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Synthesis and structure-activity relationship studies of novel 3,9-substituted α-carboline derivatives with high cytotoxic activity against colorectal cancer cells

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Abstract

In our continued focus on 1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole (YC-1) analogs, we synthesized a novel series of 3,9-substituted α-carboline derivatives and evaluated the new compounds for antiproliferactive effects. Structure activity relationships revealed that a $COOCH_3$ or CH_2OH group at position-3 and substituted benzyl group at position-9 of the α-carboline nucleus were crucial for maximal activity. The most active compound, **11**, showed high levels of cytotoxicity against HL-60, COLO 205, Hep3B, and H460 cells with IC_{50} values of 0.3, 0.49, 0.7, and 0.8 μM, respectively. The effect of compound **11** on the cell cycle distribution demonstrated G2/M arrest in COLO 205 cells. Furthermore, mechanistic studies indicated that compound **11** induced apoptosis by activating death receptor and mitochondria dependent apoptotic signaling pathways in COLO 205 cells. The new 3,9-substituted α-carboline derivatives exhibited excellent anti-proliferative activities, and compound **11** can be used as a promising pro-apoptotic agent for future development of new antitumor agents.

Graphical abstract

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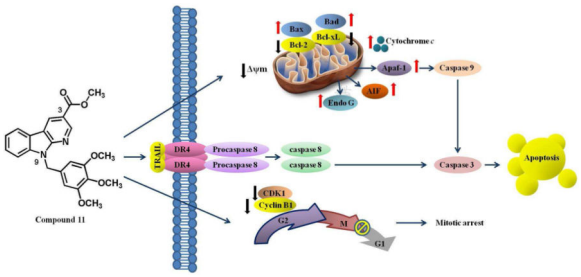
Appendix A. Supplementary data Supplementary data related to this article can be found at http://.



Keywords

α-Carboline derivatives; Apoptosis; YC-1 analogs; Structure-activity relationships (SARs); Antiproliferative activity

1. Introduction

The benzyl indazole derivative YC-1 (Fig. 1) is a well-known activator of soluble guanylyl cyclase (sGC) [1, 2]. Besides its action on sGC, YC-1 also demonstrates multiple activities, including antiplatelet action [3], neuroprotection [4], anti-inflammation [5], and antiangiogenesis [6]. In recent studies, YC-1 has been investigated for its apoptotic effects against cancer cells [7–9]. In our ongoing search for new YC-1-related potential anticancer agents, we have developed several YC-1 analogs, such as 3,9-substituted carbazole (**A**) (Fig. 1) [10], 3,9-substituted β-carboline (**B**) (Fig. 1) [11], and 6,9-substituted α-carboline (**C**) derivatives (Fig. 1) [12–14], in which a tricyclic ring system has replaced the bicyclic indazole ring system of YC-1. Carbolines have a tricyclic pyridoindole structure and are classified as α-, β-, γ-, or δ-carbolines, depending on the position of the pyridine nitrogen relative to the indole. The naturally occurring compounds show a range of biological activities, prompting the synthesis and study of related derivatives [15–17]. In comparison with the well-known β-carbolines, an α-carboline (1-azacarbazole) skeleton is rarely present in alkaloids found in nature. Only a few isolated natural products contain a pyrido[2,3-*b*]indole (α-carboline) core, for example, cryptotackieine [18], neocryptolepine [19], grossularine-1, grossularine-2 [20, 21] and mescengricin [22]. α-Carboline related derivatives have been synthesized and demonstrated to show biologically important anticancer activities [23–27].

In our previous research on YC-1 analogs, we identified certain 1,6,8,9-substituted α-carboline derivatives as anticancer agents [12]. In addition, both 3,9-substituted carbazole derivatives [10] and 3,9-substituted β-carboline derivatives [11] displayed remarkable anticancer effects. Our results revealed that introducing appropriate substituents into position-3 and 9 of the two latter tricyclic core nuclei could enhance the antitumor activity. Accordingly, we proposed that a shift from 6-substitution to 3-substitution on the α-carboline skeleton might produce better antitumor agents (Fig. 2). Consequently, we designed and synthesized a series of novel 3,9-substituted α-carboline derivatives (**D**) (Fig. 2) and screened the new compounds for cytotoxic activity against five types of human cancer cell lines. The general structures of target compounds are depicted in Fig. 2. Additional biological studies were performed to analyse the cell cycle distribution and

apoptosis characteristics of the novel α-carboline derivatives. In this study, we also investigated the mechanism of apoptotic induction in COLO 205 cells by the promising compound **11**.

2. Results and discussion

2.1. Chemistry

The synthetic route to the α-carboline scaffold is illustrated in Scheme 1. The initial synthesis of the 3-substituted α-carbolines proceeded through the acid-catalyzed decomposition of a 1-(2-pyridyl)benzotriazole and cyclization of the indole ring, a modification of the Graebe–Ullmann carbazole synthesis [28, 29]. The starting material 1,2,3-benzotriazole (**1**) was reacted with methyl 6-chloronicotinate (**2**) to form methyl 6-(1,2,3-benzotriazol-1-yl)nicotinate (**3**). Then, methyl α-carboline-3-carboxylate (**4**) was generated by a Graebe–Ullmann reaction via heating **3** in polyphosphoric acid. Subsequently, compound **4** was alkylated with various arylmethyl halides to obtain the corresponding methyl 3-carboxylate-9-substituted α-carboline derivatives (**6**–**30**). The esters in compounds **4** and **6**–**30** were also reduced with calcium borohydride to afford the corresponding carbinols (**5** and **31**–**55**). Finally, compounds **6**, **10**, and **11** were hydrolyzed with sodium hydroxide to obtain the corresponding carboxylic acid derivatives (**56**–**58**). All of the newly synthesized products were characterized by IR, ^{1}H, and ^{13}C-NMR, and mass spectroscopy.

2.2. Biological evaluation

All of the above synthesized α-carboline derivatives (**4**–**58**) were evaluated for cytotoxicity against the Detroit 551 (human normal skin fibroblast) and four cancer cell lines, including HL-60 (leukemia), Hep 3B (hepatoma), H460 (non-small-cell-lung carcinoma), and COLO 205 (colorectal adenocarcinoma). The results are summarized in Table 1. The *N*-9 nonsubstituted α-carbolines (**4** and **5**) exhibited almost no cytotoxicity. Moreover, compound **6** with a benzyl group added at the *N*-9 position was also inactive (IC_{50} > 50 μM). To potentially increase potency of the α-carboline derivatives, we introduced various substituents on the *N*-9 benzyl ring attached to the α-carboline scaffold. Initially, we evaluated the effects of methoxy moieties. Compounds **7** and **8** bearing a 2- or 3-methoxybenzyl moiety, respectively, demonstrated moderate antiproliferative effects (IC_{50} 4.0–43.1 μM), against some cancer cell lines, whereas compound **9** bearing a 4-methoxybenzyl moiety was completely inactive (IC_{50} > 50 μM). Compounds **10** (3,5-dimethoxybenzyl) and **11** (3,4,5-trimethoxybenzyl) exhibited significant activity against HL-60, COLO 205, Hep 3B, and H460 cancer cell lines (IC_{50} 0.3–5.56 μM). Subsequently, we investigated the effects of halogen atoms on the *N*-9 benzyl ring. In contrast to compounds with methoxy groups, compounds with either chloro (**12**–**18**) or fluoro (**19**–**25**) substituents were mostly inactive against the tested cancer cell lines. Only a few compounds (**13**, 3-Cl, 7.1 μM; **15**, 2,3-Cl, 4.1 μM; **18**, 3,4-Cl, 8.2 μM) displayed some potency against the HL-60 cell line. In further modifications, the *N*-9 benzyl ring was replaced with hetero-aromatic methyl groups (−CH_2Ar), including 3,4-methylenedioxy benzyl (**26**), furan-2-yl methyl (**27**), furan-3-yl methyl (**28**), thiophen-2-yl methyl (**29**), and thiophen-3-yl methyl

(**30**). However, these hetero-aromatic methyl substitutions led to dramatically diminished antitumor activities (IC_{50} > 50 μM).

Interestingly, replacing the $COOCH_3$ group (R^3) of compounds **6**–**25** with a CH_2OH group (**31**–**50**) led to enhanced inhibitory effects in some cases, particularly with the halogenated compounds (compare **12** vs. **37**, **25** vs. **50**). This finding suggested that the CH_2OH group (R^3) of α-carboline derivatives might play a pivotal role in the anti-proliferate activities against cancer cells. These results corresponded with those of previous structure-activity relationship (SAR) studies on furoindole derivatives [30], which revealed that a CH_2OH substituent and *N*-benzyl group on the core skeleton played important roles in boosting the anticancer activity of YC-1 analogs. However, even with a CH_2OH group on the 3-position, the *N*-9 hetero-aromatic α-carboline derivatives **51**–**55** remained inactive. In prior investigations on certain 3,9-substituted β-carboline derivatives, a free carboxylic acid group on the 3-position of the β-carboline core improved the antiproliferative activity [11]. However, replacing the $COOCH_3$ group (R^3) of compounds **6**, **10**, and **11** with a COOH group (**56**–**58**) did not have the same effect and considerably reduced the potency of **10** and **11**.

Among all of the new 3,9-substituted α-carboline derivatives, compound **11** [methyl-9-(3,4,5-trimethoxybenzyl)-9*H*-pyrido[2,3-*b*]indole-3-carboxylate] demonstrated the greatest cytotoxicity with submicrolar IC_{50} values against HL-60 (0.3 μM), COLO 205 (0.49 μM), Hep3B (0.7 μM), and H460 (0.8 μM) cells. In addition, compound **11** showed low cytotoxicity toward the Detroit 551 cell line (IC_{50} > 50 μM). This result suggested that α-carboline derivatives selectively suppressed tumor growth without causing toxicity to normal somatic cells.

In the present work, the above findings can be summarized into the following two SAR conclusions.

1. An *N*-9 methylaryl moiety (R^9) is an essential functional group for maintaining the potency of α-carboline derivatives against cancer cells. Generally, regardless of the substituent ($-COOCH_3$ or $-CH_2OH$) on the 3-position of the α-carboline, methoxy substitutions on an *N*-9 benzyl ring led to greater potency than halogen substitutions (Cl or F). More specifically, based on the *N*-9 methylaryl moiety, the following rank order of *in vitro* anticancer potency was found: 3,4,5-trimethoxybenzyl (**11**, **36**) ≧ 3,5-dimethoxybenzyl (**10**, **35**) > mono-methoxybenzyl (**7**–**9**, **32**–**34**) ≧ halogen substituted benzyl (**12**–**25**, **37**–**50**) ≧ benzyl (**6**, **31**) ≧ hetero-aromatic methyl (**26**–**30**, **51**–**55**).
2. With three different α-carboline R_3 substitutions, the *in vitro* anticancer activities were ranked in the following order of decreasing activity: CH_2OH (**31**–**55**) ≧ $COOCH_3$ (**6**–**30**) > COOH (**56**–**58**).

2.3. Growth inhibitory activity of compound 11 against a panel of human cancer cell lines

To further survey the potential activity spectrum of 3,9-substituted α-carbolines, compound **11** was sent to the National Cancer Institute for evaluation in the NCI-60 human cancer cell lines panel available through the Developmental Therapeutics Program. Data was obtained

for 56 human cancer cell lines (Table 2). Compound **11** displayed positive cytotoxic effects (negative value in the cell growth percent) toward 5 out of 56 cell lines. At a single high dose (10 μM), the colon carcinoma COLO 205 cell line was most sensitive (cell growth percentage = −52.54%) to the growth inhibitory effects of **11**. This finding encouraged us to investigate the mechanism of action of **11** in COLO 205 cells.

2.4. Compound 11 inhibited cell-growth and produced morphological change and apoptosis in human colon carcinoma COLO 205 cells

Among all 55 new compounds, compound **11** (Fig. 3A) was the most potent compound against human colon carcinoma COLO 205 cells. As shown in Fig. 3B, exposure of COLO 205 cells to various concentrations of **11** (0.1, 0.5, and 1.0 μM) for 48 h resulted in cell number decreases of COLO 205 cells relative to control in a dose-dependent manner. To confirm the effects of **11** on cell morphology, COLO 205 cells were stained with a fluorescent DNA-staining dye (Hoechst 33258). As shown in Fig. 3C, control cells exhibited uniformly dispersed chromatin (homogeneous blue fluorescence in the nuclei). After treatment with compound **11** at 0.5 μM for 12, 24, 36, and 48 h, the nuclei became fragmented and condensed, and cells showed the appearance of apoptotic bodies (arrows indicate apoptotic nuclei). These results indicated typical characteristics of apoptosis in the **11**-treated COLO 205 cells.

Annexin V/propidium iodide (PI) staining was also used to confirm the apoptotic characteristics produced by **11** in COLO 205 cells. As shown in Fig. 3D, cells incubated in the absence of **11** for 12, 24, 36, and 48 h were undamaged and were negative for both annexin V-FITC and PI staining (Q3). Upon treatment with **11** at 0.5 μM for 24 h to 48 h, the numbers of advanced apoptotic cells stained by positive annexin V-FITC and negative PI (Q4) significantly increased as the incubation time grew longer. The numbers of advanced apoptotic cells stained by positive annexin V-FITC and PI (Q2) also increased significantly with incubation time. These data demonstrate that compound **11** induced cell apoptosis in COLO 205 cells.

2.5. Compound 11 interfered with the cell-cycle distribution and changed expression of G2/M regulatory proteins in COLO 205 cells

Next, we investigated cell cycle arrest and apoptotic mechanisms caused by compound **11**-induced inhibition of COLO 205 cell growth. COLO 205 cells were treated with 0.5 μM of **11** for 0, 12, 24, 36, and 48 h, followed by flow cytometry analysis to determine the cell cycle distribution of treated cells. As shown in Fig. 4A, compound **11** induced a time-dependent accumulation of G_2/M cells and apoptotic (sub-G_1) cells.

Analysis of cell cycle-related protein expression elucidated the mechanisms by which compound **11** induced G_2/M arrest. Cyclin B1 and CDK1 are markers for induction of mitotic arrest [30]. COLO 205 cells treated with 0.5 μM of **11** exhibited decreased cyclin B1 and CDK1 protein levels (Fig. 4B).

2.6. Compound 11 stimulated caspase-3, caspase-8, caspase-9 and PARP cleavage in COLO 205 cells

To confirm the possibility that **11**-induced apoptosis is related to contributions from the intrinsic or extrinsic signal pathway, COLO 205 cells were treated with 0.5 μM of **11** for 0, 12, 24, 36, and 48 h, and then the activities of caspase-3, caspase-8, caspase-9, and PARP were determined using a Western blot assay. PARP cleavage is an important apoptosis marker; caspase-3 cleaves PARP between Asp214 and Glyn215 to yield p85 and p25 fragments [30]. As shown in Fig. 5, compound **11** induced significant caspase-3, caspase-8, caspase-9, and PARP cleavage.

