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Design, Synthesis, *In-silico* and Cytotoxic Studies of Indole Derivatives as Potent BCL-2 inhibitors

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ABSTRACT

Indole-based compounds have emerged as a potentially game-changing category of molecules that specifically target Bcell lymphoma-2 (BCL-2) protein, offering an innovative approach to the management of breast cancer. Breast cancer is a major public health concern globally, necessitating continued research into innovative therapeutic approaches. One such strategy involves inhibiting (BCL-2) protein, which is overexpressed in cancer cells and inhibits apoptosis. A series of indole derivatives **(b1-b12)** were synthesized using indium chloride as a catalyst in a solvent free conditions to investigate their potential to interfere with BCL-2 mediated survival pathways. Additionally, *In silico* modeling was employed to identify novel BCL-2 inhibitors and made structural alterations to enhance the selectivity and potency of indole compounds. The efficacy of indole derivatives was determined using an *In vitro* model that utilizes the MCF cell line. The findings obtained demonstrated that the compound **b11** possessed a considerable amount of anticancer activity.

Keywords: Indole, $InCl_3$, Solvent free conditions, *In silico* modeling, BCL-2 protein, Anticancer activity.

INTRODUCTION

The heterocyclic compound indole is a key structural moiety that occurs in a wide range of natural products and synthetic medicinal compounds[1,2]. Indole exhibits a wide range of biological activities like anticancer[3,4], antioxidant[5-7], antirheumatoidal[8,9] and anti-HIV[10-12]. The versatile nature of Indole, which enables it to function as both as a hydrogen bond donor and acceptor, facilitates a wide range of interaction with biological targets[13-14]. Due to its abundant variety and substantial pharmacological characteristics, Indole has emerged as a highly intriguing frame for the investigation of anticancer medications[15,16]. This phenomenon is exemplified by the recent approval of various indole-based anticancer medicines including Panobinostat, Alectinib, Sunitinib, Osimertinib [Fig. 1]. In recent years, it has been reported that a variety of indole-based compounds like mitraphylline, cedranib, indomethacin, indoximod, tryptophol, and Vincristine are used in the treatment of cancer[17]. These results emphasize the importance of the indole structure in the development of anticancer medications. Breast

cancer remains a significant worldwide health issue, affecting many individuals globally. An area of research that holds promise is the study of inhibiting the (BCL-2) protein, which exhibits a vital role in the survival of the cancer cells[18].

BCL-2 is a part of large family of proteins called as (family BCL-2) which plays a crucial role in preventing apoptosis, by regulating mitochondrial membrane permeability that acts as a vital defense mechanism against cancer formation[19]. The dysregulation of apoptosis, which is the abnormal regulation of cell death, is a key characteristic identified in diverse types of cancer, including breast cancer. This dysregulation is marked by the over production of anti-apoptotic proteins such BCL-2. Elevated levels of BCL-2 can provide cancer cells a clear advantage in terms of survival, allowing them to evade signals that trigger cell death and develop resistance to common treatments like chemotherapy and radiation therapy[20,21]. There has been an increasing focus in scientific research on investigating the potential of indole-based medicines as inhibitors of BCL-2 for breast cancer treatment[22]. The use of indole compounds as inhibitors of BCL-2 is a promising and novel strategy in the field of breast cancer treatment. Indoles, with their diverse chemical structures, have shown significant potential in suppressing cancer growth, making them attractive candidates for disrupting pro-survival signaling pathways controlled by BCL-2[23]. Several studies have provided compelling evidence on the efficacy of indole-based BCL-2 inhibitors in treating breast cancer[24-26].

Furthermore, there are several reports on the synthesis of structurally diverse indole analogues, synthesized to enhance their specificity and effectiveness as inhibitors of BCL-2[27,28]. To summarize, the use of indole-based medications to specifically target BCL-2 shows immense potential as a therapeutic method for breast cancer. The indole based medications show promising increase of the cancer cells to undergo apoptosis and overcome the resistance to therapeutic treatments.

MCRs-Multicomponent reactions, in which two or more substrates are added to a single pot to yield a variety of biologically active heterocyclic compounds which play a crucial role in the synthetic organic chemistry[29]. Numerous investigations have demonstrated the high efficiency, fast reaction times and atom economy of MCRs. We here in report the synthetic route for the synthesis of indole substituted derivatives using indium chloride as the catalyst without a solvent and explore their activity as BCL-2 inhibitors. The molecular docking and molecular dynamic studies were perfomed and all the newly synthesized derivatives were assessed for anticancer actitivty towards human cancer cell lines-MCF-7.

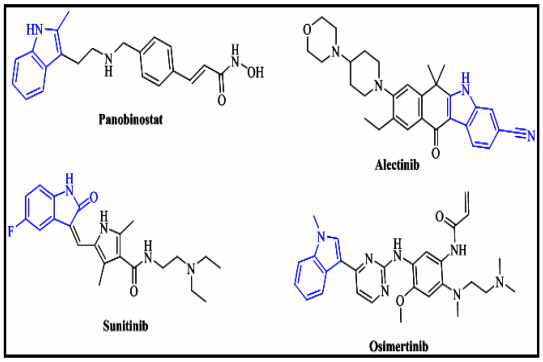


Fig. 1. Anticancer drugs containing substituted indoles in their structure

MATERIALS AND METHODS

The chemicals and reagents utilised in the laboratory are purchased from reputable suppliers such as Merck and SD fine chemicals. Melting points were determined using uncorrected open ended glass capillaries. The purity of the product was routinely assessed using Merck Silica G thin layer chromatography plates. Visualization of TLC spots was done using an iodine chamber or UV lamp. Infra red spectra of the analysed substances was obtained using a FTIR spectrophotometer KBr particles (BIO-RAD FTS). Proton-^{1}H-NMR and carbon-^{13}C-NMR spectral data were acquired using 400 MHz-Bruker Avance II nuclear magnetic resonance spectrometer. Dimethyl sulfoxide-d6 was used as solvent for proton and carbon NMR spectrum. The chemical shift of the test substances were reported on a parts per million (ppm) scale. The JEOL-SX-102 instrument uses electron ionization bomb to obtain the mass spectral analysis. The PerkinElmer Model 2400 CHN elemental analyser was used for analysis.

