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.04 (s, 2H), 9.25 (d, J = 3.3 Hz, 1H), 8.57 (d, J = 4.9 Hz, 1H), 8.41 (d, J = 4.9 Hz, 1H), 8.32 (d, J = 7.7 Hz, 1H), 8.12 (d, J = 2.2 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.61 (t, J = 7.7 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 7.31–7.30 (m, 1H), 6.92–6.91 (m, 1H), 3.86 (s, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.2, 155.6, 141.6, 138.5, 137.9, 136.9, 135.0, 130.7, 130.7, 128.6, 128.0, 121.6, 120.0, 119.8, 117.9, 114.1, 113.0, 113.0, 112.7, 103.3, 55.2; IR (KBr, cm^{-1}): 3412, 3157, 2925, 2854, 1602, 1511, 1474, 1212, 1140, 739; HRMS: m/z calcd. for $C_{21}H_{16}N_3O_2^+$, 342.1243; found: 342.1238.

4.3.5. (1H-indol-3-yl)-(6-hydroxy-9H-pyrido[3,4-b]indol-1-yl)-methanone (**19**)

Yield, 29%; deep yellow powder; mp 324–326 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.11 (s,1H), 11.73 (s, 1H), 9.26 (d, J = 2.7 Hz, 1H), 9.23 (s, 1H), 8.56–8.55 (m, 1H), 8.49 (d, J = 5.0 Hz, 1H), 8.30 (d, J = 4.6 Hz, 1H), 7.65 (d, J = 8.7 Hz, 1H), 7.59–7.58 (m, 2H), 7.29–7.28 (m, 2H), 7.12 (dd, J = 2.3 Hz and 8.7 Hz, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.4, 151.3, 138.2, 137.7, 136.1, 135.9, 135.6, 135.5, 130.5, 127.2, 122.8, 122.0, 121.6, 120.7, 118.6, 117.9, 114.2, 113.5, 112.3, 105.6; IR (KBr, cm^{-1}): 3421, 3269, 3236, 1609, 1564, 1429, 1230, 1125, 751; HRMS: m/z calcd. for $C_{20}H_{14}N_3O_2^+$, 328.1086; found: 328.1076.

4.3.6. (5-Bromo-1H-indol-3-yl)-(6-hydroxy-9H-pyrido[3,4-b] indol-1-yl)-methan- one (**20**)

Yield, 20%; orange powder; mp 313–315 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.28 (d, J = 1.6 Hz, 1H), 11.75 (s, 1H), 9.32 (d, J = 3.3 Hz, 1H), 9.24 (s, 1H), 8.74 (d, J = 1.6 Hz, 1H), 8.50 (d, J = 4.9 Hz, 1H), 8.32 (d, J = 4.9 Hz, 1H), 7.65 (d, J = 8.8 Hz, 1H), 7.59 (d, J = 2.2 Hz, 1H), 7.56 (d, J = 8.8 Hz, 1H), 7.43–7.42 (m, 1H), 7.13 (dd, J = 2.2 Hz and 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.3, 151.4, 138.6, 137.7, 136.2, 135.6, 135.6, 134.7, 130.6, 129.1, 125.3, 124.0, 120.7, 118.7, 118.3, 114.8, 114.3, 113.7, 113.5, 105.7; IR (KBr, cm^{-1}): 3438, 3303, 3152, 1604, 1511, 1424, 1230, 1130, 757, 721, 667; HRMS: m/z calcd. for $C_{20}H_{13}N_3O_2Br^+$, 406.0191; found: 406.0187.

4.3.7. (5-Nitro-1H-indol-3-yl)-(6-hydroxy-9H-pyrido[3, 4-b]indol-1-yl)-methanone (**21**)

Yield, 23%; deep yellow powder; mp 336–338 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.68 (brs, 1H), 11.87 (s, 1H), 9.53 (s, 1H), 9.52 (d, J = 2.5 Hz, 1H), 9.27 (s, 1H), 8.53 (d, J = 4.7 Hz, 1H), 8.36 (d, J = 5.1 Hz, 1H), 8.20 (dd, J = 2.2 Hz and 8.8 Hz, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.67 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 2.2 Hz, 1H), 7.14 (dd, J = 2.2 Hz and 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.2, 151.4, 142.8, 140.5, 139.0, 137.2, 136.3, 135.6, 135.6, 130.7, 126.6, 120.6, 118.7, 118.5, 118.2, 115.4, 113.5, 112.9, 105.7; IR (KBr, cm^{-1}): 3416, 3323, 3167, 1613, 1536, 1460, 1339, 1145, 738; HRMS: m/z calcd. for $C_{20}H_{13}N_4O_4^+$, 373.0937; found: 373.0936.