2.6. Compound 11 induced mitochondria signaling pathways in COLO 205 cells

The mitochondria are key organelles in the control of apoptosis [27]. We investigated whether compound **11** was capable of inducing depolarization of the mitochondrial membrane potential (Δψm) using JC-1, a lipophilic fluorescent cation dye. When JC-1 incorporates into the energized mitochondrial membrane, it spontaneously forms a complex, known as the JC-1 polymer, with intense red fluorescence, while monomeric JC-1 shows green fluorescence. COLO 205 cells were treated with 0.5 μM of **11** for 6, 12, 24, and 36 h, followed by staining with JC-1 to confirm apoptosis as the cause of decreased Δψm. As shown in Fig. 6A, in healthy cells with high mitochondrial Δψm, a significant percentage of red fluorescence (P2) was found relative to the green fluorescence (P3) of the uncomplexed monomeric dye (0 h). The percentage of red fluorescence decreased significantly over time (6–36 h), indicative of a change in Δψm occurring in the population in which apoptosis is induced. Moreover, it is well known that the dissipation of Δψm causes release of apoptosis-inducing factor (AIF), Endo G, Apaf-1, and cytochrome *c* into the cytosol, with consequent activation of the execution phase of apoptosis. In this study, we also demonstrated that mitochondrial AIF, Endo G, Apaf-1, and cytochrome *c* were released into the cytosol during compound **11**-induced apoptosis (Fig. 6B).

The Bcl-2 family proteins are key regulators of mitochondrial-related apoptotic pathways [27]. Some of these proteins (such as Bcl-xL and Bcl-2) are anti-apoptotic, whereas others (such as Bad, and Bax) are pro-apoptotic. The balance of pro- and anti-apoptotic Bcl-2 proteins influences the sensitivity of cells to apoptotic stimuli [27]. Exposure of COLO 205 cells to 0.5 μM of **11** for 6, 12, 24, 36, and 48 h verified the involvement of Bcl-2 protein activity in compound **11**-induced apoptosis. As shown in Fig. 6C, results indicated that compound **11** reduced anti-apoptotic Bcl-2 and Bcl-xL levels, as well as increased pro-apoptotic Bax levels and the release of Endo G, AIF, Apaf-1, cytochrome *c*, and procaspase 9 from the mitochondria to the cytosol. Release of Apaf-1, and cytochrome *c* leads to the activation of caspase-9. Activated caspase-9, in turn, cleaves and activates caspase-3. These results suggest that the mitochondrial signaling pathways of COLO 205 cells mediate compound **11**-induced apoptosis.

2.7. Compound 11 induced the death receptor-dependent apoptotic signaling pathways in COLO 205 cells

On binding to their ligands, death receptors trigger apoptosis by stimulating the caspase-8 mediated caspase cascade [27]. In this study, expression of several death receptors (Fas, DR4, and DR5) and their ligands (FasL and TRAIL) were detected in COLO 205 cells. COLO 205 cells were treated with 0.5 μM of **11** for 6, 12, 24, 36, and 48 h and the effects of compound **11** on death receptors and their ligands were investigated. As shown in Fig. 7A and B, compound **11** induced most increase in DR4 and TRAIL. These results suggest that DR4 up-regulation plays an important role in compound **11**-mediated apoptosis in COLO 205 cells.

2.8. Compound 11-induced apoptosis is mediated via JNK signaling pathway

Mitogen-activated protein kinases (MAPK) respond to extracellular stimuli and regulate cellular activitives, such as gene expression, mitosis, differentiation, and cell survival/apoptosis [27]. COLO 205 cells were treated with 0.5 μM of **11** for 6, 12, 24, 36, and 48 h and the effects of compound **11** on extracellular signal-regulated kinases (ERK1/2), JNK and p38 signaling pathway were investigated. As shown in Fig. 8, compound **11** decreased phosphor-ERK1/2 and phospho-p38 expression. Compound **11** induced JNK phosphorylation after 12 h incubation at 0.5 μM. These observations suggest that JNK activation is involved in compound **11**-induced apoptosis. It has been reported that JNK is activated by TRAIL in colon cancer cells [27]. In our study activated JNK might play a mediate role in TRAIL-induced COLO 205 cells apoptosis.

3. Conclusion

In this study, we continued our investigation on YC-1 analogs by synthesizing a new series of 3,9-substituted α-carboline derivatives designed as potential anticancer agents. All the products **4**–**58** were screened for cytotoxic activity against five human cancer cell lines, and some of them showed promising activity at micromolar concentration. Particularly, compound **11** displayed promising anticancer effects on COLO 205 cells by inducing cell apoptosis. Our studies have clearly identified that the death receptor protein DR4 and the mitochondrial environment are the targets of compound **11**. The apoptotic effects of compound **11** on COLO 205 cells occur through both intrinsic and extrinsic signaling pathways. Therefore, novel 3,9-substituted α-carbolines were identified as apoptosis inducers, and compound **11** could be considered as a lead compound for development of clinical trial candidates for cancer chemotherapy.

4. Experimental section

4.1. Materials and physical measurements

All of the solvents and reagents were obtained commercially and used without further purification. The progress of all reactions was monitored by TLC (thin layer chromatography) on 2 × 6 cm pre-coated silica gel 60 F_{254} plates of thickness 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm. The column chromatography was performed using silica gel 60 (Merck, particle size 0.063–0.200 mm).

Melting points (mp) were determined with a Yanaco MP-500D melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu IR-Prestige-21 spectrophotometers as KBr pellets. The 1D nuclear magnetic resonance (NMR, ^{1}H and ^{13}C) spectra were obtained on a Bruker Avance DPX-200 or DPX-400 FT-NMR spectrometer at room temperature. The 2D NMR spectra were obtained on a Bruker Avance DPX-400 FT-NMR spectrometer, and chemical shifts were expressed in parts per million (ppm, δ). The following abbreviations are used: s, singlet; d, doublet; t, triplet; dd, double doublet; and m, multiplet. Mass spectra were performed in the Instrument Center of National Science Council at National Chung Hsing University, (Taichung City, Taiwan R.O.C.), using Finnigan ThermoQuest MAT 95 XL (EI-MS).

4.2. Chemistry

4.2.1. Preparation of methyl α-carboline-3-carboxylate (4)—The α-carboline derivatives were prepared according to published methods [12]. A mixture of 1*H*-1,2,3,-benzotriazole (**1**) (5 g, 0.04 mol) and methyl 6-chloronicotinate (**2**) (8.64 g, 0.05 mol) was heated at 150–160 °C for 1.5 h. The reaction mixture was cooled and quenched with 10% Na_2CO_3 solution. The crude product was extracted with CH_2Cl_2 and washed with saturated Na_2CO_3, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane: EtOAc = 4:1) and recrystallized from MeOH to yield methyl 6-(1,2,3-benzotriazol-1-yl)nicotinate (**3**) as a white solid. Yield: 65%; mp: 121–122 °C; IR (KBr) ν (cm^{-1}): 1716 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.89 (s, 3H, −COOCH_3), 7.51 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.68 (t, *J* = 8.0 Hz, 1H, Ar*H*), 8.15 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.27 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.44–8.51 (m, 2H, Ar*H*), 9.02 (d, *J* = 1.9 Hz, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 52.92, 114.05, 115.05, 120.14, 124.54, 126.01, 130.03, 131.12, 140.67, 146.51, 150.11, 153.60, 164.73; MS (EI, 70 eV) *m/z*: 254.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{13}H_{10}N_4O_2$: 254.0804; found: 254.0808. Then, compound **3** (5 g, 0.02 mole) and polyphosphoric acid (15.42 g) were heated at 150–160°C until N_2 gas evolution ceased, and then heated to 180°C for 30 min. After cooling, 10% NaOH solution was poured into the reaction mixture to adjust the pH to 7–8. The resulting precipitate was collected and washed with water. The crude product was isolated and purified by silica gel column chromatography (*n*-hexane: EtOAc = 1:1) and recrystallized from MeOH to give methyl α-carboline-3-carboxylate (**4**) as white needles. Yield: 11%; mp: 202–204 °C; IR (KBr) ν (cm^{-1}): 1712 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.89 (s, 3H, −COOCH_3), 7.28 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.48–7.56 (m, 2H, Ar*H*), 8.29 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.98 (s, 1H, Ar*H*), 9.02 (s, 1H, Ar*H*), 12.25 (s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 52.37, 112.10, 115.30, 117.35, 120.80, 120.87, 122.22, 127.88, 129.97, 139.91, 148.21, 154.21, 166.59; MS (EI, 70 eV) *m/z*: 226.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{13}H_{10}N_2O_2$: 226.0742; found: 226.0745.

4.2.2. Preparation of methyl 9-substituted-9H-pyrido[2,3-b]indole-3-carboxylate (6–30)—A mixture of methyl α-carboline-3-carboxylate (**4**) (1 equiv) and KOH (4 equiv) in dry THF (50 mL) was heated at 50 °C for 10 min. The appropriate aryl halide (1 – 1.4 equiv) was added, and the mixture was stirred refluxing for 4 h. Reaction completion was confirmed by TLC monitoring. The mixture was poured into ice water (200 mL) and extracted with CH_2Cl_2, dried over MgSO4 and evaporated. The residue was

isolated by column chromatography (silica gel, *n*-hexane: EtOAc = 1:1), and then recrystallized to give the corresponding pure products (**6–30**).

4.2.2.1. Methyl-9-benzyl-9H-pyrido[2,3-b]indole-3-carboxylate (6): Yield: 17%; mp: 142–143 °C; IR (KBr) ν (cm^{-1}): 1718 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.92 (s, 3H, −COOCH_3), 5.57 (s, 2H, N-CH_2), 7.23–7.37 (m, 6H, Ar*H*), 7.54 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.68 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.40 (d, *J* = 8.0 Hz, 1H, Ar*H*), 9.08 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.15 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 44.45, 52.12, 110.72, 114.92, 117.64, 120.01, 121.13, 122.14, 127.12 (2C), 127.48, 127.75, 128.66 (2C), 130.01, 137.16, 139.77, 147.90, 152.84, 166.10; MS (EI, 70 eV) *m/z*: 316.3 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{16}N_2O_2$: 316.1212; found: 316.1215.

4.2.2.2. Methyl 9-(2-methoxybenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (7): Yield: 13%; mp: 126–127 °C; IR (KBr) ν (cm^{-1}): 1712 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.86 (s, 3H, −OCH_3), 3.90 (s, 3H, −COOCH_3), 5.64 (s, 2H, N-CH_2), 6.55 (d, *J* = 8.0 Hz, 1H, Ar*H*), 6.66 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.02 (d, *J* = 8.2 Hz, 1H, Ar*H*), 7.19 (t, *J* = 7.0 Hz, 1H, Ar*H*), 7.28–7.36 (m, 1H, Ar*H*), 7.50–7.52 (m, 2H, Ar*H*), 8.35 (d, *J* = 7.6 Hz, 1H, Ar*H*), 8.99 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.08 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 52.06, 54.97, 55.47, 110.53, 110.93, 114.91, 117.52, 119.94, 120.34, 121.04, 122.03, 124.46, 126.89, 127.73, 128.69, 129.80, 140.02, 147.75, 152.95, 156.60, 166.10; MS (EI, 70 eV) *m/z*: 346.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{21}H_{18}N_2O_3$: 346.1317; found: 346.1310.

4.2.2.3. Methyl 9-(3-methoxybenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (8): Yield: 37%; mp: 131–132 °C; IR (KBr) ν (cm^{-1}): 1707 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.64 (s, 3H, −OCH_3), 3.91 (s, 3H, −COOCH_3), 5.69 (s, 2H, N-CH_2), 6.73–6.86 (m, 3H, Ar*H*), 7.15 (t, *J* = 7.8 Hz, 1H, Ar*H*), 7.34 (t, *J* = 7.0 Hz, 1H, Ar*H*), 7.53 (t, *J* = 7.0 Hz, 1H, Ar*H*), 7.66 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.36 (d, *J* = 7.8 Hz, 1H, Ar*H*), 9.05 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.10 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 44.39, 52.14, 55.01, 110.74, 112.51, 113.26, 114.92, 117.67, 119.15, 120.00, 121.16, 122.12, 127.78, 129.84, 129.99, 138.74, 139.80, 147.89, 152.84, 159.42, 166.12; MS (EI, 70 eV) *m/z*: 346.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{21}H_{18}N_2O_3$: 346.1317; found: 346.1317.

4.2.2.4. Methyl 9-(4-methoxybenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (9): Yield: 17%; mp: 143–144 °C; IR (KBr) ν (cm^{-1}): 1720 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.64 (s, 3H, −OCH_3), 3.90 (s, 3H, −COOCH_3), 5.64 (s, 2H, N-CH_2), 6.82 (dd, *J* = 2.6, 8.0 Hz, 2H, Ar*H*), 7.20–7.27 (m, 2H, Ar*H*), 7.31 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.52 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.66 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.33 (d, *J* = 8.0 Hz, 1H, Ar*H*), 9.05 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.07 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.97, 52.15, 55.09, 110.78, 114.07 (2C), 114.92, 117.57, 120.04, 121.11, 122.09, 127.74, 128.71 (2C), 129.13, 129.92, 139.71, 147.90, 152.80, 158.69, 166.18; MS (EI, 70 eV) *m/z*: 346.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{21}H_{18}N_2O_3$: 346.1317; found: 346.1323.

4.2.2.5. Methyl-9-(3,5-dimethoxybenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (10): Yield: 49%; mp: 134–136 °C; IR (KBr) ν (cm^{-1}): 1720 (C=O); ^{1}H NMR (200 MHz,

DMSO-d_6) δ (ppm): 3.59 (s, 6H, 2 × −OCH_3), 3.86 (s, 3H, −COOCH_3), 5.61 (s, 2H, N-CH_2), 6.33 (s, 3H, Ar*H*), 7.34 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.52 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.62 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.30 (d, *J* = 8.0 Hz, 1H, Ar*H*), 9.01 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.04 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 44.66, 52.41, 55.35 (2C), 98.72, 105.57 (2C), 110.93, 115.14, 117.90, 120.16, 121.49, 122.26, 128.09, 130.16, 139.70, 140.02, 148.08, 153.02, 160.91 (2C), 166.42; MS (EI, 70 eV) *m/z*: 376.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{22}H_{20}N_2O_4$: 376.1423; found: 376.1428.

