Design, Synthesis and Biological Evaluation of Novel N-Substituted Benzimidazole Derivatives as Potential Anticancer Agents

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Abstract

This study presents the design, synthesis, characterization and anticancer evaluation of novel N-substituted benzimidazole derivatives targeting the Topoisomerase II receptor. A series of ten derivatives (SA1–SA10) were synthesized and screened for cytotoxicity against MDA-MB-231 human breast cancer cells using the Sulforhodamine B (SRB) assay. Computational studies, including Swiss ADME, PyRx, and Marvin Sketch predicted favorable drug-likeness properties, with compliance to Lipinski's rule of five. Molecular docking studies performed using AutoDock 4® TOOLS 1.5.6 confirmed strong binding affinities of the synthesized derivatives with Topoisomerase II. The synthesized compounds were characterized by percentage yield, solubility, melting point, and molecular formula. Purity and structural identity were confirmed through TLC, IR, NMR, and mass spectrometry. Among the derivatives, SA5 demonstrated the highest anticancer activity (IC50: 123.39 ± 76.23 µM), while SA7 also exhibited significant cytotoxicity. Other derivatives showed moderate activity, with IC_{50} values ranging from 30 to 250 µg/ml. These findings highlight the potential of SA5 and SA7 as promising anticancer agents. SA5 and SA7 emerged as promising lead compounds for anticancer drug development, highlighting the therapeutic potential of benzimidazole derivatives as Topoisomerase II inhibitors. Further biological evaluation is required to assess their complete pharmacological profile.

Keywords: Benzimdazole derivatives, Anti Cancer Activity, Topoisomerase II inhibitors, Molecular docking.

INTRODUCTION

Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and multiply (through a process called cell division) to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place [1]. Sometimes this orderly process breaks down, and abnormal or damaged cells grow and multiply when they shouldn't. These cells may form tumors, which are lumps of tissue [2]. Tumors can be cancerous or not cancerous (benign).Cancerous tumors spread into, or invade, nearby tissues and can travel to distant places in the body to form new tumors (a process called metastasis). Cancerous tumors may also be called malignant tumors [3]. Many cancers form solid tumors, but cancers of the blood, such as leukemia, generally do not.

There are more than 100 types of cancer. Types of cancer are usually named for the organs or tissues where the cancers form. Diagnosing cancer at its earliest stages often provides the best chance for a cure. Many cancer treatments are available. Treatment options will depend on several factors, such as the type and stage of cancer [4], general health, and preferences. Cancer treatment includes targeted drug therapy, immunotherapy, bone marrow transplant, radiation therapy, chemotherapy, surgery, hormone therapy etc.,

Benzimidazole derivatives have emerged as a significant class of organic compounds due to their diverse biological activities and versatile applications in medicinal chemistry, agriculture, and material science [5]. The benzimidazole ring structure, consisting of benzene ring fused with to an imidazole ring imparts unique chemical properties, making these derivatives highly valuable in the design of molecules with targeted activities [6]. The broad spectrum of pharmacological effects, including antimicrobial [7], antitumor, anti-inflammatory and antiviral activities has garnered significant interest in the development of novel therapeutics. Moreover, benzimidazole derivatives have shown promise as agrochemicals, particularly fungicides, owing to their ability to disrupt fungal cell membrane synthesis [8].

In addition to their biological relevance benzimidazole have gained attention for their potential applications in materials science, such as their use in the synthesis of compounds. These compounds have also been explored for their catalytic and electronic properties, contributing to their utility in advanced technological applications [9]. Given their multifaceted potential, the synthesis, modification, and exploration of new Benzimidazole derivatives continue to be a central focus of research [10]. This article aims to review the recent advancements in the design, synthesis, and biological evaluation of Benzimidazole derivatives, highlighting their therapeutic potential and their role in developing innovative solutions to contemporary challenges in drug discovery and agrochemical development [11].

Heterocycles are an important class of compounds, making up more than half of all known organic compounds [12]. Heterocycles are present in a wide variety of drugs, most vitamins, many natural products, biomolecules, and biologically active compounds, including antitumor, antibiotic, anti-inflammatory, antidepressant, antimalarial, anti-HIV, antimicrobial, antibacterial, antifungal, antiviral, antidiabetic, herbicidal, fungicidal, and insecticidal agents [13]. Also, they have been frequently found as a key structural unit in synthetic pharmaceuticals and agrochemicals. Some of these compounds exhibit a significant solvatochromic, photochromic, and biochemi-luminescence properties [14].

