

# Synthesis and Cytotoxic Activity of Novel 3-methyl-1-[(4-substitutedpiperazin-1-yl)methyl]-1*H*-indole Derivatives

## Authors

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## Key words

- anticancer
- apoptosis
- indole
- Mannich base
- 1,4-disubstitutedpiperazines

## Abstract

▼  
 A series of novel 3-methyl-1-[(4-substitutedpiperazin-1-yl)methyl]-1*H*-indoles (**3a–l**) were synthesized and their cytotoxicities were analyzed against 3 different human cell lines, including liver (HUH7), breast (MCF7) and colon (HCT116). The Mannich reaction of 3-methylindole (**1**) with 4-substitutedpiperazines (**2**) and formaldehyde resulted to the 3-methyl-1-[(4-substitutedpiperazin-1-yl)methyl]-1*H*-indoles (**3a–l**) in 38–69% yields. The investigation of anticancer screening

revealed that the tested compounds showed comparable activity to the reference drug 5-fluorouracil and compounds **3g**, **3h**, **3i** and **3k**, had lower 50% inhibition (IC<sub>50</sub>) concentration than reference drug. Moreover, the cytotoxic effect of the most potent compound **3h** on HUH7 and MCF7 cells through apoptosis was visualized by Hoechst staining and compared with paclitaxel, which is a mitotic inhibitor acting on microtubules. The morphological features of apoptosis were observed as condensed and fragmented nuclei that are similar to paclitaxel.

## Introduction

▼  
 Cancer treatment has been a major attempt of research in academia and pharmaceutical industry for many years as it is one of the leading causes of death [1,2]. Recent drug discovery efforts are highly focused towards design and synthesis of small molecules as anticancer agents due to the advantages of easier synthesis and lower cost. A wide variety of heterocyclic systems have been explored for the development of novel chemical entities as a lead molecule in anticancer drug discovery [3–5].

Microtubules, one of the basic components of cell structure, are involved in a wide number of vital cellular functions, such as motility, division, shape maintenance and cellular transport [6]. The research for novel drugs that can modulate the microtubule assembly either by inhibition of tubulin polymerization or by blocking microtubule disassembly are of great interest in anticancer therapy. Vincristine and vinblastine are among the earliest antitumor agents recognized as tubulin polymerization inhibitors since 1965 [7]. The indole ring is represented as the core nucleus of several tubulin polymerization inhibitors [7,8]. In the last decade, an increasing number of small synthetic molecules with indole ring as

potent anticancer agents have been reported [7,9–11]. A series of small indole containing drugs and clinical candidates are given in ○ Fig. 1. Despite the fact that indole is core structure for inhibition of tubulin polymerization, numerous papers have also shown that Mannich base analogs of heterocyclic rings exhibited potent cytotoxicity against several human tumor cell lines [12–18]. Dimmock and Kumar reviewed anticancer and cytotoxic properties of Mannich bases and outlined the effects of these compounds on anticancer activity [19].

Based on these prior observations, we designed new Mannich base analogues of 3-methylindole and aimed to evaluate their in vitro anticancer screening data against different cancer cell lines.

## Materials and Methods

### Chemistry

▼  
 All chemicals and reagents used in current study were analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points were determined by using a Mettler Toledo FP62 capillary melting point apparatus (Mettler-Toledo, Greifensee, Switzerland) and are

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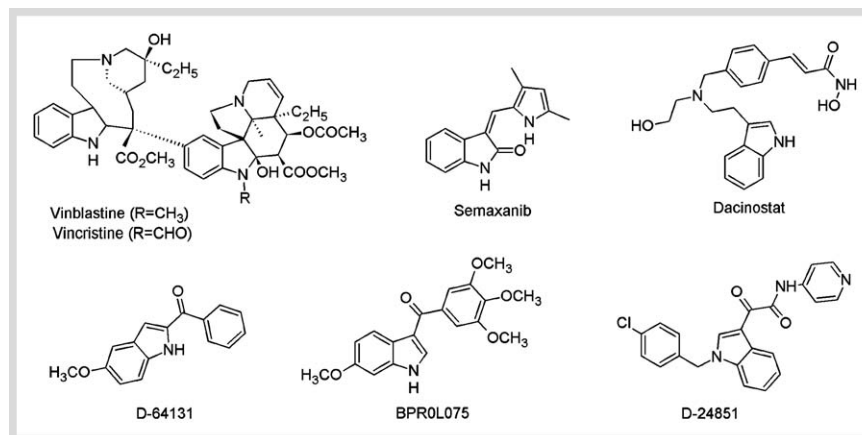
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**Fig. 1** Structure comparison of small indole molecules as anticancer agents.

uncorrected. Infrared spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1) (Perkin Elmer, Norwalk, CT, USA), using potassium bromide pellets, the frequencies were expressed in  $\text{cm}^{-1}$ . The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian, Palo Alto, CA, USA), using tetramethylsilane as the internal reference, with chloroform- $\text{CDCl}_3$  as solvent, the chemical shifts were reported in parts per million (ppm) and coupling constants ( $J$ ) were given in hertz (Hz). Elemental analyses were performed on LECO 932 CHNS instrument (Leco-932, St. Joseph, MI, USA) and were within  $\pm 0.4\%$  of the theoretical values.