4.2.2.6. Methyl-9-(3,4,5-trimethoxybenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (11): Yield: 13%; mp: 168–169 °C; IR (KBr) ν (cm^{-1}): 1712 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.56 (s, 3H, −COOCH_3), 3.61 (s, 6H, 2 × −OCH_3), 3.92 (s, 3H, −OCH_3), 5.65 (s, 2H, N-CH_2), 6.69 (s, 2H, Ar*H*), 7.33 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.55 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.76 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.36 (d, *J* = 7.6 Hz, 1H, Ar*H*), 9.13 (s, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 45.13, 52.46, 56.22 (2C), 60.33, 105.38 (2C), 111.18, 115.30, 117.99, 120.37, 121.48, 122.45, 128.11, 130.34, 133.21, 137.32, 140.19, 148.21, 153.21, 153.35 (2C), 166.48; MS (EI, 70 eV) *m/z*: 406.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{23}H_{22}N_2O_5$: 406.1529; found: 406.1537.

4.2.2.7. Methyl 9-(2-chlorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (12): Yield: 62%; mp: 171–172 °C; IR (KBr) ν (cm^{-1}): 1703 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.89 (s, 3H, −COOCH_3), 5.73 (s, 2H, N-CH_2), 6.48 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.07 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.21–7.37 (m, 2H, Ar*H*), 7.46–7.52 (m, 3H, Ar*H*), 8.35 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.96 (d, *J* = 1.8 Hz, 1H, Ar*H*), 9.08 (d, *J* = 1.8 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 42.55, 52.24, 110.48, 115.12, 117.98, 120.15, 121.45, 122.31, 127.37, 127.63, 128.04, 129.27, 129.74, 130.09, 131.81, 134.15, 139.90, 147.94, 152.89, 166.14; MS (EI, 70 eV) *m/z*: 350.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{15}ClN_2O_2$: 350.0822; found: 350.0821.

4.2.2.8. Methyl 9-(3-chlorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (13): Yield: 67%; mp: 162–163 °C; IR (KBr) ν (cm^{-1}): 1726 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.73 (s, 2H, N-CH_2), 7.12–7.19 (m, 1H, Ar*H*), 7.26–7.36 (m, 4H, Ar*H*), 7.55 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.67 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.35 (d, *J* = 7.8 Hz, 1H, Ar*H*), 9.03 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.09 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.92, 52.18, 110.61, 115.01, 117.86, 120.07, 121.33, 122.22, 125.80, 127.01, 127.58, 127.92, 130.08, 130.68, 133.31, 139.69, 139.74, 147.95, 152.77, 166.11; MS (EI, 70 eV) *m/z*: 350.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{15}ClN_2O_2$: 350.0822; found: 350.0826.

4.2.2.9. Methyl 9-(4-chlorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (14): Yield: 67%; mp: 178–179 °C; IR (KBr) ν (cm^{-1}): 1714 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.70 (s, 2H, N-CH_2), 7.22–7.35 (m, 5H, Ar*H*), 7.52 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.63 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.34 (d, *J* = 7.8 Hz, 1H, Ar*H*), 9.02 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.07 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.84, 52.18, 110.62, 114.99, 117.77, 120.07, 121.28, 122.18, 127.86, 128.69 (2C), 129.07

(2C), 130.01, 132.18, 136.21, 139.66, 147.92, 152.76, 166.12; MS (EI, 70 eV) *m/z*: 350.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{15}ClN_2O_2$: 350.0822; found: 350.0819.

4.2.2.10. Methyl 9-(2,3-dichlorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (15): Yield: 70%; mp: 198–199 °C; IR (KBr) ν (cm^{-1}): 1707 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.87 (s, 2H, N-CH_2), 6.38 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.10 (d, *J* = 7.9 Hz, 1H, Ar*H*), 7.32–7.40 (m, 1H, Ar*H*), 7.50–7.55 (m, 3H, Ar*H*), 8.40 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.96 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.12 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.16, 52.17, 110.47, 115.13, 118.05, 120.11, 121.46, 122.29, 125.62, 128.02, 128.46, 129.48, 129.65, 130.10, 132.25, 136.87, 139.80, 147.88, 152.56, 166.02; MS (EI, 70 eV) *m/z*: 384.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{14}Cl_2N_2O_2$: 384.0432; found: 384.0428.

4.2.2.11. Methyl 9-(2,4-dichlorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (16): Yield: 67%; mp: 168–169 °C; IR (KBr) ν (cm^{-1}): 1720 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.70 (s, 2H, N-CH_2), 6.51 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.17 (dd, *J* = 2.1, 8.4 Hz, 1H, Ar*H*), 7.31–7.39 (m, 1H, Ar*H*), 7.51–7.53 (m, 2H, Ar*H*), 7.67 (d, *J* = 2.1 Hz, 1H, Ar*H*), 8.38 (d, *J* = 7.7 Hz, 1H, Ar*H*), 8.96 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.09 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 42.19, 52.18, 110.43, 115.13, 118.03, 120.13, 121.45, 122.31, 127.74, 128.01, 128.77, 129.14, 130.12, 132.72, 132.81, 133.39, 139.76, 147.89, 152.78, 166.03; MS (EI, 70 eV) *m/z*: 384.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{14}Cl_2N_2O_2$: 384.0432; found: 384.0434.

4.2.2.12. Methyl 9-(2,6-dichlorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (17): Yield: 25%; mp: 225–226 °C; IR (KBr) ν (cm^{-1}): 1714 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.91 (s, 3H, −COOCH_3), 5.96 (s, 2H, N-CH_2), 7.23–7.34 (m, 2H, Ar*H*), 7.37–7.53 (m, 4H, Ar*H*), 8.36 (d, *J* = 7.7 Hz, 1H, Ar*H*), 9.03 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.09 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 41.71, 52.15, 110.24, 114.80, 117.66, 120.31, 121.04, 122.22, 127.72, 129.23 (2C), 129.81, 130.80, 131.08, 135.68 (2C), 139.61, 147.54, 153.14, 166.11; MS (EI, 70 eV) *m/z*: 384.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{14}Cl_2N_2O_2$: 384.0432; found: 384.0441.

4.2.2.13. Methyl 9-(3,4-dichlorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (18): Yield: 62%; mp: 170–171 °C; IR (KBr) ν (cm^{-1}): 1718 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.72 (s, 2H, N-CH_2), 7.16 (d, *J* = 8.0 Hz, 1H, Ar*H*), 7.32 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.46–7.58 (m, 3H, Ar*H*), 7.68 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.35 (d, *J* = 7.6 Hz, 1H, Ar*H*), 9.02 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.09 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.37, 52.15, 110.54, 115.03, 117.90, 120.07, 121.34, 122.22, 127.40, 127.91, 129.27, 130.09, 130.20, 130.93, 131.23, 138.36, 139.56, 147.91, 152.69, 166.03; MS (EI, 70 eV) *m/z*: 384.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{14}Cl_2N_2O_2$: 384.0432; found: 384.0439.

4.2.2.14. Methyl 9-(2-fluorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (19): Yield: 24%; mp: 154–155 °C; IR (KBr) ν (cm^{-1}): 1701 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.76 (s, 2H, N-CH_2), 6.88–7.05 (m, 2H, Ar*H*), 7.15–7.37

(m, 3H, Ar*H*), 7.49–7.62 (m, 2H, Ar*H*), 8.36 (d, *J* = 7.8 Hz, 1H, Ar*H*), 9.02 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.09 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 41.17, 52.53, 110.78, 115.39, 115.97 (d, $^{2}J_{CF}$ = 20.5 Hz), 118.18, 120.45, 121.63, 122.57, 124.20 (d, $^{2}J_{CF}$ = 14.5 Hz), 125.09 (d, $^{4}J_{CF}$ = 2.5 Hz), 128.24, 129.34 (d, $^{3}J_{CF}$ = 3.5 Hz), 130.08 (d, $^{3}J_{CF}$ = 8.5 Hz), 130.36, 140.15, 148.24, 153.19, 160.39 (d, $^{1}J_{CF}$ = 243.5 Hz), 166.46; MS (EI, 70 eV) *m/z*: 334.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{15}FN_2O_2$: 334.1118; found: 334.1110.

4.2.2.15. Methyl 9-(4-fluorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (20): Yield: 39%; mp: 139–140 °C; IR (KBr) ν (cm^{-1}): 1718 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.71 (s, 2H, N-CH_2), 7.03–7.13 (m, 2H, Ar*H*), 7.28–7.36 (m, 3H, Ar*H*), 7.53 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.68 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.36 (d, *J* = 7.6 Hz, 1H, Ar*H*), 9.05 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.10 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.73, 52.10, 110.63, 114.93, 115.44 (d, $^{2}J_{CF}$ = 21.5 Hz, 2C), 117.69, 120.02, 121.16, 122.13, 127.77, 129.27 (d, $^{3}J_{CF}$ = 8.0 Hz, 2C), 129.97, 133.39, 139.62, 147.89, 152.73, 161.48 (d, $^{1}J_{CF}$ = 241.5 Hz), 166.07; MS (EI, 70 eV) *m/z*: 334.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{15}FN_2O_2$: 334.1118; found: 334.1112.

4.2.2.16. Methyl 9-(2,4-difluorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (21): Yield: 39%; mp: 134–135 °C; IR (KBr) ν (cm^{-1}): 1718 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.73 (s, 2H, N-CH_2), 6.87–7.12 (m, 2H, Ar*H*), 7.19–7.37 (m, 2H, Ar*H*), 7.50–7.63 (m, 2H, Ar*H*), 8.37 (d, *J* = 7.7 Hz, 1H, Ar*H*), 9.02 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.09 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 39.91, 52.14, 104.18 (dd, $^{2}J_{CF}$ = 26.0, 25.8 Hz), 110.36, 111.76 (dd, $^{2}J_{CF}$ = 21.0 Hz, $^{4}J_{CF}$ = 3.5 Hz), 115.02, 117.82, 120.06, 120.38 (d, $^{4}J_{CF}$ = 3.0 Hz), 121.27, 122.19, 127.87, 129.99, 130.41 (dd, $^{3}J_{CF}$ = 6.0, 9.5 Hz), 139.64, 147.83, 152.73, 159.93 (d, $^{1}J_{CF}$ = 246.5 Hz), 161.73 (d, $^{1}J_{CF}$ = 231.5 Hz), 166.05; MS (EI, 70 eV) *m/z*: 352.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{14}F_2N_2O_2$: 352.1023; found: 352.1032.

4.2.2.17. Methyl 9-(2,5-difluorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (22): Yield: 36%; mp: 148–149 °C; IR (KBr) ν (cm^{-1}): 1701 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.75 (s, 2H, N-CH_2), 6.73–6.82 (m, 1H, Ar*H*), 7.07–7.38 (m, 3H, Ar*H*), 7.51–7.64 (m, 2H, Ar*H*), 8.36 (d, *J* = 7.6 Hz, 1H, Ar*H*), 9.01 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.08 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 39.92, 52.13, 110.33, 115.07, 115.39 (dd, $^{2}J_{CF}$ = 21.5 Hz, $^{3}J_{CF}$ = 4.0 Hz), 116.09 (dd, $^{2}J_{CF}$ = 24.5 Hz, $^{3}J_{CF}$ = 8.5 Hz), 117.29 (dd, $^{2}J_{CF}$ = 24.0 Hz, $^{3}J_{CF}$ = 9.0 Hz), 117.90, 120.10, 121.32, 122.21, 125.90 (dd, $^{2}J_{CF}$ = 17.5 Hz, $^{3}J_{CF}$ = 8.0 Hz), 127.89, 130.02, 139.61, 147.83, 152.70, 156.17 (d, $^{1}J_{CF}$ = 239.5 Hz), 158.08 (d, $^{1}J_{CF}$ = 239.5 Hz), 166.03; MS (EI, 70 eV) *m/z*: 352.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{14}F_2N_2O_2$: 352.1023; found: 352.1021.

4.2.2.18. Methyl 9-(2,6-difluorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (23): Yield: 55%; mp: 192–193 °C; IR (KBr) ν (cm^{-1}): 1707 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −COOCH_3), 5.79 (s, 2H, N-CH_2), 7.02–7.09 (m, 2H, Ar*H*), 7.27–7.42 (m, 2H, Ar*H*), 7.53–7.55 (m, 2H, Ar*H*), 8.34 (d, *J* = 7.7 Hz, 1H, Ar*H*), 9.02 (d, *J*

= 2.0 Hz, 1H, Ar*H*), 9.07 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 33.77, 52.16, 109.99, 111.91 (d, $^{2}J_{CF}$ = 24.0 Hz, 2C), 112.16, 114.89, 117.69, 120.10, 121.14, 122.17, 127.78, 129.83, 130.67, 139.59, 147.69, 152.73, 161.06 (dd, $^{1}J_{CF}$ = 248.0 Hz, $^{3}J_{CF}$ = 8.0 Hz, 2C), 166.11; MS (EI, 70 eV) *m/z*: 352.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{14}F_2N_2O_2$: 352.1023; found: 352.1029.

4.2.2.19. Methyl 9-(3,4-difluorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (24): Yield: 52%; mp: 119–120 °C; IR (KBr) ν (cm^{-1}): 1724 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.91 (s, 3H, −COOCH_3), 5.72 (s, 2H, N-CH_2), 7.02–7.09 (m, 1H, Ar*H*), 7.24–7.45 (m, 3H, Ar*H*), 7.55 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.71 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.37 (d, *J* = 7.6 Hz, 1H, Ar*H*), 9.05 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.11 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.45, 52.13, 110.58, 115.01, 116.49 (d, $^{2}J_{CF}$ = 17.5 Hz), 117.80 (d, $^{2}J_{CF}$ = 17.5 Hz), 117.82, 120.07, 121.27, 122.21, 123.97 (dd, $^{3}J_{CF}$ = 6.0 Hz, $^{4}J_{CF}$ = 3.5 Hz), 127.85, 130.09, 134.93 (dd, $^{3}J_{CF}$ = 4.0 Hz, $^{4}J_{CF}$ = 3.5 Hz), 139.56, 147.90, 148.77 (d, $^{1}J_{CF}$ = 256.0 Hz), 149.32 (d, $^{1}J_{CF}$ = 256.5 Hz), 152.69, 166.04; MS (EI, 70 eV) *m/z*: 352.1 $[M]^+$; HRMS (EI) *m/z*: $C_{20}H_{14}F_2N_2O_2$: 352.1023; found: 352.1021.

4.2.2.20. Methyl 9-(3,5-difluorobenzyl)-9H-pyrido[2,3-b]indole-3-carboxylate (25): Yield: 67%; mp: 175–176 °C; IR (KBr) ν (cm^{-1}): 1720 (C=O); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.91 (s, 3H, −COOCH_3), 5.75 (s, 2H, N-CH_2), 6.90–6.99 (m, 1H, Ar*H*), 7.03–7.15 (m, 1H, Ar*H*), 7.34 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.55 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.69 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.38 (d, *J* = 7.6 Hz, 1H, Ar*H*), 9.04 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.11 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.72, 52.11, 103.08 (dd, $^{2}J_{CF}$ = 26.0, 25.5 Hz), 110.28 (d, $^{2}J_{CF}$ = 23.5 Hz, 2C), 110.51, 115.03, 117.92, 120.07, 121.34, 122.21, 127.89, 130.10, 139.57, 141.79 (dd, $^{3}J_{CF}$ = 8.5, 9.5 Hz), 147.90, 152.70, 162.46 (dd, $^{1}J_{CF}$ = 245.0 Hz, $^{3}J_{CF}$ = 13.0 Hz, 2C), 166.01; MS (EI, 70 eV) *m/z*: 352.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{14}F_2N_2O_2$: 352.1023; found: 352.1021.