4.3.8. (5-Methoxy-1H-indol-3-yl)-(6-hydroxy-9H-pyrido [3,4-b] indol-1-yl)-meth- anone (**22**)

Yield, 21%; yellow powder; mp 277–279 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.00 (d, J = 2.6 Hz, 1H), 11.75 (s, 1H), 9.24 (d, J = 3.3 Hz, 1H), 9.22 (s, 1H), 8.48 (d, J = 5.1 Hz, 1H), 8.29 (d, J = 4.7 Hz, 1H), 8.11 (d, J = 2.6 Hz, 1H), 7.66 (d, J = 8.8 Hz, 1H), 7.58 (d, J = 2.2 Hz, 1H), 7.47 (d, J = 8.8 Hz, 1H), 7.11 (dd, J = 2.2 Hz and 8.8 Hz, 1H), 6.91–6.90 (m, 1H), 3.86–3.85 (m, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.3, 155.6, 151.3, 138.3, 137.8, 136.1, 135.6, 135.5, 130.7, 130.5, 128.1, 120.7, 118.6, 117.9, 114.2, 113.6, 112.9, 112.7, 105.6, 103.3, 55.2; IR (KBr, cm^{-1}): 3463, 3194, 2914, 2821, 1611, 1521, 1421, 1268, 1142, 771, 746; HRMS: m/z calcd. for $C_{21}H_{16}N_3O_3^+$, 358.1192; found: 358.1194.

4.3.9. 1-(1H-indol-3-ylcarbonyl)-9H-pyrido[3,4-b]indo- le-3-carboxylic acid (**23**)

Yield, 23%; pale yellow powder; mp 256–258 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 13.17 (brs, 1H), 12.42 (s, 1H), 12.28 (s, 1H), 9.76 (d, J = 3.2 Hz, 1H), 9.15 (s, 1H), 8.61–8.60 (m, 1H), 8.48 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 8.2 Hz, 1H), 7.66–7.65 (m, 1H), 7.58–7.57 (m, 1H), 7.38–7.37 (m, 1H), 7.32–7.30 (m, 2H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 186.4, 166.8, 142.1, 138.4, 137.1, 135.9, 135.8, 131.3, 129.1, 127.3, 123.0, 122.2, 122.1, 121.7, 120.7, 120.5, 119.9, 114.2, 113.4, 112.3; IR (KBr, cm^{-1}): 3410, 3269, 3241, 3213, 1704, 1599, 1498, 1429, 1241, 1163, 737; HRMS: m/z calcd. for $C_{21}H_{12}N_3O_3^-$, 354.0879; found: 354.0896.

4.3.10. 1-(5-Methoxy-1H-indol-3-ylcarbonyl)-9H-pyrido [3,4-b] indole-3-carboxyl- ic acid (**24**)

Yield, 26%; pale yellow powder; mp 218–220 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 13.14 (brs, 1H), 12.43 (s, 1H), 12.17 (d,

J = 2.7 Hz, 1H), 9.72 (d, J = 3.2 Hz, 1H), 9.14 (s, 1H), 8.47 (d, J = 8.2 Hz, 1H), 8.15 (d, J = 2.7 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.65–7.64 (m, 1H), 7.46 (d, J = 8.7 Hz, 1H), 7.37–7.36 (m, 1H), 6.93 (dd, J = 2.8 Hz and 8.7 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 186.2, 166.8, 155.7, 142.1, 138.5, 137.1, 135.9, 135.8, 131.3, 130.6, 129.1, 128.2, 122.1, 120.7, 120.4, 119.8, 114.1, 113.4, 112.9, 112.8, 103.4, 55.2; IR (KBr, cm^{-1}): 3460, 3258, 3118, 2916, 2848, 1708, 1592, 1491, 1426, 1213, 1123, 743; HRMS: m/z calcd. for $C_{22}H_{14}N_3O_4^-$, 384.0984; found: 384.0996.