#### General procedure for the synthesis of 3-methyl-1-[(4-substitutedpiperazin-1-yl)methyl]-1H-indoles (3a–3l)

A mixture of 3-methylindole (**1**) (0.39 g, 0.003 mol), the appropriate N-substituted amine (**2**) (0.003 mol) and 37% formaldehyde solution (1 mL) in ethanol (15 mL), was refluxed for 3–5 h. The crude products were either precipitated or it was necessary to add water in case not precipitated. The crude products were filtered, washed with water, dried and crystallized from appropriate solvents.

#### 3-Methyl-1-[(4-phenylpiperazin-1-yl)methyl]-1H-indole (3a)

Yield: 69%; m.p.: 160.2 °C. IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3054–2797 (C-H).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 7.56 (d, 1H, indole  $\text{H}_4$ ,  $J=11.6$ ), 7.43 (d, 1H, indole  $\text{H}_7$ ,  $J=8$ ), 7.25–7.20 (m, 3H, indole  $\text{H}_5$ +phenyl  $\text{H}_2$ ,  $\text{H}_6$ ), 7.12 (t, 1H, indole  $\text{H}_6$ ,  $J=8$ ), 6.95 (s, 1H, indole  $\text{H}_2$ ), 6.88–6.82 (m, 3H, phenyl  $\text{H}_3$ ,  $\text{H}_4$ ,  $\text{H}_5$ ), 4.81 (s, 2H, N- $\text{CH}_2$ -N), 3.17 (t, 4H, piperazine  $\text{H}_3$ ,  $\text{H}_5$ ,  $J=5.2$ ), 2.69 (t, 4H, piperazine  $\text{H}_2$ ,  $\text{H}_6$ ,  $J=5.2$ ), 2.33 (s, 3H, - $\text{CH}_3$ ). Anal. calcd. for  $\text{C}_{20}\text{H}_{23}\text{N}_3$  (305.42): C, 78.65; H, 7.59; N, 13.76. Found: C, 78.57; H, 7.56; N, 13.60.

#### 1-[[4-(2-Fluorophenyl)piperazin-1-yl]methyl]-3-methyl-1H-indole (3b)

Yield: 60%; m.p.: 120.2 °C. IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3045–2788 (C-H).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 7.56 (d, 1H, indole  $\text{H}_4$ ,  $J=7.6$ ), 7.43 (d, 1H, indole  $\text{H}_7$ ,  $J=8.4$ ), 7.22 (t, 1H, indole  $\text{H}_5$ ,  $J=8.0$ ), 7.12 (t, 1H, indole  $\text{H}_6$ ,  $J=6.8$ ), 7.05–6.88 (m, 5H, indole  $\text{H}_2$ +phenyl), 4.81 (s, 2H, N- $\text{CH}_2$ -N), 3.08 (t, 4H, piperazine  $\text{H}_3$ ,  $\text{H}_5$ ,  $J=4.8$ ), 2.72 (t, 4H, piperazine  $\text{H}_2$ ,  $\text{H}_6$ ,  $J=4.8$ ), 2.33 (s, 3H, - $\text{CH}_3$ ). Anal. calcd. for  $\text{C}_{20}\text{H}_{22}\text{FN}_3$  (323.41): C, 74.28; H, 6.86; N, 12.99. Found: C, 74.24; H, 6.89; N, 12.98.

#### 1-[[4-(4-Fluorophenyl)piperazin-1-yl]methyl]-3-methyl-1H-indole (3c) [20]

Yield: 51%; m.p.: 109.5 °C. IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3046–2788 (C-H).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 7.55 (d, 1H, indole  $\text{H}_4$ ,  $J=8$ ), 7.43 (d, 1H, indole  $\text{H}_7$ ,  $J=8.8$ ), 7.21 (t, 1H, indole  $\text{H}_5$ ,  $J=6.8$ ), 7.12 (t, 1H, indole  $\text{H}_6$ ,  $J=6.8$ ), 6.93 (dd, 2H, phenyl  $\text{H}_3$ ,  $\text{H}_5$ ,  $J=6.0$ ,  $J'=2.8$ ), 6.90 (s, 1H, indole  $\text{H}_2$ ), 6.81 (dd, 2H, phenyl  $\text{H}_2$ ,  $\text{H}_6$ ,  $J=9.4$ ,  $J'=4.4$ ), 4.80 (s, 2H, N- $\text{CH}_2$ -N), 3.08 (t, 4H, piperazine  $\text{H}_3$ ,  $\text{H}_5$ ,  $J=5.2$ ), 2.69 (t, 4H, piperazine  $\text{H}_2$ ,  $\text{H}_6$ ,  $J=5.2$ ), 2.33 (s, 3H, - $\text{CH}_3$ ).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 158.65, 156.27, 148.14, 137.48, 129.17, 126.33, 121.92, 119.20, 118.25, 115.72, 111.15, 110.01 (aromatics), 67.86 (C- $\text{CH}_2$ -N), 50.72 (piperazine  $\text{C}_3$ ,  $\text{C}_5$ ), 50.33 (piperazine  $\text{C}_2$ ,  $\text{C}_6$ ), 9.83 (- $\text{CH}_3$ ). Anal. calcd. for  $\text{C}_{20}\text{H}_{22}\text{FN}_3$  (323.41): C, 74.28; H, 6.86; N, 12.99. Found: C, 74.25; H, 6.75; N, 12.96.