Most of the heterocycles possess important applications in materials science such as dyestuff, fluorescent sensor, brightening agents, information storage, plastics, and analytical reagents [15]. In addition, they have applications in supra molecular and polymer chemistry, especially in conjugated polymers [16].

For medicinal chemists, the primary advantage of heterocyclic structures lies in their ability to generate diverse compound libraries from a single core scaffold. These libraries can be screened against various receptors, leading to the identification of multiple active compounds [17]. The vast possibilities of fused heterocycles enable the design of novel polycyclic frameworks with diverse physical, chemical, and biological properties [18].

Therefore, efficient methodologies resulting in polycyclic structures from biologically active heterocyclic templates are always of interest to both organic and medicinal chemists [19]. The primary objective of medicinal chemistry is to design and discovery of new drug compounds [20].

MATERIALS AND METHODS

Reagents and Instrumentation

- Oven dried glass wares were used to perform all the reactions. Procured reagents were of analytical grade and solvents of laboratory grade and purified as necessary according to techniques mentioned in Vogel's Textbook of Practical Organic Chemistry.
- In an open glass capillary tubes using Veego VMP-1 apparatus, melting points have been determined in ^{0}C and are uncorrected.
- Ascending TLC on precoated silica-gel plates (MERCK 6 F254) visualized under UV light was utilized to routinely monitor the progress and purity of the synthesized compounds. Solvents used during TLC are n-hexane, ethyl acetate, methanol, petroleum ether, chloroform and dichloromethane.
- The Infrared Spectra was plotted by Perkin-Elmer Fourier Transform-Infrared Spectrometer and in reciprocal centimetres the band positions are noted.
- Nuclear magnetic spectra (^{1}H NMR) were obtained from Bruker DRX-300 (300 MHz FT-NMR) spectrophotometer using DMSO as solvent with TMS as the internal

standard ^{13}C NMR have been recorded utilizing Bruker with Dimethyl sulphoxide as solvent. Shimadzu LC-MS was employed to record Mass Spectra [21].

General Procedure for the Synthesis of Compounds:

Procedure for the preparation of 1-(1H-benzo[d]imidazol-2-yl) ethanol

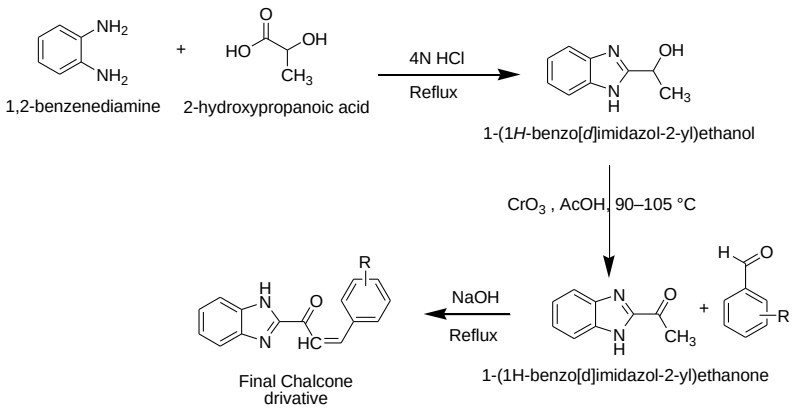
O- phenylene diamine (0.1 mmol) condensed in 25 mL of 4N HCl with 2-hydroxypropanoic acid (0.05 mmol). Reflux was used with stirring for 4 hours at 85^0C. TLC observed the reaction in a mobile phase with Ethyl acetate: n-Hexane (7:3). Ammonia was added to the reaction mixture after completing the reaction (monitored by TLC). The resulting substance was filtered and dried. After recrystallization of the crude product with ethanol, the corresponding product was produced in high purity [22].

Procedure for the preparation of 1-(1H-benzo[d]imidazol-2-yl) ethanone

1-(1H-benzo[d]imidazol-2-yl) ethanol (1 g, 6.17 mmol) was dissolved in 10 mL of acetic acid and heated to 90 °C. After 10 minutes, chromium trioxide (4.62 mmol) in 2 mL of water was slowly added dropwise to the reaction mixture and it was stirred at 105 °C for 30 min. After completion of the reaction (monitored by TLC), the crude residue was cooled to room temperature and poured into ice cold water. A brick-red colored solid compound was formed and filtered. The desired solid compound was recrystallized with ethanol and dried on a rotary evaporator to obtain the pure compound in 62% yield [23].

