RESEARCH ARTICLE | Yeditepe Journal of Health Sciences, 13(2):77-85

Synthesis and Biological Evaluation of Novel N-Substituted 3-Methylindole Derivatives

Rawan Albhaisi[1], Dilruba Tırpanlar[2], Hülya Akgün[1], Burçin Güngör[2]

[1] Department of Pharmaceutical Chemistry, Yeditepe University Faculty of Pharmacy, İstanbul, Türkiye; [2] Department of Biochemistry, Yeditepe University Faculty of Pharmacy, İstanbul, Türkiye

Abstract

Objective: Ten novel compounds with the general structure of 1-[(substituted-1-piperidinyl)methyl)]-3-methyl-1H-indole and 1-[(4-(substituted-1-piperazinyl)methyl)]-3-methyl-1H-indole, were synthesized using one-pot reaction method and investigated for their cytotoxic activity against MCF-7 and noncancerous human umbilical vein endothelial cells (HUVECs).

Materials and Methods: The synthesis of the target compounds was carried out using a one-pot reaction method, in a typical procedure, 3-methylindole (2.2 mmol, 300 mg) was dissolved in 20 mL of ethanol in a round bottom flask, then formaldehyde 37% (3 mmol) and substituted piperidine or piperazine derivatives (2.2 mmol) were added. The reaction mixture was then refluxed for 4–6 hours.

Results: *In vitro* cytotoxic activity screening of the compounds was performed against breast cancer (MCF-7) and noncancerous HUVEC lines. Compounds **1, 2, 3, 9** and **10** exhibited selective inhibitory effect on MCF-7 cells with IC_{50} values of 27, 53, 35, 32 and 31 µM respectively.

Conclusion: The anticancer activity of the target compounds was examined against breast cancer cell line MCF-7 and noncancerous HUVEC cell line using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay to investigate their selective cytotoxicity effect, tamoxifen was used as reference drug. Compounds **1, 2, 3, 9** and **10** exhibited moderate cytotoxic activity comparing to the standard and showed a selective inhibitory effect on MCF-7 cells with selectivity index (SI) values of 3.21, 1.08, 2.90, 1.48 and 2.29 respectively. The IC_{50} values of these compounds on MCF-7 cell line were determined as follows: 27, 53, 35, 32 and 31 µM. These compounds' IC_{50} values on HUVECs were obtained as 85, 57, 100, 48 and 71 µM.

Keywords: Indole, piperidine, piperazine, 3-Methyl Indole, anticancer.

Received August 21, 2025 **Accepted** August 29, 2025 **Published** August 31, 2025 **DOI** 10.36519/yjhs.2025.751

Suggested Citation Albhaisi R, Tırpanlar D, Akgün H, Güngör B. Synthesis and biological evaluation of novel N-substituted 3-methylindole derivatives. Yeditepe JHS. 2025;2:77-85.

Correspondence Hülya Akgün **E-mail** hakgun@yeditepe.edu.tr

INTRODUCTION

Cancer is a serious and critical life-threatening illness that was responsible for 9.7 million deaths in 2022, and it is the main cause of death universally after ischaemic heart diseases (1).

Cancer Cancer can be treated and managed by various methods like chemotherapy, radiation therapy, immunotherapy and local treatments like surgery (2). Although studies are continuously updating, chemotherapy remains the most effective and commonly applied treatment against various cancers. However, this effectiveness is impacted by its side effects on patient's physical and psychological health which might limit its clinical potential (3). Therefore, the development of new and safer anticancer agents with minimum toxicity and high potency should be continuously pursued (4).

Due to the rising academic interest in the indole ring as a key scaffold in pharmaceutical chemistry (5), numerous studies over the past decade have been investigating the anticancer properties of various indole-based compounds (6). This increasing attention is supported by the fact that the indole ring is one of the most versatile medicinally important heterocyclic scaffolds, recognized as a privileged moiety with a wide range of pharmacological activities (7). It has become a fundamental nucleus in many synthesized anticancer candidates, as reflected in numerous published studies (8).

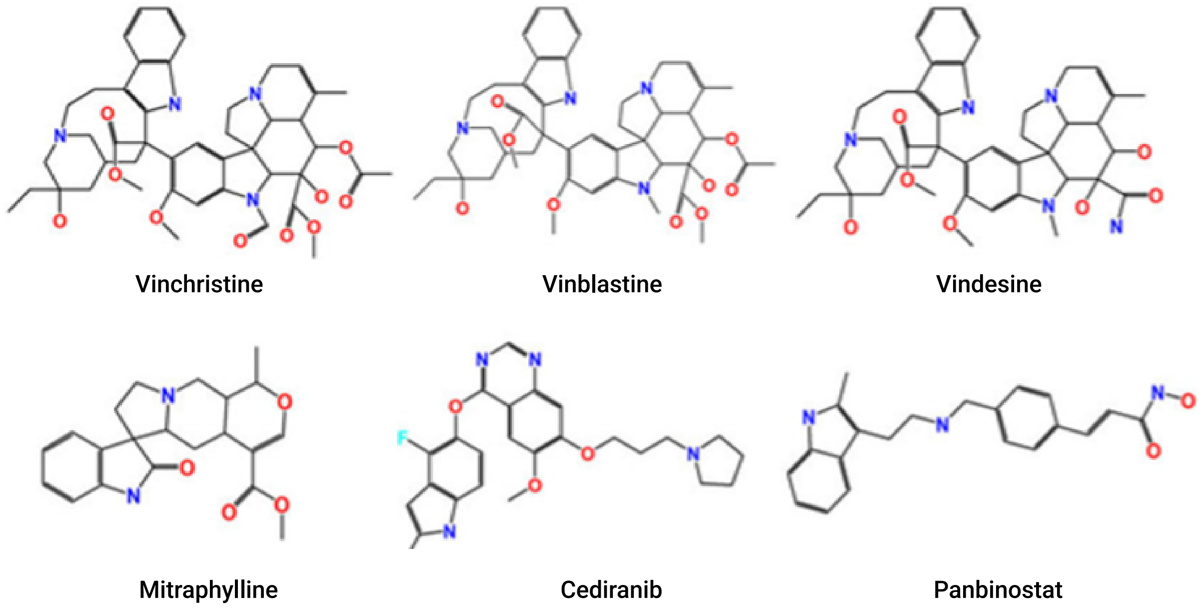
Recently, the indole ring has been used as a main scaffold for several U.S. Food and Drug Administration (FDA) approved anticancer agents such as vincristine, vinblastine etc. (Figure 1). Due to the indole ring's aromaticity and weak acidity on N-H bond, it can have different substitutions which allows variety of derivatives. As already mentioned, vinblastine and vincristine that bear an indole ring in their structure, have been widely used in clinic for the treatment of several different cancer types including breast cancer, through tubulin polymerization inhibition (9-11).