#### 1-[[4-(3-Chlorophenyl)piperazin-1-yl]methyl]-3-methyl-1H-indole (3d)

Yield: 42%; m.p.: 95.4 °C. IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3043–2718 (C-H).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 7.55 (d, 1H, indole  $\text{H}_4$ ,  $J=8.0$ ), 7.41 (d, 1H, indole  $\text{H}_7$ ,  $J=8.4$ ), 7.21 (t, 1H, indole  $\text{H}_5$ ,  $J=8.0$ ), 7.14–7.09 (m, 2H, indole  $\text{H}_6$ +phenyl  $\text{H}_2$ ), 6.93 (s, 1H, indole  $\text{H}_2$ ), 6.81–6.69 (m, 3H, phenyl), 4.78 (s, 2H, N- $\text{CH}_2$ -N), 3.14 (t, 4H, piperazine  $\text{H}_3$ ,  $\text{H}_5$ ,  $J=5.2$ ), 2.65 (t, 4H, piperazine  $\text{H}_2$ ,  $\text{H}_6$ ,  $J=5.2$ ), 2.32 (s, 3H, - $\text{CH}_3$ ). Anal. calcd. for  $\text{C}_{20}\text{H}_{22}\text{ClN}_3$  (339.86): C, 70.68; H, 6.52; N, 10.43. Found: C, 70.41; H, 6.65; N, 10.38.

#### 1-[[4-(4-Chlorophenyl)piperazin-1-yl]methyl]-3-methyl-1H-indole (3e)

Yield: 58%; m.p.: 144.1 °C. IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3041–2794 (C-H).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 7.56 (d, 1H, indole  $\text{H}_4$ ,  $J=7.6$ ), 7.41 (d, 1H, indole  $\text{H}_7$ ,  $J=8.4$ ), 7.24–7.10 (m, 4H, indole  $\text{H}_5$ ,  $\text{H}_6$ +phenyl  $\text{H}_3$ ,  $\text{H}_5$ ), 6.93 (s, 1H, indole  $\text{H}_2$ ), 6.76 (d, 2H, phenyl  $\text{H}_2$ ,  $\text{H}_6$ ,  $J=9.2$ ), 4.79 (s, 2H, N- $\text{CH}_2$ -N), 3.11 (t, 4H, piperazine  $\text{H}_3$ ,  $\text{H}_5$ ,  $J=5.2$ ), 2.67 (t, 4H, piperazine  $\text{H}_2$ ,  $\text{H}_6$ ,  $J=5.2$ ), 2.32 (s, 3H, - $\text{CH}_3$ ). Anal. calcd. for  $\text{C}_{20}\text{H}_{22}\text{ClN}_3$  (339.86): C, 70.68; H, 6.52; N, 10.43. Found: C, 70.56; H, 6.56; N, 10.39.

#### 1-[[4-(2-Methoxyphenyl)piperazin-1-yl]methyl]-3-methyl-1H-indole (3f)

Yield: 53%; m.p.: 114.5 °C. IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 3046–2788 (C-H).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  (ppm): 7.56 (d, 1H, indole  $\text{H}_4$ ,  $J=8.0$ ), 7.43 (d, 1H, indole  $\text{H}_7$ ,  $J=8.4$ ), 7.21 (t, 1H, indole  $\text{H}_5$ ,  $J=7.6$ ), 7.11 (t, 1H, indole  $\text{H}_6$ ,  $J=6.8$ ), 7.00–6.89 (m, 4H, indole  $\text{H}_2$ +phenyl  $\text{H}_3$ ,  $\text{H}_4$ ,  $\text{H}_5$ ), 6.82 (d, 1H, phenyl  $\text{H}_6$ ,  $J=7.2$ ), 4.82 (s, 2H, N- $\text{CH}_2$ -N),

3.80 (s, 3 H, O-CH<sub>3</sub>), 3.05 (bs, 4 H, piperazine H<sub>3</sub>, H<sub>5</sub>), 2.74 (t, 4 H, piperazine H<sub>2</sub>, H<sub>6</sub>, *J*=4.8), 2.32 (s, 3 H, -CH<sub>3</sub>). Anal. calcd. for C<sub>21</sub>H<sub>25</sub>N<sub>3</sub>O (335.44): C, 75.19; H, 7.51; N, 12.53. Found: C, 75.12; H, 7.40; N, 12.43.

