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In vitro antiproliferative/cytotoxic activity of 2,3′-biindole against various cancer cell lines

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Abstract: 2,3'-Biindole (**2**) was synthesized via bromination of indole (**1**) with molecular bromine and underwent simultaneous dimerization. Antiproliferative and cytotoxic activity of **2** was investigated in vitro on C6 (rat brain tumor), HeLa (human cervix carcinoma), and HT29 (human colon carcinoma) cells lines by using BrdU cell proliferation ELISA and lactate dehydrogenase (LDH) assays. In contrast to 5-fluorouracil (5-FU), 2,3'-biindole (**2**) significantly inhibited proliferation of HeLa and HT29 cell lines. According to LDH assay, the cytotoxicity of compound **2** was low on HT29 cell lines and high on HeLa and C6 cell lines. Moreover, **2** did not cause any DNA laddering on the DNA of tested cells; therefore, it is suggested that the mechanism of action of this compound may not involve apoptosis. In addition, **2** inhibited relaxation of supercoiled plasmid DNA by topoisomerase activity. Results of the present study indicates that biindole (**2**) may have promising anticancer and antitopoisomerase potential with an unknown mechanism of action.

Key words: 2,3'-Biindole, anticancer, cytotoxicity, apoptosis, antitopoisomerase, C6 cell line, HeLa cell line, HT29 cell line

1. Introduction

Indole (**1**), an electron-rich aromatic heterocyclic molecule (Nakai et al., 2003), reacts with various kinds of electrophiles (Sundberg, 1996; Sala et al., 2002; Zolfigol et al., 2010; Shing et al., 2014). The reactive pyrrole ring of indole (**1**) in Figure 1 with the relatively unreactive benzene ring can react with various kinds of electrophiles. In contrast to the accepted wisdom, the indole (**1**) nucleus has not been challenged to realize a nucleophilic substitution reaction (Yamada et al., 2003, 2012). In **1**, electrophilic substitution is preferred in position 3 with almost all reagents including halogens (Tang et al., 2007), nitrate (Gribble, 2003), sulfur (Gilow et al., 1991), Friedel–Crafts acyls (Ottoni et al., 2001), and alkyls (Zhang et al., 2012). Bromination of indole poses a dimeric product (**2**; Figure 1); as a result, 3-haloindoles and 2-haloindoles are unstable structures (Robertson et al., 2000; Joule and Mills, 2010; Kianmehr et al., 2012). Since the dimeric structures can react with transition metals as ligands in order to give metal complexes, they have high potential for use in catalytic processes (Omura et al., 1977; Black, 1993; Saundane et al., 2013).

N-containing heterocycles like azaindole and azanaphthalene ring systems are common moieties in a vast number of biologically active natural and unnatural compounds with pharmaceutically important molecules (Sundberg, 1996; Higasio and Shoji, 2001; Sala et al., 2002; Kawasaki and Higuchi, 2005; Şahin et al., 2012; Ökten et al., 2013). Biheterocycles like biindole (**2**) show a wide range of biological activities, and in many cases enhanced activity is observed compared to the single parent heterocycle (Hadden and Blagg, 2008).

Some dimeric or substituted indole derivatives, such as indole-3-carbinol and its metabolic products, have been shown to be antiproliferative against various cancer lines such as breast, colon, hepatocellular, and prostate cell lines (Katdare et al., 1998; Chinni and Sarkar, 2002; Frydoonfar et al., 2002, 2003; Hong et al., 2002; Howells et al., 2002; Zheng et al., 2002; Nachshon-Kedmi et al., 2003; Rahman et al., 2003; Weng et al., 2008; Ji et al., 2013; Parvatkar et al., 2013; Ali et al., 2014). 3,3'-Diindolylmethane, a biindole derivative, can inhibit the growth and also the invasion of breast cancer, glioma, and nonsmall lung cancer cells (Rahimi et al., 2010).

For this reason, heterocyclic compounds like indole constitute the framework of pharmacologically active compounds (Şahin et al., 2012; Ökten et al., 2013), and

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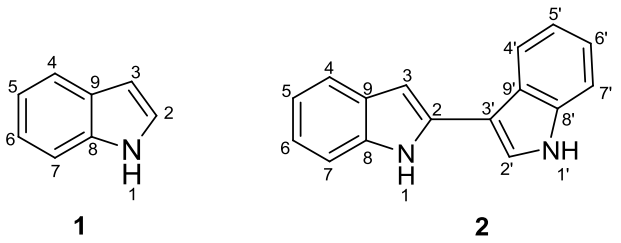


Figure 1. Structure of indole (**1**) and 2,3'-biindole (**2**).

we have studied the synthesis and anticancer potential of indole derivatives. In the current study, we investigated a method of synthesis for biindole (**2**) by using indole (**1**) treated with molecular bromine and determined its structure by 1D nuclear magnetic resonance (NMR) (^{1}H-NMR and ^{13}C-NMR) and 2D NMR (HETCOR, HMBC, NOESY, DEPT-90, and DEPT-135) techniques and elemental analysis. Furthermore, we have investigated antiproliferative activity of 2,3'-biindole (**2**) against HeLa, HT29, and C6 cell lines and its mechanism of action. Although **2** is a well-known compound, this is the first investigation revealing the antiproliferative and antitopoisomerase potential of **2** on several cancer cell lines in vitro. Revealing the anticancer potential of **2** may contribute to the investigation of a cure for cancer or therapy in the future.

2. Materials and methods

Thin layer chromatography was carried out on Merck silica F_{254} 0.255-mm plates, and spots were visualized by UV fluorescence at 254 nm. Classic column chromatography was performed using Merck 60 (70-230 mesh) silica gel. Melting point was determined on a Thomas-Hoover capillary melting point apparatus. Solvents were concentrated at reduced pressure. NMR spectra were recorded on a Bruker 400 MHz for ^{1}H-NMR and at 100 MHz for ^{13}C-NMR. IR spectrum was recorded on a JASCO 430 FT/IR instrument. Elemental analysis was recorded on an Elementar Vario MICRO Cube instrument.

Cell proliferation ELISA BrdU (colorimetric) kits were obtained from Roche Diagnostics GmbH (Mannheim, Germany). The antitumor drug 5-fluorouracil (5-FU) was purchased from Sigma. Other antiproliferative chemicals were used in analytical grade and obtained from Sigma-Aldrich, Merck, and Roche.

2.1. Synthesis of 2,3'-biindole (2)

To a solution of indole (0.3 g, 2.56 mmol, 1 eq.) in $CHCl_3$ (25 mL), bromine solution (0.43 g, 1.28 mmol, 0.5 eq.) in $CHCl_3$ (5 mL) was added over 5 min. The mixture was stirred at room temperature for 20 min in the dark. After completion of the reaction, the resulting dark solution was washed with 5% $NaHCO_3$ solution (3 × 15 mL) and dried over Na_2SO_4. The solvent was removed, and the crude product was passed through a short silica gel (5 g) column eluted with hexane-AcOEt (6/1) to yield a pale brown solid (0.24 g, 82%). NMR analysis (Figures S1–S8 on journal's website; Table) of the product showed the formation of **2**. Mp: 203–204 °C (lit[Bocci and Palla, 1984], 203–205 °C). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 6.80 (s, 1H, H_3), 7.06 (t, 1H, H_6, J = 6.8 Hz), 7.00 (d, 1H, H_5, J = 6.8 Hz), 7.21 (t, 2H, H_5, H_6, J = 7.2 Hz, J = 7.6 Hz), 7.41 (d, 1H, H_7, J = 6.8 Hz), 7.53 (t, 2H, H_4, H_7, J = 7.6 Hz, J = 9.6 Hz), 7.91 (s, 1H, H_2), 8.04 (d, 1H, H_4, J = 6.4 Hz), 11.25 (s, 1H, NH_1), 11.44 (s, 1H, NH_1). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 97.3 (q), 109.0, 110.9, 112.4, 119.3, 119.6, 120.1, 120.3, 120.8, 122.2, 123.6, 125.2 (q), 129.8 (q), 134.7 (q), 136.5 (q), 137.2 (q). IR (KBr, cm^{-1}): 3120, 1621, 1576, 1493, 1436, 1372, 1303, 1252, 1229, 1014, 984, 874, 832. Anal. Calcd. for $C_{16}H_{12}N_2$ (232.28): C, 82.73%; H, 25.21%. Found: C, 83.01%; H, 25.17%.

