# *In-silico* molecular screening of benzimidazole scaffolds as potential anticancer agents

Seekarajapuram Vachala Dinakaran*, Alex Joseph, Safwan Mohammed, Nabin Joshi

Department of Pharmaceutical Chemistry, RR college of Pharmacy, RR Institutions, Chikkabanawara, Bangalore-560 090, Karnataka, India.

*Address for Correspondence:
Dr. S. D. Vachala,
HOD and Professor,
RRCOP, RR Institutions,
Chikkabanawara, Bangalore-560 107, Karnataka, India
Telephone: +91 8668016793
Email: sdvachu@gmail.com
ORCID iD: 0000-0002-7725-424X

# Abstract

*Benzimidazole is an aromatic heterocyclic organic molecule. It is an important pharmacophore and a privileged structure in medicinal chemistry. The development of modern drug discovery which is computer aided in silico screening helps better to understand the drug receptor interactions. Based on this, an attempt was done to synthesize and evaluate some benzimidazole derivatives as anticancer agents.The study was aimed to prepare and in silico evaluation of varied 2-substituted benzimidazole scaffolds.Newer 2-substitued benzimidazoles were synthesized by using carboxylic acids, o-phenylene diamine and sodium hydroxide through the condensation reaction. All these synthesized compounds were confirmed by spectral analysis. In silico screening was performed by using PyRx software along with the different other soft wares for anticancer screening. The molecular interactions of the compounds with the receptor 3ERT were studied. The docking score values were considered to represent the binding efficiency of the compounds. Among the compounds tested, BI02 to BI010 showed better binding efficiency than the unsubstituted benzimidazole (BI01). Compound BI09, having bromobenzyl substitution in the 2$^{nd}$ positions of the benzimidazole scaffold was found to be more active and it showed the highest docking score value -8.3. As the size of the substituted group in 2$^{nd}$ position increases, the activity was also increasing. When the benzimidazole and the phenyl ring were separated by methylene unit, the binding efficiency was found to be improved much.*


***Keyword:*** *o-phenylene diamine, benzimidazole, In-silico screening, 3ERT receptor, anticancer activity.*

## 1. Introduction

Benzimidazole is considered as a most privileged pharmacophore in the field of medicinal chemistry. It is bicyclic aromatic heterocyclic molecule containing benzene and imidazole fused ring systems. Currently, it is a drug of choice because of its varied therapeutic activities like antibacterial [1], antifungal [2], Anti-malarial [3], antimicrobial and anti-protozoal [4], antihypertensive [5], activity against breast cancer [6], Antiparasitic [7], Antiulcer activity [8], anti-inflammatory [9], antioxidant property [10], anti HIV [11]. Literature survey showed that among the benzimidazoles (BZD) reported, 2-substituted derivatives are found to be pharmacologically more potent and hence the design and synthesis of 2-substituted benzimidazoles are the potential area of research.

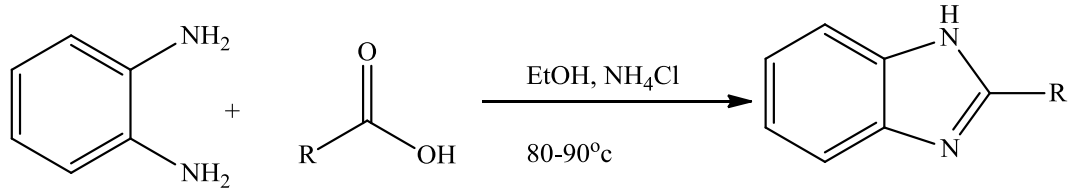
In recent times, BZD is more emphasized on complementary and alternative medicines to manage and treat various cancer diseases. It is achieved very easily through the development of computer aided drug design and developmental studies [12]. It helps us to understand the drug interactions with the specific target receptors. In the study of breast cancer in human, the estrogen signalling was found to be an important underlying mechanism in the initiation stage. The study of estrogen influence in cancer is remarkably a challenging task and also considered very important as it is involved in the construction of tissues and various biological spectacles [13,14]. It was tried extensively to understand the mechanism behind the growth of breast cancer cells. As a result of this, the anti-oestrogen therapy was emerged which was the first targeted therapy for human cancer. Thus, the present study was thought to be worthwhile to design and develop BZDs as newer anticancer agents.