Other versatile pharmacologically important heterocyclic moieties especially in the rational drug design field, are piperidine and piperazine, which both exhibit various pharmacological activities (12,13). For instance, the piperidine structure has been reported to target cancer progression by either inhibiting farnesyl transferase activity or modulating cell signalling and cell mobility through Ras protein family modifications (14).

MATERIALS AND METHODS

Materials

3-Methylindole, 4-phenylpiperidine, 4-hydroxy-4-phenylpiperidine, 3,5-dimethylpiperidine, 4-methylpiperidine, bis(4-fluorophenyl) methylpiperazine, trifluoromethylphenylpiperazine, 4-bromophenylpiperazine, 4-methoxyphenylpiperazine, 2,3-dichlorophenylpiperazine, 4-*tert*-butylpiperazine-1-carboxylate, ethanol,


Vinchristine

Vinblastine

Vindesine

Mitraphylline

Cediranib

Panbinostat

Figure 1. FDA approved anticancer drugs containing indole scaffold.

methanol, formaldehyde, toluene, ethyl acetate, and chloroform were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Synthesis Methods

General Synthesis Method of Compounds 1-4

3-Methylindole (2.2 mmol, 300 mg) was dissolved in 20 mL of ethanol in a round bottom flask, then formaldehyde 37% (3 mmol) and substituted piperidine (2.2 mmol) were added. The mixture was refluxed for 4–6 hours, and the reaction was controlled by thin-layer chromatography (TLC) with silica gel plate as a stationary phase and toluene:ethyl acetate (10:1) as a mobile phase. After the reaction was completed, the mixture was placed in the freezer for 24 hours, and the resulting precipitate was filtrated and dried. Purification of the synthesized compounds was carried out using a combination of crystallization with either ethanol or methanol and column chromatography, using toluene: ethyl acetate (10:1) solvent system.

General Synthesis Method of Compounds 5-10

3-Methylindole (2.2 mmol, 300 mg) was dissolved in 20 mL of ethanol in a round bottom flask, then formaldehyde 37% (3 mmol) and substituted piperazine (2.2 mmol) were added. The mixture was refluxed for 4–6 hours, and the reaction was controlled by TLC with silica gel plate as a stationary phase and toluene: ethyl acetate (10:1) as a mobile phase. After the reaction was completed, the mixture was placed in the freezer for 24 hours, and the resulting precipitate was filtrated and dried. Purification of the synthesized compounds was carried out using a combination of crystallization with either ethanol or methanol and column chromatography, using toluene:ethyl acetate (10:1) solvent system.

Analytical Methods

Melting point (°C) determination of compounds were carried out using a FP62 capillary melting point apparatus (Mettler Toledo, Columbus, OH, USA) and were uncorrected. The reactions were controlled by TLC on aluminium sheets 20x20 cm silica gel 60 F254 (Merck, Darmstadt, Germany) plates. Compounds **4**, **5**, **6** and **7** were purified by column chromatography using silica gel 60 mesh (Merck, Darmstadt, Germany) as stationary phase and toluene:ethyl acetate (10:1) solution as mobile phase.

Infrared (IR) spectra were recorded on a Spectrum One FT-IR spectrometer (PerkinElmer, Waltham, MA, USA; Version 5.0.1), by applying potassium bromide (KBr) as a background, and the frequencies were shown in cm^{-1}.

^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Mercury-500 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA) using tetramethylsilane (TMS; Sigma-Aldrich, St. Louis, MO, USA) as the internal reference. Deuterated dimethyl sulfoxide (DMSO-d6) or deuterated chloroform ($CDCl_3$) were used as solvents, and the chemical shifts were reported in parts per million (ppm).

Full FT-IR and NMR results are provided in Supplementary Material.

BIOLOGICAL STUDY

Cell Culture

MCF-7 and HUVEC cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA; Cat. No.11875093) with 10% (v/v) fetal bovine serum, 1% streptomycin-penicillin at 37°C in an incubator containing 5% CO_2.

Cytotoxic Activity

To assess the viability of MCF-7 and HUVEC cell lines in response to compound treatment, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was conducted. Cells were seeded into 96-well plates at a density of 3,500 cells/well in 100 µL of medium. After a 24-hour incubation, the cells were treated with six different concentrations (6.25, 12.5, 25, 50, 100, and 200 µM) of the compounds, each diluted in 100 µL of medium. Control wells received the same volume of medium without the compounds.

After 72 hours of incubation, 10 µL of 5 mg/mL MTT in phosphate-buffered saline (PBS) was added to each well. After 3 hours of incubation at 37°C, formazan crystals were dissolved in 150 µL of 2-propanol and incubated at room temperature for an additional 30 minutes. Absorbance values were determined at 570 nm using a Multiskan Ascent microplate reader (Thermo Electron Corporation, Vantaa, Finland). Tamoxifen was used as a reference drug to compare the cytotoxic activity on cells.