2.2. Cell culture and cell proliferation assay

HeLa (human cervix carcinoma), HT29 (human colon carcinoma), and C6 (rat brain tumor) cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM; Sigma), supplemented with 10% (v/v) fetal bovine serum (Sigma) and PenStrep solution (Sigma) at 37 °C in a 5% CO_2 humidified atmosphere. For the proliferation assay, cells were plated in 96-well culture plates (COSTAR, Corning, USA) at a density of 30,000 cells per well. Stock solution of the 2,3'-biindole (**2**) and 5-FU were solved in DMSO and diluted with DMEM. The final concentration of DMSO was below 0.5% in all tests. The vehicle (DMSO), 5-FU, and the samples at various concentrations (0–100 µg/mL) were added to each well.

Cells were then incubated overnight before applying the BrdU cell proliferation ELISA assay reagent (Roche) according to the manufacturer's procedure. Briefly, cells were pulsed with BrdU labeling reagent for 4 h followed by fixation in FixDenat solution for 30 min at room temperature. Thereafter, cells were incubated with a 1:100 dilution of anti-BrdU-POD for 1.5 h at room temperature. The amount of cell proliferation was assessed by determining the 450 nm value of the culture media after the addition of substrate solution using a microplate reader (RAYTO RT2100C, China). Results were reported as percentage of inhibition of cell proliferation, where the optical density measured from vehicle-treated cells was 100% of proliferation. Percentage inhibition of cell proliferation was calculated as follows: $[1 - (A_{treatments} / A_{vehiclecontrol})] \times 100$. Percent inhibition was reported as mean value ± SEM of 3 independent assays.

2.3. Cytotoxic activity assay

The cytotoxic activities of 2,3'-biindole (**2**) and the control compound were tested using the LDH Cytotoxicity Detection Kit (Roche) based on the detection of lactate dehydrogenase (LDH) activity released from the cells into the supernatant, according to the manufacturer's

Table. ^{1}H-NMR and ^{13}C-NMR data of 2,3'-biindole (400 MHz, DMSO-d_6).

C/H	DEPT	δ_C ppm (Hz)	δ_H ppm (Hz) coupling constants (*J*)	HMBC
1	-	-	11.23 s	
2	C	134.6	-	H_3, $H_{2'}$
3	CH	97.3	6.80 s	H_4, H_5
4	CH	119.6	7.53 t (7.54 d) *J* = 7.6 Hz, *J* = 9.6 Hz	H_5
5	CH	119.3	7.00 d *J* = 6.8 Hz	H_3, H_6, H_7
6	CH	120.8	7.06 t *J* = 6.8 Hz	H_4
7	CH	110.9	7.41 d *J* = 6.8 Hz	H_5, H_6
8	C	129.7	-	H_3, H_4, H_5, H_6, H_7
9	C	136.5	-	H_3, H_4, H_5, H_6, H_7
1'	-	-	11.44 s	
2'	CH	123.6	7.91 s	
3'	C	109.0	-	H_3, H_2, $H_{4'}$
4'	CH	120.1	8.04 d *J* = 6.4 Hz	H_5, $H_{6'}$
5'	CH	122.2	7.21 t *J* = 7.2 Hz, *J* = 7.6 Hz	$H_{4'}$
6'	CH	120.3	7. 21 t *J* = 7.2 Hz, *J* = 7.6 Hz	H_5, $H_{7'}$
7'	CH	112.4	7.53 t (7.51 d) *J* = 7.6 Hz, *J* = 9.6 Hz	H_2, H_4, H_5, $H_{6'}$
8'	C	125.2	-	H_2, H_4, H_5, $H_{6'}$
9'	C	137.2	-	H_2, H_4, H_5, $H_{6'}$

instructions. Briefly, 3×10^4 cells in 100 µL were seeded into 96-well microtiter plates as triplicates and treated with an IC_{50} concentration of **2** at 37 °C with 5% CO_2 overnight. LDH activity was determined by measuring absorbance at 492–630 nm using a microplate reader (RAYTO RT2100C). Percentage cytotoxicity was calculated as follows: cytotoxicity (%) = [(experimental value – low control) / (high control – low control)] × 100.

2.4. DNA laddering assay

The DNA laddering effect of the test compounds was measured according to the method of Gong et al. (1994) with some modifications. Briefly, 7.5×10^5 cells were seeded into 25-cm^2 culture flasks and treated with IC_{50} concentrations of C1 and C2 at 37 °C with 5% CO_2 overnight. Treated cells were harvested using a sterile plastic scraper, transferred to a 15-mL sterile Falcon tube, washed with 1 mL of sterile DPBS, and pelleted by spinning at 1500 × g for 5 min. The cell pellet was resuspended with 200 µL of ice-cold DPBS by gently pipetting and then fixed with 5 mL of ice-cold 70% ethanol, vortexed briefly, and incubated at –20 °C for 24 h. The cells were centrifuged at 1500 × g for 5 min, the supernatant was removed, and the remaining ethanol was removed by air drying. The cell pellet was resuspended in 50 µL of phosphate-citrate buffer (consisting of 192 parts 0.2 M Na_2HPO_4 and 8 parts 0.1 M citric acid, pH 7.8), incubated at 37 °C for 30 min in a shaker incubator, and centrifuged at 1500 × g for 5 min. Next, 40 µL of supernatant was transferred to a 1.5-mL microcentrifuge tube, mixed with 5 µL of Tween 20 solution (0.25% in ddH_2O) and 5 µL of RNase A solution, and incubated at 37 °C for 30 min in a shaker incubator. Then 5 µL of proteinase K was added to each tube and tubes were incubated at 37 °C for 10 min. Eventually, the content of the microcentrifuge tube was mixed with 4 µL of 6X loading buffer, loaded to 1.5% agarose gel containing 0.5 µg/mL ethidium bromide (EtdBR), and electrophoresed at 200 mA for 40 min. DNA fragmentation in the gels was visualized using a gel documentation system (UVP, UK).

2.5. Topoisomerase I assay

The DNA topoisomerase I activity of **2** was determined by testing the relaxation of supercoiled plasmid DNA (pHOT) using a topoisomerase I assay kit (TopoGEN, Inc., USA). A mixture containing 0.25 µg of the plasmid pHOT DNA and 1–2 units of recombinant human DNA topoisomerase I (TopoGEN) was incubated with an IC_{50} concentration of **2** (test) or EtdBr (positive control). No enzyme was added for the negative control. All reactions were incubated in the reaction buffer (10 mM Tris-HCl, pH 7.9; 1 mM EDTA; 0.15 M NaCl; 0.1% BSA; 0.1 mM spermidine; 5% glycerol) at 37 °C for 30 min. Reactions

were terminated with 5 µL (per 20 µL of reaction volume) of stop buffer (5% sarkosyl, 0.0025% bromophenol blue, 25% glycerol). Reaction products were analyzed on 1% agarose gel at 60 V for 1.5 h. The gels were stained with EtdBr for 10 min, destained with water for 20 min after electrophoresis, and visualized using a gel documentation system (UVP).

2.6. Statistical analysis

The results of in vitro proliferation assays are the means ± SEMs of 2 individual experiments; a total of 6 measurements were performed for each cell type. Differences between treatment groups were tested by 2-way ANOVA, and P-values of 0.05 were considered significant.

3. Results

3.1. Synthesis of biindole (2)

A variety of methods have been reported for the synthesis of **2** and its derivatives (Bergman, 1973; Bergman and Eklund, 1980; Bocchi and Palla, 1982, 1983, 1984; Saito et al., 1982; Robertson et al., 2000; Wahlström et al., 2007; Dupeyre et al., 2011). The reaction of indole with molecular bromine yielded a pale brown solid (82%). Facile one-step synthesis of **2** was achieved by bromination of indole (**1**), which underwent simultaneous dimerization (Figure 2). The preparation of the asymmetric dimer structure (**2**) shows a mechanism that is likely to yield 3-bromoindole (**3**), as **3** can react with another molecule of indole to provide coupling in a 2,3-manner (Bocchi and Palla, 1982; Robertson et al., 2000). The structure of **2** was identified by 1D and 2D NMR techniques (HETCOR, HMBC, and NOESY) and multiple-pulse NMR experiments (DEPT-90 and DEPT-135). NMR spectra are available in the supplementary figures.