### 1-[[4-(3-Methoxyphenyl)piperazin-1-yl]methyl]-3-methyl-1H-indole (3g)

Yield: 57%; m. p.: 111.6 °C. IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3050–2842 (C-H). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  (ppm): 7.56 (d, 1 H, indole H<sub>4</sub>, *J*=8.0), 7.42 (d, 1 H, indole H<sub>7</sub>, *J*=8.4), 7.24–7.10 (m, 3 H, indole H<sub>5</sub>, H<sub>6</sub>+phenyl H<sub>2</sub>), 6.94 (s, 1 H, indole H<sub>2</sub>), 6.49–6.38 (m, 3 H, phenyl), 4.80 (s, 2 H, N-CH<sub>2</sub>-N), 3.76 (s, 3 H, O-CH<sub>3</sub>), 3.16 (t, 4 H, piperazine H<sub>3</sub>, H<sub>5</sub>, *J*=5.2), 2.67 (t, 4 H, piperazine H<sub>2</sub>, H<sub>6</sub>, *J*=4.8), 2.32 (s, 3 H, -CH<sub>3</sub>). Anal. calcd. for C<sub>21</sub>H<sub>25</sub>N<sub>3</sub>O (335.44): C, 75.19; H, 7.51; N, 12.53. Found: 75.18; H, 7.53; N, 12.45.

### 1-[[4-(4-Cyanophenyl)piperazin-1-yl]methyl]-3-methyl-1H-indole (3h)

Yield: 48%; m. p.: 139.5 °C. IR (KBr)  $\nu$  (cm<sup>-1</sup>): 2938–2832 (C-H), 2211 (C≡N). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  (ppm): 7.56 (d, 1 H, indole H<sub>4</sub>, *J*=7.6), 7.44–7.39 (m, 3 H, indole H<sub>7</sub>+phenyl H<sub>3</sub>, H<sub>5</sub>), 7.21 (t, 1 H, indole H<sub>5</sub>, *J*=7.2), 7.12 (t, 1 H, indole H<sub>6</sub>, *J*=7.6), 6.91 (s, 1 H, indole H<sub>2</sub>), 6.74 (d, 2 H, phenyl H<sub>2</sub>, H<sub>6</sub>, *J*=8.8), 4.78 (s, 2 H, N-CH<sub>2</sub>-N), 3.26 (t, 4 H, piperazine H<sub>3</sub>, H<sub>5</sub>, *J*=4.8), 2.63 (t, 4 H, piperazine H<sub>2</sub>, H<sub>6</sub>, *J*=4.8), 2.32 (s, 3 H, -CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  (ppm): 153.44, 137.44, 133.71, 129.20, 126.32, 122.08, 120.30 (aromatics), 119.32 (-CN), 114.47, 111.30, 109.98, 100.49 (aromatics), 67.74 (C-CH<sub>2</sub>-N), 50.21 (piperazine C<sub>3</sub>, C<sub>5</sub>), 47.21 (piperazine C<sub>2</sub>, C<sub>6</sub>), 9.87 (-CH<sub>3</sub>). Anal. calcd. for C<sub>21</sub>H<sub>22</sub>N<sub>4</sub> (330.43): C, 76.33; H, 6.71; N, 16.96. Found: C, 76.29; H, 6.46; N, 16.93.

### 3-Methyl-1-[[4-(4-nitrophenyl)piperazin-1-yl]methyl]-1H-indole (3i)

Yield: 48%; m. p.: 134 °C. IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3042–2852 (C-H), 1332 (NO<sub>2</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  (ppm): 8.08 (d, 2 H, phenyl H<sub>3</sub>, H<sub>5</sub>, *J*=9.6), 7.56 (d, 1 H, indole H<sub>4</sub>, *J*=7.6), 7.42 (d, 1 H, indole H<sub>7</sub>, *J*=8.4), 7.22 (t, 1 H, indole H<sub>5</sub>, *J*=7.2), 7.15 (t, 1 H, indole H<sub>6</sub>, *J*=7.6), 6.93 (s, 1 H, indole H<sub>2</sub>), 6.74 (d, 2 H, phenyl H<sub>2</sub>, H<sub>6</sub>, *J*=9.6), 4.82 (s, 2 H, N-CH<sub>2</sub>-N), 3.40 (t, 4 H, piperazine H<sub>3</sub>, H<sub>5</sub>, *J*=4.8), 2.68 (t, 4 H, piperazine H<sub>2</sub>, H<sub>6</sub>, *J*=4.8), 2.33 (s, 3 H, -CH<sub>3</sub>). Anal. calcd. for C<sub>20</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub> (350.41): C, 68.55; H, 6.33; N, 15.99. Found: 68.71; H, 6.27; 15.94.