4.2.2.21. Methyl 9-((benzo[d][1,3]dioxol-5-yl)methyl)-9H-pyrido[2,3-b] indole-3-carboxylate (26): Yield: 13%; mp: 129–130 °C; IR (KBr) ν (cm^{-1}): 1708 (C=O); ^{1}H NMR (40 MHz, DMSO-d_6) δ (ppm): 3.91 (s, 3H, −COOCH_3), 5.63 (s, 2H, N-CH_2), 5.91 (s, 2H, −OCH_2O–), 6.78 (s, 2H, Ar*H*), 6.88 (s, 1H, Ar*H*), 7.32 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.54 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.70 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.35 (d, *J* = 7.6 Hz, 1H, Ar*H*), 9.06 (s, 1H, Ar*H*), 9.08 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 44.59, 52.46, 101.39, 108.22, 108.68, 111.13, 115.27, 117.97, 120.38, 121.07, 121.46, 122.44, 128.08, 130.30, 131.29, 140.01, 147.01, 147.80, 148.24, 153.11, 166.45; MS (EI, 70 eV) *m/z*: 360.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{21}H_{16}N_2O_4$: 360.1110; found: 360.1117.

4.2.2.22. Methyl 9-((furan-2-yl)methyl)-9H-pyrido[2,3-b]indole-3-carboxylate (27): Yield: 28%; mp: 154–155 °C; IR (KBr) ν (cm^{-1}): 1718 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.91 (s, 3H, −COOCH_3), 5.72 (s, 2H, N-CH_2), 6.35 (d, *J* = 4.1 Hz, 1H, Ar*H*), 6.48 (d, *J* = 3.1 Hz, 1H, Ar*H*), 7.33 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.48 (s, 1H, Ar*H*), 7.56 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.77 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.33 (d, *J* = 7.7 Hz, 1H, Ar*H*), 9.05 (s, 1H, Ar*H*), 9.06 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 38.19, 52.47, 109.09, 110.95, 111.11, 115.28, 118.06, 120.33, 121.54, 122.36, 128.05, 130.23, 139.98,

143.26, 148.12, 150.24, 152.87, 166.44; MS (EI, 70 eV) *m/z*: 306.2 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{18}H_{14}N_2O_3$: 306.1004; found: 306.1008.

4.2.2.23. Methyl 9-((furan-3-yl)methyl)-9H-pyrido[2,3-b]indole-3-carboxylate (28): Yield: 20%; mp: 125–126 °C; IR (KBr) ν (cm^{-1}): 1716 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.91 (s, 3H, −COOCH_3), 5.55 (s, 2H, N-CH_2), 6.32 (s, 1H, Ar*H*), 7.32 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.50 (d, *J* = 0.9 Hz, 1H, Ar*H*), 7.55 (t, *J* = 7.9 Hz, 1H, Ar*H*), 7.73 (s, 1H, Ar*H*), 7.77 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.33 (d, *J* = 7.8 Hz, 1H, H-5), 9.06 (s, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 35.84, 52.08, 110.34, 110.57, 114.91, 117.48, 119.98, 121.04 (2C), 122.02, 127.64, 129.82, 139.54, 140.88, 143.77, 147.77, 152.53, 166.11; MS (EI, 70 eV) *m/z*: 306.2 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{18}H_{14}N_2O_3$: 306.1004; found: 306.0999.

4.2.2.24. Methyl 9-((thiophen-2-yl)methyl)-9H-pyrido[2,3-b]indole-3-carboxylate (29): Yield: 23%; mp: 145–146 °C; IR (KBr) ν (cm^{-1}): 1718 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.91 (s, 3H, −COOCH_3), 5.89 (s, 2H, N-CH_2), 6.91 (t, *J* = 4.0 Hz, 1H, Ar*H*), 7.21 (d, *J* = 2.8 Hz, 1H, Ar*H*), 7.31–7.34 (m, 2H, Ar*H*), 7.56 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.80 (d, *J* = 8.4 Hz, 1H, Ar*H*), 8.33 (d, *J* = 7.6 Hz, 1H, Ar*H*), 9.06 (*s*, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 39.49, 52.10, 110.66, 115.01, 117.74, 120.04, 121.19, 122.09, 125.94, 126.82, 127.03, 127.70, 129.91, 139.17, 139.33, 147.74, 152.30, 166.05; MS (EI, 70 eV) *m/z*: 322.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{18}H_{14}N_2O_2S$: 322.0776; found: 322.0782.

4.2.2.25. Methyl 9-((thiophen-3-yl)methyl)-9H-pyrido[2,3-b]indole-3-carboxylate (30): Yield: 41%; mp: 140–141 °C; IR (KBr) ν (cm^{-1}): 1716 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.91 (s, 3H, −COOCH_3), 5.69 (s, 2H, N-CH_2), 6.98–7.00 (m, 1H, Ar*H*), 7.32 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.39–7.41 (m, 2H, Ar*H*), 7.54 (t, *J* = 7.9 Hz, 1H, Ar*H*), 7.75 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.34 (d, *J* = 7.8 Hz, 1H, Ar*H*), 9.06 (d, *J* = 2.0 Hz, 1H, Ar*H*), 9.08 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 39.73, 52.11, 110.65, 114.93, 117.55, 119.99, 121.08, 122.06, 123.16, 126.96, 127.22, 127.71, 129.89, 137.70, 139.63, 147.83, 152.54, 166.12; MS (EI, 70 eV) *m/z*: 322.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{18}H_{14}N_2O_2S$: 322.0776; found: 322.0778.

4.2.3. Preparation of 3-hydroxymethyl-9-substituted-9H-pyrido[2,3-b]indoles (5 and 31–55)—Compound **4** or **6**–**30** (1 equiv) was dissolved in a homogenous solution of $Ca(BH_4)_2$ (10 equiv) in dry THF (20 mL). The mixture was stirred at room temperature for 30 min. The mixture was poured into ice water (200 mL) and extracted with EtOAc, dried over MgSO4 and evaporated. The residue was isolated by column chromatography (silica gel, *n*-hexane: EtOAc = 1:1) to give the corresponding pure products (**5** and **31**–**55**).

4.2.3.1. Methyl 9H-pyrido[2,3-b]indole-3-carboxylate (5): Yield: 15%; mp: 199–200 °C; IR (KBr) ν (cm^{-1}): 3128 (NH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.43 (s, 2H, −CH_2OH), 2.58 (s, 1H, −CH$_2$O*H*), 7.17 (t, 1H, *J* = 7.2 Hz, Ar*H*), 7.39–7.47 (m, 2H, Ar*H*), 8.09 (d, 1H, *J* = 7.7 Hz, Ar*H*), 8.24 (s, 1H, Ar*H*), 8.26 (s, 1H, Ar*H*), 11.62 (s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 18.07, 111.20, 114.98, 119.19, 120.18, 121.08,

123.57, 126.46, 128.50, 139.18, 146.53, 150.52; MS (EI, 70 eV) *m/z*: 195.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{12}H_{10}N_2O$: 198.0793; found: 198.0799.

4.2.3.2. (9-Benzyl-9H-pyrido[2,3-b]indol-3-yl)methanol (31): Yield: 29%; mp: 141–142 °C; IR (KBr) ν (cm^{-1}): 3392 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.68 (d, *J* = 5.2 Hz, 2H, −C*H*$_2$OH), 5.34 (t, *J* = 5.4 Hz, 1H, −CH$_2$O*H*), 5.68 (s, 2H, N-C*H*$_2$), 7.17–7.28 (m, 6H, Ar*H*), 7.49 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.58 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.20 (d, *J* = 7.6 Hz, 1H, Ar*H*), 8.46 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.51 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 44.26, 61.48, 110.21, 114.91, 120.03, 120.10, 121.48, 126.95, 127.14 (2C), 127.41, 127.91, 128.68 (2C), 129.60, 137.87, 139.47, 145.78, 150.61; MS (EI, 70 eV) *m/z*: 288.2 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{16}N_2O$: 288.1263; found: 288.1265.

4.2.3.3. (9-(2-Methoxybenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (32): Yield: 19%; mp: 167–168 °C; IR (KBr) ν (cm^{-1}): 3242 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.90 (s, 3H, −OC*H*$_3$), 4.67 (d, *J* = 4.5 Hz, 2H,−C*H*$_2$OH), 5.31 (t, *J* = 4.5 Hz, 1H, −CH$_2$O*H*), 5.63 (s, 2H, N-C*H*$_2$), 6.40 (d, *J* = 7.5 Hz, 1H, Ar*H*) 6.65 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.04 (d, *J* = 8.2 Hz, 1H, Ar*H*) 7.15–7.29 (m, 2H, Ar*H*), 7.40–7.47 (m, 2H, Ar*H*), 8.23 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.41 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.52 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 40.33, 55.51, 61.37, 109.97, 110.82, 114.81, 119.86, 119.90, 120.28, 121.37, 125.10, 126.58, 126.84, 127.74, 128.42, 129.49, 139.64, 145.62, 150.60, 156.53; MS (EI, 70 eV) *m/z*: 318.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{18}N_2O_2$: 318.1368; found: 318.1362.

4.2.3.4. (9-(3-Methoxybenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (33): Yield: 5%; mp: 164–165 °C; IR (KBr) ν (cm^{-1}): 3284 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.64 (s, 3H, −OC*H*$_3$), 4.68 (d, *J* = 4.0 Hz, 2H, −C*H*$_2$OH), 5.29 (t, *J* = 4.0 Hz, 1H, −CH$_2$O*H*), 5.66 (s, 2H, N-C*H*$_2$), 6.71–6.83 (m, 3H, Ar*H*) 7.14 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.24 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.46 (t, *J* = 7.0 Hz, 1H, Ar*H*), 7.59 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.21 (d, *J* = 7.8 Hz, 1H, Ar*H*), 8.46 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.52 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 44.04, 54.91, 61.34, 110.11, 112.15, 113.21, 114.72, 119.06, 119.89 (2C), 121.32, 126.74, 127.71, 129.51, 129.65, 139.35 (2C), 145.61, 150.48, 159.32; MS (EI, 70 eV) *m/z*: 318.2 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{18}N_2O_2$: 318.1368; found: 318.1371.

4.2.3.5. (9-(4-Methoxybenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (34): Yield: 28%; mp: 163–165 °C; IR (KBr) ν (cm^{-1}): 3385 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.64 (*s*, 3H, −OC*H*$_3$), 4.68 (d, *J* = 5.2 Hz, 2H, −C*H*$_2$OH), 5.32 (t, *J* = 5.6 Hz, 1H, −CH$_2$O*H*), 5.61 (s, 2H, N-C*H*$_2$), 6.80 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.19–7.27 (m, 3H, Ar*H*), 7.46 (t, *J* = 8.2 Hz, 1H, Ar*H*), 7.61 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.19 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.47 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.50 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.60, 55.03, 61.39, 110.18, 113.93 (2C), 114.76, 119.90 (2C), 121.33, 126.72, 127.72, 128.55 (2C), 129.44, 129.74, 139.27, 145.65, 150.47, 158.51; MS (EI, 70 eV) *m/z*: 318.2 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{18}N_2O_2$: 318.1368; found: 318.1376.

4.2.3.6. (9-(3,5-Dimethoxybenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (35): Yield: 50%; mp: 140–141 °C; IR (KBr) ν (cm^{-1}): 3356 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.61 (s, 6H, 2 × −OCH_3), 4.68 (d, J = 4.4 Hz, 2H, −CH_2OH), 5.33 (t, J = 5.0 Hz, 1H, −CH_2OH), 5.61 (s, 2H, N-CH_2), 6.35 (s, 3H, ArH) 7.25 (t, J = 7.2 Hz, 1H, ArH), 7.46 (t, J = 7.8 Hz, 1H, ArH), 7.59 (d, J = 8.0 Hz, 1H, ArH), 8.21 (d, J = 7.6 Hz, 1H, ArH), 8.46 (d, J = 2.0 Hz, 1H, ArH), 8.51 (d, J = 2.0 Hz, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 44.17, 55.11 (2C), 61.40, 98.31, 105.36 (2C), 110.20, 114.79, 119.91, 120.01, 121.39, 126.84, 127.81, 129.57, 139.45, 140.15, 145.66, 150.54, 160.63 (2C); MS (EI, 70 eV) *m/z*: 348.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{21}H_{20}N_2O_3$: 348.1474; found: 348.1479.

4.2.3.7. (9-(3,4,5-Trimethoxybenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (36): Yield: 27%; mp: 146–147 °C; IR (KBr) ν (cm^{-1}): 3385 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 3.56 (s, 3H, −OCH_3), 3.60 (s, 6H, 2 × −OCH_3), 4.69 (d, 2H, J = 5.2 Hz, −CH_2OH), 5.32 (t, J = 5.4 Hz, 1H, −CH_2OH), 5.60 (s, 2H, N-CH_2), 6.66 (s, 2H, ArH), 7.24 (t, J = 7.2 Hz, 1H, ArH), 7.47 (t, J = 7.7 Hz, 1H, ArH), 7.68 (d, J = 8.2 Hz, 1H, ArH), 8.20 (d, J = 7.6 Hz, 1H, ArH), 8.48 (d, J = 2.0 Hz, 1H, ArH), 8.51 (d, J = 2.0 Hz, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 44.49, 55.88 (2C), 60.02, 61.36, 104.93 (2C), 110.23, 114.80, 119.92 (2C), 121.34, 126.80, 127.74, 129.49, 133.51, 136.83, 139.45, 145.55, 150.53, 152.93 (2C); MS (EI, 70 eV) *m/z*: 378.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{22}H_{22}N_2O_4$: 378.1580; found: 378.1588.

4.2.3.8. (9-(2-Chlorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (37): Yield: 24%; mp: 184–185 °C; IR (KBr) ν (cm^{-1}): 3221 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.67 (d, J = 4.4 Hz, 2H, −CH_2OH), 5.33 (t, J = 5.1 Hz, 1H, −CH_2OH), 5.75 (s, 2H, N-CH_2), 6.40 (d, J = 7.6 Hz, 1H, ArH), 7.07 (t, J = 7.4 Hz, 1H, ArH), 7.21–7.32 (m, 2H, ArH), 7.41–7.54 (m, 3H, ArH), 8.26 (d, J = 7.6 Hz, 1H, ArH), 8.39 (s, 1H, ArH), 8.54 (s, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 42.16, 61.33, 109.91, 114.93, 120.03, 120.25, 121.56, 127.04, 127.17, 127.48, 127.93, 128.98, 129.58, 129.86, 131.66, 134.74, 139.47, 145.71, 150.43; MS (EI, 70 eV) *m/z*: 322.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{15}ClN_2O$: 322.0873; found: 322.0878.