The DEPT-135 spectra (Figure S5) of **2** revealed signals at δ_C 109.0, 134.6, which were assigned quaternary carbons of C-3' and C-2, respectively. In HMBC spectra (Figure S6), the one from the quaternary C atom at C-2 (δ_C 134.6) correlated with 2 singlet protons, H-3 (δ_H 6.80) and H-2' (δ_H 7.91), while C-3' (δ_C 109.0) correlated with H-3 (δ_H 6.80), H-2' (δ_H 7.91), and C-4' (δ_C 8.04). In addition, the signal at δ_C 123.6 did not correlate with any protons, indicating that it belonged to C-2'. The NOESY (Figure S7) and HETCOR (Figure S8) experiments also supported this evidence. In NOESY spectra (Figure S7), correlation was observed between H-3 (δ_H 6.80), H-4 (δ_H 7.54), and H-4' (δ_H 8.04). The signal at δ_C 97.3 correlated with a singlet (δ_H 6.80) in the HETCOR (Figure S8) experiment as well as 2 doublets (δ_H 7.00, 7.54) in the HMBC (Figure S6) experiment. These results indicated that singlet (δ_H 6.80) and doublet (δ_H 7.54, 7.00) protons were at C-3, C-4, and C-5, respectively. Long-range couplings between signals observed in ^{13}C-NMR (Figure S3) and ^{1}H-NMR (Figures S1 and S2) spectra were explained by HMBC (Figure S6) experiments. The HMBC cross peaks between methine protons and carbons showed that methine groups (δ_H 6.80, 7.91, 8.04) and quaternary carbon (δ_C 109.0) were at C-3, C-2', C-4', and C-3', respectively. A doublet at δ_H 7.54 at C-4 correlated to δ_H 7.00 at C-5 and δ_H 6.80 singlet at C-3; a singlet at 11.23 (NH) correlated to 7.91 at C-2' and 7.41 at C-7; and another singlet 11.44 (NH) correlated to 7.91 at C-2' and 7.51 at C-7' in the NOESY (Figure S7) experiment. All NMR data (Figures S1–S7) support the proposed structure (**2**).

3.2. Antiproliferative activity of 2,3'-biindole (2) against HeLa, HT29, and C6 cell lines

The antiproliferative activity of **2** against HeLa, HT29, and C6 cell lines was investigated using the BrdU cell proliferation ELISA assay (Roche) at several concentrations. In contrast to the 5-FU control used as a cancer drug, **2** significantly inhibited ($P < 0.05$) proliferation of HeLa, HT29, and C6 cell lines at especially high concentrations (30–75 µg/mL and 50–75 µg/mL, respectively) (Figures 3A–3C).

3.3. Effect of 2,3'-biindole (2) on the morphology of HeLa, HT29, and C6 cell lines

Treatment of cells with **2** led to blebbing, disruption, and disintegration of cells in general, indicating its cytotoxic effect. At high concentrations, **2** caused cell detachment from the plate surface (Figure 4).

3.4. Cytotoxic activity of 2,3'-biindole (2) on HeLa, HT29, and C6 cell lines

Cytotoxic activity of **2** on HeLa, HT29, and C6 cell lines was determined using the LDH Cytotoxicity Detection Kit (Roche) at IC_{50} concentrations. Results showed that the

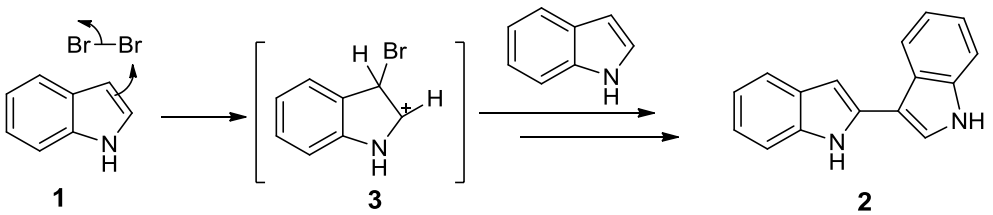


Figure 2. Formation of 2,3'-biindole (**2**).

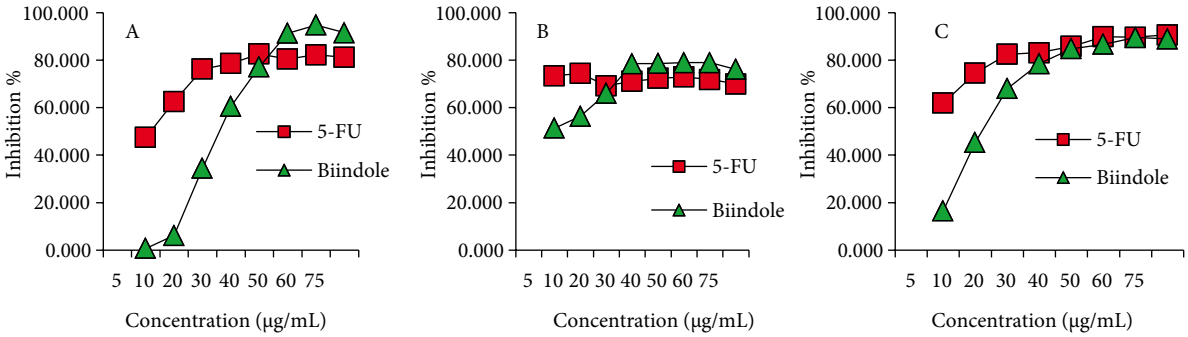


Figure 3. Antiproliferative activity of **2** and the control drug, 5-FU, against HeLa (A), HT29 (B), and C6 (C) cell lines. Antiproliferative activity of **2** against HeLa (A) and HT29 (B) cell lines was significantly higher ($P < 0.05$) than 5-FU at higher concentrations. Each substance was tested 3 times in triplicate against all cell lines. Data show average of 3 individual experiments.

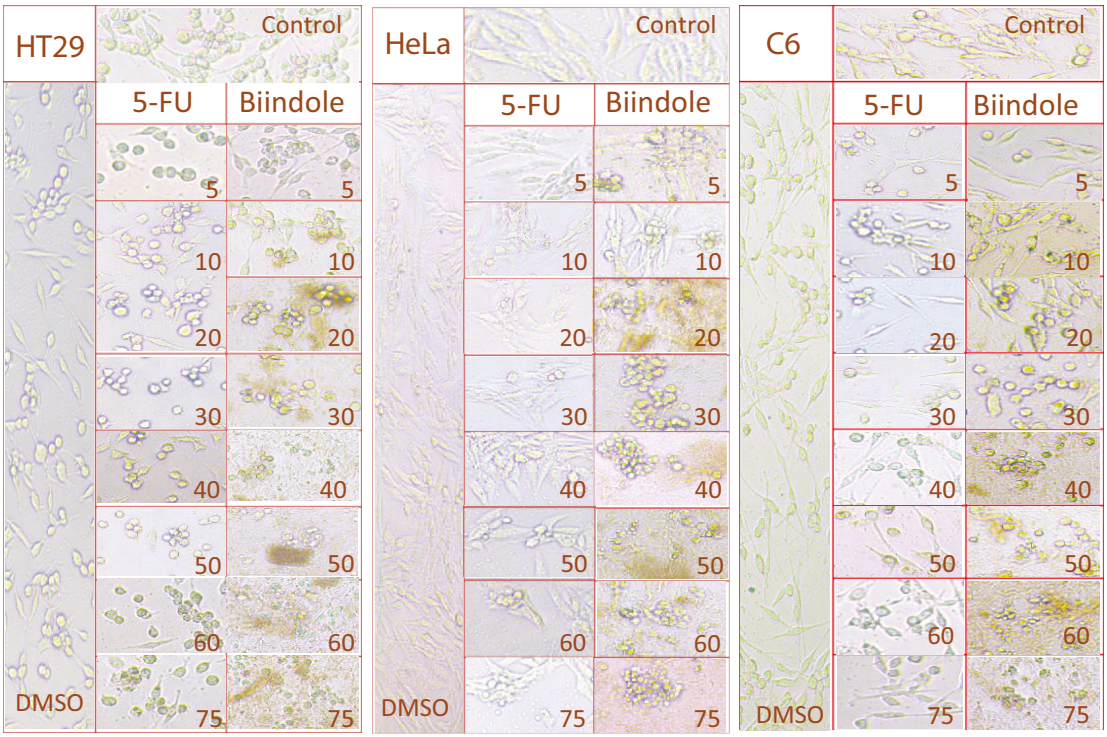


Figure 4. Effect of 2,3'-biindole (**2**) and control drug, 5-FU, on the morphology of HeLa, HT29, and C6 cell lines. Photographs display blebbing, disruption, and disintegration of cells treated with **2** and control (5-FU). Photographs captured by inverted microscope (Leica DM IL LED, Germany) at 20× magnification.

cytotoxic activity of **2** was lower on HT29 cell lines and higher on HeLa and C6 cells lines than that of 5-FU ($P < 0.05$) (Figure 5).