General method for the preparation of compounds b1-b12

To indole [1 mmol], aromatic substituted aldehydes [1 mmol] and aromatic amines [1.5 mmol], $InCl_3$(10 mol percent) was added and then

it was refluxed for stipulated time (Table 2). After completion, crushed ice was added and the crude was obtained as a solid precipitate. The precipitate was filtered and purified by column chromatography with 50% ethylacetate-hexane as eluents to get the pure compounds **(b1-b12)**.

Spectral data

Compound b1

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 8.02 (s, 1H, NH), 7.96 (s, 1H), 7.71-7.65 (m, 5H), 7.48-7.27 (m, 9H), 5.70 (s, 1H), 2.85 (s, 3H), ^{13}C-NMR (Dimethyl sulfoxide-d_6) δ: 147.52, 144.67, 136.65, 132.91, 129.65, 128.88, 128.11, 127.03, 125.89, 123.92, 121.86, 120.48, 119.17, 112.37, 110.93, 47.91, 29.66. IR (KBr) υ: 3346, 2920, 2855, 2214, 1635, 1420, 758 cm^{-1}. EI-MS *m/z*: 313 [M+1]. Colour: Brown solid, Yield: 90%, m.p.: 141-145°C, Elemental Analysis for $C_{22}H_{20}N_2$-: Calculated% (Found%): C, 84.58 (84.56); H, 6.45 (6.47); N, 8.97 (8.99).

Compound b2

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 8.04 (s, 1H, NH), 7.76 (1H, s), 7.40-7.33 (4H, m), 7.15-7.25 (m, 7H), 6.55 (d, *J* = 7.9 Hz, 2H), 5.83 (s, 1H), 2.83 (s, 3H); ^{13}C-NMR (Dimethyl sulfoxide-d_6) δ: 147.43, 142.88, 142.56, 137.37, 132.44, 131.18, 130.78, 130.08, 129.65, 128.43, 126.05, 123.58, 122.45, 121.16, 119.56, 111.17, 112.85, 47.84, 28.96. IR (KBr) υ: 3325, 2975, 1615, 1385, 845, 734 cm^{-1}. EI-MS *m/z*: 348 [M+2]. Colour: Yellow solid, Yield: 89%, m.p.: 112-115°C, Elemental Analysis for $C_{22}H_{19}N_2Cl$:-Calculated% (Found%): C, 76.18 (76.16); H, 5.52 (5.54); N, 8.08 (8.06).

Compound b3

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 8.14 (s, 1H, NH),7.94 (s,1 H), 7.47 (d, *J* = 7.91 Hz, 1H), 7.39-7.29 (m, 3H), 7.20-7.13 (m, 2H), 7.10-6.97 (m, 3H), 6.82-6.55 (m, 4H), 5.81 (s, 1H), 3.83 (s, 3H), 2.82(s, 3H). ^{13}C-NMR (Dimethyl sufoxide-d_6) δ: 157.82, 148.61, 137.62, 136.37, 134.95, 128.76, 127.52, 123.65, 122.91, 121.84, 118.97, 117.82, 114.57, 113.37, 112.22, 54.19, 41.08, 32.95. IR (KBr) υ: 3488, 2895, 1545, 1478, 1287, 1157, 1043, 858, 643 cm^{-1}. EI-MS *m/z*: 343 [M+1]. Colour: Brown solid, m.p.: 167-170°C, Yield: 91%, Elemental Analysis for $C_{23}H_{22}N_2O$: Calculated% (Found%): C, 80.67 (80.65); H, 6.48 (6.46); N, 8.18 (8.16).

Compound b4

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 9.55 (s, 1H, OH), 8.84 (s, 1H, NH), 8.79 (s, 1H), 8.54 (t, 2H, *J* = 7.81 Hz), 8.47-8.17 (m, 3H), 7.34-7.12 (m, 4H), 7.08-6.77 (m, 4 H), 5.48 (s, 1H). ^{13}C-NMR (Dimethyl sufoxide-d_6) δ: 162.46, 151.28, 148.24, 141.09, 139.65, 138.74, 137.56, 132.63, 121.96, 121.81, 121.53, 119.84, 115.63, 115.33, 115.21, 111.34, 108.17, 106.42, 52.13. IR (KBr) υ: 3486, 3355, 2928, 1619, 1538, 1265, 758 cm^{-1}. EI-MS *m/z*: 315 [M+1], Colour: Brown Solid, Yield: 89%, m.p.: 143-145°C, Elemental Analysis for $C_{23}H_{18}N_2O$:-Calculated% (Found%): C, 80.23 (80.21); H, 5.77 (5.75); N, 8.91 (8.89).

