*Section A -Research paper*

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# Synthesis, Characterization, *in Silico* and Cytotoxic Studies of Benzotriazole Derivatives

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**Abstract**

Using Schrodinger 2021-3, *in silico* molecular docking and ADMET investigations of the proposed compounds were carried out upon the binding cavity standardized for Legumain. In order to analyses the newly synthesized benzotriazole (6a–p) mannich base derivatives, 1HNMR, 13CNMR, and mass spectrum data were collected. These substances (6a-p) were tested for their anticancer properties on four different human cancer cell lines using the MTT method. The test compounds' binding to the legumain binding site has been confirmed by the IFD results, which concur with those of XP docking experiments. Compounds' anticipated ADMET characteristics are within acceptable bounds. As compared to the control medication, the majority of the compounds showed excellent to moderate anticancer activity. Positive control values ranged from 0.13 0.017 to 3.08 0.135 M, and the drugs' IC50 values ranged from 0.012 0.001 to 22.9 9.11 M. These 6a, 6c, 6e, 6f, 6j, 6n, and 6p were shown to have more powerful anticancer properties than etoposide among synthetic chemicals. The chemical 6a, which has an electron-donor (3,5-dimethoxy) group on the phenyl ring, had the most anticancer effects when tested against four cancer cell lines, with IC50 values of MCF-7 = 0.012 0.001, A549 = 0.18 0.076, Colo-205 = 0.34 0.083, and A2780 = 0.07 0.006 M, respectively.

**Keywords:** A549, Benzotriazole, A2780, Colo-205, ADMET, MCF-7, MTT assay.

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## 1. Introduction

Medical chemists have paid close attention to the distinctive nitrogen-containing heterocycles known as benzotriazoles because they represent a promising class of bioactive heterocyclic products with a variety of biological properties, against cancer, microorganisms especially bacterias and fungi, inflammation, malarial, and tuberculosis[1]. A variety of proteins react agonistically to benzotriazole derivatives. In addition to peptides, acid azides, 3-hydroxymethyl-2,3- dihydrobenzofurans, and 3-hydroxymethylbenzofurans, benzotriazole is a precursor utilised in chemical synthesis[2]. Three tautomers of benzotriazole exist: two 1H-forms and one 2H-form. The 1H-forms benefit from the equilibrium in solution [4]. Benzotriazole has a PKa of 8.2, making it a far weaker base than 1,2,3-triazole, indazole, or benzimidazole [5-7]. Benzotriazoles are said to have a range of biological activities, including antiviral, antibacterial, anticonvulsant, antifungal, anti-inflammatory, and anticancer action, according to the literature. As anticancer, antibacterial, antifungal, and antiviral medicines, organic molecules containing nitrogen and sulphur and their metal complexes have a broad variety of biological action. [8]. In addition to its other functions, benzotriazole serves as a precursor in the production of 3hydroxymethyl-2,3-dihydrobenzofurans, acid azides, peptides, and 3-N-substituted benzotriazoles, which come in two isomers: 1H- and 2H-substituted. It is commonly accepted that in solid and solution, 1H-substituted compounds predominated, but in the gas phase, the fraction of 2H-tautomers rose [10]. The energy disparity between the two isomers is negligible, albeit [11]. In a similar vein, benzotriazoles with Mannich bases have recently been made from the N, N-dimethyl amino propiophenone hydrochlorides and benzotriazole, respectively, via amine exchange processes[12]. 1,2,3-Benzotriazole ($C_6H_5N_3$), is one of the most researched chemicals in the triazole family. For more than 50 years, researchers have been interested in determining the defending nature of BTA including its derivatives[13]. They have received some fascinating evaluations during the previous ten years [14–20]. Numerous substances containing the 1,2,3-benzotriazole system have been studied for a wide range of activities, against corrosion[21], viruses[22], inflammation[23], convulsion[24], enzyme inhibition[25], DNA cleavage[26], herbs[27], fungi[26], microbes[29], and proliferation[30]. In addition, replacing the isoCA-4's 3,4,5-trimethoxyphenyl ring with a quinazoline or quinoline nucleus produced tubulin inhibitors with significant

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antiproliferative effects against a number of malignant cell types.