3.5. Apoptotic potential of 2,3'-biindole (2)

Apoptotic potential of **2** on C6 and HeLa cell lines was investigated using a DNA fragmentation assay at its IC_{50} concentration. In this study, SO22, an apoptotic quinoline derivative (Köprülü et al., 2013), was used as a positive control because the apoptotic action of SO22 is higher than that of camptothecin, a quinoline alkaloid used for apoptotic potential studies. Although **2** induced DNA fragmentation at a very low level, especially on C6 cell lines, camptothecin did not induce fragmentation of cell DNA that was as high as SO22 (Figure 6). Therefore, we suggest that the antiproliferative activity of **2** may not involve induction of apoptosis in general.

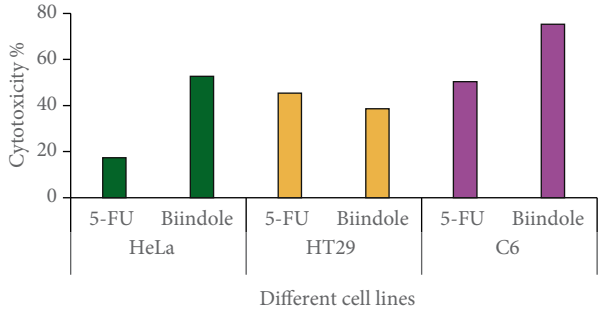


Figure 5. Cytotoxic activity of 2,3'-biindole (**2**) and 5-FU on HeLa, HT29, and C6 cell lines. Cytotoxic activity of **2** was higher than 5-FU on HeLa and C6 cell lines ($P < 0.05$); **2** was less cytotoxic on HT29 cells than on HeLa and C6 cell lines. Data show average of 3 individual experiments performed in triplicate.

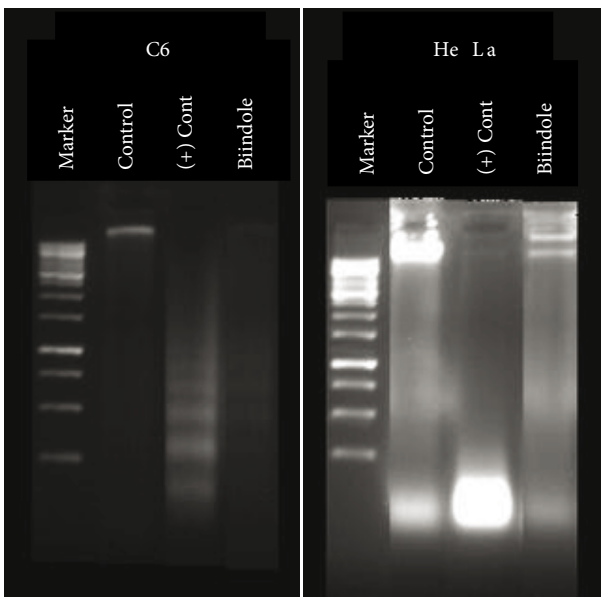
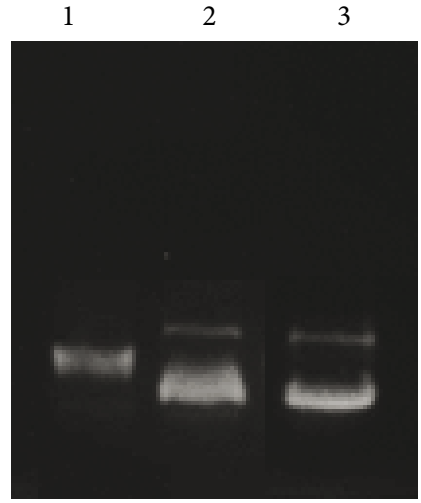


Figure 6. Apoptotic potential of 2,3'-biindole (**2**) on C6 and HeLa cell lines; **2** did not induce fragmentation of cell DNA as high as apoptotic compound SO22 (control: no apoptotic compound; (+) Cont: SO22, an apoptotic quinoline derivative).

3.6. Antitopoisomerase activity of 2,3'-biindole (2)

To understand if antiproliferative activity of **2** involves inhibition of topoisomerases, we tested the antitopoisomerase activity of compound **2** using a TOPO isomerase I assay kit (TopoGEN). Results showed that compound **2** inhibited topoisomerase activity as the positive control, EtdBr (Figure 7).



TOPO I	-	+	+
SC DNA	+	+	+
EtdBr	-	+	+
Biindole	-	-	+

Figure 7. Antitopoisomerase I activity of 2,3'-biindole (**2**); **2** inhibited topoisomerase activity as positive control, EtdBr (TOPO I: topoisomerase I, SC DNA: supercoiled plasmid DNA, EtdBr: ethidium bromide).

4. Discussion

The antiproliferative activity of some substituted indole and biindole derivatives against breast, colon, and lung cancer cells has been reported (Katdare et al., 1998; Chinni and Sarkar, 2002; Frydoonfar et al., 2002, 2003; Hong et al., 2002; Howells et al., 2002; Zheng et al., 2002; Hudson et al., 2003; Nachshon-Kedmi et al., 2003; Rahman et al., 2003; Weng et al., 2008; Ji et al., 2013; Ali et al., 2014). The antiproliferative and cytotoxic activities of **2** against HeLa, C6, and HT29 cell lines were tested and documented for the first time in this study. We compared the antiproliferative activity of **2** with a known anticancer drug, 5-FU, which has strong antiproliferative activity in various tumor cell lines (Chen et al., 1995; Ferguson et al., 1999; Ahmed and Jamil, 2011). We found that **2** was as antiproliferative against tumor cell lines HeLa, C6, and HT29 as 5-FU (Figures 3–5). Compound **2** was more antiproliferative against HeLa and HT29 cell lines than C6 cell lines at higher concentrations (30–75 µg/mL) (Figures 3 and 4). These results were similar to previous studies done with indole and biindole derivatives (Katdare et al., 1998; Frydoonfar et al., 2002, 2003; Hong et al., 2002; Howells et al., 2002; Rahman et al., 2003; Weng et al., 2008; Ji et al., 2013; Ali et al., 2014).

We also tested the cytotoxic activity of **2** on HeLa, C6, and HT29 cell lines. Cytotoxicity of **2** was low on HT29 cell lines and high on HeLa and C6 cell lines. The blebbing, disruption, and disintegration of HeLa, HT29, and C6 cell lines treated with **2** somewhat proved the cytotoxicity

results of compound **2** (Figure 4). Moreover, DNA laddering assay results showed that **2** did not induce DNA fragmentation in cells tested, indicating that it may act through a mechanism other than apoptosis. In addition, as EtdBr, compound **2** inhibited relaxation of supercoiled plasmid DNA by topoisomerase I activity, suggesting that compound **2** may act by inhibiting topoisomerase I action to inhibit cell proliferation.

In conclusion, our present work demonstrated that the potential anticancer compound **2** worked on C6 (rat brain tumor), HeLa (human cervix carcinoma), and HT29 (human colon carcinoma) cells lines via inhibiting proliferation of those cell lines and topoisomerase activity. However, further in vitro and in vivo investigations are needed to reveal the anticancer potential and mechanism of action of **2**.

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References

Ahmed M, Jamil K (2011). Cytotoxicity of neoplastic drugs Gefitinib, Cisplatin, 5-FU, Gemcitabine, and Vinorelbine on human cervical cancer cells (HeLa). Biology and Medicine 3: 60–71.

Ali AQ, Teoha SG, Salhin A, Eltayeb NE, Ahamed MBK, Majid AMSA (2014). Synthesis of isatin thiosemicarbazones derivatives: in vitro anti-cancer, DNA binding and cleavage activities. Spectrochim Acta A 125: 440–448.

Bergman J (1973). Synthesis of 2,3′-biindolyl from indole in one-step reactions. J Heterocycl Chem 10: 121.

Bergman J, Eklund N (1980). Synthesis of 2,2'-biindolyls by coupling reactions. Tetrahedron 36: 1439–1445.

Black DSC (1993). New dimensions in indole chemistry: from ligand design to natural products. Synlett 1993: 246–252.

Bocchi V, Palla V (1982). High yield selective bromination and iodination of indoles in N,N-dimethylformamide. Synthesis 1982: 1096.

Bocchi V, Palla V (1983). A new procedure for the synthesis of 2-(2-indolyl)pyrroles, 2,3-bi-indolyls, and 2-(3-indolyl)-3-(2-indolyl)indoles. J Chem Soc Chem Commun 1983: 1074.

Bocchi V, Palla V (1984). Synthesis and spectroscopic characteristics of 2,3,-biindolyl and 2,2-indolylpyrroles. Tetrahedron 40: 3251–3256.

Chen MF, Chen LT, Boyce HW Jr (1995). 5-Fluorouracil cytotoxicity in human colon HT-29 cells with moderately increased or decreased cellular glutathione level. Anticancer Res 15: 163–167.