Compound b5

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 9.45 (s, 1H, OH), 8.97 (s, 1H, NH), 8.88 (s, 1H), 8.67 -8.27 (m, 3H), 8.01-7.46 (m, 6H), 7.31 (d, 2H, *J* = 7.25 Hz), 6.98 (d, 2H, *J* = 7.12 Hz), 5.44 (s,1H), 3.35 (s, 3H). ^{13}C-NMR δ (Dimethyl sulfoxide): 163.47, 152.19, 148.03, 140.09, 139.79, 138.85, 137.47, 131.68, 121.87, 121.71, 121.33, 118.53, 115.94, 115.50, 115.30, 110.49, 107.67, 107.41, 51.05, 36.02. IR (KBr) υ: 3495, 3375, 2949, 1609, 1508, 1241, 756 cm^{-1}. EI-MS *m/z*: 329 [M+1], Colour: Light Brown Solid, Yield: 95%, m.p.: 162-164°C, Elemental Analysis for $C_{23}H_{20}N_2O$:-Calculated% (Found%): C, 80.46 (80.42); H, 6.14 (6.10); N, 8.53 (8.49).

Compound b6

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 9.65 (s, 1H, OH), 8.93 (s, 1H), 8.82 (s, 1H), 8.72-8.58 (m, 2H), 7.98-7.84 (m, 5H), 7.70-7.62 (m, 3H), 7.44 (d, 2H, *J* = 7.23 Hz), 7.01 (s, 1H), 5.42 (s, 1H), 3.31 (s, 3H). ^{13}C-NMR (Dimethyl sulfoxide) δ: 163.55, 151.11, 149.21, 148.00, 137.68, 131.86, 131.77, 126.16, 123.51, 119.11, 116.07, 116.04, 115.61, 114.63, 113.42, 107.79, 107.52, 103.16, 61.43, 58.0, 35.07IR (KBr) υ: 3457, 3357.96, 2929.63, 1609.63, 1508.60, 1240.89, 807.14, 739.99 cm^{-1}. EI-MS *m/z*: 329 [M+1], Colour: Brown Solid, Yield: 93%, m.p.:138-142°C, Elemental Analysis for $C_{23}H_{20}N_2O$:-Calculated% (Found%): C, 80.46 (80.42); H, 6.14 (6.10); N, 8.53 (8.49).

Compound b7

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 8.98 (s, 1H, NH), 7.96 (s, 1H), 7.38-7.32 (m, 2H), 7.27-7.18 (m, 2H), 7.17-7.13 (m, 3H), 7.05- 6.98 (m, 2H), 6.64-6.58 (m, 2H), 6.55-6.51 (m, 2H), 5.86 (s, 1H), 2.81 (s, 3H). ^{13}C-NMR (Dimethyl sulfoxide-d_6) δ: 148.78, 144.28, 130.35, 130.12, 129.73, 128.43, 127.12, 123.45, 121.98, 119.78, 119.54, 119.20, 113.02, 111.10, 48.73, 29.45. IR(KBr) υ: 3385, 2983, 1515, 1478, 1276, 788, 652 cm^{-1}. EI-MS *m/z*: 358 [M+1]. Colour: Light brown solid, Yield: 88%,

m.p.: 67-70°C, Elemental Analysis for $C_{22}H_{19}N_3O_2$:- Calculated% (Found%): C, 73.93 (73.95); H, 5.36 (5.35); N, 11.76 (11.73).

Compound b8

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 8.92 (s, 1H, NH) 7.97-7.38 (m, 5H), 7.19-6.98 (m, 5H), 6.64-6.59 (m, 2H), 6.55-6.51 (m, 2H), 5.65(s, 1H), 2.83 (s, 3H). ^{13}C-NMR (Dimethyl sulfoxide-d_6) δ: 148.71, 146.8, 136.28, 134.95, 130.12, 129.68, 127.12, 126.32, 124.54, 122.88, 119.67, 119.44, 119.25, 113.35, 111.17, 47.84, 29.76. IR (KBr) υ: 3392, 2987, 1576, 1426, 1296, 778, 658 cm-1. EI-MS *m/z*: 358 [M+1]. Colour: Brown solid, Yield: 89%, m.p.: 75-78°C, Elemental Analysis for $C_{22}H_{19}N_3O_2$:- Calculated% (Found%): C, 73.93 (73.95); H, 5.36(5.35); N, 11.76 (11.73).

Compound b9

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 8.75 (s, 1H, NH) 7.80-7.56 (m, 2H), 7.36-7.32 (m, 2H), 7.29-7.04 (m, 6H), 6.90-6.42 (m, 4H), 5.74 (s, 1H), 2.75 (s, 3H). ^{13}C-NMR (Dimethyl sulfoxide-d_6) δ: 149.71, 147.93, 138.16, 136.94, 132.22, 130.78, 129.15, 127.32, 124.59, 122.14, 119.65, 119.42, 118.78, 112.37, 111.16, 45.48, 31.65. IR (KBr) υ: 3392, 2987, 1576, 1426, 1296, 778, 658 cm^{-1}. EI-MS *m/z*: 358 [M+1]. Colour: Brown solid, Yield: 91%, m.p.: 71-75°C, Elemental Analysis for $C_{22}H_{19}N_3O_2$:- Calculated% (Found%): C, 73.93 (73.95); H, 5.36 (5.35); N, 11.76 (11.73).