These findings indicated that when binding to the colchicine site, the benzotriazole moiety might act as a substitute for the conventional 3,4,5-trimethoylphenyl moiety. One of the most powerful anti-microbial medications used either alone or in combination for cancer therapy is heterocyclic compounds with nitrogen atoms[35-36]. Using the ChEMBL target prediction algorithm, the biological target associated with the anticancer action of the drugs was predicted; (https://www.ebi.ac.uk/chembl/). Legumain, which has been linked to a number of human tumours and is thought to be overexpressed, is the most plausible target for these substances. To shed some light on whether the binding of the compounds in the binding site of the legume is thermodynamically favourable and, if yes, the molecular interactions between the chemicals and amino acid residents of the binding site, in silico molecular docking studies were carried out. GLIDE looks for advantageous chemical–target–protein interactions. We have committed ourselves to creating and launching innovative anticancer drugs in light of these encouraging outcomes. By using the Mannich base reaction, we presented a series of N-(1H-Benzo[d][1,2,3]triazol-1-yl)alkyl substituted benzenamines (6a–6p) as novel heterocyclic analogues of benztriazoles. By substituting substituted aromatic amines and substituted aldehydes for the 1NH hydrogen, an unique strategy for the production of a new series of innovative benzotriazoles derivatives was presented. We discuss insilico design, their synthesis, and their effective anticancer activity against human cancer cell lines in this work.

## 2. Materials and Methods

### *2.1. In silico* Studies

Chemdraw Professional 16 was used to do preliminary optimization on the developed hybrids. All of the ligand geometries have been optimized using the Maestro v11.3 (v4.1, Schrodinger 2020-21).[37–38]. For input structure, Ligprep 2020-2 was chosen as an energetically minimal equivalent. With the help of the protein preparation wizard (Epik v4.1, Schrodinger suite 2020-2), the optimised legumain (PDB ID: 5LUB) was created[37–38]. The water molecules were eliminated from the crystal structure prior to protein optimisation. Glide Integrated Maestro 11.3 carried out the docking investigation in accordance with the literature-recommended methodology [39]. For the binding pocket of legumain, the proposed hybrids' interaction and

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selectivity were seen.

### *2.2.* ADMET Studies

For forecasting the pharmaceutically important features of organic molecules, including as absorption, distribution, metabolism, excretion, and toxicity (ADMET), Schrödinger offers QikProp[40]. In order to compare a test compound's qualities to those of 95% of known medications, it offers numerical values or a range.

### *2.3.* Chemistry

Reagent-class chemicals that have undergone any necessary purification were employed. Lab India's digital melting point equipment was used to calculate melting points. Shimadzu FTIR spectrometer model utilized to capture compound's IR spectra. NMR spectra in DMSO solvent were determined using a Bruker DRX-300 spectrometer with an internal standard acting as a TMS. The mass spectra of several substances were examined using a Shimadzu LCMS 2010A spectrometer. Thin layer chromatography was used to verify the responses (TLC).

#### *2.3.1.* General Procedure for the synthesis of N-(1-(1H-Benzo[d][1,2,3] triazol-1-yl)alkyl)- substituted benzenamine (6a-6p)

Benzotriazole (3) (1 g, 8.39 mmol), formaldehyde (4a) (3 ml, 10 mmol), and 3,5-dimethoxyaniline (5a) (1.3 g, 8.39 mmol) were combined and agitated in an ethanol solvent at room temperature for an hour. The reaction mixture was cooled to 25 °C and agitated for 5 hours after the TLC reaction was finished. Then, it was chilled at -5°C for a desired period of 16 hours (Figure 1). The precipitate was removed, washed with diethyl ether, and dried in a vacuum in order to get pure compounds 6a–6p with a yield of 46-75%.