The absorbance of the control group (no compound) was considered as 100%. The percentage inhibition (%) of cell proliferation was determined with the following equation:

$$\text{Percentage inhibition (\%)} = (\text{Compound}_{Abs}\text{-Blank}_{Abs}) \times 100/(\text{Control}_{Abs}\text{-Blank}_{Abs}).$$

Cytotoxic measurement parameter IC_{50}, inhibitory concentration by 50%, was obtained using curve fitting method on excel and R^2 values were considered. Each compound was studied with 5 replicates in two independent experiments. Graphs were generated and statistics were calculated using Microsoft Excel.

The SI measures a drug's or compound's activity specifically against targeted cancer cells compared to healthy cells. The SI is calculated as the ratio of the IC_{50} value of the compound in healthy cells to its IC_{50} value in cancer cells. An SI value greater than 1 indicates a desirable level of selectivity toward cancer cells (15).

RESULTS

Chemical data

The desired compounds were synthesized by Mannich reaction between 3-methylindole, and various substituted piperidine and piperazine derivatives, following a procedure adapted from previously published literatures (16). The general synthetic procedure of the ten target compounds is illustrated in the scheme below (Scheme 1).

The FTIR, ^{1}H-NMR, and ^{13}C-NMR spectra of the newly synthesized compounds furnished compelling evidence regarding their structural characteristics. The IR spectra revealed key functional group vibrations, with aromatic and aliphatic C-H stretching bands observed between 3061-2828 cm^{-1}, indicating the presence of both aromatic and aliphatic hydrogens in the compounds. The aromatic C=C stretching bands were observed within the range of 1618-1459 cm^{-1}. In ^{1}H-NMR spectra, the indole CH_3 singlet peaks were detected at 2.35 ppm, which is consistent with the methyl group attached to the indole ring. Additionally, piperidine hydrogens showed peaks between 1.72-2.92 ppm, while the piperazine hydrogens were detected between 2.53-3.45 ppm, reflecting the incorporation of these heterocyclic rings into the structure. The N-CH_2-N singlet peaks recorded at 4.82-4.90 ppm further corroborate the presence of indole-piperidine and piperazine linkages. Aromatic hydrogens in the indole and phenyl rings were observed between 6.95-7.60 ppm.

3-Methyl-1-[(4-phenylpiperidin-1-yl)methyl]-1H-indole (1)

Yield: 40 %. mp: 108ºC. IR (KBr, cm^{-1}): 1465 (aromatic C=C), 2948 (aliphatic C-H), 3020 (aromatic C-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.78-1.87 (m, 4H, piperidine), 2.31-2.42 (m, 6H, CH_3 + 3H piperidine), 3.11 (d, 2H, piperidine, *J* = 11.2 Hz), 4.90 (s, 2H, N-CH_2-N), 6.98 (s, 1H, indole), 7.16-7.17 (dd, 1H, indole, J_1 = 7.9 H_Z, J_2 = 0.9 H_Z), 7.20-7.25 (m, 4H, phenyl), 7.28-7.33 (m, 2H, 1H indole + 1H phenyl), 7.49 (d, 1H, indole, *J* = 8.25 H_Z), 7.61 (d, 1H, indole, *J* = 7.85 Hz). ^{13}C-NMR (DMSO, δ ppm): 9.65 (CH_3), 33.32, 42.18, 51.62 (piperidine-C), 67.96 (CH_2), 109.88, 110.61, 118.88, 118.89, 121.63, 126.21, 126.46, 126.82, 128.45, 128.78, 137.45, 146.13 (Ar-C).

1-[(3-Methyl-1H-indol-1-yl)methyl]-4-phenylpiperidin-4-ol (2)

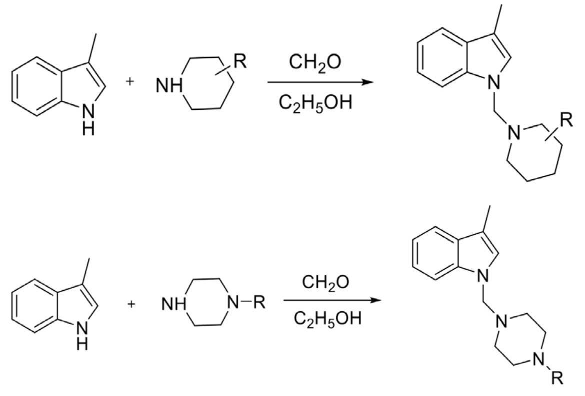
Yield: 42%. mp:129°C. IR (KBr, cm^{-1}): 1459 (aromatic C=C), 2912 (aliphatic C-H), 3053 (aromatic C-H), 3292 (O-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.75-1.78 (dd, 2H, piperidine, J_1 = 14.1 H_Z, J_2 = 2.4 H_Z), 2.17 (t, 2H, piperidine, *J* = 11.4 H_Z), 2.36 (s, 3H, CH_3), 2.70 (t, 2H, piperidine, *J* = 11.35 H_Z), 2.88 (d, 2H, piperidine, *J* = 10.95 H_Z), 4.86 (s, 2H, N-CH_2-N), 7.01 (s, 1H, indole), 7.14-7.17 (dd, 1H, indole, J_1 = 14.9 Hz, J_2 = 0.75 H_Z), 7.23-7.30 (m, 2H, phenyl), 7.35-7.38 (m, 2H, phenyl), 7.47-7.51 (m, 3H, indole + phenyl), 7.60 (d, 1H, indole, *J* = 7.8 H_Z). ^{13}C-NMR (DMSO, δ ppm): 9.64 (CH_3), 38.29, 46.97 (piperidine-C), 67.88 (CH_2), 71.12 (C-OH), 109.83, 110.79, 118.90, 121.61, 124.48, 126.29, 127.09, 128.39, 128.90, 137.35, 148.20 (Ar-C).