4.3.11. 1-(1H-indol-3-ylcarbonyl)-6-hydroxy-9H-pyrido [3,4-b] indole-3-carboxylic acid (**25**)

Yield, 27%; deep yellow powder; mp 311–313 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 13.07 (brs, 1H), 12.28 (d, J = 2.3 Hz, 1H), 12.19 (s, 1H), 9.77 (d, J = 3.2 Hz, 1H), 9.41 (brs, 1H), 9.03 (s, 1H), 8.62–8.61 (m, 1H), 7.75–7.74 (m, 2H), 7.73 (s, 1H), 7.58–7.57 (m, 1H), 7.33–7.32 (m, 2H), 7.19 (dd, J = 2.3 Hz and 8.7 Hz, 1H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 186.5, 166.9, 152.0, 138.4136.9, 136.3, 136.0135.8, 134.9, 130.9, 127.3, 122.9, 122.2121.7121.3119.8, 119.1, 114.2, 114.0, 112.3, 105.9; IR (KBr, cm^{-1}): 3250, 1751, 1599, 1507, 1472, 1217, 1151, 731; HRMS: m/z calcd. for $C_{21}H_{12}N_3O_4^-$, 370.0828; found: 370.0839.

4.4. General procedure for compounds (**26** and **27**)

To a stirred solution of amino acid (100 mmol, L-tryptophan **13** for **26**, 5-Hydroxy-L-tryptophan **14** for **27**) in H_2SO_4 (2 mL) and H_2O (200 mL), was added indole-3-glyoxal **9** (100 mmol). The reaction mixture was stirred at room temperature for about 10 h and monitored by TLC. The precipitate was adjusted to pH 6–7 with concentrated ammonia liquor. The mixture was kept at 0 °C for 12 h and the formed precipitate was collected by filtration, washed well with water, dried in vacuum. The crude product was purified quickly by flash chromatography on silica gel (EtOAc/Methanol = 3/1, v/v) to afford the desired compound **26** and **27**.

4.4.1. 1-(1H-indole-3-carbonyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carb- oxylic acid (**26**)

Compound **26** was obtained as white solid in 81% yield; HRMS: m/z calcd. for $C_{21}H_{16}N_3O_3^-$, 358.1192; found: 358.1196.

4.4.2. 6-Hydroxy-1-(1H-indole-3-carbonyl)-2,3,4,9-tetr-ahydro-1H-pyrido[3,4-b] indole-3-carboxylic acid (**27**)

Compound **27** was obtained as white solid in 80% yield; HRMS: m/z calcd. for $C_{21}H_{16}N_3O_4^-$, 374.1141; found: 374.1156.

4.5. General procedure for compounds (**28** and **29**)

Freshly distilled $SOCl_2$ (14.6 mL, 200 mmol) was added dropwise to a stirred methanol (100 mL) cooled to below 0 °C under N_2. After the addition, the mixture was stirred at room temperature for 15 min, and then substituted 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (20 mmol, **26** and **27**) was added in portion. The reaction mixture was stirred at room temperature overnight and monitored by TLC. Quantity sufficient water was added to dilute the reaction mixture, and then the pH was adjusted to 9–10 by adding 10% Na_2CO_3 water solution. The resulting mixture was extracted with EtOAc (30 mL × 3). The combined organic phase was washed with water, brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified quickly by flash chromatography on silica gel (PE/EtOAc = 1/2, v/v) to afford the desired compound 1,2,3,4-tetrahydro-β-carboline-3-carboxylate (**28** and **29**).

4.5.1. Methyl 1-(1H-indole-3-carbonyl)-2,3,4,9-tetrahy-dro-1H-pyrido[3,4-b] indole-3-carboxy late (**28**)

Compound **28** was obtained as white solid in 95% yield; HRMS: m/z calcd. for $C_{22}H_{19}N_3O_3Na^+$, 396.1324; found: 396.1319.

4.5.2. Methyl 6-hydroxy-1-(1H-indole-3-carbonyl)-2,3,4,9-tetrahydro-1H-pyrido [3,4-b]indole -3-carboxylate (**29**)

Compound **29** was obtained as white solid in 92% yield; HRMS: m/z calcd. for $C_{22}H_{18}N_3O_4^-$, 388.1298; found: 388.1291.