Chinni SR, Sarkar FH (2002). Experimental therapeutics, preclinical pharmacology akt inactivation is a key event in indole-3-carbinol-induced apoptosis in PC-3 cells. Clin Cancer Res 8: 1228–1236.

Dupeyre G, Lemoine P, Ainseba N, Michela S, Cachet X (2011). A one-pot synthesis of 7-phenylindolo[3,2-a]carbazoles from indoles and b-nitrostyrenes, via an unprecedented reaction sequence. Org Biomol Chem 9: 7780–7790.

Ferguson PJ, Collins O, Dean NM, DeMoor J, Li CS, Vincent D, Koropatnick J (1999). Antisense down-regulation of thymidylate synthase to suppress growth and enhance cytotoxicity of 5-FUdR, 5-FU and Tomudex in HeLa cells. Brit J Pharmacol 127: 1777–86.

Frydoonfar HR, McGrath DR, Spigelman AD (2002). Inhibition of proliferation of a colon cancer cell line by indole-3-carbinol. Colorectal Dis 4: 205–207.

Frydoonfar HR, McGrath DR, Spigelman AD (2003). The effect of indole-3-carbinol and sulforaphane on a prostate cancer cell line. ANZ J Surg 73: 154–156.

Gilow HM, Brown CS, Copeland JN, Kelly KE (1991). Sulfenylation of some pyrroles and indoles. J Heterocyclic Chem 28: 1025–1034.

Gong J, Traganos F, Darzynkiewicz Z (1994). A selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. Anal Biochem 218: 314–319.

Gribble G (2003). Novel chemistry of indole in the synthesis of heterocycles. Pure Appl Chem 75: 1417–1432.

Hadden MK, Blagg BSJ (2008). Dimeric approaches to anti-cancer chemotherapeutics. Anti-Cancer Agent Me 7: 807–816.

Higasio YS, Shoji T (2001). Heterocyclic compounds such as pyrroles, pyridines, pyrollidins, piperdines, indoles, imidazol and pyrazins. Appl Catal A-Gen 221: 197–207.

Hong C, Firestone GL, Bjeldanes LF (2002). Bcl-2 family-mediated apoptotic effects of 3,3′-diindolylmethane (DIM) in human breast cancer cells. Biochem Pharmacol 63: 1085–1097.

Howells LM, Gallacher-Horley B, Houghton CE, Manson MM, Hudson EA (2002). Indole-3-carbinol inhibits protein kinase B/Akt and induces apoptosis in the human breast tumor cell line MDA MB468 but not in the nontumorigenic hbl100 line. Mol Cancer Ther 1: 1161–1172.

Ji YY, Zhu YM, Wang JW (2013). GS-2, a pyrazolo[1,5-a]indole derivative with inhibitory activity of topoisomerases, exerts its potent cytotoxic activity by ROS generation. Environ Toxicol Phar 36: 1186–1196.

Joule JA, Mills K (2010). Heterocyclic Chemistry. 5th ed. Oxford, UK: Wiley.

Katdare M, Osborne MP, Telang NT (1998). Inhibition of aberrant proliferation and induction of apoptosis in pre-neoplastic human mammary epithelial cells by natural phytochemicals. Oncol Rep 5: 311–315.

Kawasaki T, Higuchi K (2005). Simple indole alkaloids and those with a nonrearranged monoterpenoid unit. Nat Prod Rep 22: 761–793.

Kianmehr E, Ghanbari M, Faghiha N, Rominger F (2012). A palladium-catalyzed one-pot procedure for the regioselective dimerization and cyanation of indoles. Tetrahedron Lett 53: 1900–1904.

Köprülü TK, Ökten S, Duman S, Tekin Ş, Çakmak O (2013). Determination of anticancer activity and mechanism of action of several new quinoline compounds. In: Proceedings of the 44th World Chemistry Congress. İstanbul, Turkey: IUPAC, p. 781.

Nachshon-Kedmi M, Yannai S, Haj A, Fares FA (2003). Indole-3-carbinol and 3,3′-diindolylmethane induce apoptosis in human prostate cancer cells. Food Chem Toxicol 41: 745–752.

Nakai Y, Goto A, Yamada F, Somei M (2003). Nucleophilic substitution reaction of N-2-(1-hydroxyindol-3-yl)ethylindole-3-acetamide and -1-hydroxyindole-3-acetamide. Heterocycles 60: 1589.

Ökten S, Çakmak O, Erenler R, Tekin Ş, Yüce Ö (2013). Simple and convenient preparation of novel 6,8-disubstituted quinoline derivatives and their promising anticancer activities. Turk J Chem 37: 896–908.

Omura S, Iwai Y, Hirano A, Nakagawa A, Awaya J, Tsuchiya H, Takahashi Y, Masuma R (1977). A new alkaloid AM-2282 of *Streptomyces* origin. Taxonomy, fermentation, isolation and preliminary characterization. J Antibiot (Tokyo) 30: 275–282.

Ottoni O, Neder AVF, Dias AKB, Cruz RPA, Aquino LB (2001). Acylation of indole under Friedel-Crafts conditions—an improved method to obtain 3-acylindoles regioselectively. Org Lett 3: 1005–1007.

Parvatkar PT, Ajay AK, Bhat MK, Parameswaran PS, Tilve SG (2013). Iodine catalyzed one-pot synthesis of chloro-substituted linear and angular indoloquinolines and in vitro antiproliferative activity study of different indoloquinolines. Med Chem Res 22: 88–93.

Rahimi M, Huang KL, Tang CK (2010). 3,3′-Diindolylmethane (DIM) inhibits the growth and invasion of drug-resistant human cancer cells expressing EGFR mutants. Cancer Lett 295: 59–68.

Rahman KM, Aranha O, Sarkar FH (2003). Indole-3-carbinol (I3C) induces apoptosis in tumorigenic but not in nontumorigenic breast epithelial cells. Nutr Cancer 45: 101–112.

Robertson N, Parsons S, MacLean EJ, Coxall RA, Mount AR (2000). Preparation, X-ray structure and properties of a hexabrominated, symmetric indole trimer and its TCNQ adduct: a new route to functional molecular systems. J Mater Chem 10: 2043–2047.

Şahin OY, Okten S, Tekin S, Cakmak O (2012). Determination of anticancer activities of some quinoline derivatives against C6 tumor cells. J Biotechnol 161: 24.

Saito I, Morii T, Matsuura S (1982). A new procedure for the synthesis of 2-substituted indoles and 2,2′-bi-indolyls. J Chem Soc Chem Commun 1982: 977.

Sala GD, Capozzo D, Izzo I, Giordano A, Iommazzo A, Spinella A (2002). Synthesis of antifungal n-isoprenyl-indole alkaloids from the fungus *Aporpium caryae*. Tetrahedron Lett 43: 8839–8841.

Saundane AR, Vijaykumar K, Vaijinath AV, Walmik P (2013). Synthesis, antimicrobial and antioxidant activities of some new indole derivatives containing pyridopyrimidine and pyrazolopyridine moieties. Med Chem Res 22: 806–817.

Singh NG, Kathing C, Rani JWS, Nongkhlaw RL (2014). Synthesis of pharmacologically active bis(indolyl) and tris(indolyl) derivatives using chlorotrimethylsilane. J Chil Chem Soc 61: 442–446.

Sundberg RJ (1996). Indoles. 1st ed. London, UK: Academic Press.

Tang S, Li JH, Xie YX, Wang NX (2007). Halogenation of indoles with copper(II) halides: selective synthesis of 2-halo-, 3-halo-, and 2,3-dibromoindoles. Synthesis 10: 1535–1541.

Wahlström N, Slätt J, Stensland B, Ertan A, Bergman J, Janosik T (2007). Synthetic applications of cyanoacetylated bisindoles: synthesis of novel cycloheptadiindoles, indolocarbazoles, and related aza analogue. J Org Chem 72: 5886–5889.

Weng JR, Tsai CH, Kulp SK, Chen CS (2008). Down regulation of c-Myc and induction of an angiogenesis inhibitor, thrombospondin-1, by 5-FU in human colon cancer KM12C cells. Cancer Lett 262: 153–163.

Yamada F, Goto A, Peng W, Hayashi T, Saga Y, Somei M (2003). Nucleophilic substitution reaction at the 1-position of 1-hydroxytryptamine and -tryptophan derivatives. Heterocycles 61: 163–172.

Yamada F, Shinmyo D, Nakajou M, Somei M (2012). Nucleophilic substitution reaction of 1-methoxyindole-3-carbaldehyde. Heterocycles 86: 435–453.