1-[(3,5-Dimethylpiperidin-1-yl)methyl]-3-methyl-1H-indole (3)

Yield: 39%. mp: 87°C. IR (KBr, cm^{-1}): 1461 (aromatic C=C), 2916 (aliphatic C-H), 3056 (aromatic C-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 0.86 (d, 6H, piperidine $(CH_3)_2$, *J* = 5.9 H_Z), 1.70-1.73 (dd, 6H, piperidine, J_1 = 12.8 H_Z, J_2 = 5.1 H_Z), 2.36 (s, 3H, CH_3), 2.92 (d, 2H, piperidine, *J* = 6.6 H_Z), 4.83 (s, 2H, N-CH_2-N), 6.95 (s, 1H, indole), 7.14 (t, 1H, indole, *J* = 7.8 H_Z), 7.22-7.28 (m, 1H, indole), 7.45 (d, 1H, indole, *J* = 8.2 H_Z), 7.59 (d, 1H, indole, *J* = 7.85 H_Z). ^{13}C-NMR (DMSO, δ ppm): 9.64 (CH_3), 19.58 $(CH_3)_2$, 31.29, 41.75, 58.68 (piperidine-C), 67.94 (CH_2), 109.93, 110.43, 118.75, 118.77, 121.57, 126.49, 128.72, 137.43 (Ar-C).

3-Methyl-1-[(4-methylpiperidin-1-yl)methyl]-1H-indole (4)

Yield: 43%. mp: 46°C. IR (KBr, cm^{-1}): 1460 (aromatic C=C), 2920 (aliphatic C-H), 3054 (aromatic C-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 0.92 (d, 3H, CH_3, *J* = 5.7 H_Z), 1.28-1.26 (m, 3H, piperidine), 1.64 (d, 2H, piperidine, *J* = 9.4 H_Z), 2.19 (t, 2H, piperidine, *J* = 10.85 H_Z), 2.35 (s, 3H, CH_3), 2.95 (d, 2H, piperidine, *J* = 11.3 H_Z), 4.82 (s, 2H, N-CH_2-N), 6.95 (s, 1H, indole), 7.15 (t, 1H, indole, *J* = 7.15 H_Z), 7.28-7.22 (m, 1H, indole), 7.45 (d, 1H, indole, *J* = 8.2 H_Z), 7.59 (d, 1H,



Scheme 4.1. General synthesis procedure of compounds.

indole, J = 7.8 H_Z). ^{13}C-NMR (DMSO, δ ppm): 9.62 (CH_3), 21.86 (piperidine-CH_3), 30.35, 34.18, 51.25 (piperidine-C), 67.99 (CH_2), 109.88, 110.47, 118.77, 118.80, 121.52, 126.47, 128.72, 137.44 (Ar-C).

1-{4-[bis(4-fluorophenylmethyl)piperazin-1-yl]methyl}-3-methyl-1H-indole (5)

Yield: 56%. mp: 145°C. IR (KBr, cm^{-1}): 1504-1599 (aromatic C=C), 2920 (aliphatic C-H), 3060 (aromatic C-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.36-2.42 (m, 7H, 3H CH_3 + 4H piperazine), 2.62 (s, 4H, piperazine), 4.20 (s, 1H, CH), 4.85 (s, 2H, N-CH_2-N), 6.93-6.97 (m, 5H, 4H phenyl + 1H indole), 7.13-7.16 (dd, 1H, indole, J_1 = 14.8 H_Z, J_2 = 0.75 H_Z), 7.21-7.24 (m, 1H, indole), 7.28-7.31 (m, 4H, phenyl), 7.41 (d, 1H, indole, J = 8.2 H_Z), 7.60 (d, 1H, indole, J = 7.8 H_Z). ^{13}C-NMR (DMSO, δ ppm): 9.62 (CH_3), 50.58, 51.55 (piperazine-C), 67.32 (CH_2), 74.40 (CH), 109.75, 110.71, 115.29, 115.46, 118.88, 118.91, 121.65, 126.41, 128.80, 129.17, 129.23, 137.41, 138.14, 138.16, 160.82, 162.77 (Ar-C).

3-Methyl-1-{4-[(4-trifluoromethylphenyl)piperazin-1-yl]methyl}-1H-indole (6)

Yield: 64%. mp: 147°C. IR (KBr, cm^{-1}): 1462-1618 (aromatic C=C), 2838 (aliphatic C-H), 3027 (aromatic C-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.35 (s, 3H, CH_3), 2.73 (s, 4H, piperazine), 3.30 (s, 4H, piperazine), 4.86 (s, 2H, N-CH_2-N), 6.89 (d, 2H, phenyl, J = 8.75 H_Z), 6.99 (s, 1H, indole), 7.16 (t, 1H, indole, J = 7.1 H_Z), 7.25-7.28 (m, 1H, indole), 7.44-7.48 (m, 3H, 2H phenyl + 1H indole), 7.60 (d, 1H, indole, J = 7.85 H_Z). ^{13}C-NMR (DMSO, δ ppm): 9.61 (CH_3), 47.90, 50.60 (piperazine-C), 67.60 (CH_2), 109.74, 111.07, 114.66, 119.01, 119.10, 120.49, 120.75, 121.78, 123.63, 125.78, 126.07, 126.35, 126.38, 126.40, 128.97, 137.24, 153.19 (Ar-C).

1-{4-[(4-Bromophenyl)piperazin-1-yl]methyl}-3-methyl-1H-indole (7)

Yield: 47%. mp: 146°C. IR (KBr, cm^{-1}): 1586 (aromatic C=C), 2828 (aliphatic C-H), 3038 (aromatic C-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.35 (s, 3H, CH_3), 2.72 (s, 4H, piperazine), 3.17 (t, 4H, piperazine, J = 4.6 H_Z), 4.85 (s, 2H, N-CH_2-N), 6.75-6.76 (dd, 2H, phenyl, J_1 = 6.95 H_Z, J_2 = 2.15 Hz), 6.98 (s, 1H, indole), 7.14-7.17 (dd, 1H, indole, J_1 = 14.9 Hz, J_2 = 0.85 Hz), 7.23-7.28 (m, 1H, indole), 7.33-7.34 (dd, 2H, phenyl, J_1 = 6.95 Hz, J_2 = 2.2 Hz), 7.45 (d, 1H, indole, J = 8.25 Hz), 7.60 (d, 1H, indole, J = 7.8 Hz) ^{13}C-NMR (DMSO, δ ppm): 9.61 (CH_3), 48.92, 50.31 (piperazine-C), 67.62 (CH_2), 109.76, 111.00, 111.95, 117.79, 118.98, 119.05, 121.73, 126.09, 128.96, 131.86, 137.25, 150.24 (Ar-C).