4.6. General procedure for compounds (**30** and **31**)

To a stirred solution of the corresponding 1,2,3,4-tetrahydro-β-carboline-3-carboxylate (3 mmol, **28** and **29**) in xylene (50 mL), was added sulfur powder (0.2 g). The solution was heated at reflux for 6 h. After cooling to room temperature, some solvent was removed under reduced pressure and filtered through Celite to afford a yellow crude product. The crude product was purified by flash chromatography on silica gel (PE/EtOAc = 1/1, v/v) to afford the desired compound β-carboline-3-carboxylate (**30** and **31**).

4.6.1. Methyl 1-(1H-indole-3-carbonyl)-9H-pyrido[3,4-b]indole-3-carboxylate (**30**)

Yield, 71%; yellow powder; mp 251–252 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.25 (s, 1 H), 12.21 (s, 1 H), 9.66 (d, J = 3.2 Hz, 1 H), 9.39 (s, 1 H), 9.04 (s, 1 H), 8.59–8.58 (m, 1 H), 7.73–7.72 (m, 2 H), 7.58–7.57 (m, 1 H), 7.30–7.29 (m, 2 H), 7.16 (dd, J = 2.3, 9.2 Hz, 1 H), 4.02 (s, 3 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 186.9, 166.3, 152.6, 138.9, 137.7, 136.9, 136.5, 136.4, 134.4, 131.5, 127.8, 123.5, 122.7, 122.2, 121.8, 120.5, 119.8, 114.7, 114.6, 112.8, 106.6, 52.9; HRMS: m/z calcd. for $C_{22}H_{15}N_3O_3Na^+$, 392.1011; found: 392.1026.

4.6.2. Methyl 6-hydroxy-1-(1H-indole-3-carbonyl)-9H-pyrido[3,4-b]indole-3-carboxylate (**31**)

Yield, 70%; yellow powder; mp 267–268 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.45 (s, 1 H), 12.27 (s, 1 H), 9.67 (d, J = 3.2 Hz, 1 H), 9.14 (s, 1 H), 8.60–8.59 (m, 1 H), 8.46 (d, J = 7.8 Hz, 1 H), 7.90 (d, J = 7.8 Hz, 1 H), 7.63–7.61 (m, 1 H), 7.58–7.57 (m, 1 H), 7.35–7.34 (m, 1 H), 7.31–7.30 (m, 2 H), 4.02 (s, 3 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 186.8, 166.1, 142.6, 138.9, 137.8, 136.6, 136.4, 135.3, 131.8, 129.7, 127.8, 123.6, 122.8, 122.7, 122.2, 121.3, 121.0, 120.4, 114.7, 114.0, 112.9, 52.9; HRMS: m/z calcd. for $C_{22}H_{14}N_3O_4^-$, 384.0985; found: 384.0978.

4.7. General procedure for compounds (**32**–**42**)

A solution of compounds **30**, **31** (1 mmol) and alkyl diamine (2 mL) was stirred at room temperature under N_2 for 6 h and the completion of the reaction was monitored by TLC. The resulting reaction solution was concentrated under reduced pressure. Then the residue was purified by flash chromatography on silica gel (MeOH) to afford the desired compounds **32**–**42**.

4.7.1. N-(2-aminoethyl)-1-(1H-indole-3-carbonyl)-9H-pyrido[3,4-b]indole-3-carb- oxamide (**32**)

Yield, 98%; pale yellow powder; mp 258–259 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 9.08 (s, 1 H), 9.07 (s, 1 H), 8.66 (t, J = 5.9 Hz, 1 H), 8.55–8.54 (m, 1 H), 8.46 (d, J = 7.7 Hz, 1 H), 7.85 (d, J = 8.2 Hz, 1 H), 7.64–7.57 (m, 2 H), 7.35–7.30 (m, 3 H), 3.41 (quartet, J = 5.9 Hz, 2 H), 2.84 (t, J = 5.9 Hz, 2 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.4, 165.2, 142.7, 139.3, 138.7, 137.2, 136.7, 136.4, 132.1, 129.7, 127.6, 123.6, 122.7, 122.2, 121.1, 117.1, 114.4, 113.8, 112.0, 42.4, 41.6; HRMS: m/z calcd. for $C_{23}H_{20}N_5O_2^+$, 398.1617; found: 398.1615.