## 2. Materials and methods

### 2.1. Experimental: General procedure for Synthesis of 2-substituted benzimidazole

Equimolar mixture of *o*-phenylenediamine (0.01 M) and carboxylic acids (0.01 M) in ethanol was stirred with ammonium chloride for 2-hour at $80^0$C on a hot plate. Thin layer chromatographic technique was performed to confirm the completion of the reaction. After

that, the reaction mixture was cooled and poured in ice-cold water. The granular solid was separated and recrystallized from the alcohol [15].




*o*-phenylenediamine carboxylic acid 2-substituted benzimidazole derivative BI 01-10

**Figure 1. Synthesis of 2-substitued benzimidazole derivatives.**

All the chemicals used were of AR grade. The reaction was monitored using TLC method. The melting point of all synthesized compound were recorded and are uncorrected. IR Spectra were recorded on Perkin-Elmer-1800 FTIR Spectrophotometer. $^{1}$H NMR spectra ($CDC1_3$) were recorded on Bruker Advance 400 NMR spectrophotometer using TMS as internal standard. Mass spectra were recorded on LC-MS Q-T of Micro Mass analyzer (shimadzu).

**2.2.*Insilco* studies on 3ERT:**

Molecular docking is a tool of computational investigation of ligand binding to a receptor. It predicts the affinity and activity of the molecules by finding the binding orientation of compounds to their protein targets.

**2.2.1. Preparation of ligands:**

The synthesized benzimidazole derivatives were used as ligands, which were first drawn in ChemDraw software and it was saved in SDF format. Afterwards, they were converted to pdb format.

**2.2.2. Molecular Docking:**

The crystallographic structure of the target protein in complex with 4-hydroxy tamoxifen was obtained from the Protein Data Bank (PDB) with the PDB ID 3ERT. The protein was prepared for docking using the UCSF Chimera 1.14.1 DockPrep tool [16]. Additionally, the prepare_receptor4.py script from MGLTools 1.5.6 was used to add AutoDock atom types and add Gasteiger charges to the protein structure.

The prediction of potential binding sites was performed first using the DoG Site Scorer tool of the Proteins Plus server. Subsequently, a blind molecular docking was performed. For blind molecular docking, the receptor was defined as rigid and PyRx software was used, which works with AutoDock vina 1.1.2 (vina) [17]. For docking at the binding site, the conformational search space was determined by establishing the coordinates in the center of residues at the interface using PyRx software.

The following steps were involved in the docking process. To get the docking score value, at first ligand structure were drawn on the chemdraw software in mdl (.sdf) file format. And next, PDB format of protein structure were downloaded from the RCSB website i.e., 3ERT. Then pre-process the protein in Swiss PDB Viewer software and make a new folder in the desktop with attaching both the ligand along with the pre- processed macromolecule in the same. Open PyRx software and click the following (Edit-preferences-workspace- browse-ok) and close the software. Again, open PyRx software and click the following (file-import-chemical table file-next) and then choose the ligand which will display on the screen. At the bottom row, the selected option will be seen just right click and choose to minimize all and again do right click and choose to convert all to auto dock option. Then choose vina wizard option and press start, add macromolecule. Afterwards, select the ligand and macromolecule shown onto the screen, choose forward option the docking process will start and shows the highestscore value at the first and can be saved in .csv format.

# 3. Results and discussion

## 3.1.Chemistry

Totally ten novel benzimidazole derivatives BI01-BI10 were synthesized from *o*-phenylene diamine. The reaction of *o*-phenylenediamine with different carboxylic acids in presence of 10% sodium hydroxide resulted in the formation of 2-substituted benzimidazoles (Table 1). The crude product was filtered and recrystallized. The recrystallization was carried out by dissolving the synthesized product in boiling water. Charcoal was added and digested for 15 minutes. It was filtered while hot. The filtrate was cooled and the crystals of benzimidazole was separated, washed with 25ml of cold water and dried at 100°C. The yield of benzimidazole derivatives was calculated and the melting point was determined.

## 3.2. Spectral characterization of compound 4-(1H-benzimidazol-2-yl)phenol (BI04):

Structure of the compound was confirmed by spectral analysis. In the IR spectra of compound BI04, the following absorption peaks were observed and the results were satisfactory. Aromatic -CH: 3357cm$^{-1}$ (2900-2800); -NH: 3427cm$^{-1}$ (3500-3350); Aromatic C=C: 1574cm$^{-1}$ (1680-1400); Alkene -C=C: 1633cm$^{-1}$ (1680-1400). Further, in the NMR spectra, the aromatic protons were observed as multiplets in the region of 7.78 - 7.6 ppm, the -CH=CH group produced double doublet at 6.4 - 6.5 ppm and 6.8 - 6.9 ppm, the hetero-aromatic NH as singlet at 7.9 ppm. The mass spectroscopy of the compounds revealed m/z peak at 220 and molecular ion peak at ($M^+$) 221.