1-{4-[(4-methoxyphenyl)piperazin-1-yl]methyl}-3-methyl-1H-indole (8)

Yield: 50%. mp: 115°C. IR (KBr, cm^{-1}): 1513 (aromatic C=C), 2831 (aliphatic C-H), 2934 (aromatic C-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.35 (s, 3H, CH_3), 2.75 (s, 4H, piperazine), 3.11 (t, 4H, piperazine, J = 4.6 H_Z), 3.78 (s, 3H, O-CH_3), 4.85 (s, 2H, N-CH_2-N), 6.83-6.90 (m, 4H, phenyl), 6.99 (s, 1H, indole), 7.14-7.17 (dd, 1H, indole, J_1 = 14.9 H_Z, J_2 = 0.85 H_Z), 7.23-7.28 (m, 1H, indole), 7.47 (d, 1H, indole, J = 8.25 H_Z), 7.60 (d, 1H, indole, J = 7.85 H_Z). ^{13}C-NMR (DMSO, δ ppm): 9.62 (CH_3), 50.60, 50.63 (piperazine-C), 55.56 (O-CH_3), 67.67 (CH_2), 109.81, 110.89, 114.42, 118.44, 118.94, 118.98, 121.68, 126.16, 128.95, 137.29, 145.66, 153.92 (Ar-C).

1-{4-[(2,3-dichlorophenyl)piperazine-1-yl]methyl}-3-methyl-1H-indole (9)

Yield: 56%. mp: 110°C. IR (KBr, cm^{-1}): 1448-1579 (aromatic C=C), 2832 (aliphatic C-H), 3061 (aromatic C-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.36 (s, 3H, CH_3), 2.78 (s, 4H, piperazine), 3.08 (s, 4H, piperazine), 4.86 (s, 2H, N-CH_2-N), 6.94-6.95 (dd, 1H, phenyl, J_1 = 7.55 H_Z, J_2 = 2 H_Z), 7.00 (s, 1H, indole), 7.13-7.18 (m, 3H, indole + phenyl), 7.24-7.28 (m, 1H, indole), 7.47 (d, 1H, indole, J = 8.25 H_Z), 7.61 (d, 1H, indole, J = 7.85 H_Z). ^{13}C-NMR (DMSO, δ ppm): 9.64 (CH_3), 50.62, 51.15 (piperazine-C), 67.74 (CH_2), 109.80, 110.97, 118.64, 118.96, 119.02, 121.72, 124.69, 126.15, 127.43, 127.57, 128.98, 134.05, 137.26, 151.15 (Ar-C).

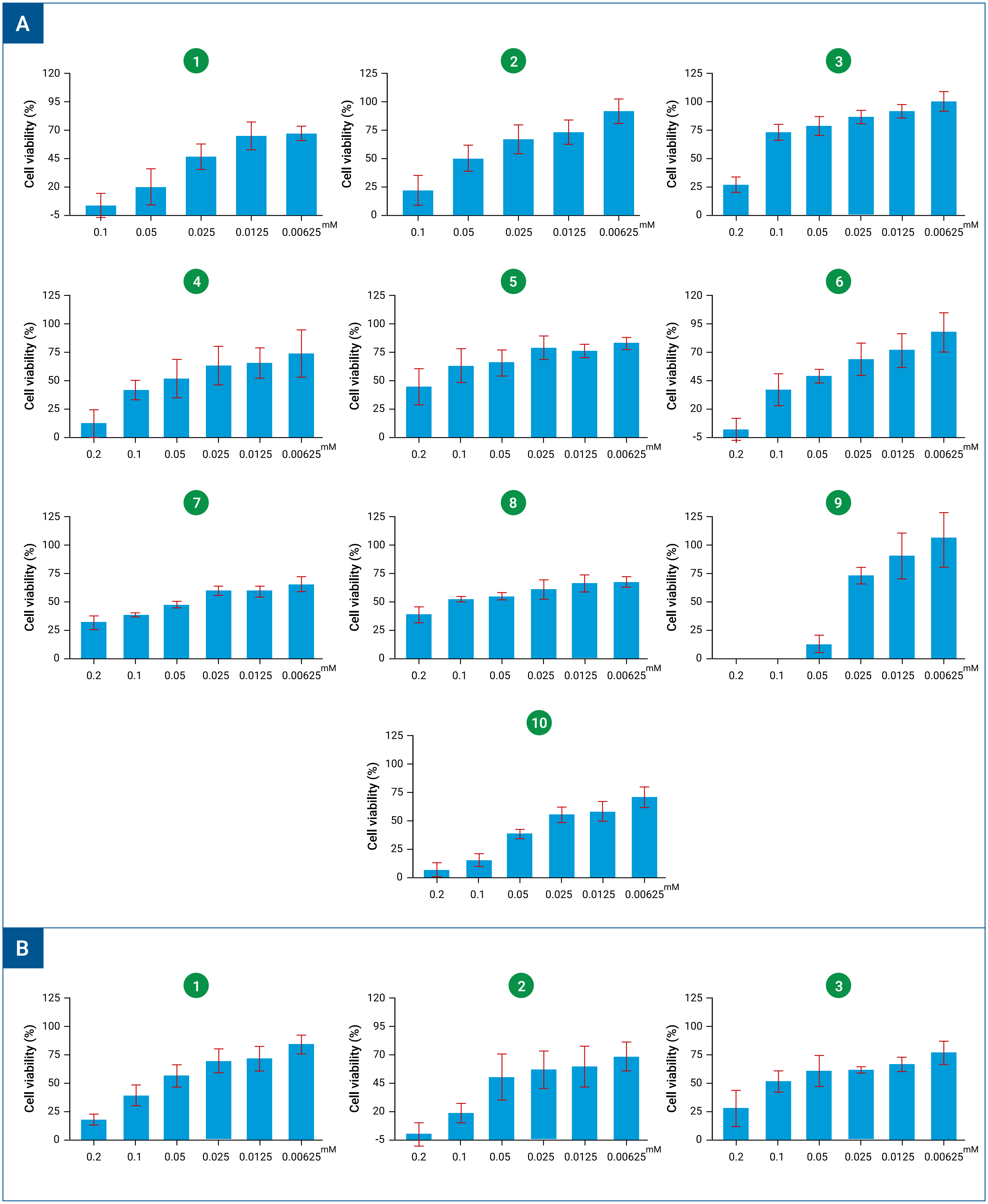
tert-Butyl-4-[(3-methyl-1H-indol-1-yl)methyl]piperazine-1-carboxylate