Compound b10

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 8.96 (s, 1H, NH), 8.27 (s, 1H), 8.62-8.43 (m, 2H), 7.86-7.58 (m, 7H), 7.34 (d, 2H, *J* = 7.38 Hz), 6.94-6.83 (m, 2H), 5.64 (s, 2H, NH_2), 5.56 (s, 1H), 2.93 (s, 3H). ^{13}C-NMR (Dimethyl sulfoxide-d_6) δ: 150.18, 147.16, 140.96, 137.69, 134.34, 133.78, 131.92, 129.98, 128.59, 128.17, 127.78, 127.26, 127.12, 126.84, 124.46,119.57, 118.15, 117.32, 53.14, 38.01. IR (KBr) υ: 3425.68, 3385.12, 3145.13,2965.83, 1617.34, 787.18 cm^{-1}. EI-MS *m/z*: 328 [M+1], Colour: Light bown Solid, Yield: 92%, m.p.: 170-172°C, Elemental Analysis for $C_{22}H_{21}N_3$:- Calculated% (Found%): C, 80.70 (78.88); H, 6.46 (6.32);N, 12.83 (10.74).

Compound b11

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 8.92 (s, 1H, NH), 8.20 (s, 1H), 8.56-8.34 (m, 2H), 7.98-7.77 (m, 6H), 7.68-7.42 (m, 3H), 6.97 (d, 2H, *J* = 7.62 Hz), 5.43 (s, 1H), 5.54 (s, 2H, NH_2), 2.86 (s, 3H). ^{13}C-NMR (Dimethyl sulfoxide-d_6): 152.29, 145.06, 139.96, 136.66,135.21, 133.62, 132.99, 129.51, 128.89, 128.87, 128.68, 128.17, 128.03, 126.58, 124.84, 119.17, 119.10, 117.33, 55.54, 33.51. IR(KBr) υ: 3402.57, 3375.00, 3130.43, 2955.91, 1627.46, 767.68 cm^{-1}. EI-MS *m/z*: 328 [M+1], Colour: Yellow Solid, Yield: 93%, m.p.: 176-178°C, Elemental Analysis for $C_{22}H_{21}N_3$:-Calculated% (Found%): C, 80.70 (78.88); H, 6.46 (6.32);N, 12.83 (10.74).

Compound b12

^{1}H-NMR (Dimethyl sulfoxide-d_6) δ: 8.77 (s, 1H, NH), 8.16 (s, 1H), 8.03-7.85 (m, 2H), 7.76-7.36 (m, 7H), 7.15-6.77 (m, 4H), 5.62 (s, 2H, NH_2), 5.46 (s, 1H), 3.02 (s, 3H). ^{13}C-NMR (Dimethyl sulfoxide-d_6): 149.48, 148.18, 142.67, 139.95, 135.14, 133.69, 132.23, 129.54, 129.12, 128.87, 128.58, 127.35, 127.18, 126.98, 124.65, 119.87, 118.53, 117.24, 54.45, 37.32. IR (KBr) υ: 3425.52, 3394.62, 3185.19, 2928.74, 1634.52, 757.15 cm^{-1}. EI-MS *m/z*: 328 [M+1], Colour: Light bown Solid, Yield: 90%, m.p.: 170-172°C, Elemental Analysis for $C_{22}H_{21}N_3$: Calculated% (Found%): C, 80.70 (78.88); H, 6.46 (6.32);N, 12.83(10.74).

Molecular docking

The *In-silico* modeling of designed compounds was performed by Auto Dock Vina Version 1.2.0. by POAP. BCL-2 receptor X-ray crystal structure (PDB:6WH0) was retrieved Research Collaboratory for structural Bioinformatics protein Data Bank. The virtual screening programm PyRx was used to carry out the docking procedures. Pymol 8 and Discovery Studio Visualizer were used to visualise the ligand-target protein interactions. The outcomes are presented as images.

Molecular dynamics

Molecular dynamics simulation analysis[30-32] was used to analyse protein-ligand complex stability and fluctuation. The goal was to assess potential drug candidate's protein target binding. The MD simulations were conducted using the Desmond module, which was developed by D.E.Shaw Research. The complex protein-ligand interaction was solved using the TIP3 water model with a cubic box boundary constraint. The system became inert at a concentration of 0.15 M Na^+ and Cl^- counter ions. In MD simulations, an NPT ensemble was used, maintaining constant pressure (1.01325 bar) and temperature (300k). In the simulations, energies were minimized using the OPLS-4 force field 1.2 and 50 picoseconds recording intervals. The molecular

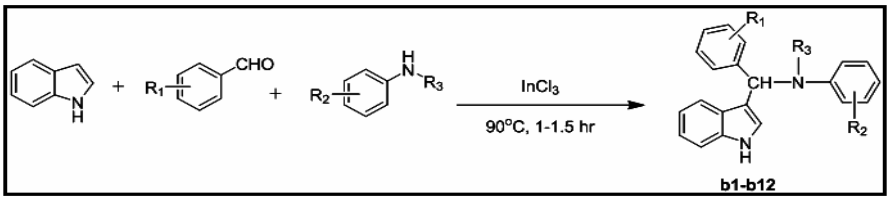
dynamics simulation lasted 100 nanoseconds. The MD simulation results were evaluated using Simulation Interaction Diagram of the Schrödinger desmond module.The trajectories and 3D structures were visually examined using the Meastro graphical interface subsequent to Simulation.