*4.7.2. N-(3-aminopropyl)-1-(1H-indole-3-carbonyl)-9H-pyrido [3,4-b]indole-3-carb- oxamide (**33**)*

Yield, 97%; pale yellow powder; mp 214–216 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 9.07 (s, 1 H), 9.02 (s, 1 H), 8.77 (t, J = 5.9 Hz, 1 H), 8.55–8.54 (m, 1 H), 8.45 (d, J = 7.8 Hz, 1 H), 7.85 (d, J = 8.3 Hz, 1 H), 7.64–7.58 (m, 1 H), 7.35–7.29 (m, 3 H), 3.52 (quartet, J = 5.9, 6.4 Hz, 2 H), 2.73 (t, J = 6.4 Hz, 2 H), 1.71 (quintet, J = 6.4 Hz, 2 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.5, 165.3, 142.6, 139.6, 138.7, 137.4, 136.8, 136.4, 132.0, 129.7, 127.6, 123.6, 122.7, 122.1, 121.0, 117.2, 114.5, 113.8, 112.9, 38.2, 33.0; HRMS: m/z calcd. for $C_{24}H_{22}N_5O_2^+$, 412.1774; found: 412.1785.

*4.7.3. N-(4-aminobutyl)-1-(1H-indole-3-carbonyl)-9H-pyrido[3,4-b]indole-3-carb-oxamide (**34**)*

Yield, 95%; pale yellow powder; mp 141–142 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 9.07 (s, 1 H), 9.03 (s, 1 H), 8.77 (t, J = 5.9 Hz, 1 H), 8.55–8.54 (m, 1 H), 8.45 (d, J = 7.8 Hz, 1 H), 7.85 (d, J = 8.2 Hz, 1 H), 7.64–7.59 (m, 2 H), 7.35–7.29 (m, 3 H), 3.52 (quartet, J = 5.9, 6.4 Hz, 2 H), 2.79 (t, J = 6.4 Hz, 2 H), 1.76 (quintet, J = 6.4 Hz, 2 H), 1.65 (m, 2 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.4, 165.4, 142.6, 139.5, 138.9, 137.4, 136.8, 136.4, 132.0, 129.6, 127.6, 123.6, 122.7, 122.1, 121.0, 117.2, 114.5, 113.9, 113.8, 113.6, 112.9, 58.9, 55.5, 37.8, 31.9; HRMS: m/z calcd. for $C_{25}H_{24}N_5O_2^+$, 426.1930; found: 426.1931.

*4.7.4. N-(2-(dimethylamino)ethyl)-1-(1H-indole-3-carbonyl)-9H-pyrido[3,4-b] indole-3-carboxamide (**35**)*

Yield, 85%; pale yellow powder; mp 125–127 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.27 (s, 1 H), 12.21 (brs, 1 H), 9.09 (s, 1 H), 8.91 (s, 1 H), 8.57–8.56 (m, 1 H), 8.49 (t, J = 5.5, 1 H), 8.45 (d, J = 8.2 Hz, 1 H), 7.86 (d, J = 8.2 Hz, 1 H), 7.63–7.62 (m, 2 H), 7.34–7.31 (m, 3 H), 3.52 (quartet, J = 5.5, 6.0 Hz, 2 H), 2.51 (t, J = 6.0 Hz, 2 H), 2.26 (s, 6 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.4, 165.1, 142.6, 139.0, 138.0, 137.3, 136.7, 136.4, 132.1, 129.5, 127.6, 123.7, 122.8, 122.2, 121.1, 117.1, 114.7, 113.7, 112.7, 58.4, 45.6, 37.3; HRMS: m/z calcd. for $C_{25}H_{24}N_5O_2^+$, 426.1930; found: 426.1935.