Yield: 35%. mp: 83°C. IR (KBr, cm^{-1}): 1421-1461 (aromatic C=C), 1693 (C=O), 2916 (aliphatic C-H), 2932 (aromatic C-H). ^{1}H-NMR ($CDCl_3$, δ ppm): 1.43 (s, 9H, tert-butyl), 2.34 (s, 3H, CH_3), 2.53 (s, 4H, piperazine), 3.45 (s, 4H, piperazine), 4.83 (s, 2H, N-CH_2-N), 6.95 (s, 1H, indole), 7.16 (t, 1H, indole, J = 7.5 H_Z), 7.22–7.28 (m, 1H, indole), 7.41 (d, 1H, indole, J = 8.2 H_Z), 7.59 (d, 1H, indole, J = 7.85 H_Z). ^{13}C-NMR (DMSO, δ ppm): 9.59 (CH_3), 28.39 $(CH_3)_3$, 43.09, 50.28 (piperazine-C), 67.67 (CH_2), 79.72 (O-CH), 109.72, 110.98, 118.97, 119.04, 121.74, 126.10, 128.90, 137.22 (Ar-C), 154.53 (C=O).

The chemical structures of all molecules, synthesized are given in Supplementary Material.

Biological activity

The anticancer activity of the target compounds was examined against breast cancer cell line MCF-7 and non-cancerous human endothelial cell line HUVEC using MTT assay to investigate their selective cytotoxicity effect. Tamoxifen was used as standard drug. Among the investigated derivatives, compounds **1**, **2**, **3**, **9** and **10** exhibited selective inhibitory effect on MCF-7 cells with SI values of 3.21, 1.08, 2.90, 1.48 and 2.29 respectively, suggesting that these compounds are acting selectively on cancerous cells. Also, these compounds exerted a greater selective cytotoxic activity than tamoxifen that exhibited an SI value of 1.15. The IC_{50} values of these compounds on MCF-7 cell line were determined as follows: 27, 53, 35, 32 and 31 μM. These compounds' IC_{50} values on HUVECs were obtained as 85, 57, 100, 48 and 71 μM.


A
1
120
95
70
45
20
-5
Cell viability (%)
0.1
0.05
0.025
0.0125
0.00625
mM
2
125
100
75
50
25
0
Cell viability (%)
0.1
0.05
0.025
0.0125
0.00625
mM
3
125
100
75
50
25
0
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
4
125
100
75
50
25
0
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
5
125
100
75
50
25
0
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
6
120
95
70
45
20
-5
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
7
125
100
75
50
25
0
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
8
125
100
75
50
25
0
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
9
125
100
75
50
25
0
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
10
125
100
75
50
25
0
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
B
1
125
100
75
50
25
0
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
2
120
95
70
45
20
-5
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM
3
125
100
75
50
25
0
Cell viability (%)
0.2
0.1
0.05
0.025
0.0125
0.00625
mM