Cytotoxic activity

The compound **b11** was tested against MCF-7 cancer cell line for anticancer activity (*In vitro*) using the sulforhodamine B(SRB) assay. To supplement the cell culture media 10% foetal bovine serum and 2 mM L-glutamine were added. Injection of 5000 cells per well in 90 µL onto 96-well microtiter plates was the current screening procedure. The incubation of microtiter plates was for 24 h, with 95% air, 5% CO_2 and 100% relative humidity at 37°C. Different concentrations of test compounds or control solutions are used to treat the cells and are incubated for 72 hours. The cells are fixed with 100 µL of cold 10% TCA and incubated for 1 hours. Traces of TCA are removed by washing the cells with tap water and medium. It is carried out by the addition of 100µL of 0.4% of sulphorhodamine solution in 1% acetic acid. The solubilisation of the bound dye was performed using 10mM tris base solution. At 540nm using ELISA plate readerthe absorbance of the eluted dye was measured. To establish the drug concentration that inhibited development completely, Ti=Tz was used. The formula [(Ti-Tz)/Tz]x100=-50 calculates the medication concentration that reduces protein levels by 50% at the conclusion of the rapy which is represented as LC_{50}.

RESULTS AND DISCUSSION

The route for the synthesis of novel indole derivatives **(b1-b12)** was shown in Scheme 1. In order to screen reaction conditions, different mole percentages of $InCl_3$ was used in different solvents like EtOH, THF, CH_3CN, toluene, DMF, $CHCl_3$ at different temperatures (Table 1) for the reaction between indole, benzaldehyde and N-methyl aniline to synthesize compound b1. On the basis of these results (Table 1) the optimised reaction conditions were found using $InCl_3$ (10 mol%) under solvent free conditions at 80°C. Hence, we here in describe an efficient and high yielding process from the reaction between indole, substituted benzaldehyde and substituted aniline in the presence of $Incl_3$ under neat conditions to yield 3-alkylated indole derivatives **(b1-b12)** (Table 2).



Scheme 1. Preparation of 3-substituted indole derivatives b1-b12 under solvent free conditions

The synthesized compounds **(b1-b12)** were analysed using ^{1}H-NMR, IR, and Mass spectra, and elemental analysis, which confirmed the ascribed structures. The synthesized compounds **(b1-b12)** exhibited distinctive peaks corresponding to Ar-NH at 3217-3597 cm^{-1}, Ar-CH at 2961-3422 cm^{-1} and Ali-CH at 2872-3001 cm^{-1} suggesting the existence of specific functional groups in the synthesized compounds. This was further verified by the appearence of distinct peaks in the ^{1}H-NMR spectra, specifically demonstrating the presence of -NH at 7.94-8.91ppm, Aromatic-C-H at 6.92 -7.97ppm, Benzylic C-H at 5.41-5.86ppm and Aliphatic C-H at 2.31-2.95ppm, which corroborated the corresponding structures of the produced compounds **(b1-b12)**. The molecular weight of compounds **(b1-b12)** was Validated by mass spectrometry analysis. The elemental analysis of all synthesized compounds exhibited a deviation of not more than +0.4% from the calculated value, there by confirming the purity of the produced compounds.

Table 1: Effect of catalyst, solvent and temperatue for the synthesis of b1

Sr. No	Catalyst	Solvent	Temperature (°C)	Yield(%)
1	$InCl_3$ (20%)	EtOH	80	68
2	$InCl_3$ (20%)	THF	70	71
3	$InCl_3$ (20%)	CH_3CN	85	76
4	$InCl_3$ (20%)	Toluene	120	70
5	$InCl_3$ (20%)	DMF	120	65
6	$InCl_3$ (20%)	$CHCl_3$	70	52
7	$InCl_3$ (10%)	SF	80	90
8	$InCl_3$ (30%)	SF	80	82
9	$InCl_3$ (20%)	SF	80	79
10	$InCl_3$ (20%)	SF	100	81

SF-Solvent Free

Table 2: $InCl_3$ catalysed synthesis of b1-b12

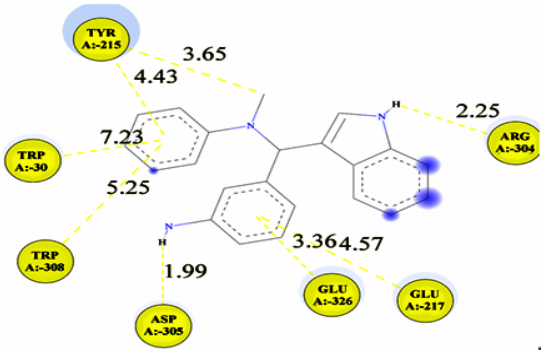
Entry	R_1	R_2	R_3	Time (h)	Yield (%)[a]
b1	H	H	CH_3	1.5	90
b2	*p*-Cl	H	CH_3	1.0	89
b3	*o*-OCH_3	H	CH_3	1.0	91
b4	*p*-OH	H	H	1.0	89
b5	*m*-OH	H	CH_3	1.25	95
b6	*o*-OH	H	CH_3	1.0	93
b7	*p*-NO_2	H	CH_3	1.25	88
b8	*m*-NO_2	H	CH_3	1.5	89
b9	*o*-NO_2	H	CH_3	1.25	91
b10	*p*-NH_2	H	CH_3	1.0	92
b11	*m*-NH_2	H	CH_3	1.5	93
b12	*o*-NH_2	H	CH_3	1.0	90

a: Isolated yield

Molecular Docking Study

In elucidating the binding affinity of ligands to the target proteins, *In silico* molecular docking studies were performed which aids in the design of new drugs[33]. This study involved the utilization of ligands sourced from the titled compounds. These ligands were subjected to molecular docking examinations against the BCL-2 protein using Protein Data Bank, (PDB:ID-6WH0) and investigated their interactions with different orientations of BCL-2. Venetoclax is used as a standard anti apoptotic BCL-2 reference drug. Over expression of BCL-2 can lead to tumour growth and therefore targeting BCL-2 has emerged as adynamic therapeutic approach to induce apoptosis in cancerous cells[34,35].