4.2.3.9. (9-(3-Chlorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (38): Yield: 21%; mp: 112–113 °C; IR (KBr) ν (cm^{-1}): 3354 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.68 (d, J = 5.5 Hz, 2H, −CH_2OH), 5.33 (t, J = 5.5 Hz, 1H, −CH_2OH), 5.71 (s, 2H, N-CH_2), 7.12–7.30 (m, 5H, ArH), 7.48 (t, J = 7.8 Hz, 1H, ArH), 7.63 (d, J = 8.1Hz, 1H, ArH), 8.22 (d, J = 7.6 Hz, 1H, ArH), 8.46 (d, J = 2.0 Hz, 1H, ArH), 8.53 (d, J = 2.0 Hz, 1H, ArH); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.98, 61.75, 110.44, 115.24, 120.36, 120.56, 121.89, 126.13, 127.31 (2C), 127.74, 128.30, 130.16, 130.97, 133.58, 139.65, 140.81 (2C), 146.12, 150.80; MS (EI, 70 eV) *m/z*: 322.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{15}ClN_2O$: 322.0873; found: 322.0879.

4.2.3.10. (9-(4-Chlorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (39): Yield: 20%; mp: 163–164 °C; IR (KBr) ν (cm^{-1}): 3221 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.68 (d, J = 5.4 Hz, 2H, −CH_2OH), 5.32 (t, J = 5.6 Hz, 1H, −CH_2OH), 5.69 (s, 2H, N-CH_2), 7.22–7.34 (m, 5H, ArH), 7.46 (t, J = 7.2 Hz, 1H, ArH), 7.60 (d, J = 8.2 Hz, 1H, ArH), 8.22

(d, *J* = 7.7 Hz, 1H, Ar*H*), 8.46 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.52 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.45, 61.32, 110.02, 114.79, 119.94, 120.03, 121.41, 126.83, 127.80, 128.53 (2C), 128.93 (2C), 129.65, 131.87, 136.82, 139.20, 145.65, 150.37; MS (EI, 70 eV) *m/z*: 322.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{15}ClN_2O$: 322.0873; found: 322.0878.

4.2.3.11. (9-(2,3-Dichlorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (40): Yield: 13%; mp: 176–177 °C; IR (KBr) ν (cm^{-1}): 3221 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.67 (d, *J* = 4.9 Hz, 2H, −CH_2OH), 5.36 (t, *J* = 1.4 Hz, 1H, −CH$_2$O*H*), 5.77 (s, 2H, N-CH_2), 6.30 (d, *J* = 7.7 Hz, 1H, Ar*H*), 7.09 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.29 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.42–7.53 (m, 3H, Ar*H*), 8.26 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.38 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.54 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 42.89, 61.34, 109.96, 115.03, 120.09, 120.42, 121.62, 125.54, 127.15, 127.99, 128.41, 129.34, 129.62, 130.02, 132.22, 137.55, 139.45, 145.74, 150.36; MS (EI, 70 eV) *m/z*: 356.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{14}Cl_2N_2O$: 356.0483; found: 356.0487.

4.2.3.12. (9-(2,4-Dichlorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (41): Yield: 19%; mp: 153–154 °C; IR (KBr) ν (cm^{-1}): 3209 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.67 (d, *J* = 6.0 Hz, 2H, −CH_2OH), 5.34 (t, *J* = 6.0 Hz, 1H, −CH$_2$O*H*), 5.73 (s, 2H, N-CH_2), 6.43 (d, *J* = 8.4 Hz, 1H, Ar*H*), 7.18 (dd, *J* = 8.0, 2.0 Hz, 1H, Ar*H*), 7.25–7.33 (m, 1H, Ar*H*), 7.42–7.49 (m, 2H, Ar*H*), 7.07 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.26 (d, *J* = 7.7 Hz, 1H, Ar*H*), 8.40 (s, 1H, Ar*H*), 8.54 (s, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 41.84, 61.30, 109.87, 114.97, 120.07, 120.34, 121.58, 127.08, 127.67, 127.94, 128.60, 129.05, 129.96, 132.62 (2C), 134.02, 139.35, 145.70, 150.33; MS (EI, 70 eV) *m/z*: 356.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{14}Cl_2N_2O$: 356.0483; found: 356.0480.

4.2.3.13. (9-(2,6-Dichlorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (42): Yield: 6%; mp: 101–102 °C; IR (KBr) ν (cm^{-1}): 3331 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.66 (d, *J* = 5.1 Hz, 2H, −CH_2OH), 5.30 (t, *J* = 1.8 Hz, 1H, −CH$_2$O*H*), 5.90 (s, 2H, N-CH_2), 7.17–7.23 (m, 2H, Ar*H*), 7.33–7.52 (m, 4H, Ar*H*), 8.19 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.41 (s, 1H, Ar*H*), 8.47 (s, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 41.45, 61.33, 109.72, 114.60, 119.84, 120.27, 121.41, 126.70, 127.54, 129.12 (2C), 129.47, 130.55, 131.63, 135.68 (2C), 139.23, 145.36, 150.74; MS (EI, 70 eV) *m/z*: 356.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{14}Cl_2N_2O$: 356.0483; found: 356.0488.

4.2.3.14. (9-(3,4-Dichlorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (43): Yield: 23%; mp: 159–160 °C; IR (KBr) ν (cm^{-1}): 3329 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.68 (d, *J* = 4.7 Hz, 2H, −CH_2OH), 5.34 (*t*, *J* = 4.7 Hz, 1H, −CH$_2$O*H*), 5.70 (s, 2H, N-CH_2), 7.12 (dd, *J* = 1.9, 8.3 Hz, 1H, Ar*H*), 7.26 (t, *J* = 7.4 Hz, 1H, Ar*H*), 7.44–7.66 (m, 4H, Ar*H*), 8.22 (d, *J* = 7.7 Hz, 1H, Ar*H*), 8.46 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.53 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.06, 61.32, 109.99, 114.88, 119.98, 120.23, 121.51, 126.99, 127.35, 127.93, 129.16, 129.84, 129.96, 130.87, 131.12, 139.05, 139.15, 145.70, 150.31; MS (EI, 70 eV) *m/z*: 356.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{14}Cl_2N_2O$: 356.0483; found: 356.0492.

4.2.3.15. (9-(2-Fluorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (44): Yield: 15%; mp: 104–105 °C; IR (KBr) ν (cm^{-1}): 3228 (OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.68 (d, *J* = 5.2 Hz, 2H, −C*H*$_2$OH), 5.30 (t, *J* = 5.6 Hz, 1H, −CH$_2$O*H*), 5.74 (s, 2H, N-C*H*$_2$), 6.86 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.99 (t, *J* = 7.2 Hz, 1H, Ar*H*), 7.19–7.28 (m, 3H, Ar*H*), 7.47 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.55 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.22 (d, *J* = 7.6 Hz, 1H, Ar*H*), 8.43 (s, 1H, Ar*H*), 8.51 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 38.26, 61.31, 109.78, 114.84, 115.44 (d, $^2J_{CF}$ = 21.0 Hz), 119.96, 120.06, 121.40, 124.38 (d, $^2J_{CF}$ = 15.0 Hz), 124.55 (d, $^4J_{CF}$ = 4.0 Hz), 126.87, 127.74, 128.74 (d, $^3J_{CF}$ = 4.0 Hz), 129.38 (d, $^3J_{CF}$ = 9.0 Hz), 129.68, 139.34, 145.62, 150.41, 159.93 (d, $^1J_{CF}$ = 243.0 Hz); MS (EI, 70 eV) *m/z*: 306.2 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{15}FN_2O$: 306.1168; found: 306.1165.

4.2.3.16. (9-(4-Fluorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (45): Yield: 9%; mp: 153–154 °C; IR (KBr) ν (cm^{-1}): 3298 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.68 (d, *J* = 3.5 Hz, 2H, −C*H*$_2$OH), 5.34 (t, *J* = 4.0 Hz, 1H, −CH$_2$O*H*), 5.68 (s, 2H, N-C*H*$_2$), 7.03–7.12 (m, 2H, Ar*H*), 7.21–7.33 (m, 3H, Ar*H*), 7.46 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.62 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.21 (d, *J* = 7.5 Hz, 1H, Ar*H*), 8.47 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.52 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.45, 61.39, 110.10, 114.85, 115.37 (d, $^2J_{CF}$ = 21.0 Hz, 2C), 119.97, 120.03, 121.44, 126.87, 127.84, 129.19 (d, $^3J_{CF}$ = 8.0 Hz, 2C), 129.64, 134.04 (d, $^4J_{CF}$ = 2.5 Hz), 139.25, 145.72, 150.43, 161.48 (d, $^1J_{CF}$ = 241.5 Hz); MS (EI, 70 eV) *m/z*: 306.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{15}FN_2O$: 306.1168; found: 306.1165.

4.2.3.17. (9-(2,4-Difluorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (46): Yield: 13%; mp: 150–151 °C; IR (KBr) ν (cm^{-1}): 3327 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.67 (d, *J* = 5.1 Hz, 2H, −C*H*$_2$OH), 5.34 (t, *J* = 4.5 Hz, 1H, −CH$_2$O*H*), 5.71 (s, 2H, N-C*H*$_2$), 6.85–7.03 (m, 2H, Ar*H*), 7.20–7.31 (m, 2H, Ar*H*), 7.44–7.59 (m, 2H, Ar*H*), 8.22 (d, *J* = 7.7 Hz, 1H, Ar*H*), 8.44 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.52 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 38.03, 61.37, 103.63, 104.14 (dd, $^2J_{CF}$ = 26.0, 25.5 Hz), 109.86, 111.76 (dd, $^2J_{CF}$ = 21.5 Hz, $^4J_{CF}$ = 3.0 Hz), 114.95, 120.05, 120.23, 120.88 (dd, $^2J_{CF}$ = 15.0 Hz, $^4J_{CF}$ = 3.5 Hz), 121.52, 127.01, 127.88, 129.81, 130.24 (dd, $^3J_{CF}$ = 4.0, 10.0 Hz), 139.30, 145.71, 150.41, 159.91 (d, ^1J = 246.0 Hz), 161.77 (d, $^1J_{CF}$ = 246.0 Hz); MS (EI, 70 eV) *m/z*: 324.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{14}F_2N_2O$: 324.1074; found: 324.1079.

4.2.3.18. (9-(2,5-Difluorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (47): Yield: 11%; mp: 110–111 °C; IR (KBr) ν (cm^{-1}): 3385 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.68 (d, *J* = 5.2 Hz, 2H, −C*H*$_2$OH), 5.32 (t, *J* = 5.0 Hz, 1H, −CH$_2$O*H*), 5.73 (s, 2H, N-C*H*$_2$), 6.62–6.71 (m, 1H, Ar*H*), 7.09–7.35 (m, 3H, Ar*H*), 7.45–7.61 (m, 2H, Ar*H*), 8.24 (d, *J* = 7.6 Hz, 1H, Ar*H*), 8.44 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.53 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 38.03, 61.03, 109.79, 114.92, 115.66 (dd, $^2J_{CF}$ = 23.5 Hz, $^3J_{CF}$ = 4.5 Hz), 115.76 (dd, $^2J_{CF}$ = 24.5 Hz, $^3J_{CF}$ = 8.0 Hz), 117.22 (dd, $^2J_{CF}$ = 24.5 Hz, $^3J_{CF}$ = 9.0 Hz), 120.01, 120.24, 121.50, 126.56 (dd, $^2J_{CF}$ = 18.0 Hz, $^3J_{CF}$ = 8.0 Hz), 126.98, 127.87, 129.86, 139.22, 145.66, 150.30, 156.11 (d, $^1J_{CF}$ = 239.5 Hz), 158.01 (d, $^1J_{CF}$ = 239.0 Hz); MS (EI, 70 eV) *m/z*: 324.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{14}F_2N_2O$: 324.1074; found: 324.1076.

4.2.3.19. (9-(2,6-Difluorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (48): Yield: 29%; mp: 156–157 °C; IR (KBr) ν (cm^{-1}): 3317 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.66 (d, *J* = 5.3 Hz, 2H, −C*H*$_2$OH), 5.30 (t, *J* = 5.6 Hz, 1H, −CH$_2$O*H*), 5.75 (s, 2H, N-C*H*$_2$), 7.05 (t, *J* = 7.9 Hz, 2H, Ar*H*), 7.19–7.53 (m, 4H, Ar*H*), 8.19 (d, *J* = 8.0 Hz, 1H, Ar*H*), 8.43 (d, *J* = 2.0 Hz, 1H, Ar*H*), 8.48 (d, *J* = 2.0 Hz, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 33.38, 61.31, 109.44, 111.75 (d, $^2J_{CF}$ = 24.0 Hz, 2C), 112.64 (dd, $^2J_{CF}$ = 17.5, 18.0 Hz), 114.69, 119.91, 120.03, 121.33, 126.72, 127.51, 129.52, 130.33 (dd, $^3J_{CF}$ = 10.0, 10.5 Hz), 139.18, 145.48, 150.32, 161.07 (dd, $^1J_{CF}$ = 247.5 Hz, $^3J_{CF}$ = 8.0 Hz, 2C); MS (EI, 70 eV) *m/z*: 324.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{14}F_2N_2O$: 324.1074; found: 324.1064.

4.2.3.20. (9-(3,4-Difluorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (49): Yield: 12%; mp: 139–140 °C; IR (KBr) ν (cm^{-1}): 3311 (OH); ^{1}H NMR (200 MHz, DMSO-d_6) δ (ppm): 4.68 (d, *J* = 4.1 Hz, 2H, −C*H*$_2$OH), 5.36 (t, *J* = 4.9 Hz, 1H, −CH$_2$O*H*), 5.68 (s, 2H, N-C*H*$_2$), 6.99–7.02 (t, *J* = 6.5 Hz, 1H, Ar*H*), 7.22–7.52 (m, 4H, Ar*H*), 7.65 (d, *J* = 8.1 Hz, 1H, Ar*H*), 8.22 (d, *J* = 7.7 Hz, 1H, Ar*H*), 8.46 (s, 1H, Ar*H*), 8.52 (s, 1H, Ar*H*); ^{13}C NMR (50 MHz, DMSO-d_6) δ (ppm): 43.20, 61.37, 110.06, 114.92, 116.36 (d, $^2J_{CF}$ = 17.5 Hz), 117.75 (d, $^2J_{CF}$ = 17.5 Hz), 120.01, 120.21, 121.52, 123.89 (dd, $^3J_{CF}$ = 6.0 Hz, $^4J_{CF}$ = 3.5 Hz), 126.99, 127.95, 129.80, 135.64 (dd, $^3J_{CF}$ = 4.0 Hz, $^4J_{CF}$ = 3.5 Hz), 139.19, 145.74, 148.70 (d, $^1J_{CF}$ = 255.0 Hz), 149.02 (d, $^1J_{CF}$ = 261.5 Hz), 150.36; MS (EI, 70 eV) *m/z*: 324.1 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{14}F_2N_2O$: 324.1074; found: 324.1076.