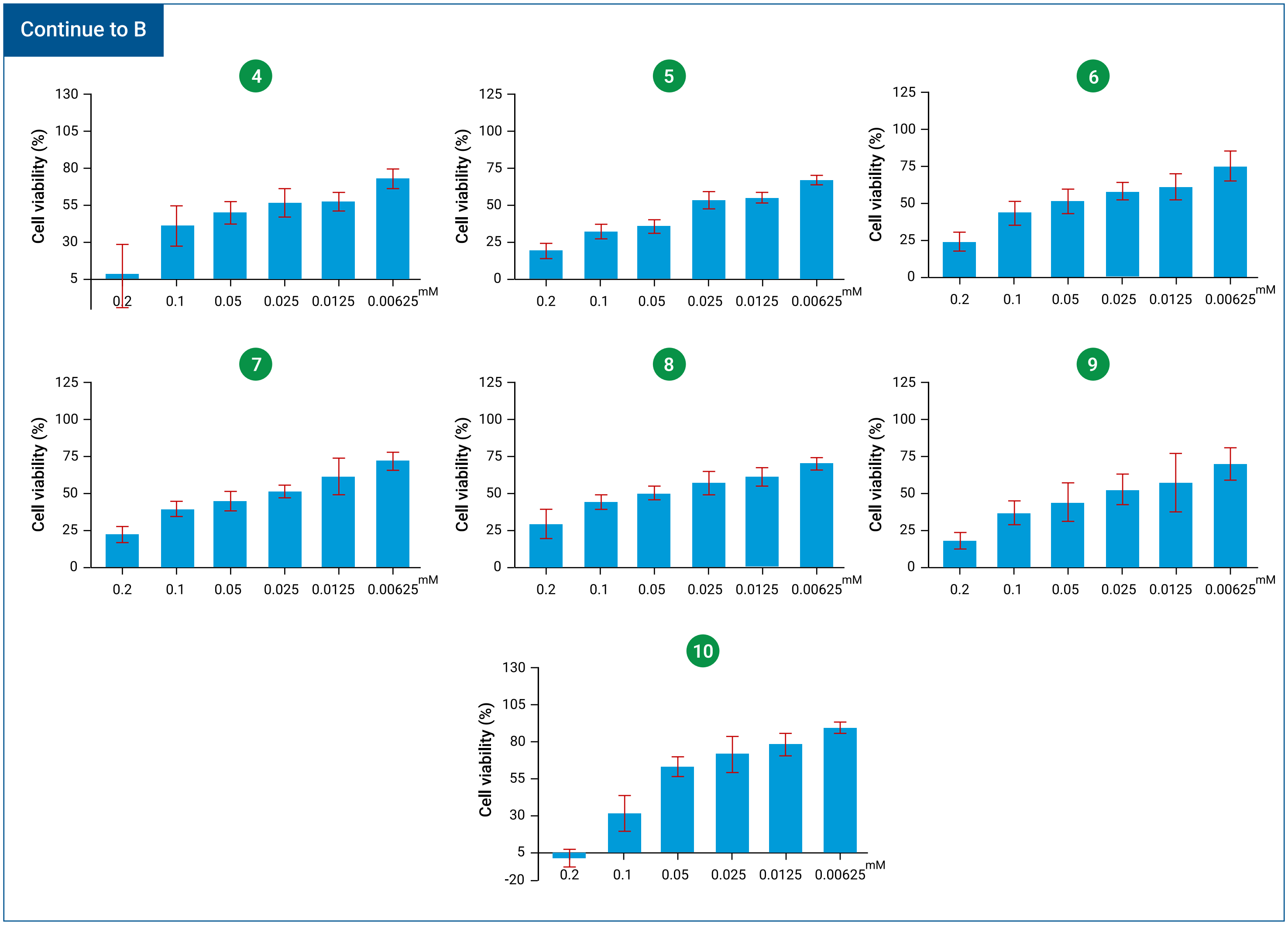


Figure 2. Cell viability graphs of MCF-7 (A) and HUVEC (B) cells treated with the target compounds. SD±data mean. At least 2 independent study with 5 technical replicates.

The compounds tested, exhibit dose dependent cytotoxic effect on both MCF-7 and HUVEC cell lines (Figure 2). The effect of the compounds was more prominent even at low concentrations when administered on MCF-7 compared to HUVECs. This suggested that the breast cancer cell line MCF-7 was more vulnerable to applied compounds.

DISCUSSION

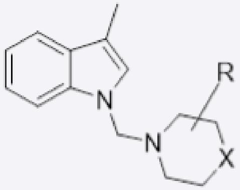
Literature has extensively investigated the role of the indole scaffold in development of anticancer agents (17-20) and the cytotoxic activity of piperidine and piperazine derivatives (21,22).

Even though, the indole derivatives have been described as quite potent molecular structures targeting cancer cells, only a few studies have investigated the cytotoxic activity of 3-methyl indole (23). Therefore, in this work, we synthesized hybrid molecules with the general structure of 1-[(substituted-1-piperidinyl)methyl)]-3-methyl-1H-indole and 1-[(4-(substituted-1-piperazinyl) methyl)]-3-methyl-1H-indole, and investigated their cytotoxic activities using MTT assay, the results indicated that compounds **1**, **2**, **3**, **9** and **10** exhibited selective inhibitory effect on MCF-7 cells suggesting that these compounds are acting on cancerous cells, selectively.

CONCLUSION

Ten novel N-substituted 3-methylindole derivatives were synthesized with moderate yields. Their structural elucidation was confirmed using IR, ^{1}H-NMR, ^{13}C-NMR spectroscopic methods. The target compounds were investigated for their anticancer activity against MCF-7 and HUVEC cell lines. Among the tested compounds, compounds **1**, **2**, **3**, **9** and **10** exhibited selective inhib-

Table 1. Cytotoxicity and selectivity of the target compounds on MCF-7 and HUVEC cell lines.



Compound No.	X	R	HUVEC		MCF-7		SI ≥ 1
			IC_{50}	R^2	IC_{50}	R^2	
1	C	4-phenyl	**85**	**0.95**	**27**	**0.92**	**3.21**
2	C	4-hydroxy-4-phenyl	**57**	**0.95**	**53**	**0.95**	**1.08**
3	C	3,5-dimethyl	**100**	**0.95**	**35**	**0.98**	**2.90**
4	C	4-methyl	47	0.95	141	0.95	0.33
5	N	Bis(4-fluorophenyl) methyl	33	0.82	158	0.94	0.21
6	N	4-trifluoromethylphenyl	74	0.91	78	0.94	0.95
7	N	4-bromophenyl	56	0.85	61	0.85	0.93
8	N	4-methoxyphenyl	74	0.92	105	0.95	0.71
9	N	2,3-dichlorophenyl	**48**	**0.91**	**32**	**0.99**	**1.48**
10	N	4-*tert*. butoxy carbonyl	**71**	**0.97**	**31**	**0.85**	**2.29**
Tamoxifen	-	-	11	0.98	10	0.99	1.15

itory effect on MCF-7 cells with SI values of 3.21, 1.08, 2.90, 1.48 and 2.29 respectively. The IC_{50} values of these compounds were of 27, 53, 35, 32 and 31 μM. Comparing to the standard, compounds showed moderate cytotoxic activity. Moreover, in future studies, these compounds can be further structurally modified and investigated for their biological activity to obtain remarkable agents.

Ethical Approval: N.A.

Informed Consent: N.A.