**Table 1. List of the compounds synthesized (BI01-BI10)**

| S.No | Compound code | IUPAC Name | R | Molecular formula | Molecular weight |
|---|---|---|---|---|---|
| 1 | BI01 | 1H-Benzimidazole | -H | $C_7H_6N_2$ | 118.14 |
| 2 | BI02 | 2-methylbenzimidazole | $-CH_3$ | $C_8H_8N_2$ | 132.16 |
| 3 | BI03 | 2-Phenyl benzimidazole | $-C_6H_6$ | $C_{13}H_{10}N_2$ | 194.23 |
| 4 | BI04 | 2-(1H-benzimidazol-2-yl) phenol | $-C_6H_5OH$ | $C_{13}H_{10}N_2O$ | 210.23 |
| 5 | BI05 | 2-[(Z)-2-phenylethenyl]- 1H-benzimidazole | $-CH=CHC_6H_6$ | $C_{15}H_2N_2$ | 220.27 |
| 6 | BI06 | 2-(1H-benzimidazol-2-yl) aniline | $-C_6H_5NH_2$ | $C_{13}H_{11}N_3$ | 209.25 |
| 7 | BI07 | 2-(4-chloro-benzyl)-1H-benzimidazole | $-C_6H_5Cl$ | $C_{14}H_{11}ClN_2$ | 242.70 |
| 8 | BI08 | 2-(1H-Benzimidazol-2-yl)phenyl acetate | $OC_6H_5OC=OCH_3$ | $C_{15}H_{12}N_2O_3$ | 268.27 |
| 9 | BI09 | 2-(4-bromo-benzyl)-1H-benzimidazole | $-CH_2C_6H_5Br$ | $C_{14}H_{11}BrN_2$ | 287.15 |
| 10 | BI10 | 3-amino-4-1H-benzimidazol-2-yl-5-nitrophenol | $C_6H_3NO_2NH_2OH$ | $C_{13}H_{10}N_4O_3$ | 270.24 |

**Table 2. Physico-chemical properties of the synthesized compounds**

| Compound code | Melting point (°C) | Yield (%) |
|---|---|---|
| BI01 | 129 | 82.20 |
| BI02 | 133 | 73.14 |
| BI03 | 138 | 67.46 |
| BI04 | 126 | 75.77 |
| BI05 | 136 | 72.43 |
| BI06 | 141 | 59.83 |
| BI07 | 183 | 60.21 |
| BI08 | 172 | 66.29 |
| BI09 | 189 | 79.27 |
| BI10 | 167 | 89.57 |

### 3.3.*Insilico anticancer* screening of the 2-substited benzimidazoles:

*In silico* screening of the synthesized compounds was carried out to analyses the binding efficiency of the compounds on 3ERT (Figure 2) which is a known target in human breast cancer cells. PyRx software was used for this study along with thedifferent other soft wares like, BIOIVIA discovery studio, Swiss PDB viewer, Chemdraw software. The study results showed good docking score value and interaction of the compounds with the amino acids residues in the binding site of the receptor (Table 3). The molecular docking results were demonstrated in terms of negative energy. The lower the energy value, best would be the binding affinity of the compound with the target [18]. This indicates the anticancer activity of the compounds. The results were given in Table 3