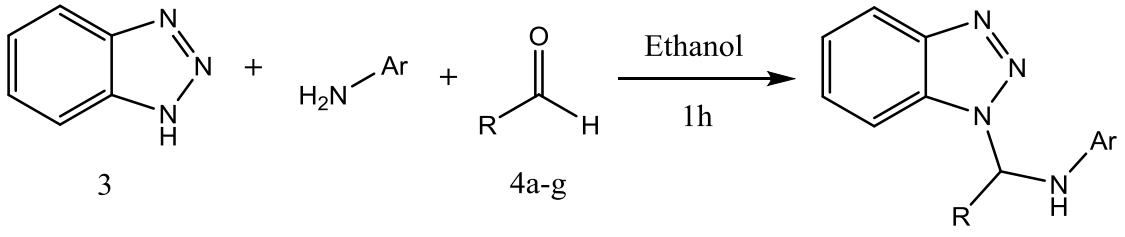


**Figure 1.** Scheme for the synthesis of N-(1-(1H-Benzo[d][1,2,3]triazol-1-yl)alkyl)-substituted benzenamine (6a-6p)

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**Table 1.** List of derivatives with R and Ar substitutions

| Compound | R | Ar |
|---|---|---|
| 4a | H | - |
| 4b | 4-fluorophenyl | - |
| 4c | 4-(N,N'-dimethylamino)phenyl | - |
| 4d | 4-chlorophenyl | - |
| 4e | 3-chlorophenyl | - |
| 4f | ethyl | - |
| 4g | methyl | - |
| 5a | - | 3,5-dimethoxyphenyl |
| 5b | - | 4-chlorophenyl |
| 5c | - | 4-nitrophenyl |
| 5d | - | 3-chlorophenyl |
| 5e | - | 3,5-dinitrophenyl |
| 5f | - | 4-aminopyridyl |
| 5g | - | 3,5-dimethoxy-4-methylphenyl |
| 5h | - | phenyl |
| 5i | - | 4-methoxyphenyl |
| 6a | H | 3,5-dimethoxyphenyl |
| 6b | H | 3,5-dinitrophenyl |
| 6c | H | 4-aminopyridyl |
| 6d | H | 3,5-dimethoxy-4-methylpheny |
| 6e | ethyl | 4-chlorophenyl |
| 6f | methyl | 3-chlorophenyl |
| 6g | 3-chlorophenyl | 4-nitrophenyl |
| 6h | 4-chlorophenyl | 4-nitrophenyl |
| 6i | 4-(N,N'-dimethylamino)phenyl | 4-nitrophenyl |
| 6j | 4-fluorophenyl | 4-nitrophenyl |
| 6k | H | phenyl |
| 6l | H | 4-nitrophenyl |
| 6m | H | 4-chlorophenyl |
| 6n | H | 4-methoxyphenyl |
| 6o | H | 3-chlorophenyl |
| 6p | ethyl | 3-chlorophenyl |

### *2.4.* Pharmacological activity

#### *2.4.1.* Cytotoxicity Studies: MTT assay

100 L of complete media containing 1104 cells were added to each well of a 96-well tissue culture micro titer plate. Prior to the experiment, the plates were incubated for 18 hours at 37 $^{0}$C in an incubator with humidified 5% $CO_2$. Following removal of the

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medium, 100 L of fresh media containing the test substances and etoposide (Eto) at various concentrations, including 0.5, 1, and 2 M, were added to each well[41-42]. The medium was then emptied out and filled with 10 L of MTT dye. Plates were incubated at 37$^{o}$C for two hours. The crystals of formazan produced as a result were dissolved in 100 L of extraction buffer. The optical density (O.D.) was determined at 570 nm using a microplate reader (Multi-mode Varioskan Instrument-Themo Scientific). The maximum concentration of DMSO in the medium was 0.25 %.