All the synthesized molecules **(b1-b12)** interacted with the binding pocket of the target protein BCL-2 with favourable docking scores ranging between -7.1 to -9.5 kcal/mol^{-1} (Table 3). Their binding energy values are correlated with the standard ligand CoCry (Cocrystal) and reference drug Venetoclax which has a binding energy of -9.1 k.cal/mol^{-1}. It was found that compound **b5** and **b11** exhibited the highest binding scores in comparison with all the other synthesized compounds and CoCry. The amino acids which exhibited greatest degree of interaction with **b11** are GLU-326, TRP-308, ALA-307, ASP-305, ARG-305, GLU-217, TYR-215, TRP-30 and CoCry were GLU-326, ASP-305, ARG-304, GLU-217. Among all the compounds, it is clearly evident that the binding energy of **b11**(-9.2k. cal/mol^{-1}) and amino acid interactions ASP-356, LYS-355, ALA-307, and TYR-215 are very perfect fitting with the standard, Cocrystal which shows interactions as ASP-356, LYS-355, ALA-307, TYR-215, TRP 308, MET-40 so that it was suggested that the **b11** demonstrate strong suppression of the BCL-2 protein (Table 4). Consequently, these results indicate a high potential of **b11** for the treatment of breast cancer. The interactions of binding, 2D and 3D model of active compound **b11** and Cocry was shown in the Figure 2 and 3.



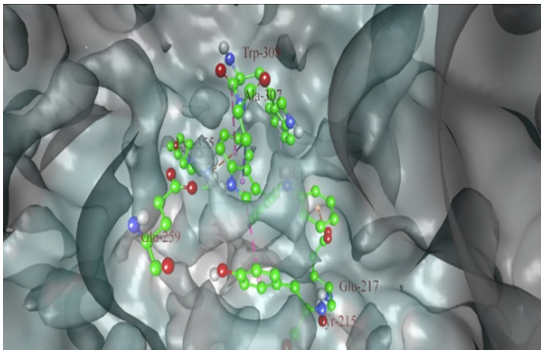
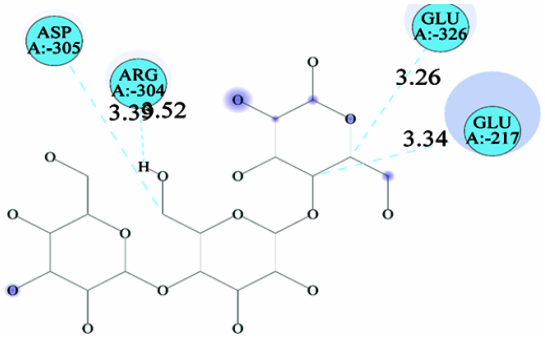


Fig. 2. The binding orientations 2D and 3D model of test compound b11



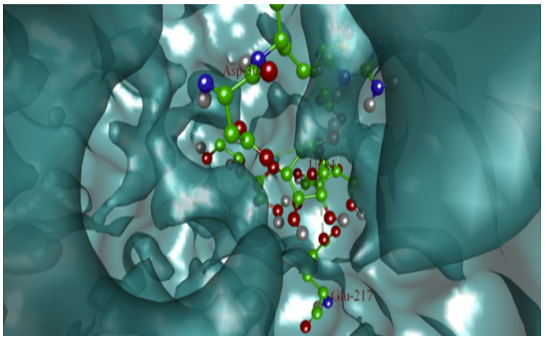


Fig. 3. The binding orientations 2D and 3D model of Cocrystal

Table 3: Binding energies of compounds b1-b12

Sr. No	Compound Code	Binding energy (kcal/mol)
1	venetoclax	-9.1
2	b5	-9.5
3	b11	-9.2
4	b8	-9.1
5	b7	-8.5
6	b9	-8.3
7	b1	-8.2
8	b3	-8
9	b10	-7.9
10	b6	-8.2
11	b2	-8
12	b4	-8
13	b12	-7.8
14	Co Cry (standard)	-7.1

Table 4: Molecular Docking results of compounds (b1-b12) with PDB ID-6WHO amino acids

Sr. No	Compound Code	Degree of interaction (H-bond) with amino acids
1	b5	ASP-356, LYS-355, ALA-307, TYR-215, MET-40, TRP-30
2	**b11**	**GLU-326, TRP-308, ALA-307, ASP-305, ARG-304, GLU-217, TYR-215, TRP-30**
W	b8	TRP-308, ALA-307, ASP-305, GLU-217, PRO-216, MET-40, TRP-30, TYR-29
4	b7	GLU-326, GLU-325, ARG-304, GLU-217, MET-40, SER-33, TRP-30
5	b9	GLU 326, TRP-308, ALA-307, ASP-305, ARG-304, GLU-217, TRP-30
6	b1	ALA-307, ASP-305, ARG-304, GLU-217, TRP-30
7	b2	TRP-308, ALA-307, ASP-305, ARG-304, GLU-217, TYR-215, TRP-30
8	b3	GLU-326, TRP-308, GLU-217, PRO-216, TYR-215, TRP-30, TYR-29
9	b4	GLU-326, TRP-308, ASP-305, GLU-217, TYR-215, SER-33, TRP-30, TYR-29
10	b10	LYS-355, TRP-308, ALA-307, ASP-305, GLU-259, GLU-217, TYR-215
11	b12	GLU-326, GLU-325, GLU-217, MET-40, TRP-30
12	b6	GLU-326, TRP-308, ALA-307, GLU-217, TRP-30, TYR-29
13	Co-Cry (standard)	**GLU 326, ASP 305, ARG 304, GLU 217**