### 3-Methyl-1-[[4-(4-methylphenyl)piperazin-1-yl]methyl]-1H-indole (3j)

Yield: 45%; m. p.: 117.3 °C. IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3048–2789 (C-H). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  (ppm): 7.55 (d, 1 H, indole H<sub>4</sub>, *J*=8.0), 7.42 (d, 1 H, indole H<sub>7</sub>, *J*=8.4), 7.21 (t, 1 H, indole H<sub>5</sub>, *J*=7.6), 7.11 (t, 1 H, indole H<sub>6</sub>, *J*=6.8), 7.04 (d, 2 H, phenyl H<sub>3</sub>, H<sub>5</sub>, *J*=8.4), 6.94 (s, 1 H, indole H<sub>2</sub>), 6.78 (d, 2 H, phenyl H<sub>2</sub>, H<sub>6</sub>, *J*=8.4), 4.80 (s, 2 H, N-CH<sub>2</sub>-N), 3.11 (t, 4 H, piperazine H<sub>3</sub>, H<sub>5</sub>, *J*=4.8), 2.68 (t, 4 H, piperazine H<sub>2</sub>, H<sub>6</sub>, *J*=5.2), 2.32 (s, 3 H, -CH<sub>3</sub>), 2.25 (s, 3 H, -CH<sub>3</sub>). Anal. calcd. for C<sub>21</sub>H<sub>25</sub>N<sub>3</sub> (319.44): C, 78.96; H, 7.89; N, 13.15. Found: C, 79.28; H, 7.85; N, 12.97.

### 3-Methyl-1-[[4-(2,3-dimethylphenyl)piperazin-1-yl]methyl]-1H-indole (3k)

Yield: 43%; m. p.: 108.7 °C. IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3053–2785 (C-H). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  (ppm): 7.57 (d, 1 H, indole H<sub>4</sub>, *J*=7.6), 7.45 (d, 1 H, indole H<sub>7</sub>, *J*=8.0), 7.22 (t, 1 H, indole H<sub>5</sub>, *J*=7.2), 7.15 (t, 1 H, indole H<sub>6</sub>, *J*=7.6), 7.05 (t, 1 H, phenyl H<sub>5</sub>, *J*=6.8), 6.96

(s, 1 H, indole H<sub>2</sub>), 6.90 (d, 2 H, phenyl H<sub>4</sub>, H<sub>6</sub>, *J*=8.8), 4.84 (s, 2 H, N-CH<sub>2</sub>-N), 2.88 (t, 4 H, piperazine H<sub>3</sub>, H<sub>5</sub>, *J*=4.4), 2.72 (bs, 4 H, piperazine H<sub>2</sub>, H<sub>6</sub>), 2.34 (s, 3 H, -CH<sub>3</sub>), 2.23 (s, 3 H, -CH<sub>3</sub>), 2.14 (s, 3 H, -CH<sub>3</sub>). Anal. calcd. for C<sub>22</sub>H<sub>27</sub>N<sub>3</sub> (333.47): C, 79.24; H, 8.16; N, 12.60. Found: C, 79.10; H, 8.11; N, 12.52.

### 3-Methyl-1-[[4-(2-phenylethyl)piperazin-1-yl]methyl]-1H-indole (3l) [20]

Yield: 39%; m. p.: 122.9 °C. IR (KBr)  $\nu$  (cm<sup>-1</sup>): 3022–2763 (C-H). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  (ppm): 7.54 (d, 1 H, indole H<sub>4</sub>, *J*=8.0), 7.41 (d, 1 H, indole H<sub>7</sub>, *J*=8.0), 7.28–7.16 (m, 6 H, indol H<sub>5</sub>+phenyl), 7.11 (t, 1 H, indole H<sub>6</sub>, *J*=7.2), 6.92 (s, 1 H, indole H<sub>2</sub>), 4.76 (s, 2 H, N-CH<sub>2</sub>-N), 2.75 (m, 2 H, -CH<sub>2</sub>-C<sub>6</sub>H<sub>5</sub>), 2.60–2.54 (m, 10 H, -CH<sub>2</sub>-N), 2.31 (s, 3 H, -CH<sub>3</sub>). Anal. calcd. for C<sub>28</sub>H<sub>31</sub>N<sub>3</sub> (409.57): C, 79.24; H, 8.16; N, 12.56. Found: C, 79.20; H, 8.15; N, 12.56.

## Cytotoxicity studies

### Cell culture

The human cancer cell lines were grown in Dulbecco's Modified Eagle's Medium (DMEM), with 10% fetal bovine serum (FBS) and 1% penicillin. They were incubated in 37 °C incubators containing 5% CO<sub>2</sub> and 95% air.