## 3. Results and Discussion

### *3.1.* *In Silico* Studies

The docking scores of all the compounds were discovered to be negative and fall within a similar range, as shown in Table 2. The negative values of the docking scores imply that the compounds' thermodynamically advantageous binding to the binding site of legumain (PDB ID: 5LUB).

Supple displays the test compounds' highest-scoring poses from GLIDE XP docking, Figure 1, **(supplementary data)**. Using 2D interaction diagrams and 3D poses, the molecular interactions with the important amino acid residues in the binding site are depicted. Serine 216, Arginine 44, Histidine 45, and Tyrosine 217 are identified as the main amino acid residues in the binding site that interact with the test chemicals. Serine 216 interacts with the side chain's amino group through a hydrogen bond. Van der Waals interactions between arginine 44 and histidine 45 with benzotriazole, arginine 44 with an aromatic ring at R, and tyrosine 217 with an aromatic ring at Ar are observed in the majority of the compounds. Induced Fit Docking (IFD) is a programme that docks ligands into the flexible binding site as compared to the traditional XP docking research. Since proteins in living environments are typically flexible, binding affinities predicted by XP docking research, in which proteins are stiff, may not be correct. Since the binding site, which is 5 around the bound ligand, is variable in IFD investigations, more precise estimates of the binding affinity can be made.

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**Table 2** shows the XP docking and IFD scores of the test compounds in Induced Fit Docking studies

| Title | GlideXP Dockingscore (Kcal/mole) | IFD Score |
|---|---|---|
| **6a** | -4.909 | -596.28 |
| **6b** | -4.320 | -596.08 |
| **6c** | -2.654 | -599.62 |
| **6d** | -5.045 | -596.44 |
| **6e** | -4.944 | -598.03 |
| **6f** | -4.110 | -596.37 |
| **6g** | -4.373 | -596.38 |
| **6h** | -4.659 | -596.50 |
| **6i** | -5.104 | -597.10 |
| **6j** | -4.639 | -596.59 |
| **6k** | -4.252 | -596.11 |
| **6l** | -4.999 | -596.90 |
| **6m** | -4.717 | -597.50 |
| **6n** | -5.644 | -597.63 |
| **6o** | -4.507 | -596.74 |
| **6p** | -4.467 | -597.27 |

**Table 3:** The total binding energy of the top scored compound/protein complex and the contributions from important interactions

| Title | ΔGbind | ΔGcoulomb | ΔGcovalent | ΔGhbond | ΔGLipo | ΔGvdW |
|---|---|---|---|---|---|---|
| 6a | -19.87 | -1.43 | 1.69 | -0.79 | -13.42 | -30.43 |
| 6b | -25.15 | 3.18 | 3.49 | -0.49 | -12.01 | -30.31 |
| 6c | -55.72 | 8.5 | 1.85 | -1.15 | -11.65 | -27.27 |
| 6c | -25.06 | -8.06 | 0.25 | -1.23 | -13.24 | 27.57 |
| 6d | -21.65 | -2.55 | 2.72 | -0.50 | -13.26 | -29.41 |
| 6e | -29.68 | -4.31 | 2.59 | -0.78 | -18.67 | -31.02 |
| 6f | -26.68 | -4.17 | 2.40 | -0.82 | -18.24 | -29.65 |
| 6g | -24.78 | -5.28 | 2.67 | -0.96 | -19.43 | -30.58 |
| 6h | -27.01 | -2.19 | 4.44 | -0.91 | -21.36 | -29.13 |
| 6i | -22.59 | -3.36 | 5.56 | -0.89 | -18.66 | -36.03 |
| 6j | -19.72 | -3.22 | 3.89 | -0.87 | -13.41 | -28.27 |
| 6k | -20.7 | -3.79 | 3.33 | -0.74 | -12.44 | -27.60 |
| 6l | -24.09 | -1.24 | 3.32 | -0.75 | -12.50 | -28.49 |
| 6m | -23.84 | -4.93 | 3.24 | -0.73 | -13.33 | -27.87 |
| 6n | -21.37 | -4.01 | 3.33 | -0.75 | -12.62 | -28.18 |
| 6o | -24.16 | -3.76 | 1.76 | -0.67 | -14.24 | -28.57 |
| 6p | -26.4 | -3.88 | 3.67 | -0.81 | -19.58 | -29.88 |