Molecular Dynamic Studies

The interaction patterns exhibited by the protein-ligand complexes of N-((3-aminophenyl)(1H-indol-3-yl)methyl)-N-methylaniline **(b11)** were investigated by calculating the RMSD-Root-Mean-Square Deviation and RMSF-Root-Mean-Square Fluctuation (RMSF) values. The complex's interaction fingerprints, and the protein-ligand contacts were determined by analysing aminoacid residues interactions and determining their RMSD and RMSF, maintaining the protein-ligand complex stability[36]. To be more precise, the ligands were evaluated by measuring the CA (C-α) protein. Furthermore, the acquired data showed that the RMSF of **b11** did not change throughout the period of 100 nanoseconds. Results from molecular dynamics simulation for period of 100 ns of the protein back bone and ligand in relation its protein were obtained. There were 3.11 ligands per protein, 2.52 protein backbone, and 2.45 protein CA values in the **b11** complex. The range of contacts revealed by the **(b11)** in the protein was 1.87 Å to 7.82 Å and in the ligand it was 2.14 Å to 6.23 Å, according to the MD simulations [Fig. 4]. The RMSF values of **(b11)** protein-ligand complexes remained consistent throughout the molecular dynamic's simulation. Based on the molecular dynamic's simulation results, it is found that compound's stability is outstanding, and the fluctuation curves show that the interactions between the 23 amino acids are constant. Additionally, the remaining amino acids in the RMSF residual index also do not fluctuate. Among the amino acid interactions with respect to **b11**, we find that GLU 259 amino acid has direct interaction of 72% with NH_2 group of **b11**, TYR 215 amino acid has 71% interaction with NH_2 of **b11**, and LYS 353 with 44% and 30% of direct interactions with the indole ring of **(b11)** respectively [Fig. 5]. The docking contact of **(b11)** suggests that the interaction between the amino acid TYR 215 and the MD simulation was congruent. The radial coordinate denotes the torsion potential of the rotatable bond, whereas the angular coordinate denotes the torsional angle. The torsional conformation of **(b11)**, together with the flexible bonds in 3-aminophenyl and methylaniline is shown in Figure 6.