*4.7.5. 1-(1H-indole-3-carbonyl)-N-(2-(methylamino)ethyl)-9H-pyrido[3,4-b]indole -3-carboxamide (**36**)*

Yield, 82%; pale yellow powder; mp 131–132 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.27 (brs, 1 H), 9.08 (s, 1 H), 9.01 (s, 1 H), 8.61 (t, J = 5.5 Hz, 1 H), 8.54–8.53 (m, 1 H), 8.46 (d, J = 8.3, 1 H), 7.85 (d, J = 8.3 Hz, 1 H), 7.63–7.59 (m, 2 H), 7.35–7.29 (m, 3 H), 3.52 (quartet, J = 5.9 Hz, 2 H), 2.81 (t, J = 5.9 Hz, 2 H), 2.40 (s, 3 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.4, 165.3, 142.7, 139.2, 138.6, 137.3, 136.8, 136.4, 132.0, 129.6, 127.6, 123.7, 122.7, 122.2, 121.1, 117.1, 114.5, 113.8, 112.9, 50.6, 38.5, 35.8; HRMS: m/z calcd. for $C_{24}H_{22}N_5O_2^+$, 412.1774; found: 412.1774.

*4.7.6. N-(3-(dimethylamino)propyl)-1-(1H-indole-3-carbonyl)-9H-pyrido[3,4-b] indole-3-carboxamide (**37**)*

Yield, 81%; pale yellow powder; mp 134–135 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.32 (brs,1 H), 12.23 (s, 1 H), 9.07 (s, 1 H), 8.91 (s, 1 H), 8.69 (t, J = 5.9 Hz, 1 H), 8.53–8.52 (m, 1 H), 8.45 (d, J = 8.2 Hz, 1 H), 7.84 (d, J = 8.3 Hz, 1 H), 7.64–7.58 (m, 2 H), 7.33–7.30 (m, 3 H), 3.46 (quartet, J = 6.9 Hz, 2 H), 2.34 (t, J = 6.9 Hz, 2 H), 2.08 (s, 6 H), 1.75 (quintet, J = 6.9 Hz, 2 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.6, 165.3, 142.6, 139.5, 138.6, 137.5, 136.8, 136.3, 131.9, 129.6, 127.5, 123.7, 122.8, 122.7, 122.1, 121.1, 121.0, 117.1, 114.7, 113.7, 112.9, 57.7, 45.5, 38.4, 27.6; HRMS: m/z calcd. for $C_{26}H_{26}N_5O_2^+$, 440.2087; found: 440.2082.

*4.7.7. N-(2-aminoethyl)-6-hydroxy-1-(1H-indole-3-carbonyl)-9H-pyrido[3,4-b] indole-3-carboxamide (**38**)*

Yield, 91%; pale yellow powder; mp 265–267 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.02 (brs, 1 H), 9.08 (s, 1 H), 8.94 (s, 1 H), 8.66–8.65 (m, 1 H), 8.53–8.52 (m, 1 H), 7.71–7.67 (m, 2 H), 7.57–7.56 (m, 1 H), 7.29–7.28 (m, 2 H), 7.15 (dd, J = 2.3, 8.7 Hz, 1 H), 3.49 (quartet, J = 5.9 Hz, 2 H), 2.91 (t, J = 5.9 Hz, 2 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.5, 165.5, 152.5, 138.9, 138.4, 137.1, 136.8, 136.6, 131.7, 127.6, 123.5, 122.7, 122.1, 121.8, 119.7, 117.1, 114.4, 112.9, 106.5, 41.1, 40.9; HRMS: m/z calcd. for $C_{23}H_{18}N_5O_3^-$, 412.1410; found: 412.1409.

*4.7.8. N-(4-aminobutyl)-6-hydroxy-1-(1H-indole-3-carbonyl)-9H-pyrido[3,4-b] indole-3-carboxamide (**39**)*

Yield, 85%; pale yellow powder; mp 181–182 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 11.96 (brs, 1 H), 8.98 (s, 1 H), 8.90 (s, 1 H), 8.51–8.44 (m, 2 H), 7.69–7.64 (m, 3 H), 7.25–7.14 (m, 3 H), 3.43–3.41 (m, 2 H), 2.85–2.84 (m, 2 H), 1.71–1.70 (m, 2 H), 1.65–1.64 (m, 2 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.4, 165.5, 152.4, 139.0, 138.4, 137.2, 136.9, 136.8, 136.6, 131.6, 127.6, 123.3, 122.5, 121.8, 119.7, 116.9, 114.3, 113.3, 106.5, 73.1, 63.6, 26.9, 25.7; HRMS: m/z calcd. for $C_{25}H_{22}N_5O_3^-$, 440.1723; found: 440.1728.