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### *3.2.* ADMET Studies

Table 4 displays the test compounds' drug-like characteristics. Compounds' anticipated attributes are within the permitted bounds. As none of the chemicals were expected to exhibit CNS action from this in silico investigation, they do not have any CNS effects. All of the compounds' octanol/water partition coefficients (QPlogPo/w) and aqueous solubilities (QPlogS) were discovered to be within the permissible range, indicating that there are no solubility-related problems with these compounds. When the results fell above the value of 500, suggesting they have excellent permeability, the compounds were also anticipated to show no problems with Caco-2 cell permeability (QPPCaco) and MDCK cell permeability (QPPMDCK). The chemicals' projected brain/blood partition coefficients (QPlogBB) likewise fall within the acceptable range, indicating that there won't be any immediate problems with the blood-brain barrier. Similar to the positive results of the expected values of ligand binding with serum albumin, this indicates that the ligands won't engage in non-selective protein binding with human albumin in the circulatory system. Based on the percentages of human oral absorption, it was likewise projected that the ligands would exhibit high oral absorption in humans. The estimated values for HERG toxicity (QPlogHERG) of 6a-p do, however, exceed the advised range, suggesting that these substances may have harmful effects on the heart.

**Table 4.** Prediction of drug like properties of the test compounds using QikProp

| Title | #rotor | CNS | QPlogPo/w | Qplogs | QPlogHERG | QPPCaco | QPlogBB | QPPMDCK | #metab | QPlogKhsa | Percent Human Oral Absorption |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cpd 6a | 5 | 0 | 3.11 | -3.93 | -5.42 | 2183.59 | -0.39 | 1150.68 | 5 | 0.09 | 100.00 |
| Cpd 6b | 5 | -2 | 1.52 | -3.76 | -5.47 | 32.61 | -2.25 | 12.23 | 5 | -0.03 | 62.92 |
| Cpd 6c | 3 | 0 | 2.01 | -2.85 | -5.36 | 1218.95 | -0.47 | 612.76 | 4 | -0.24 | 93.93 |
| Cpd 6c | 3 | 0 | 1.99 | -2.84 | -5.35 | 1167.86 | -0.49 | 585.05 | 4 | -0.24 | 93.48 |
| Cpd 6d | 5 | 0 | 3.51 | -4.70 | -5.60 | 2186.29 | -0.43 | 1152.22 | 5 | 0.27 | 100.00 |
| Cpd 6e | 4 | 1 | 4.08 | -4.69 | -5.31 | 3196.17 | 0.02 | 4289.35 | 2 | 0.39 | 100.00 |
| Cpd 6f | 3 | 1 | 3.61 | -4.16 | -5.00 | 2757.51 | 0.02 | 3265.45 | 3 | 0.27 | 100.00 |
| Cpd 6g | 5 | -1 | 4.30 | -5.74 | -6.26 | 408.28 | -0.99 | 463.53 | 5 | 0.65 | 100.00 |