**Figure 2.** 3D structure of human oestrogen receptor 3ERT.

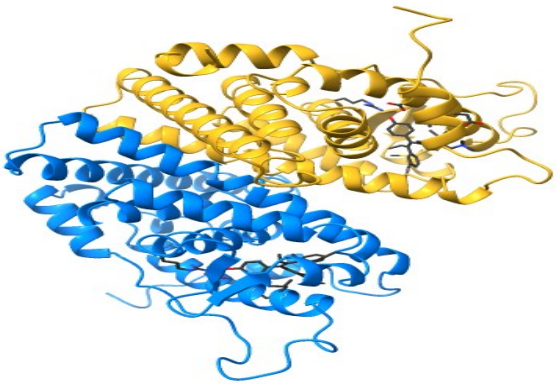


**Figure 3.** Image highlighting the binding site of BI09 on 3ERT.

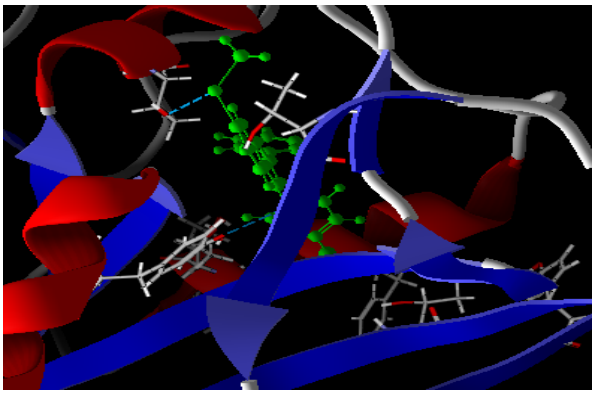


**Figure 4.** The ribbon representation of the lowest energy conformer of compound BI09 docked in the 3ERT binding domain.

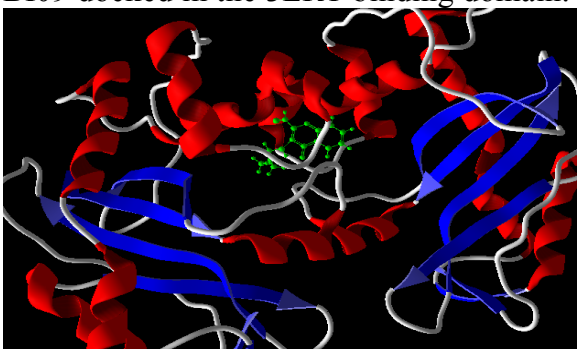
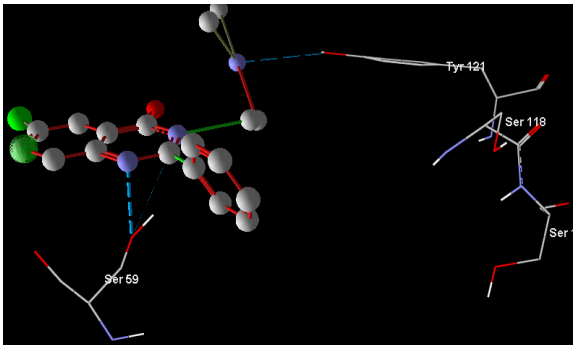


**Figure 5.** The hydrogen bond interactions of the best pose of BI09 with Tyr 121 and Ser 59 residue of 3ERT.




Among the compounds tested, BI02 to BI10 showed better binding efficiency than the unsubstituted benzimidazole (BI01). Compound BI09, having bromobenzyl substitution in the 2nd positions of the benzimidazole scaffold was found to be more active and it showed the highest docking score value -8.3. It was found to be more effectively binding with 3ERT receptor (Figure 3-Figure 5).

**Table 3.** The docking score value of compounds BI01-10 on 3ERT:

| SI.No | Compound code | Score Value |
|---|---|---|
| 1 | BI01 | -5.8 |
| 2 | BI02 | -6.3 |
| 3 | BI03 | -7.2 |
| 4 | BI04 | -7.7 |
| 5 | BI05 | -7.9 |
| 6 | BI06 | -7.5 |
| 7 | BI07 | -7.6 |
| 8 | BI08 | -7.8 |
| 9 | BI09 | -8.3 |
| 10 | BI10 | -7.8 |

It also registered that as the size of the substitutional group increases in 2nd position the activity is also increasing. Further, when the benzimidazole moiety and the phenyl ring were separated by methylene unit, the binding efficiency was found to be much improved.

## 4. Conclusion

Benzimidazole is an important hetero-cyclic moiety for the discovery of new drugs. Based on the literature survey an attempt was done to synthesize some benzimidazole derivatives using *o*-phenylene diamine and various carboxylic acids to form 2-substituted benzimidazoles (BI01-BI10). All these synthesized compounds were evaluated for, Melting point, UV spectral analysis, IR studies, NMR and Mass spectral analysis. The spectral studies confirmed the structure of the compounds. Including all these analyses, docking studies was also performed and got the docking score value of each benzimidazole derivatives. This present study revealed that 4-bromo benzyl substitution in the ring system supports its biological activities than the any other functional groups tested.

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