General procedure for the preparation of N-substituted benzimidazole derivatives

Refluxing a mixture of 1-(1H-benzo[d]imidazol-2-yl) ethanone (0.01 mmol) and the corresponding aromatic or heteroaromatic aldehyde (0.01 mmol) in absolute EtOH (10-12 mL) using NaOH as a catalyst. The Completion of reaction was monitored by TLC using N-hexane: EtOAc (5:5) as solvent and the reaction content was decanted into a Petridish. After the solvent was air dried, and the product was scraped and rinsed with weak HCl to gently to remove the base by neutralization [24].



SCHEME-I

In-silico molecular docking studies

Devices and materials

In the molecular scenario in the modern drug design, the docking is commonly used to understand the interaction between the target ligand-receptor and the target lead molecule's binding orientation with its protein receptor and is quite frequently used to detect the associations between the target components. The research work was done *in-silico* by utilizing bioinformatics tools. Also, we utilize some of the offline programming's like protein data bank (PDB) www.rcsb.org/pdb, PubChem database, Marvin sketch. The molecular docking studies were carried out through PyRx [25].

Preparation of protein

By utilizing the offline program protein data bank (PDB), we take the Topoisomerase II (PDB ID: 4FM7) was obtained from PDB website. From the protein (4FM7) we removed the crystal water, followed by the addition of missing hydrogens, protonation, ionization, energy minimization. The SPDBV (swiss protein data bank viewer) force field was applied for energy minimization. Prepared protein is validated by utilizing the Ramachandran plot [26].

Identification of active sites

The active amino acids in the protein were identified using the offline Protein-Ligand Interaction Profiler (PLIP) tool https://plip-tool.biotec.tu-dresden.de/plipweb/plip/index on Google. This analysis provided detailed insights into the key residues involved in ligand binding [27].

Preparation of Ligands

By utilizing the Marvin sketch tools the molecules are designed by two and three dimensional structures. After designed molecules the structure was obtained in 3D optimization in Marvin sketch and saved the pdb format [28].

In vitro anticancer activity

The *in vitro* cytotoxicity of the synthesized compounds was assessed against MDA-MB-231 cancer cell line using SRB assay. The monolayer culture of the cell line was trypsinized, followed by adjusting the cell count to 1.0×105 cells/ mL by means of DMEM medium containing 10% FBS. The diluted cell suspension (0.1 mL) was added to each well of the 96-well microtiter plate. The test wells were added with various concentrations (100µL) of test samples, and the control wells received media (100µL). The plates were then incubated at 37°C for 72h in 5% CO_2 atmosphere. After this duration, the cultures were fixed

with trichloroacetic acid (25µL, 10% w/v) and stained for 30 min with sulforhodamine B (0.4% w/v) in acetic acid (1% v/v). Unbound dye was cleared by four washes with acetic acid (1% v/v), and protein-bound dye was extracted with 10 mM unbuffered Trisbase [tris (hydroxyl methyl) amino methane]. The optical density of the protein-bound dye was recorded at 540 nm. The percentage cell viability (CV) was calculated using the following formula:

$$\text{Cell viability} = \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

The concentration of test samples required to inhibit cell growth by 50% was tabulated from the dose–response for each cell line**.** [29]

RESULTS

Molecular docking

The *in-silico* docking study of the designed molecules to the enzyme's active sites was performed by the pyrx to determine the binding affinities of the ligands. The designed compounds were docked towards Topoisomerase II (PDB ID: 4FM7) in order to ascertain their Topoisomerase II inhibition activity against inflammation. All the compounds were exhibited good affinity for the receptor when compared with Imatinib with Topoisomerase II inhibitory activity as an anti-cancer agent. The Docking scores of docking studies against Topoisomerase II (PDB ID: 4FM7) are shown in **Table 1**. From the in-silico docking results, it is evident that the interactions are mainly lipophilic factors due to the presence of aromatic heterocyclic rings. Among the docked compounds, compound SI2 possesses significant docking score -7.9K/cal. The remaining docked compound shows a docking score range from 7.3 to 7.9 K/cal along with one or two hydrogen bond interactions. Figure 1-10 shows the docking pose of compounds all the designed compounds.

Table 1. Molecular Docking Scores of Synthesized Compounds

Name	docking energy
SI1	-7.3
SI2	-7.9
SI3	-.7.0
SI4	-7.0
SI5	-7.5
SI6	-7.6
SI7	-7.6
SI8	-7.7
SI9	-7.6