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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cpd 6h | 5 | -1 | 4.29 | -5.73 | -6.25 | 409.74 | -0.99 | 465.65 | 4 | 0.64 | 100.00 |
| Cpd 6i | 6 | -2 | 4.24 | -5.97 | -6.48 | 339.17 | -1.39 | 153.74 | 5 | 0.71 | 100.00 |
| Cpd 6j | 5 | -2 | 4.03 | -5.35 | -6.20 | 408.78 | -1.03 | 340.46 | 4 | 0.57 | 100.00 |
| Cpd 6k | 3 | 0 | 2.90 | -3.43 | -5.56 | 2170.72 | -0.24 | 1143.35 | 3 | 0.06 | 100.00 |
| Cpd 6l | 4 | -2 | 2.22 | -3.60 | -5.51 | 269.95 | -1.21 | 120.13 | 3 | 0.02 | 83.43 |
| Cpd 6m | 3 | 0 | 3.39 | -4.18 | -5.52 | 2170.95 | -0.08 | 2823.53 | 2 | 0.17 | 100.00 |
| Cpd 6n | 4 | 0 | 3.00 | -3.68 | -5.48 | 2165.94 | -0.32 | 1140.63 | 3 | 0.08 | 100.00 |
| Cpd 6o | 3 | 0 | 3.39 | -4.18 | 5.52 | 2170.94 | -0.08 | 2818.46 | 3 | 0.17 | 100.00 |
| Cpd 6p | 4 | 1 | 4.03 | -4.53 | -5.20 | 3269.61 | 0.02 | 3928.24 | 3 | 0.38 | 100.00 |

Note: #rotor, Number of non-trivial (not CX3), non-hindered (not alkene, amide, small ring) rotatable bonds: recommended range 0-15; CNS, predicted central nervous system activity: –2 (inactive) to +2 (active) scale; QPlogPo/w, Predicted octanol/water partition coefficient: recommended value -2.0 – 6.5; QPlogS, Predicted aqueous solubility, log S. S in mol dm–3 is theconcentration of the solute in a saturated solution that is in equilibrium with the crystalline solid: recommended range -6.5 – 0.5; PlogHERG, Predicted IC50 value for blockage of HERG K+ channels: recommended value below - 5; QPPCaco, Predicted apparent Caco-2 cell permeability in nm/sec. Caco2 cells are a model for the gut-blood barrier: <25 = poor, >500 = great; QPlogBB, Predicted brain/blood partition coefficient: recommended range -3.0 – 1.2; QPPMDCK Predicted apparent MDCK cell permeability in nm/sec: <25 = poor, >500 = great; QPlogKhsa, Prediction of binding to human serum albumin: recommended range -1.5 – 1.5; Percent Human Oral Absorption, Predicted human oral absorption on 0 to 100% scale: > 80% = high, <25% = poor.

### *3.3.* Chemistry

In Scheme 1, the synthesis of novel benzotriazole (6a–p) Mannich base derivatives was shown. To produce pure final products, the commercially available chemical benzotriazole (3) performs the Mannich reaction with a variety of aryl amines (5a–i) and aliphatic and aromatic aldehydes (4a–g) at room temperature for one hour. 6a-p. Moreover, $^{1}$HNMR, $^{13}$CNMR, and mass spectral data were used to describe these molecules. The methylene proton appeared at 4.14 ppm as a singlet (s, -CH2-), the

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amine proton showed at 6.57 ppm (t, -NH-), and the proton values of compound 6a were 3.89 ppm as singlets (s, $(-OCH_3)_2$), as well as the $^{13}$CNMR value of the -CH2- peak appeared at 57.6 ppm. The mass value of compound m/z: 285 $[M+H]^+$.

### *3.3.1.* Spectral Characterization

**N-((1H-Benzo[d][1,2,3] triazol-1-yl)methyl)-3,5-dimethoxybenzenamine (6a-6p)**

$^1$H NMR (400 MHz, DMSO-d6): δ 3.92 (s, 6H), 6.10 (d, 2H, *J* = 6.2 Hz), 6.83 (s, 1H), 7.20 (s,2H), 6.90 (t, 1H), 7.35 (t, 1H), 7.49 (dd, 1H, *J* = 7.01, *J* = 8.0 Hz), 7.98 (d, 1H, *J* = 8.22Hz), 8.08(d, 1H, *J* = 8.22 Hz); $^{13}$C NMR (75 MHz, DMSO-d6): δ 57.6, 60.4, 104.6, 109.4, 112.8, 126.7,127.2, 128.7, 142.7, 147.6, 163.4; MS (ESI): m/z 285-350 $[M+H]^+$.