Zhang L, Zhu Y, Yin G, Lu P, Wang Y (2012). 3-Alkenylation or 3-alkylation of indole with propargylic alcohols: construction of 3,4-dihydrocyclopenta[*b*]indole and 1,4-dihydrocyclopenta[*b*] indole in the presence of different catalysts. J Org Chem 77: 9510–9520.

Zheng Q, Hirose Y, Yoshimi N, Murakami A, Koshimizu K, Ohigashi H (2002). Further investigation of the modifying effect of various chemopreventive agents on apoptosis and cell proliferation in human colon cancer cells. J Cancer Res Clin 128: 539–546.

Zolfigol MA, Khazaei A, Kolvari E, Koukabi N, Soltani H, Behjunia M (2010). H_5IO_6/KI: a new combination reagent for iodination of aromatic amines, and trimethylsilylation of alcohols and phenols through in situ generation of iodine under mild conditions. Helv Chim Acta 93: 587–594.

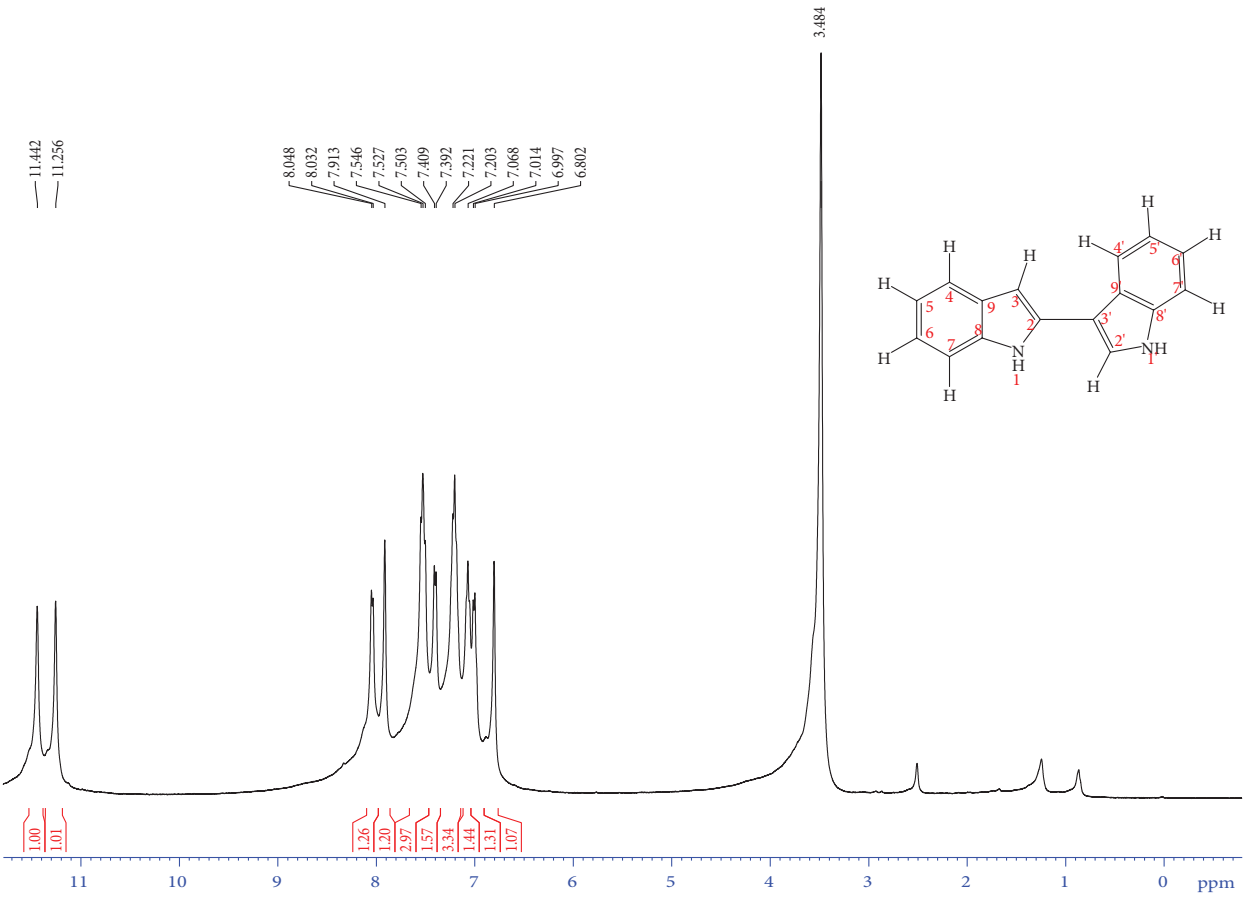

Figure S1. ^{1}H-NMR spectrum of 2,3'-Biindole (**2**).

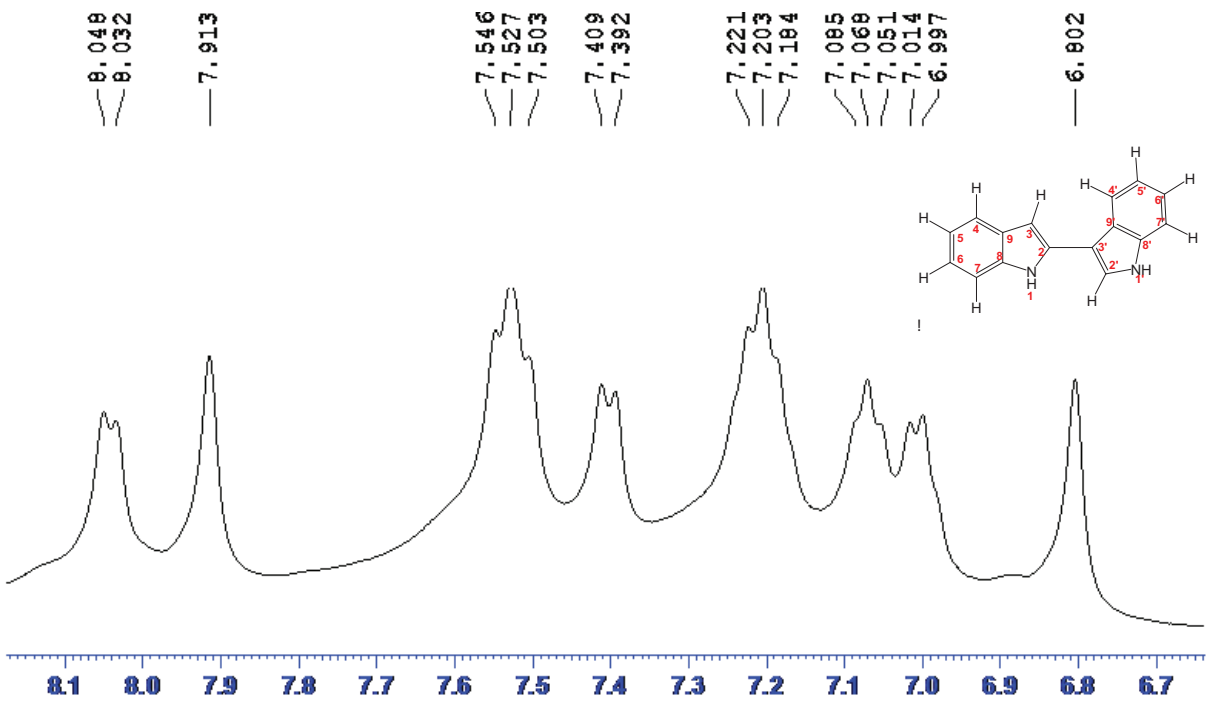

Figure S2. ^{1}H-NMR spectrum of 2,3'-biindole (**2**) (aromatic region).