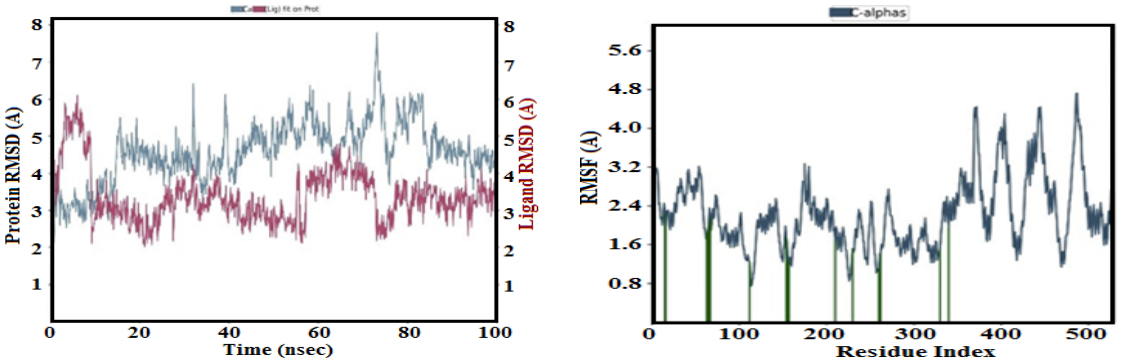


Fig. 4. RMSD, and RMSF of BCL-2b11 complex

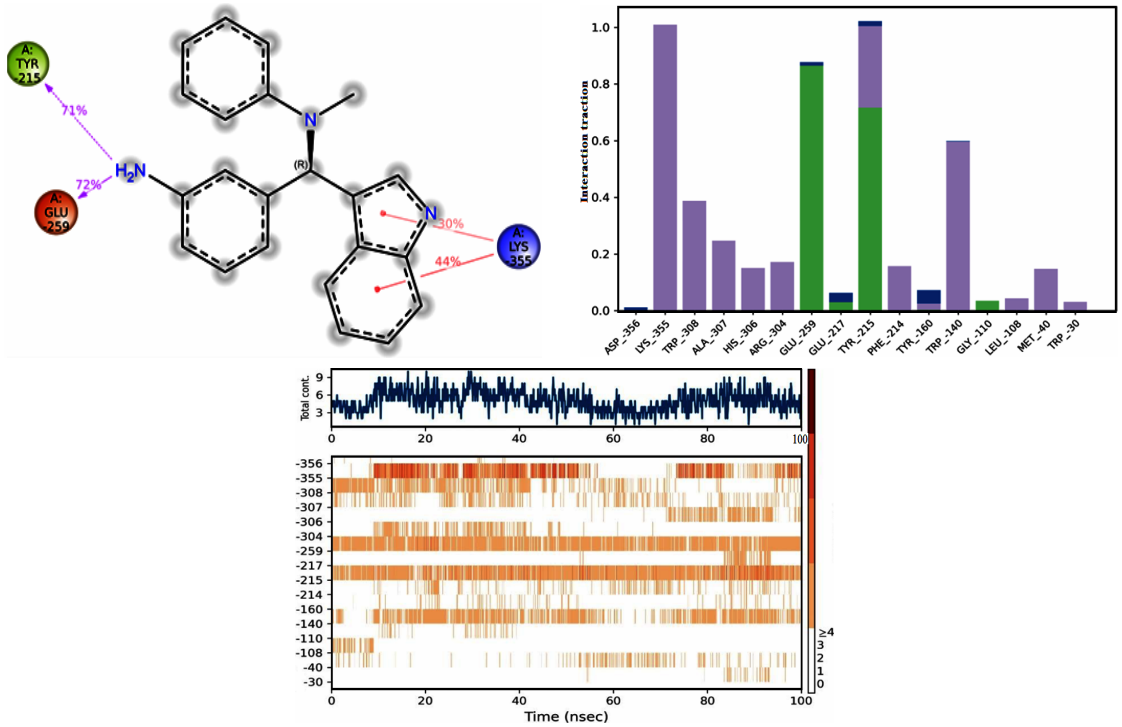


Fig. 5. The protein-ligand contact of the compound b11

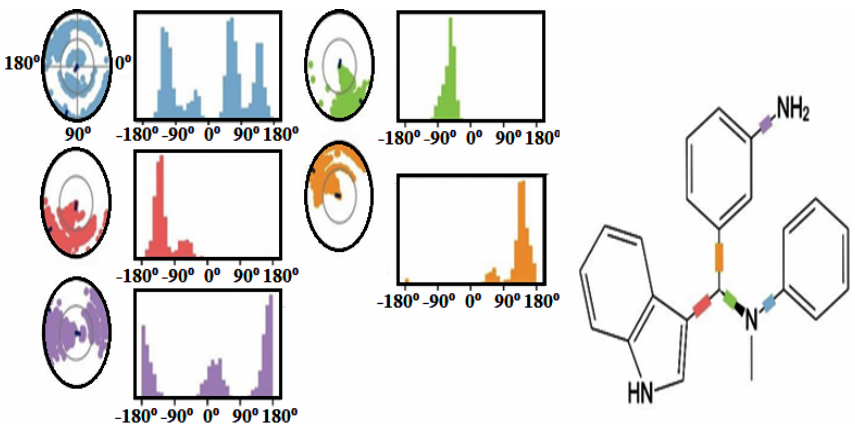


Fig. 6. The torsional conformation of b11

Cytotoxic activity

The anticancer activity of the prepared indole derivatives **(b1-b12)** were tested against breast cancer (MCF-7) (human cancer cell lines) utilising Sulforhodamine B-(SRB) assay. Based on the data obtained from the docking and dynamic studies, choosed the compound **(b11)** to be evaluated, and found that **b11** was the highly effective compound against the (MCF-7) cell line. When compared to the conventional drug Imatinib, which has IC_{50} value- 0.10 µM/mL, a TGI-0.2 µM/mL, GI50-0.02 µM/mL and LC_{50}-1.8 µM/mL, **(b11)** demonstrated a significant level of activity towards the (MCF-7) cell line. It shows IC_{50} value as 0.10 µM/mL, while its TGI was 0.11 µM/mL, GI_{50} was 0.08 µM/mL, and LC_{50} was 1.2 µM/mL as shown in Figure 7.

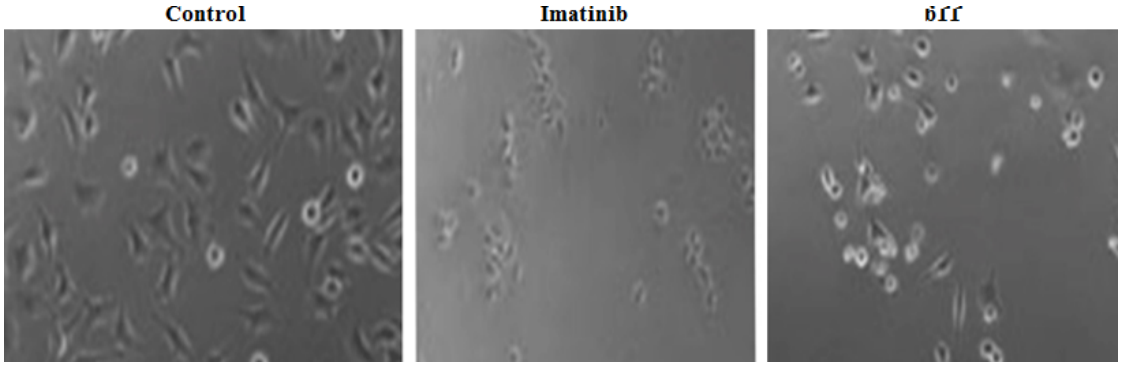


Fig. 7. The *In vitro* anticancer activity of the synthesized compound b11

CONCLUSION

The current investigation used a computational approach to identify lead moiety from the synthesized indole compounds **(b1-b12)**. The results indicate that N-((3-aminophenyl)(1H-indol-3-yl)methyl)-N-methylaniline **(b11)** have the favourable binding interactions with the BCL-2 proteins. The dynamic behaviour of the interaction between the ligands and proteins was assessed by MD simulations, providing significant insights into drug-target complexes stability and conformational changes. The findings of this work present a streamlined approach for identifying the highly effective lead compound **(b11)** and demonstrated the superior characteristics of **(b11)** as a lead moiety for inhibiting the target BCL-2 proteins.

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Conflict of interest

The authors declares there are no conflicts of interest with the publication of this manuscript.

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