### NCI-60 Sulphorhodamine B (SRB) assay

Cancer cells (range of 2000 cells/well to 5000 cells/well) were inoculated into 96-well plates in 200  $\mu$ L of media and incubated in 37 °C incubators containing 5% CO<sub>2</sub> and 95% air. After a 24 h incubation period, one plate for each cell line was fixed with 100  $\mu$ L of 10% ice-cold trichloroacetic acid (TCA). This plate represents the behavior of the cells just prior to compound treatment and is accepted as the time-zero plate. The compounds to be tested were solubilized in dimethyl sulfoxide (DMSO) to a final concentration of 40 mM and stored at +4 °C. While treating the cells with the compounds, the corresponding volume of the compound was applied to the cell to achieve the desired drug concentration and diluted through serial dilution (40, 20, 10, 5, 2.5  $\mu$ M). After drug treatment, the cells were incubated in 37 °C incubators containing 5% CO<sub>2</sub> and 95% air for 72 h. Following the termination of the incubation period after drug treatment, the cells were fixed with 100  $\mu$ L 10% ice-cold TCA and incubated in the dark at +4 °C for 1 h. Then the TCA was washed away with ddH<sub>2</sub>O 5 times and the plates were left to air dry. In the final step, the plates were stained with 100  $\mu$ L of 0.4% SRB (cat.86183-5 g from Sigma) solution in 1% acetic acid solution. Following staining, the plates were incubated in dark for 10 min at room temperature. The unbound dye was washed away using 1% acetic acid and the plates were left to air dry. To measure the absorbance results, the bound stain was then solubilized using 200  $\mu$ L of 10 mM Tris-Base. Camptothecin was the positive control and 5-Fluorouracil (5-FU) was standard drug for the cytotoxic effect. The OD values were obtained at 515 nm.

### Hoechst staining

Apoptotic morphological alterations were visualized by Hoechst 33258 staining under fluorescent microscope. Cells were inoculated into 6-well plates (60 000 cell/well) and incubated for 24 h. Then, they were treated with solvent DMSO, **3h** or paclitaxel and incubated for 72 h. Fixation of the cells were accomplished by methanol followed by Hoechst 33258 staining. Finally, cells were destained with ddH<sub>2</sub>O and observed under fluorescent microscope.

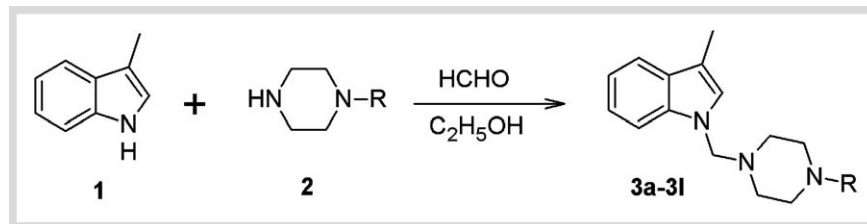


Fig. 2 Synthesis of compounds 3a–3l.

## Results and Discussion

### Chemistry

The target 3-methyl-1-[(4-substitutedpiperazin-1-yl)methyl]-1H-indole derivatives (**3a–l**) have been prepared by Mannich reaction between indole and appropriate piperazines (Fig. 2). Although 2 of compounds were registered in literature by our lab group for sigma receptor binding studies [20], for the entirety of the 3-methyl-1-substitutedindole group, data for them are given again.

The prepared Mannich bases showed IR bands at 3054–2832  $\text{cm}^{-1}$  (C-H) for all derivatives and a typical nitrile band at 2211  $\text{cm}^{-1}$  for Compound **3h**. In the  $^1\text{H}$  NMR spectra, the signals of the respective protons of the prepared compounds **3a–l** were verified on the basis of their chemical shifts, multiplicities and coupling constants. General characteristic peaks of 3-methylindole ring were observed at about  $\delta$  7.55 (d, 1H, indole H<sub>4</sub>), 7.40 (d, 1H, indole H<sub>7</sub>), 7.22 (t, 1H, indole H<sub>5</sub>), 7.20 (t, 1H, indole H<sub>6</sub>), 6.90 (s, 1H, indole H<sub>2</sub>), and 2.30 (s, 3H, -CH<sub>3</sub>). The  $^1\text{H}$  NMR spectra of compounds **3a–l** showed a singlet with 2H intensity each, in the range 4.84–4.76 ppm assigned to the methylene protons, confirming the Mannich condensation. The chemical shift of the aliphatic protons of the piperazine ring were observed in the range 3.40–3.08 (t, 4H, piperazine H<sub>3</sub>, H<sub>5</sub>) and 2.72–2.63 ppm (t, 4H, piperazine H<sub>2</sub>, H<sub>6</sub>). In  $^{13}\text{C}$ -NMR of **3c** and **3h** significant peaks at  $\delta$  67.74 (C-CH<sub>2</sub>-N), 50.21 (piperazine C<sub>3</sub>, C<sub>5</sub>), 47.21 (piperazine C<sub>2</sub>, C<sub>6</sub>), 9.87 (-CH<sub>3</sub>) were observed.

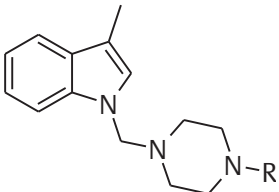
### Biological activity

The cytotoxic activity of the synthesized compounds **3a–3l** was investigated on liver (HUH7), breast (MCF7) and colon (HCT116) cancer cell lines, by means of sulphorhodamine B (SRB) assays in triplicate.