SI10	-7.7
Doxorubicin	-8.1

Figure 1. 2D docking pose of compound SI

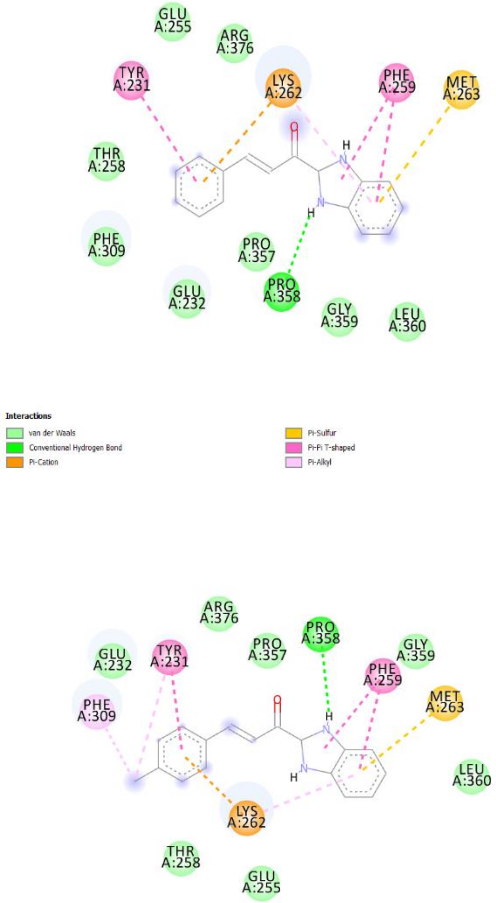


Figure 2. 2D docking pose of compound SI2

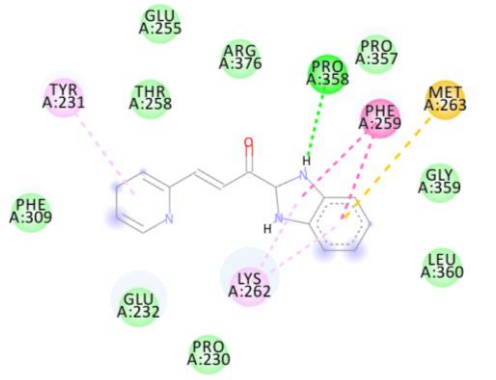


Figure 3. 2D docking pose of compound SI3

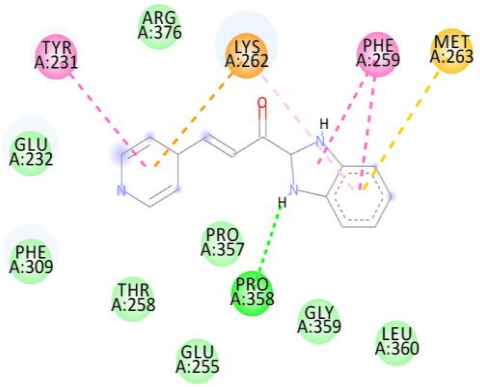


Figure 4. 2D docking pose of compound SI4

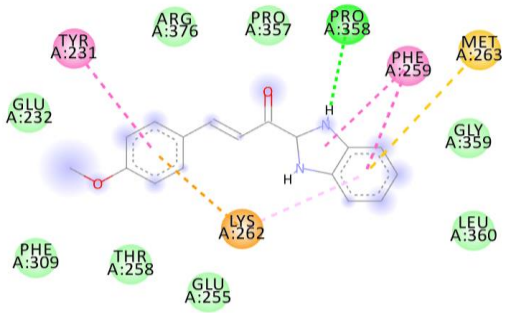


Figure 5. 2D docking pose of compound SI5

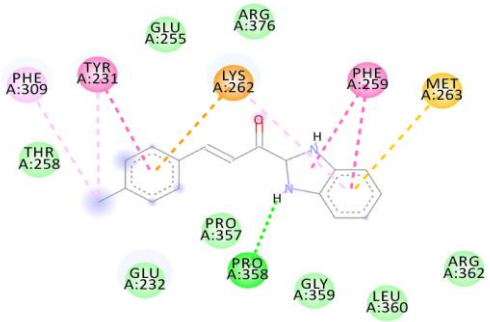


Figure 6. 2D docking pose of compound SI6

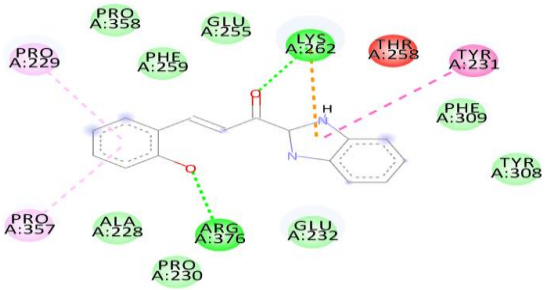


Figure 7. 2D docking pose of compound SI7

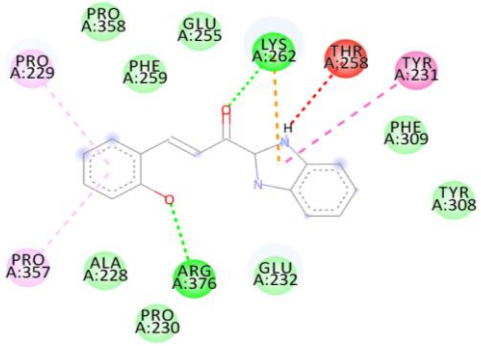


Figure 8. 2D docking pose of compound SI8

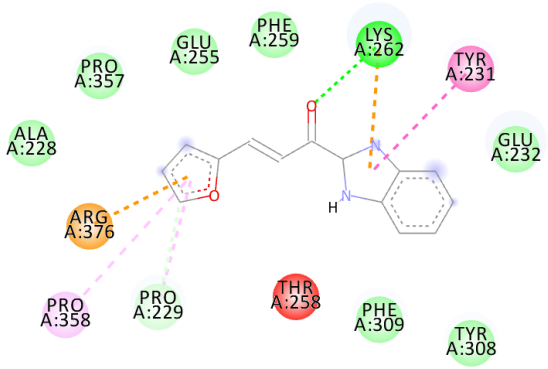


Figure 9. 2D docking pose of compound SI9

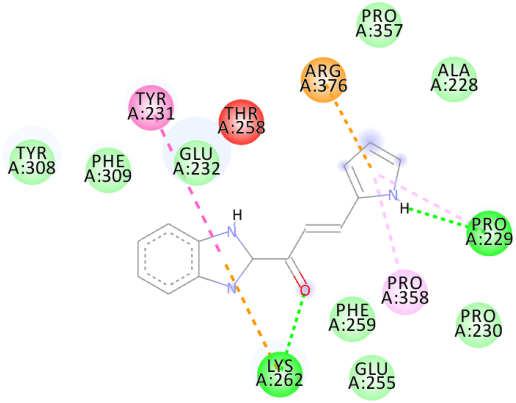


Figure 10. 2D docking pose of compound SI10

In-vitro anticancer activity

Results of anticancer activity of the compounds were expressed as IC_{50} values which were determined by plotting the percentage cell viability versus concentration of sample on a logarithmic graph and reading off the control. The experiments were performed in triplicates, and then, the final IC_{50} values were calculated by taking average of triplicate experimental results. The results of in-vitro anti-cancer activity expressed in IC_{50} (μg/mL) are expressed in **table 2** and were compared to Doxorubicin. There are 7 compounds are subjected to *in-vitro* cytotoxicity study by MTT assay method with cell lines MDA-MB-231 cell lines. All the tested compounds displayed an IC_{50} > 115 μg/mL at a concentration range of 30–250 μg/mL. Among the tested compounds, derivative BPB substituted with phenylpyrazine shows a significant IC_{50} value (78.34 μg/ml) and fallowed by compound substituted with morpholine derivative (76.23 μg/ml) shows good inhibition in breast cancer cell line. Remaining all other tested compounds shows good to moderate cytotoxic activity on tested cell line.[30]