*4.7.9. N-(2-(dimethylamino)ethyl)-6-hydroxy-1-(1H-indole-3-carbonyl)-9H-pyrido [3,4-b]indole-3-arboxamide (**40**)*

Yield, 80%; pale yellow powder; mp 173–174 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.18 (brs, 1 H), 12.01 (s, 1 H), 9.43 (brs, 1 H), 8.94 (s, 1 H), 8.89 (s, 1 H), 8.55–8.53 (m, 1 H), 8.46 (t, J = 5.5 Hz, 1 H), 7.70–7.67 (m, 2 H), 7.61–7.60 (m, 1 H), 7.31–7.30 (m, 2 H), 7.15 (dd, J = 2.3, 8.7 Hz, 1 H), 3.52–3.51 (m, 2 H), 2.52–2.50 (m, 2 H), 2.26 (s, 6 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.5, 165.1, 152.4, 138.3, 137.8, 137.1, 136.8, 136.6, 131.7, 127.6, 123.7, 122.7, 122.2, 121.8, 119.8, 117.0, 114.7, 114.4, 112.9, 106.5, 58.5, 45.6, 37.3; HRMS: m/z calcd. for $C_{25}H_{22}N_5O_3^-$, 440.1723; found: 440.1719.

*4.7.10. 6-Hydroxy-1-(1H-indole-3-carbonyl)-N-(2-(methylamino) ethyl)-9H-pyrido [3,4-b]indole-3-carboxamide (**41**)*

Yield, 82%; pale yellow powder; mp 156–157 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.01 (s, 1 H), 11.98 (brs, 1 H), 9.36 (brs, 1 H), 8.99 (s, 1 H), 8.93 (s, 1 H), 8.55–8.53 (m, 2 H), 7.69–7.66 (m, 2 H), 7.58–7.57 (m, 1 H), 7.31–7.29 (m, 2 H), 7.14 (dd, J = 2.3, 8.7 Hz, 1 H), 3.50 (quartet, J = 5.9 Hz, 2 H), 2.78 (t, J = 5.9 Hz, 2 H), 2.38 (s, 3 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.5, 165.3, 152.4, 138.4, 138.3, 137.0, 136.8, 136.7, 136.6, 131.7, 127.6, 123.6, 122.7, 122.2, 121.8, 119.7, 117.0, 114.5, 114.4, 112.9, 106.5, 50.8, 38.6, 36.1; HRMS: m/z calcd. for $C_{24}H_{20}N_5O_3^-$, 426.1566; found:426.1560.

*4.7.11. N-(3-(dimethylamino)propyl)-6-hydroxy-1-(1H-indole-3-carbonyl)-9H- pyrido[3,4-b]indole-3-carboxamide (**42**)*

Yield, 85%; pale yellow powder; mp 253–254 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ(ppm): 12.24 (brs, 1 H), 11.96 (s, 1 H), 9.40 (brs, 1 H), 8.91 (s, 1 H), 8.89 (s, 1 H), 8.65 (t, J = 5.9 Hz, 1 H), 8.53–8.51 (m, 1 H), 7.66–7.65 (m, 2 H), 7.57–7.56 (m, 1 H), 7.30–7.28 (m, 2 H), 7.14 (dd, J = 2.3, 8.7 Hz, 1 H), 3.45 (quartet, J = 6.8 Hz, 2 H), 2.30 (t, J = 6.8 Hz, 2 H), 2.07 (s, 6 H), 1.74 (quintet, J = 6.8 Hz, 2 H); ^{13}C NMR (DMSO-d_6, 151 MHz) δ(ppm): 187.6, 165.4, 152.4, 138.6, 138.4, 137.3, 136.7, 136.6, 131.5, 127.5, 123.6, 122.7, 122.1, 121.8, 119.7, 117.0, 114.7, 114.3, 112.8, 106.4, 57.8, 45.7, 38.5, 27.8; HRMS: m/z calcd. for $C_{26}H_{24}N_5O_3^-$, 454.1879; found: 454.1871.

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