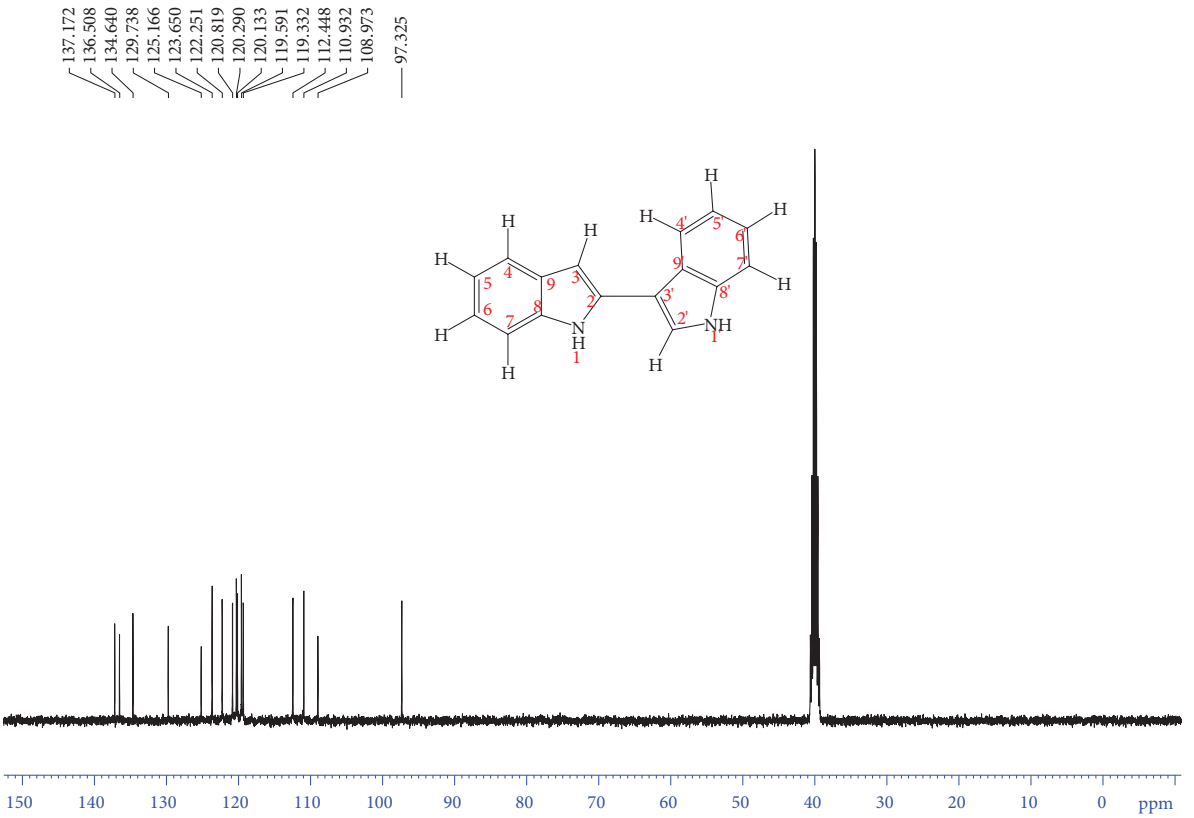

Figure S3. ^{13}C-NMR spectrum of 2,3'-biindole (**2**).

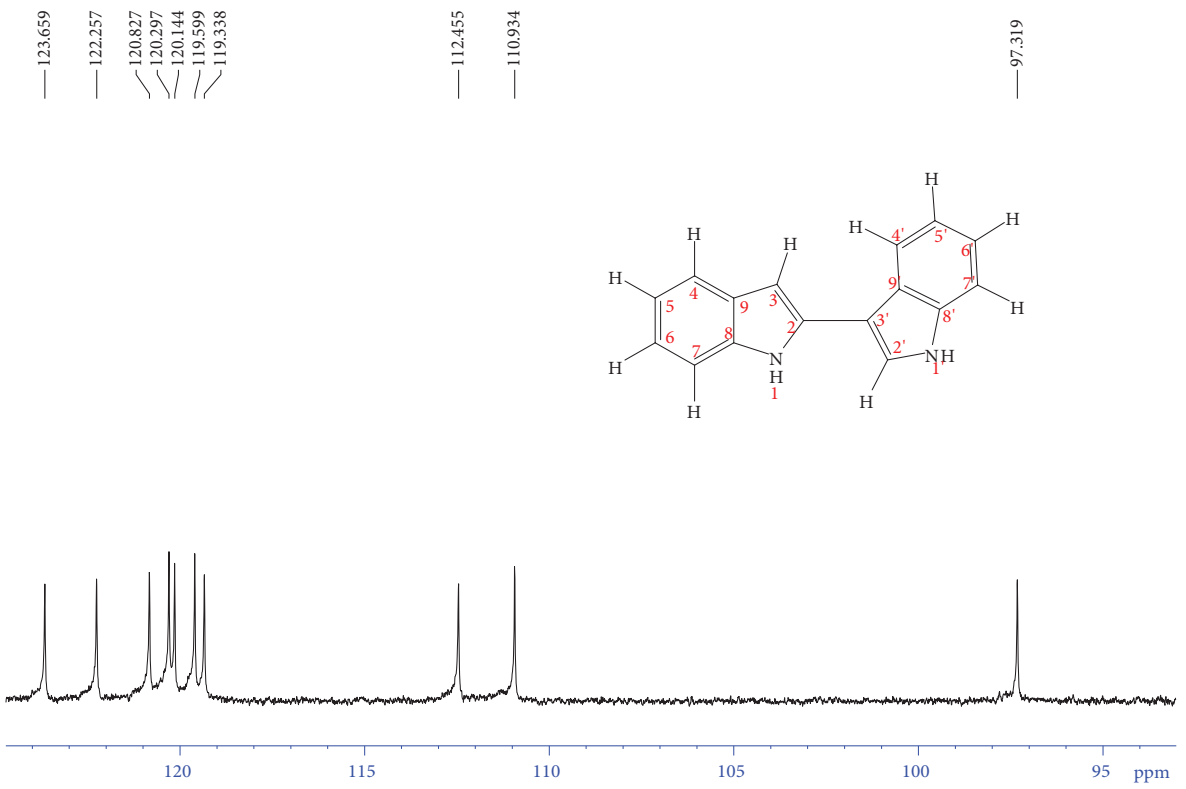

Figure S4. DEPT-90 spectrum of 2,3'-biindole (**2**).

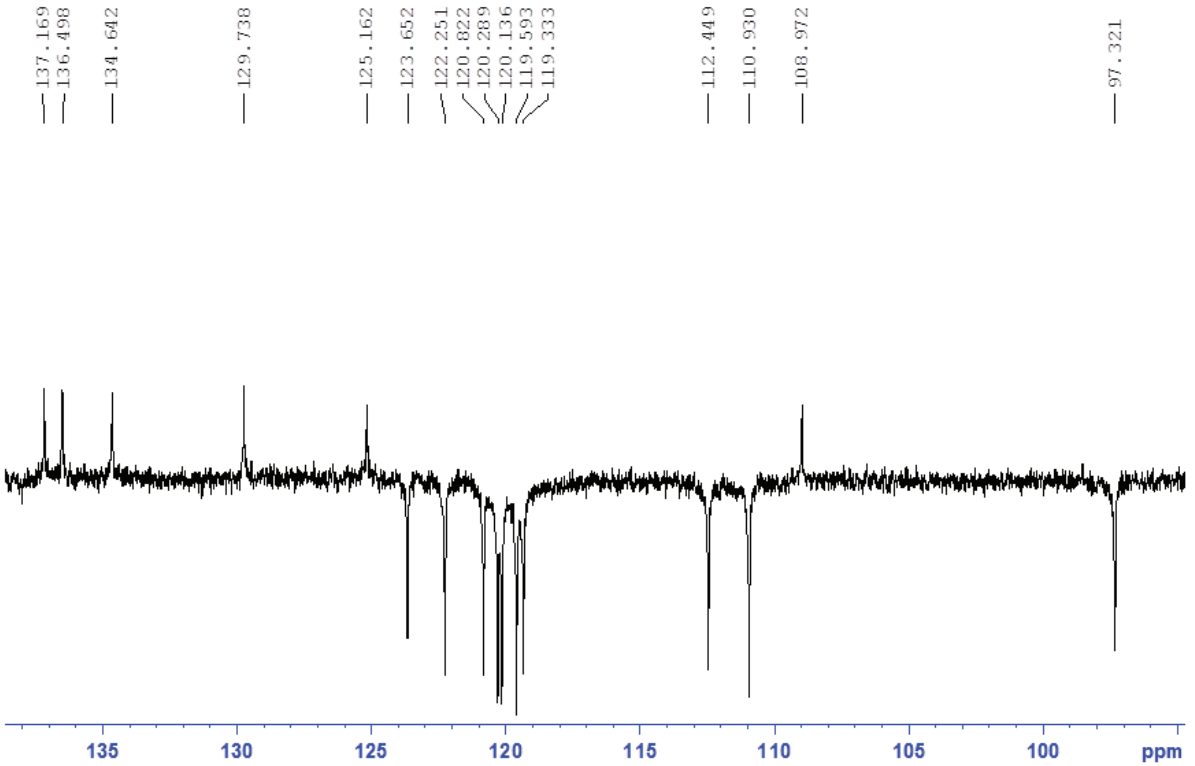

Figure S5. DEPT-135 spectrum of 2,3'-biindole (**2**).