Table 2. Data from *In vitro* Cell Line Study

S. No	**Compound code**	**MDA-MB-231 (IC_{50} μg/ml)**
1	SI1	76.23
2	SI2	88.27
3	SI3	96.54
4	SI4	89.92
5	SI5	123.39
6	SI6	78.34
7	SI7	114.16
8	SI8	74.35
9	SI9	86.94
10	SI10	99.24
11	DOX	23.14

SUMMARY AND CONCLUSION

The titled compounds were synthesized through the outlined synthetic routes, yielding 60–85% through simple and efficient techniques. The compounds were chemically stable and obtained in pure form, as confirmed by sharp melting points and distinct spots on TLC. Purification was achieved through successive recrystallization using appropriate solvents. Structural confirmation was supported by characteristic peaks in the ^{1}H-NMR spectra at corresponding δ ppm values and molecular ion peaks in the LC-MS spectra. Detailed analysis of IR, ^{1}H-NMR, and LC-MS data further validated the assigned structures of the synthesized compounds. Molecular docking studies for all the designed compounds were performed using Discovery Studio software. The compounds exhibited significant docking scores, which were compared with the standard drug Doxorubicin. Additionally, the synthesized compounds were evaluated for *in vitro* anticancer activity using the SRB assay on the MDA-MB-231 cell line.

The findings from this study suggest that the synthesized benzimidazole derivatives exhibit significant anticancer potential. The structure and chemical name of the newly synthesized compound are provided below, highlighting its potential as a promising candidate for further anticancer drug development.

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