4.2.3.21. (9-(3,5-Difluorobenzyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (50): Yield: 13%; mp: 110–111 °C; IR (KBr) ν (cm^{-1}): 3317 (OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.69 (d, *J* = 6.1 Hz, 2H, −C*H*$_2$OH), 5.31 (t, *J* = 5.5 Hz, 1H, −CH$_2$O*H*), 5.71 (s, 2H, N-C*H*$_2$), 6.91 (d, *J* = 6.6 Hz, 2H, Ar*H*), 7.07 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.27 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.49 (t, *J* = 7.9 Hz, 1H, Ar*H*), 7.63 (d, *J* = 8.2 Hz, 1H, Ar*H*), 8.22 (d, *J* = 7.7 Hz, 1H, Ar*H*), 8.46 (s, 1H, Ar*H*), 8.52 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 43.66, 61.51, 103.06 (dd, $^2J_{CF}$ = 26.0, 25.0 Hz), 110.14, 110.36 (d, $^2J_{CF}$ = 25.0 Hz, 2C), 115.08, 120.19, 120.43, 121.68, 127.17, 128.09, 130.06, 139.38, 142.70 (dd, $^3J_{CF}$ = 8.5, 9.5 Hz), 145.89, 150.52, 162.63 (dd, $^1J_{CF}$ = 246.0 Hz, $^3J_{CF}$ = 13.0 Hz, 2C); MS (EI, 70 eV) *m/z*: 324.2 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{19}H_{14}F_2N_2O$: 324.1074; found: 324.1066.

4.2.3.22. (9-((Benzo[d][1,3]dioxol-5-yl)methyl)-9H-pyrido[2,3-b]indol-3-yl) methanol (51): Yield: 13%; mp: 101–102 °C; IR (KBr) ν (cm^{-1}): 3282 (OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.69 (d, 2H, *J* = 4.8 Hz, −C*H*$_2$OH), 5.30 (t, 1H, *J* = 5.2 Hz, −CH$_2$O*H*), 5.59 (s, 2H, N-C*H*$_2$), 5.91 (s, 2H, −OC*H*$_2$O–), 6.77 (s, 2H, Ar*H*), 6.85 (s, 1H, Ar*H*), 7.24 (t, 1H, *J* = 7.2 Hz, Ar*H*), 7.47 (t, 1H, *J* = 8.0 Hz, Ar*H*), 7.63 (d, 1H, *J* = 8.0 Hz, Ar*H*), 8.20 (d, 1H, *J* = 7.6 Hz, Ar*H*), 8.47 (s, 1H, Ar*H*), 8.50 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 43.89, 61.36, 100.94, 107.77, 108.23, 110.17, 114.77, 119.91, 120.54, 121.33, 126.76, 127.74, 129.50, 131.58, 139.22, 145.64, 146.45, 147.33, 150.41; MS (EI, 70 eV) *m/z*: 332.2 $[M]^+$; HRMS (EI) *m/z*: calculated for $C_{20}H_{16}N_2O_3$: 332.1161; found: 332.1164.

4.2.3.23. (9-((Furan-2-yl)methyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (52): Yield: 11%; mp: 120–121 °C; IR (KBr) ν (cm^{-1}): 3338 (OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm):

4.68 (s, 2H, −CH_2OH), 5.32 (t, 1H, *J* = 5.0 Hz, −CH$_2$O*H*), 5.68 (s, 2H, N-CH_2), 6.34 (s, 1H, Ar*H*), 6.41 (s, 1H, Ar*H*), 7.25 (t, 1H, *J* = 7.3 Hz, Ar*H*), 7.47–7.51 (m, 2H, Ar*H*), 7.70 (d, 1H, *J* = 7.6 Hz, Ar*H*), 8.18 (d, 1H, *J* = 6.9 Hz, Ar*H*), 8.45 (s, 1H, Ar*H*), 8.47 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 37.56, 61.37, 108.31, 110.23, 110.53, 114.82, 120.06 (2C), 121.30, 126.77, 127.70, 129.61, 139.27, 142.67, 145.58, 150.21, 150.53; MS (EI, 70 eV) *m*/*z*: 278.1 [M]$^+$; HRMS (EI) *m*/*z*: calculated for $C_{17}H_{14}N_2O_2$: 278.1055; found: 278.1049.

4.2.3.24. (9-((Furan-3-yl)methyl)-9H-pyrido[2,3-b]indol-3-yl)methanol (53): Yield: 16%; mp: 115–116 °C; IR (KBr) ν (cm^{-1}): 3336 (OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.68 (d, 2H, *J* = 4.7 Hz, −CH_2OH), 5.34 (t, 1H, *J* = 5.2 Hz, −CH$_2$O*H*), 5.51 (s, 2H, N-CH_2), 6.28 (s, 1H, Ar*H*), 7.25 (t, 1H, *J* = 7.3 Hz, Ar*H*), 7.47–7.50 (m, 2H, Ar*H*), 7.67–7.70 (m, 2H, Ar*H*), 8.19 (d, 1H, *J* = 7.7 Hz, Ar*H*), 8.46 (s, 1H, Ar*H*), 8.47 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 35.53, 61.41, 110.09, 110.45, 114.87, 119.94, 121.38, 121.60, 126.78, 127.76, 129.44, 139.21, 140.69 (2C), 143.69, 145.61, 150.28; MS (EI, 70 eV) *m*/*z*: 278.1 [M]$^+$; HRMS (EI) *m*/*z*: calculated for $C_{17}H_{14}N_2O_2$: 278.1055; found: 278.1049.

4.2.3.25. (9-((Thiophen-2-yl)methyl)-9H-pyrido[2,3-b]indol-3-yl) methanol (54): Yield: 11%; mp: 113–114 °C; IR (KBr) ν (cm^{-1}): 3329 (OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.68 (d, 2H, *J* = 4.7 Hz, −CH_2OH), 5.34 (t, 1H, *J* = 5.2 Hz, −CH$_2$O*H*), 5.86 (s, 2H, N-CH_2), 6.90 (t, 1H, *J* = 3.9 Hz, Ar*H*), 7.18 (d, 1H, *J* = 2.6 Hz, Ar*H*), 7.23–7.30 (m, 2H, Ar*H*), 7.49 (t, 1H, *J* = 7.8 Hz, Ar*H*), 7.74 (d, 1H, *J* = 8.2 Hz, Ar*H*), 8.19 (d, 1H, *J* = 7.7 Hz, Ar*H*), 8.48 (s, 1H, Ar*H*), 8.49 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 39.25, 61.73, 110.53, 115.30, 120.43 (2C), 121.75, 126.04, 127.06 (2C), 127.09, 128.12, 130.03, 139.34, 140.26, 145.93, 150.39; MS (EI, 70 eV) *m*/*z*: 294.1 [M]$^+$; HRMS (EI) *m*/*z*: calculated for $C_{17}H_{14}N_2OS$: 294.0827; found: 294.0830.

4.2.3.26. (9-((Thiophen-3-yl)methyl)-9H-pyrido[2,3-b]indol-3-yl) methanol (55): Yield: 40%; mp: 124–125 °C; IR (KBr) ν (cm^{-1}): 3354 (OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.68 (d, 2H, *J* = 5.3 Hz, −CH_2OH), 5.30 (t, 1H, *J* = 5.4 Hz, −CH$_2$O*H*), 5.66 (s, 2H, N-CH_2), 6.97 (d, 1H, *J* = 4.7 Hz, Ar*H*), 7.25 (t, 1H, *J* = 7.4 Hz, Ar*H*), 7.37–7.40 (m, 2H, Ar*H*), 7.48 (t, 1H, *J* = 7.8 Hz, Ar*H*), 7.69 (d, 1H, *J* = 8.2 Hz, Ar*H*), 8.20 (d, 1H, *J* = 7.7 Hz, Ar*H*), 8.47 (s, 1H, Ar*H*), 8.49 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 39.08, 61.34, 110.05, 114.77, 119.85 (2C), 121.30, 122.74, 126.72 (2C), 127.22, 127.67, 129.44, 138.36, 139.20, 145.56, 150.21; MS (EI, 70 eV) *m*/*z*: 294.1 [M]$^+$; HRMS (EI) *m*/*z*: calculated for $C_{17}H_{14}N_2OS$: 294.0827; found: 294.0823.

4.2.4. Preparation of 9-substituted-9H-pyrido[2,3-b]indole-3-carboxylic acid (56–58)—

Compound **6**, **10**, or **11** (1 equiv) was dissolved in 50% MeOH solution (20 mL), and NaOH (2 equiv) was added. The mixture was heated under reflux for 1 h and then cooled and acidified with dilute HCl. The precipitate was collected and recrystallized from MeOH to yield the pure compound (**56**–**58**).

4.2.4.1. 9-Benzyl-9H-pyrido[2,3-b]indole-3-carboxylic acid (56): Yield: 95%; mp: 238–239 °C; IR (KBr) ν (cm^{-1}): 1683 (C=O), 2808–3066 (OH); ^{1}H NMR (400 MHz, DMSO-d_6)

δ (ppm): 5.68 (s, 2H, N-CH_2), 7.17–7.24 (m, 5H, Ar*H*), 7.29 (t, 1H, *J* = 7.3 Hz, Ar*H*), 7.49 (t, 1H, *J* = 7.9 Hz, Ar*H*), 7.57 (d, 1H, *J* = 8.2 Hz, Ar*H*), 8.27 (d, 1H, *J* = 7.7 Hz, Ar*H*), 9.02 (s, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 44.76, 110.90, 115.18, 118.93, 120.32, 121.44, 122.26, 127.39 (2C), 127.87, 128.04, 129.01 (2C), 130.39, 137.45, 140.00, 148.44, 153.05, 167.59; MS (EI, 70 eV) *m*/*z*: 302.1 $[M]^+$; HRMS (EI) *m*/*z*: calculated for $C_{19}H_{14}N_2O_2$: 302.1055; found: 302.1059.

4.2.4.2. 9-(3,5-Dimethoxybenzyl)-9H-pyrido[2,3-b]indole-3-carboxylic acid (57): Yield: 27%; mp: 263–264 °C; IR (KBr) ν (cm^{-1}): 1595 (C=O), 3136–3502 (OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.60 (s, 6H, 2 × −OCH_3), 5.61 (s, 2H, N-CH_2), 6.32 (s, 1H, Ar*H*), 6.35(s, 2H, Ar*H*), 7.25 (t, 1H, *J* = 7.4 Hz, Ar*H*), 7.44 (t, 1H, *J* = 7.8 Hz, Ar*H*), 7.56 (d, 1H, *J* = 8.2 Hz, Ar*H*), 8.20 (d, 1H, *J* = 7.6 Hz, Ar*H*), 8.91 (s, 1H, Ar*H*), 9.00 (s, 1H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 44.24, 55.11 (2C), 98.44, 105.35 (2C), 110.17, 113.90, 120.05, 120.58, 121.24, 126.50, 128.34, 129.49, 139.44, 140.19, 148.59, 151.42, 160.62 (2C), 168.46; MS (EI, 70 eV) *m*/*z*: 362.2 $[M]^+$; HRMS (EI) *m*/*z*: calculated for $C_{21}H_{18}N_2O_4$: 362.1267; found: 362.1260.

4.2.4.3. 9-(3,4,5-Trimethoxybenzyl)-9H-pyrido[2,3-b]indole-3-carboxylic acid (58): Yield: 17%; mp: > 500 °C; IR (KBr) ν (cm^{-1}): 1701 (C=O), 3319–3508 (OH); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.56 (s, 3H, −OCH_3), 3.60 (*s*, 6H, 2 × −OCH_3), 5.65 (s, 2H, N-CH_2), 6.69 (s, 2H, Ar*H*), 7.32 (t, 1H, *J* = 7.2 Hz, Ar*H*), 7.54 (t, 1H, *J* = 7.6 Hz, Ar*H*), 7.76 (d, 1H, *J* = 8.0 Hz, Ar*H*), 8.34 (d, 1H, *J* = 7.6 Hz, Ar*H*), 9.09 (s, 2H, Ar*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 45.11, 56.20 (2C), 60.33, 105.33 (2C), 111.12, 115.20, 119.07, 120.42, 121.35, 122.36, 127.97, 130.54, 133.31, 137.26, 140.15, 148.49, 153.14, 153.34 (2C), 167.57; MS (EI, 70 eV) *m*/*z*: 391.2 $[M]^+$; HRMS (EI) *m*/*z*: calculated for $C_{22}H_{20}N_2O_5$: 392.1372; found: 392.1364.

4.3. Biological evaluation

4.3.1. MTT assay for anti-proliferative activity—Human tumor cell lines of the cancer screening panel were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (GIBCO/BRL), penicillin (100 U/mL)/streptomycin (100 μg/mL) (GIBCO/BRL) and 1% L-glutamine (GIBCO/BRL) at 37 °C in a humidified atmosphere containing 5% CO_2. Human hepatoma Hep 3B and normal skin Detroit 551 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (GIBCO/BRL), penicillin (100 U/mL)/streptomycin (100 μg/mL) (GIBCO/BRL) and 1% L-glutamine (GIBCO/BRL) at 37 °C in a humidified atmosphere containing 5% CO_2. Logarithmically growing cancer cells were used for all experiments. The human tumor cell lines were treated with vehicle or test compounds for 48 h. Cell growth rate was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliun bromide] reduction assay [31]. After 48 h treatment, cell growth rate was measured on the ELISA reader at a wavelength of 570 nm and the IC_{50} values of test compounds were calculated.

4.3.2. In vitro NCI-60 panel cancer cell lines study—The anticancer activities were tested through the Developmental Therapeutic Program (DTP) of National Cancer Institute

(NCI) [32]. For more information on the anticancer activities screening protocol, please see: http://dtp.nci.nih.gov/branches/btb/ivclsp.html.

4.3.3. Cell morphology and Hoechst 33258 staining—The Hoechst 33258 staining assays were conducted according to our previous report [33]. The COLO 205 cells were plated at a density of 2.5×10^5 cells per well in 12-well plates, and then incubated with 0.5 μM of compound **11** for 12 h to 48 h. Cells were directly examined and photographed under a contrast-phase microscope. Nuclei were stained with Hoechst 33258 (bis-benzimide, Sigma) to detect chromatin condensation or nuclear fragmentation; characteristics of apoptosis. After 0, 12, 24, 36, and 48 h, compound **11**-treated cells were stained with 5 μg/mL Hoechst 33258 for 10 min. After washing twice with PBS, cells were fixed with 4% paraformaldehyde (PFA) in PBS for 10 min at 25 °C. Fluorescence of the soluble DNA (apoptotic) fragments was measured in a Varian Fluorometer at an excitation wavelength of 365 nm and emission wavelength of 460 nm.