As shown in Table 1 and Fig. 3, all tested compounds were screened on 3 different human cell lines with mean 50% inhibition (IC<sub>50</sub>) in micromolar concentration range. For liver cell line, HUH7, most of the compounds showed no activity at a concentration higher than 100  $\mu\text{M}$ . However, it is interesting that compounds with cytotoxic activity (**3g**, **3h**, **3i** and **3k**) exhibited better cell growth inhibition than standard drug 5-fluorouracil (5-FU) with IC<sub>50</sub> values of 24.76, 14.38, 25.01 and 22.20  $\mu\text{M}$ , respectively. The cytotoxic effects were not impressive against MCF7 breast cancer cells, all of the compounds showed cell viability with IC<sub>50</sub> values ranging from 13.69–68.81  $\mu\text{M}$  concentrations. It was noteworthy that the cytotoxic effects were more pronounced against colon carcinoma cell line, HCT116. Similar to HUH7 cell line, compounds **3h** (IC<sub>50</sub>=8.75  $\mu\text{M}$ ), **3i** (IC<sub>50</sub>=15.91  $\mu\text{M}$ ) and **3k** (IC<sub>50</sub>=16.62  $\mu\text{M}$ ) have better IC<sub>50</sub> values than 5-FLU (IC<sub>50</sub>=18.78  $\mu\text{M}$ ) and also compound **3h** possessed 8.75  $\mu\text{M}$  value, which represents good druggable cytotoxic activity.

Considering the indole core structure of the compounds, we showed the cytotoxic effect of the most active compound **3h** on

Table 1 Cytotoxic activity data for compounds 3a–l.



Compound	R	IC <sub>50</sub> ( $\mu\text{M}$ ) <sup>*</sup>		
		HUH7	MCF7	HCT116
<b>3a</b>	Phenyl	NI	NI	NI
<b>3b</b>	2-Fluorophenyl	NI	23.30	14.48
<b>3c</b>	4-Fluorophenyl	NI	40.95	NI
<b>3d</b>	3-Chlorophenyl	NI	68.81	63.30
<b>3e</b>	4-Chlorophenyl	NI	64.73	64.55
<b>3f</b>	2-Methoxyphenyl	NI	41.29	26.88
<b>3g</b>	3-Methoxyphenyl	24.76	13.69	19.60
<b>3h</b>	4-Cyanophenyl	14.38	23.48	8.75
<b>3i</b>	4-Nitrophenyl	25.01	NI	15.91
<b>3j</b>	4-Methylphenyl	NI	NI	28.15
<b>3k</b>	2,3-Dimethylphenyl	22.20	22.89	16.62
<b>3l</b>	2-Phenylethyl	NI	26.23	23.86
<b>CPT</b>		0.15	>0.01	>0.01
<b>5-FLU</b>		30.70	3.50	18.78

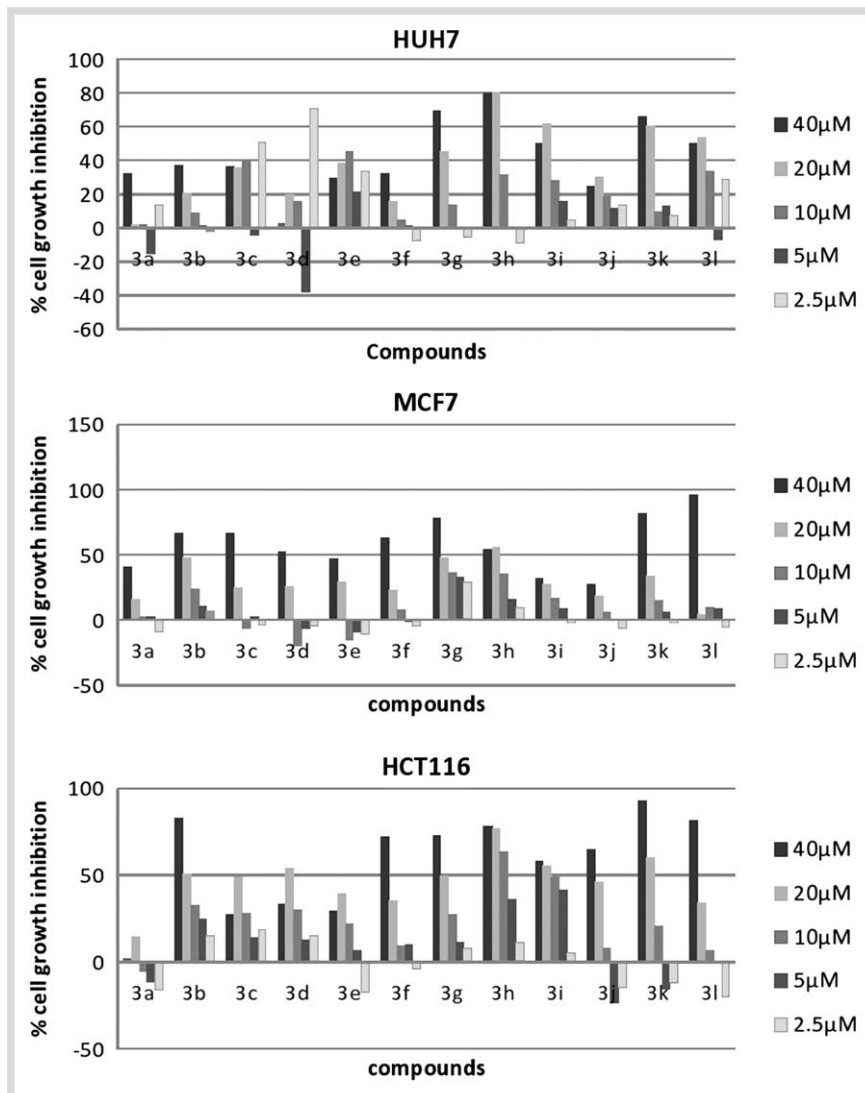
<sup>\*</sup>All the experiments were conducted in triplicate ( $1 < R^2 < 0.8$ );