Peer-review: Externally peer-reviewed

Author Contributions: Concept – H.A., B.G.; Design – H.A.; Supervision – H.A., B.G.; Materials – B.G., D.T.; Data Collection and/or Processing – R.A., D.T.; Analysis and/or Interpretation – H.A., B.G., R.A., D.T.; Literature Review – H.A., R.A.; Writer – H.A., B.G., R.A.; Critical Reviews – H.A., B.G.

Conflict of Interest: The author declares no conflict of interest.

Financial Disclosure: The author declared that this study has received no financial support.

Acknowledgement: This study was conducted as part of a Master's Thesis at the Faculty of Pharmacy, Yeditepe University. The infrastructure and laboratories of the Faculty of Pharmacy were utilized during the investigation.

REFERENCES

1 Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-63. [CrossRef]

2 Roy PS, Saikia BJ. Cancer and cure: A critical analysis. Indian J Cancer. 2016;53(3):441-2. [CrossRef]

3 Kayl AE, Meyers CA. Side-effects of chemotherapy and quality of life in ovarian and breast cancer patients. Curr Opin Obstet Gynecol. 2006;18(1):24-8. [CrossRef]

4 Furue H. [Chemotherapy cancer treatment during the past sixty years]. Gan To Kagaku Ryoho. 2003;30(10):1404-11. Japanese.

5 Sravanthi TV, Manju SL. Indoles - A promising scaffold for drug development. Eur J Pharm Sci. 2016;91:1-10. [CrossRef]

6 Sachdeva H, Mathur J, Guleria A. Indole derivatives as potential anticancer agents: a review. J Chil Chem Soc. 2020;65(3):4900-7. [CrossRef]

7 Kaushik NK, Kaushik N, Attri P, Kumar N, Kim CH, Verma AK, et al. Biomedical importance of indoles. Molecules. 2013;18(6):6620-62. [CrossRef]

8 Devi N, Kaur K, Biharee A, Jaitak V. Recent development in indole derivatives as anticancer agent: a mechanistic approach. Anticancer Agents Med Chem. 2021;21(14):1802-24. [CrossRef]

9 Vitaku E, Smith DT, Njardarson JT. Analysis of the structural diversity, substitution patterns, and frequency of nitrogen heterocycles among U.S. FDA approved pharmaceuticals. J Med Chem. 2014;57(24):10257-74. [CrossRef]

10 Dhiman A, Sharma R, Singh RK. Target-based anticancer indole derivatives and insight into structure–activity relationship: A mechanistic review update (2018-2021). Acta Pharm Sin B. 2022;12(7):3006-27. [CrossRef]

11 Hong Y, Zhu YY, He Q, Gu SX. Indole derivatives as tubulin polymerization inhibitors for the development of promising anticancer agents. Bioorg Med Chem. 2022;55:116597. [CrossRef]

12 Frolov NA, Vereshchagin AN. Piperidine derivatives: Recent advances in synthesis and pharmacological applications. Int J Mol Sci. 2023;24(3):2937. [CrossRef]

13 Sharma A, Wakode S, Fayaz F, Khasimbi S, Pottoo FH, Kaur A. An overview of piperazine scaffold as promising nucleus for different therapeutic targets. Curr Pharm Des. 2020;26(35):4373-85. [CrossRef]

14 Millet R, Domarkas J, Houssin R, Gilleron P, Goossens JF, Chavatte P, et al. Potent and selective farnesyl transferase inhibitors. J Med Chem. 2004;47(27):6812-20. [CrossRef]

15 Mass EB, de Lima CA, D'Oca MGM, Sciani JM, Longato GB, Russowsky D. Synthesis, selective cytotoxic activity against human breast cancer MCF7 cell line and molecular docking of some chalcone-dihydropyrimidone hybrids. Drugs Drug Candidates. 2022;1(1):3-21. [CrossRef]

16 Akkoç M, Yüksel M, Durmaz I, Atalay R. Design, synthesis, and biological evaluation of indole-based 1,4-disubstituted piperazines as cytotoxic agents. Turk J Chem. 2012;36(4):515-25. [CrossRef]

17 Rao VK, Chhikara BS, Shirazi AN, Tiwari R, Parang K, Kumar A. 3-substitued indoles: one-pot synthesis and evaluation of anticancer and Src kinase inhibitory activities. Bioorg Med Chem Lett. 2011;21(12):3511-4. [CrossRef]

18 Andreani A, Burnelli S, Granaiola M, Leoni A, Locatelli A, Morigi R, et al. Antitumor activity of bis-indole derivatives. J Med Chem. 2008;51(15):4563-70. [CrossRef]

19 Cai M, Hu J, Tian JL, Yan H, Zheng CG, Hu WL. Novel hybrids from N-hydroxyarylamide and indole ring through click chemistry as histone deacetylase inhibitors with potent antitumor activities. Chin Chem Lett. 2015;26(6):675-80. [CrossRef]

20 Zhang G, Tang Z, Fan S, Li C, Li Y, Liu W, et al. Synthesis and biological assessment of indole derivatives containing penta-heterocycles scaffold as novel anticancer agents towards A549 and K562 cells. J Enzyme Inhib Med Chem. 2023;38(1):2163393. [CrossRef]

21 Mitra S, Anand U, Jha NK, Shekhawat MS, Saha SC, Nongdam P, et al. Anticancer applications and pharmacological properties of piperidine and piperine: a comprehensive review on molecular mechanisms and therapeutic perspectives. Front Pharmacol. 2022;12:772418. [CrossRef]

22 Schumacher TJ, Sah N, Palle K, Rumbley J, Mereddy VR. Synthesis and biological evaluation of benzofuran piperazine derivatives as potential anticancer agents. Bioorg Med Chem Lett. 2023;93:129425. [CrossRef]

23 Koksal M, Yarim M, Durmaz I, Cetin-Atalay R. Synthesis and cytotoxic activity of novel 3-methyl-1-[(4-substitutedpiperazin-1-yl)methyl]-1H-indole derivatives. Arzneimittelforschung. 2012;62(8):389-94. [CrossRef]