4.3.4. Apoptosis studies—Determination of apoptotic cells by fluorescent staining was done as described previously [34]. The Annexin V-FITC Apoptosis Detection Kit was obtained from Strong Biotech Corporation (Strong Biotech, Taiwan). The COLO 205 cells (2×10^5 cells/well) were fluorescently labeled for detection of apoptotic and necrotic cells by adding 100 μL of binding buffer, 2 μL of annexin V-FITC, and 2 μL of PI to each sample. Samples were mixed gently and incubated at room temperature in the dark for 15 min. Binding buffer (300 μL) was added to each sample immediately before flow cytometric analysis. A minimum of 10,000 cells within the gated region were analyzed.

4.3.5. Flow cytometric analysis for cell cycle—COLO 205 cells were added to 0.5 μM compound **11** for 0, 12, 24, 36, and 48 h. Cells were fixed in 70% EtOH overnight, washed twice, and re-suspended in PBS containing 20 μg/mL PI, 0.2 mg/mL RNase A, and 0.1% Triton X-100 in the dark room. After 30 min incubation at 37 °C, cell cycle distribution was analyzed using ModFit LT Software (Verity Software House, Topsham, USA) in a BD FACSCanto flow cytometer (Becton Dickinson, San Jose, CA).

4.3.6. Mitochondrial membrane potential analysis—Cells were plated on 6 well plate at 1.0×10^6 cells/well and treated with 0.5 μM compound **11** for 6, 12, 24, and 36 h. Mitochondrial membranes were stained with 0.5 mL JC-1 working solution (BD MitoScreen Kit) added to each sample. Samples were incubated for 10–15 min at 37 °C in the dark. Mitochondrial membrane potential was measured using the BD FACSCanto flow cytometer (Becton Dickinson, San Jose, CA).

4.3.7. Western Blot Assay—The treated cells (1×10^7 cells/10 mL in 10 cm dish) were collected and washed with PBS. After centrifugation, cells were lysed in a lysis buffer. The lysates were incubated on ice for 30 min and centrifuged at 12,000 g for 20 min. Supernatants were collected, and protein concentrations were then determined using a Bradford Assay. After adding a 5× sample loading buffer containing 625 mM Tris-HCl, pH = 6.8, 500 mM dithiothreitol, 10% SDS, 0.06% bromophenol blue, and 50% glycerol, protein samples were electrophoresed on 10% SDS-polyacrylamide gel, and transferred to a

nitrocellulose membrane. Immunoreactivity was detected using the Western blot chemiluminescence reagent system (PerkinElmer, Boston, MA).

4.3.8. Statistical analysis—Statistical analysis was performed with an analysis of variance (ANOVA) followed by the Tukey's test. All data were expressed as mean ± SD from at least three independent experiments. *$P < 0.001$ was indicative of a significant difference.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- 3,9-Substituted-α-carboline derivatives of YC-1 analogs were synthesized
- Structure-activity relationships were established based on antiproliferative effects
- Compound **11** showed good potency against four cancer cell types (IC_{50} 0.3–0.8 μM)
- Compound **11** arrested cells in G2/M phase and induced apoptosis in COLO 205 cells
- The apoptotic effects were produced via intrinsic and extrinsic signaling pathways

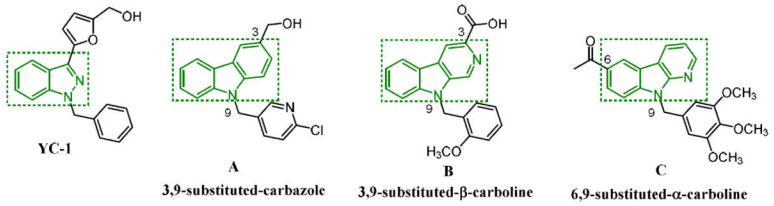


Figure 1.
Structures of YC-1 and its analogs.

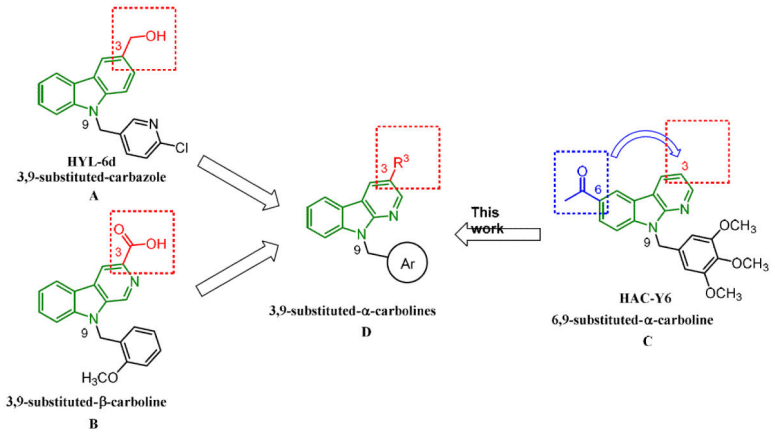


Figure 2.
Rationally designed 3,9-substituted-α-carboline derivatives of YC-1 analogs.

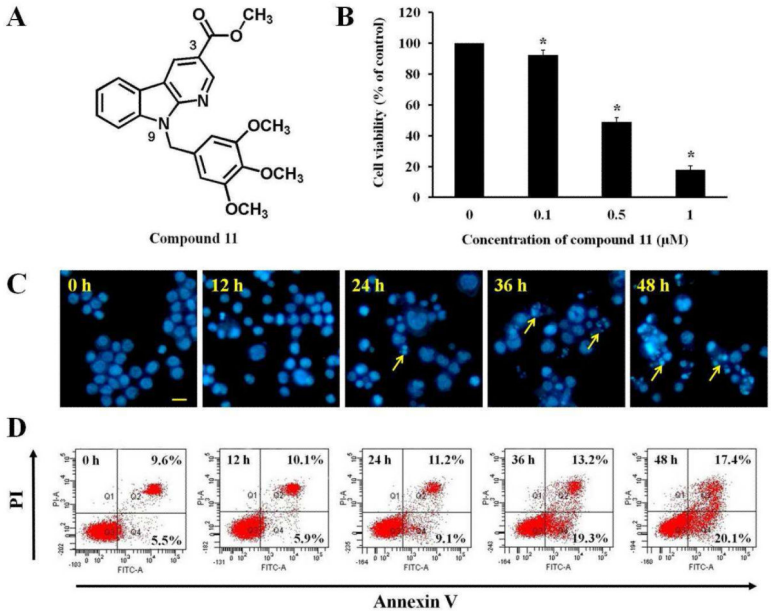


Figure 3.

(A) Chemical structure of compound **11**. (B) Effects of compound **11** on viability of COLO 205 cells. COLO 205 cells were exposed to different concentrations of compound **11** for 48 h. Cell viability was assessed using the MTT assay. The data are presented as mean ± SEM of three independent experiments. Cells without treatment served as a control. * $p<0.001$ vs. control. (C) Fluorescent images of Hoechst staining showing compound **11** induced cell death. The arrowhead indicates an apoptotic nucleus. COLO205 cells were treated with 0.5 μM of compound **11** for 0 h, 12 h, 24 h, 36 h, and 48 h. (D) Confirmation of compound **11**-induced apoptosis was assessed using annexin V/PI staining and flow cytometry. The fraction of annexin V-positive COLO 205 cells was 5.5% prior to treatment and 5.9%, 9.1%, 19.3%, and 20.1% after treatment with 0.5 μM of compound **11** for 12 h, 24 h, 36 h, and 48 h, respectively. Scale bar=20 μm.

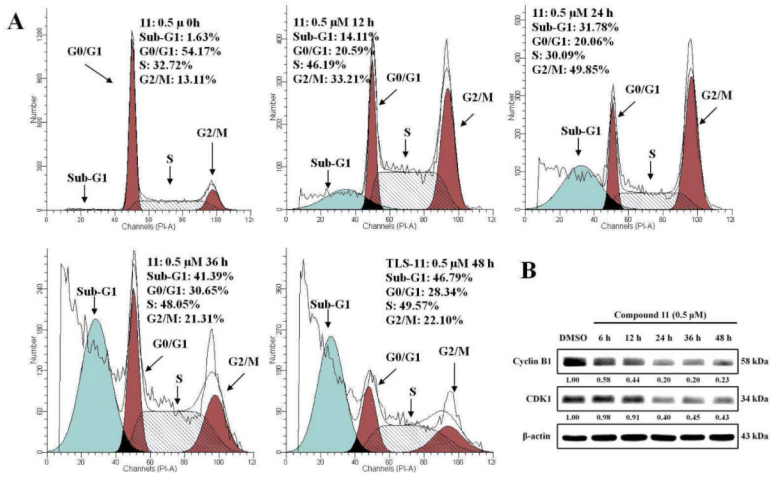


Figure 4.

Effects of compound **11** on the cell cycle in COLO 205 cells. (A) COLO 205 cells were incubated with 0.5 μM of compound **11** for 0 h, 12 h, 24 h, 36 h, and 48 h. They were then harvested and analyzed using flow cytometry. (B) compound **11** decreased cyclin B1 and CDK1 protein expression by western blot (n = 3 independent experiments). β-Actin was used as a loading control.

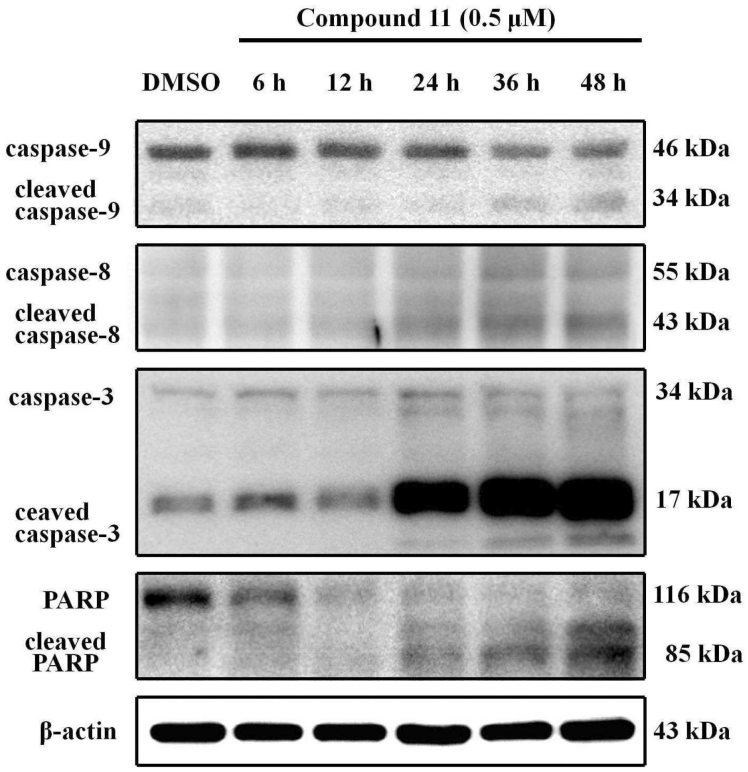


Figure 5.

Compound **11** induced caspase-3, caspase-8, and caspase-9 activity in COLO 205 cells. COLO 205 cells were treated with 0.5 μM of compound **11** for the indicated times and lysed for protein extraction. Protein samples (40 μg protein/lane) were separated using 10% SDS-PAGE and subjected to immunoblotting with antibodies specific to caspase-9, caspase-8, caspase-3, PARP, and β-actin (n = 3 independent experiments). β-Actin was used as a loading control.

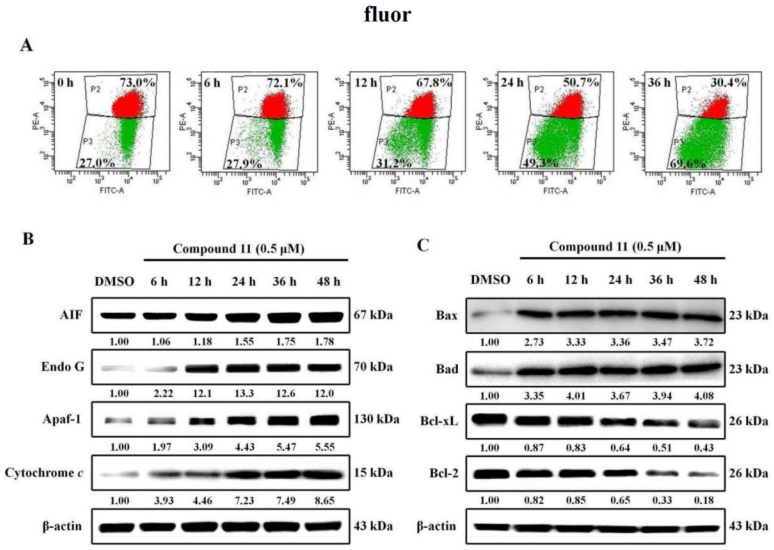


Figure 6.

Compound **11** induced the mitochondrial apoptosis pathway in COLO 205 cells. (A) Effects of compound **11** on mitochondrial membrane potential in COLO 205 cells. Cells (1×10^6 cells/mL) were untreated or treated with compound **11** (0.5 μM, 6 h-36 h) to induce apoptosis. Cells were stained with JC-1 according to the protocol on a BD™ MitoScreen as described in the section Methods for Staining Cells with JC-1 and Analyzing by Flow Cytometry. (B) COLO 205 cells were treated with 0.5 μM of compound **11** for the indicated times and lysed for protein extraction. Protein samples (40 μg protein/lane) were separated using 10% SDS-PAGE and subjected to immunoblotting with antibodies specific to AIF, Endo G, Apaf-1, cytochrome *c*, (C) Bax, Bad, Bcl-xL, Bcl-2, and β-actin (n = 3 independent experiments). β-Actin was used as a loading control.

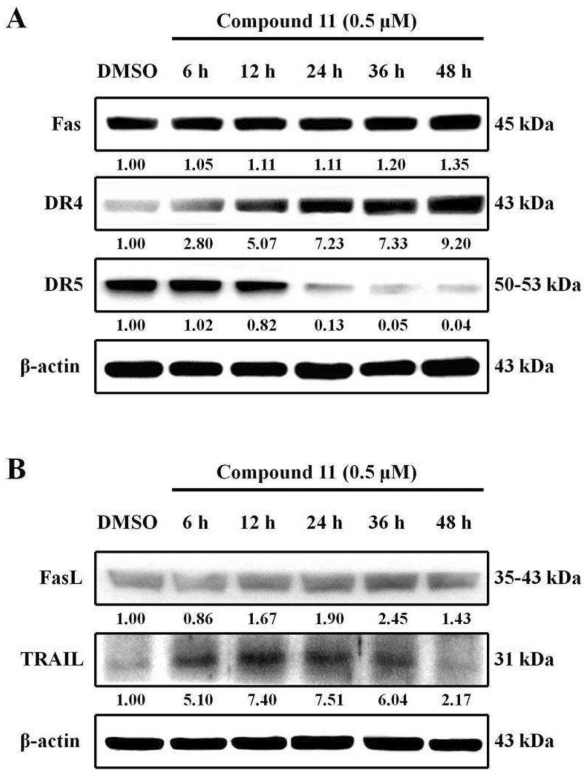


Figure 7.