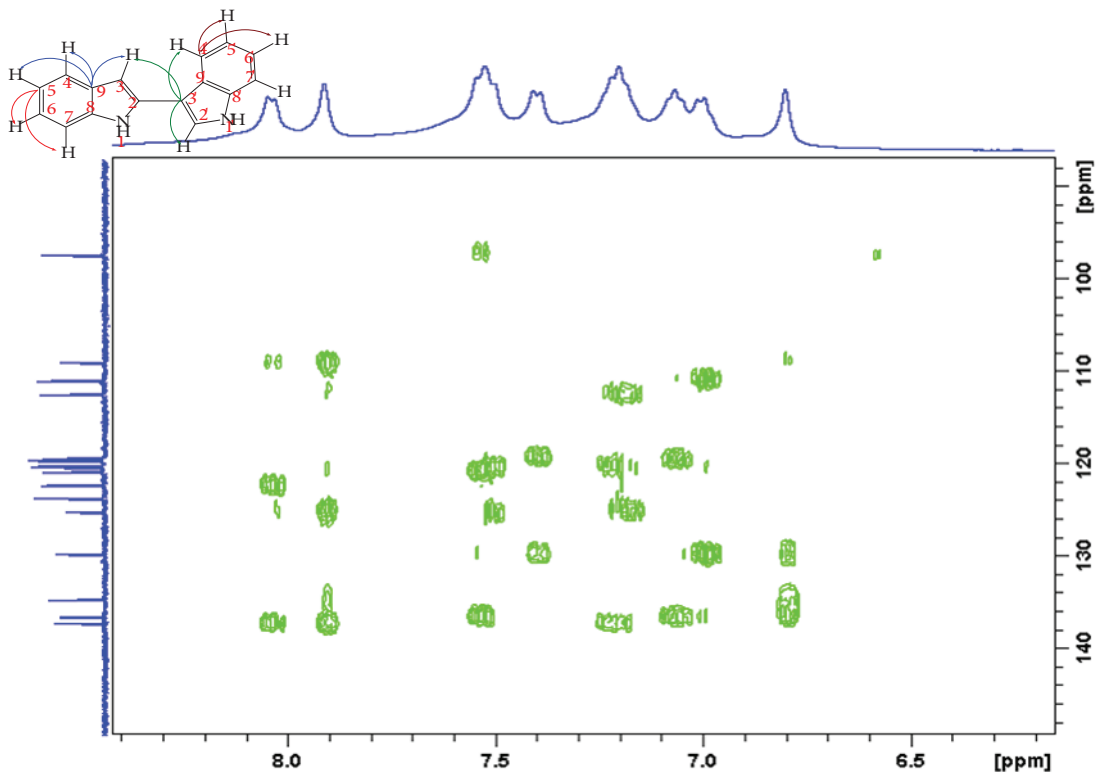

Figure S6. HMBC spectrum of 2,3'-biindole (**2**).

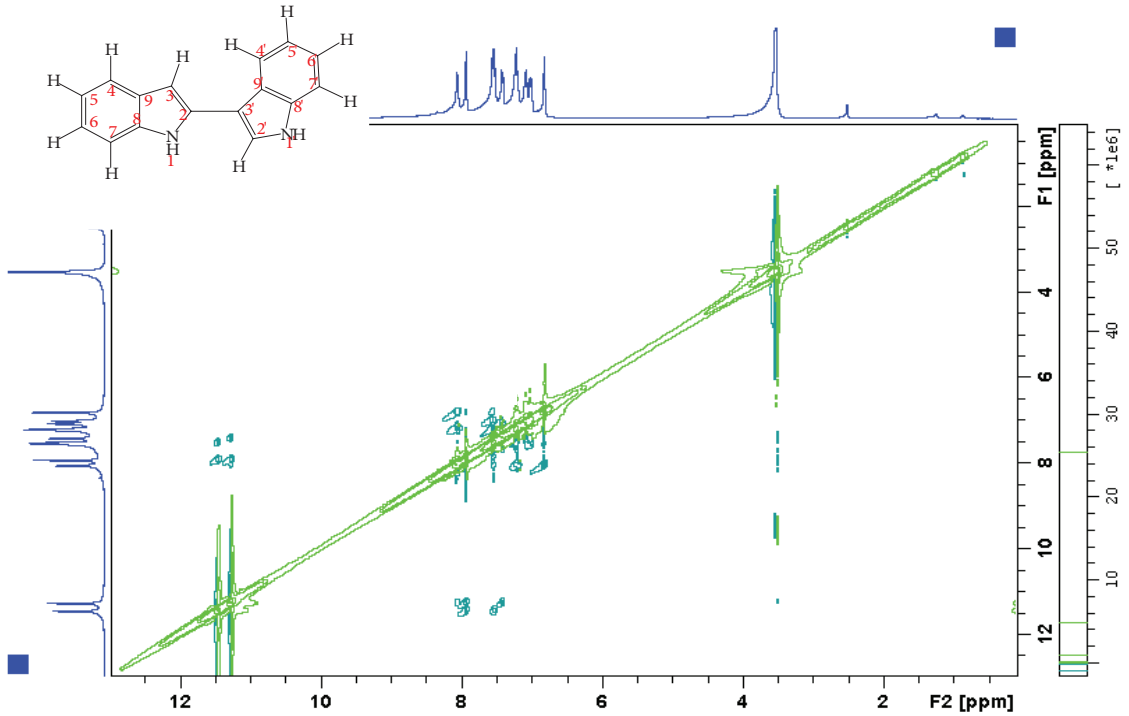

Figure S7. NOESY spectrum of 2,3'-biindole (**2**).

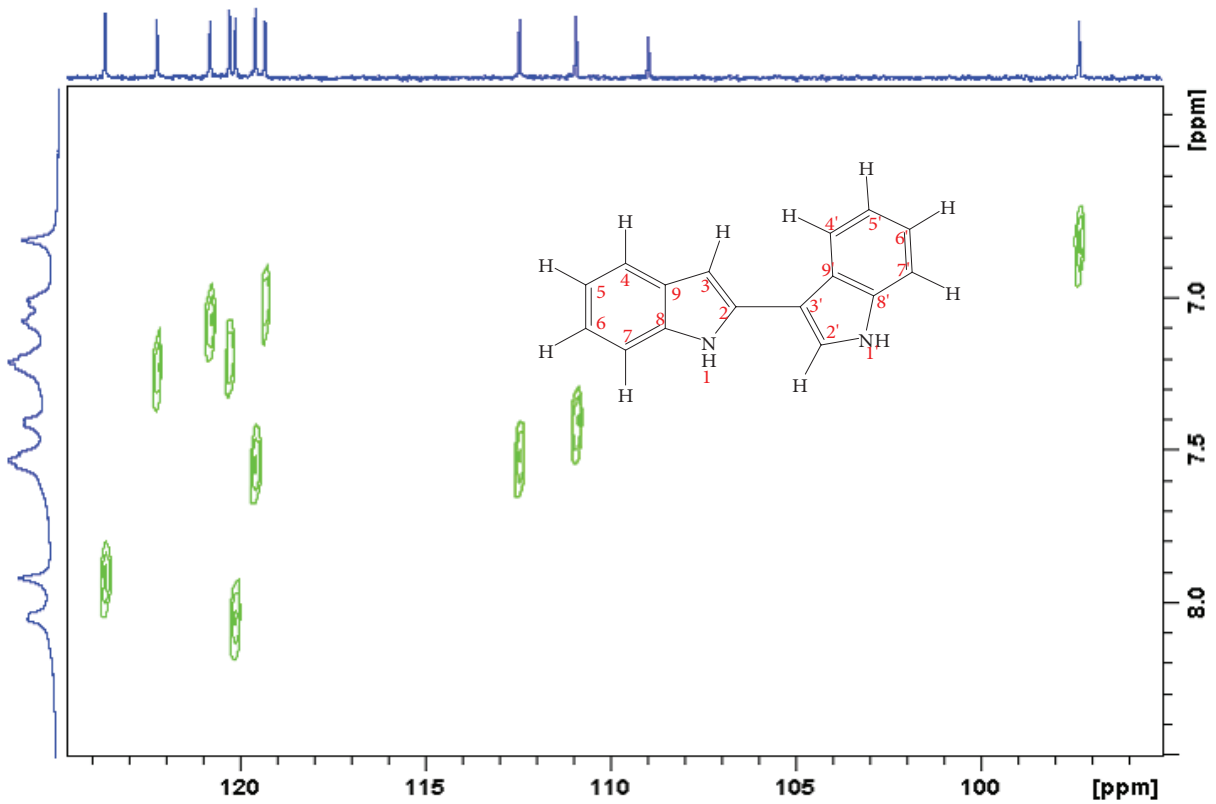

Figure S8. HETCOR spectrum of 2,3'-biindole (**2**).