NI: no inhibition at a concentration lower than 100  $\mu\text{M}$ ;

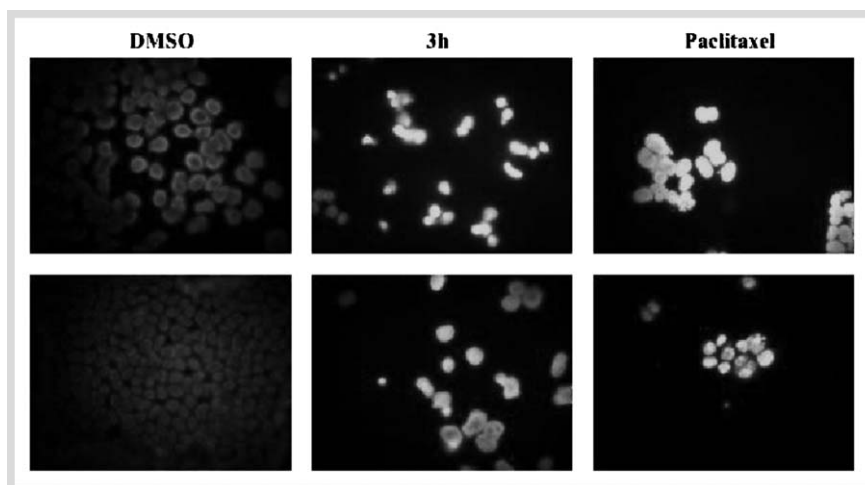
CPT: Camptothecin; 5-FLU: 5-fluorouracil

HUH7 and MCF7 cells through apoptosis and compared with paclitaxel, which is a mitotic inhibitor acting on microtubules. Apoptotic morphological alterations were visualized by Hoechst 33258 staining under fluorescent microscope. The morphological features of apoptosis, i.e., condensation of chromatin and fragmentation of the nucleus, were examined. DMSO treated control cells showed round and homogeneous nuclei, whereas **3h** and paclitaxel treated-cells showed condensed and fragmented nuclei (Fig. 4).

In order to study the structure-activity relationship (SAR), these results of activity screening are not clear enough, but on the other hand, some observations can be stated. Compound **3a** with non-substituted phenyl indicated no cytotoxic activity against all 3 cell lines. Interestingly, the SAR study reveals that substitution on the phenyl ring plays an important role. Among the compounds, **3g**, **3h**, **3i** and **3k** which have 3-OCH<sub>3</sub>, 4-CN, 4-NO<sub>2</sub> and 2,3-diCH<sub>3</sub> substituents on phenyl ring showed better activity than 5-FLU. The activity results of these 4 compounds are almost parallel for both liver HUH7 and colon HCT116 cells. Generally, the derivatives carrying halogen on different positions of phenyl exhibited moderate or low activity results; the only exception is compound **3b** carrying *o*-fluoro substituent on phenyl ring for HCT116 cell line. The findings for MCF7 repre-



**Fig. 3** Inhibitory effects of compounds on HUH7, MCF7 and HCT116 cell growth.



**Fig. 4** The apoptotic effect of inhibitors on the morphology of the nuclear chromatin in HUH7 (upper panel) and MCF7 (lower panel) cells.

sented that the compounds were inactive for breast carcinoma. Especially, in HUH7 liver cell line, although halogen substituted compounds had no cytotoxic activity, introduction of an electron rich substituent such as cyano, nitro to phenyl group have produced compounds with significant  $IC_{50}$  values.

### Conclusion

▼  
In conclusion, the present study showed that the synthesized compounds could be used as a part of a template for future development through modification and derivatization to design

more potent anticancer compounds that carry indole core structure. As a preliminary work, this group representing 1-substituted indoles can build our research interest and experience in the discovery of novel promising antitumor agents. With combination of these and future indole-based series, a QSAR study can be valuable and these results may be helpful for extensive functionalization of the substituent of the indole scaffold on a lead compound in future.

### Acknowledgement

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### Conflict of Interest

The authors have declared no conflict of interest.

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