Compound **11** induced death receptor apoptosis pathway in COLO 205 cells. COLO 205 cells were treated with 0.5 μM of compound **11** for the indicated times and lysed for protein extraction. Protein samples (40 μg protein/lane) were separated using 10% SDS-PAGE and subjected to immunoblotting with antibodies specific to Fas, DR4, DR5 (A), FasL, TRAIL (B), and β-actin (n = 3 independent experiments). β-Actin was used as a loading control.

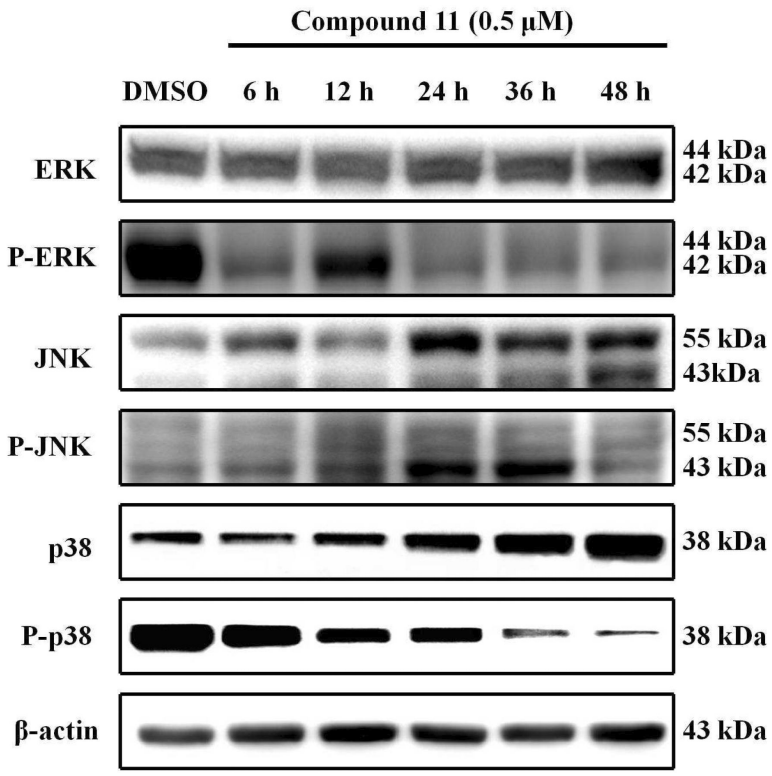
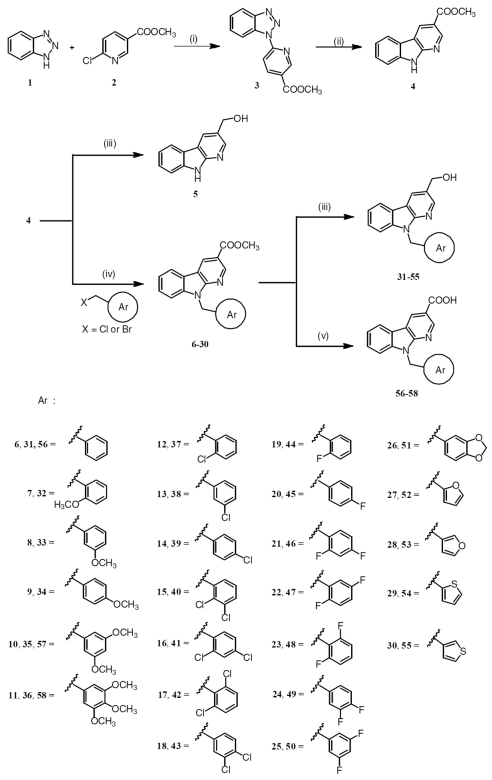


Figure 8.

Expression of MAPKs in the compound **11**-treated COLO 205 cells. COLO 205 cells were treated with 0.5 μM of compound **11** for the indicated times and lysed for protein extraction. Protein samples (40 μg protein/lane) were separated using 10% SDS-PAGE and subjected to immunoblotting with antibodies specific to ERK1/2, phosphor-ERK1/2, JNK, phosphor-JNK, p38, phosphor-p38 and β-actin (n = 3 independent experiments). β-Actin was used as a loading control.

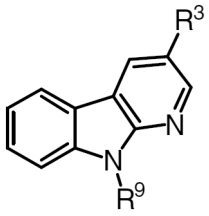


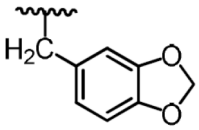
Scheme 1.

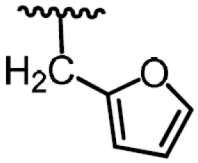
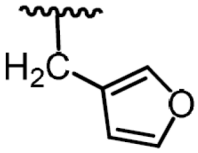
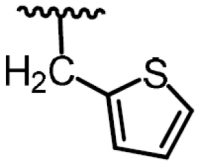
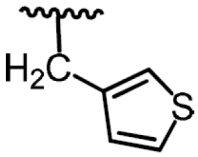
Reagents and conditions : (i) 150-160 °C, 1.5 h; (ii) 150-160 °C, PPA, then heating at 180 °C for 0.5 h. (iii) $Ca(BH_4)_2$, THF, r. t.; (iv) KOH, THF, reflux; (v) NaOH, Methanol, H_2O, reflux.

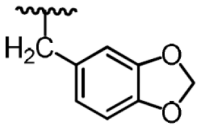
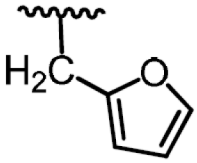
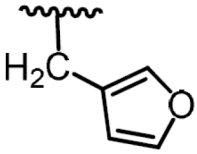
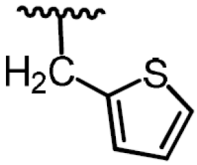
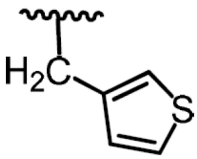
Table 1

Cytotoxic activities of 3,9-substituted-α-carbolines.



Compd	R^3	R^9	IC_{50} (μM)[a]					
			HL-60[b]	COLO205[b]	Hep3B[b]	H460[b]	A498[b]	Detroit551[b]
4	$COOCH_3$	H	50	>50	>50	27.7	46.6	>50
5	CH_2OH	H	49.7	–	–	–	–	>50
6	$COOCH_3$	benzyl	>50	>50	>50	>50	>50	>50
7	$COOCH_3$	2-methoxybenzyl	4.0	12.4	24.2	22.8	43.1	100
8	$COOCH_3$	3-methoxybenzyl	4.4	>50	19.8	33.2	>50	>50
9	$COOCH_3$	4-methoxybenzyl	>50	>50	>50	>50	>50	100
10	$COOCH_3$	3,5-dimethoxybenzyl	0.6	5.56	2.5	3.0	>50	100
11	$COOCH_3$	3,4,5-trimethoxybenzyl	0.3	0.49	0.7	0.8	>50	>50
12	$COOCH_3$	2-chlorobenzyl	25	>50	>50	>50	>50	>50
13	$COOCH_3$	3-chlorobenzyl	7.1	>50	>50	50	>50	>50
14	$COOCH_3$	4-chlorobenzyl	>50	>50	>50	>50	>50	>50
15	$COOCH_3$	2,3-dichlorobenzyl	4.1	>50	>50	>50	>50	>50
16	$COOCH_3$	2,4-dichlorobenzyl	>50	>50	>50	>50	>50	>50
17	$COOCH_3$	2,6-dichlorobenzyl	>50	>50	>50	>50	>50	>50
18	$COOCH_3$	3,4-dichlorobenzyl	8.2	>50	>50	50	>50	>50
19	$COOCH_3$	2-flurobenzyl	>50	>50	>50	>50	>50	>50
20	$COOCH_3$	4-flurobenzyl	>50	>50	>50	>50	>50	>50
21	$COOCH_3$	2,4-diflurobenzyl	>50	>50	>50	>50	>50	>50
22	$COOCH_3$	2,5-diflurobenzyl	25	>50	>50	>50	>50	75
23	$COOCH_3$	2,6-diflurobenzyl	>50	>50	>50	>50	>50	>50
24	$COOCH_3$	3,4-diflurobenzyl	50	>50	>50	>50	>50	100
25	$COOCH_3$	3,5-diflurobenzyl	>50	>50	>50	>50	>50	>50
26	$COOCH_3$		>50	>50	>50	>50	>50	>50

Compd	R^3	R^9	IC_{50} (μM)[a]					
			HL-60[b]	COLO205[b]	Hep3B[b]	H460[b]	A498[b]	Detroit551[b]
27	$COOCH_3$		>50	>50	>50	>50	>50	>50
28	$COOCH_3$		>50	>50	>50	>50	>50	>50
29	$COOCH_3$		>50	>50	>50	>50	>50	>50
30	$COOCH_3$		>50	>50	>50	>50	>50	>50
31	CH_2OH	benzyl	25	35.56	23.5	40.6	>50	80.4
32	CH_2OH	2-methoxybenzyl	5.6	7.9	10.3	18.1	16.4	90.4
33	CH_2OH	3-methoxybenzyl	8.8	9.35	50	46.7	45.2	>50
34	CH_2OH	4-methoxybenzyl	8.1	22.6	>50	>50	38.9	>50
35	CH_2OH	3,5-dimethoxybenzyl	0.4	3.8	2.5	2.5	45.6	>50
36	CH_2OH	3,4,5-trimethoxybenzyl	0.6	3.17	4.1	3.7	29.4	100
37	CH_2OH	2-chlorobenzyl	3.8	>50	6.2	6.4	24.3	25
38	CH_2OH	3-chlorobenzyl	8.8	27.0	16.9	43.9	43.4	100
39	CH_2OH	4-chlorobenzyl	50	>50	50	>50	>50	>50
40	CH_2OH	2,3-dichlorobenzyl	3.0	6.2	5.3	5.3	17.5	27.9
41	CH_2OH	2,4-dichlorobenzyl	32.0	31.5	30.8	41.5	>50	75
42	CH_2OH	2,6-dichlorobenzyl	>50	>50	50	45.8	>50	100
43	CH_2OH	3,4-dichlorobenzyl	8.7	28.8	30.1	27.3	40.8	>50
44	CH_2OH	2-flurobenzyl	13.9	30.7	10.6	21.8	49.3	75.2
45	CH_2OH	4-flurobenzyl	43.3	>50	>50	>50	>50	>50
46	CH_2OH	2,4-diflurobenzyl	34.9	48.0	>50	>50	>50	>50
47	CH_2OH	2,5-diflurobenzyl	9.5	28.8	20.3	18.3	38.2	63.6

Compd	R^3	R^9	IC_{50} (μM)[a]					
			HL-60[b]	COLO205[b]	Hep3B[b]	H460[b]	A498[b]	Detroit551[b]
48	CH_2OH	2,6-diflurobenzyl	50	>50	>50	>50	>50	75
49	CH_2OH	3,4-diflurobenzyl	29.9	38.2	>50	>50	>50	92.2
50	CH_2OH	3,5-diflurobenzyl	5.0	28.9	10.7	13.5	26.1	50
51	CH_2OH		42.2	>50	>50	>50	>50	100
52	CH_2OH		>50	>50	>50	>50	>50	>50
53	CH_2OH		>50	>50	>50	>50	>50	>50
54	CH_2OH		>50	>50	>50	>50	>50	>50
55	CH_2OH		>50	>50	50	>50	>50	>50
56	COOH	benzyl	>100	>50	>100	>100	>50	>100
57	COOH	3,5-dimethoxybenzyl	22.4	>50	41.9	>50	46.4	>100
58	COOH	3,4,5-trimethoxybenzyl	>50	–	–	–	–	>50
YC-1			25.3	–	–	–	0.3	–

[a] Human tumor cells were treated with different concentrations of samples for 48h. Data are presented as IC_{50} (μM, the concentration of 50% proliferation-inhibitory effect).

[b] Cell lines include leukemia (HL-60), liver carcinoma (Hep3B), lung carcinoma (H460), colon carcinoma (COLO205), and normal skin fibroblast (Detroit 551).

Table 2

Growth percentages of compound **11** in the NCI-60 human cancer cell lines (Drug Screen Program).

Panel/Cell line	Compounds / Growth percentage (%)[a]
	11
Leukemia	
CCRF-CEM	7.66
HL-60(TB)	5.33
K-562	12.71
MOLT-4	29.58
RPMI-8226	26.36
SR	14.90
Non-small cell lung cancer	
EKVX	63.03
HOP-62	24.83
HOP-92	115.40
NCI-H226	40.21
NCI-H23	38.91
NCI-H322M	57.89
NCI-H460	11.04
NCI-H522	4.70
Colon cancer	
COLO 205	**−52.54**[b]
HCC-2998	25.42
HCT-116	14.49
HCT-15	23.53
HT-29	2.30
KM12	22.08
SW-620	30.37
CNS cancer	
SF-268	46.66
SF-295	17.25
SF-539	7.79
SNB-19	40.52
SNB-75	27.72
Melanoma	
LOX IMVI	35.29
MALME-3M	67.63
M14	21.53
MDA-MB-435	**−38.70**[b]
SK-MEL-2	5.57
SK-MEL-28	37.89

Panel/Cell line	Compounds / Growth percentage (%)[a]
	11
SK-MEL-5	2.75
UACC-62	27.34
Ovarian cancer	
IGROV1	46.03
OVCAR-3	**−17.97** [b]
OVCAR-4	50.03
OVCAR-5	55.90
NCI/ADR-RES	18.14
SK-OV-3	9.78
Renal cancer	
786-0	26.47
A498	30.25
ACHN	45.63
CAKI-1	45.45
RXF 393	**−15.58** [b]
SN12C	37.23
TK-10	63.05
UO-31	46.03
Prostate cancer	
PC-3	34.13
DU-145	23.71
Breast cancer	
MCF7	8.38
MDA-MB-231/ATCC	27.05
HS 578T	37.35
BT-549	34.91
T-47D	55.79
MDA-MB-468	**−24.11** [b]
Mean growth	25.98
Range of growth	**−52.54** to 115.40
The most sensitive cell line	COLO 205 (**−52.54**)
Positive cytostatic effect[c]	43/56
Positive cytotoxic effect[d]	5/56

[a] Data obtained from NCI *in vitro* 60-cell screen program at 10 μM.

[b] Negative values represent compound proved lethal to the cancer cell line (cell death).

[c] Ratio between number of cell lines with percent growth from 0 to 50 and total number of cell lines.

[d] Ratio between number of cell lines with percent growth of < 0 and total number of cell lines.