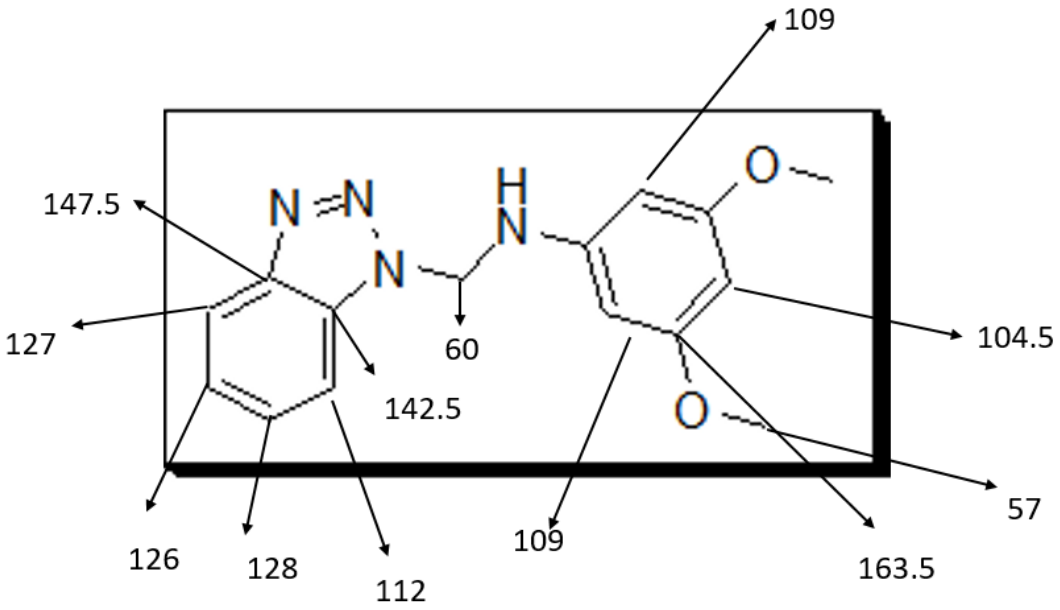


**Figure 2.** Observed $^{13}$C NMR peak for the derivatives 6a

### 3.4. Pharmacology

#### 3.4.1. Cytotoxicity Screening: MTT assay

Using the MTT technique, these substances (6a-p) were examined for their ability to inhibit the growth of four distinct human cancer cell lines: MCF-7 (breast cancer), A549 (lung cancer), Colo-205 (colon cancer), and A2780 (ovarian cancer). The data were compiled in Table 5, with etoposide being utilised as a clinical medication and a positive control. As compared to the control medication, the majority of the compounds showed excellent to moderate anticancer activity. Positive control values

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ranged from 0.13± 0.017 to 3.08± 0.135 M, and the drugs' IC50 values ranged from 0.012 ±0.001 to 22.9± 9.11 M. These 6a, 6c, 6e, 6f, 6j, 6n, and 6p were shown to have more powerful anticancer properties than etoposide among synthetic chemicals. Additionally, the structure-activity relationship of each compound was investigated. The results showed that compound 6a, which has an electron-donating (3,5-dimethoxy) group on the phenyl ring, had the strongest anticancer activity against four cancer cell lines, with IC50 values of MCF-7=0.012±0.001M, A549=0.18±0.076M, Colo-205=0.34±0.083M, and A2780= 0.07±0.006M, respectively. In comparison to compound 6a, compound 6n, which has a 4-methoxy substituent on the aryl backbone, has slightly less activity (MCF-7 = 0.97±0.06 M, A549 = 1.87±0.51 M, Colo-205 = 2.06±0.33 M, and A2780 = 2.33±1.64 M). Compound 6c, which had high action on all cell lines (MCF-7 = 1.65±0.18 M, A549 = 0.72±0.44 M, Colo-205 = 1.27±0.39 M, and A2780 = 2.08±1.82 M), was produced by replacing the phenyl ring with a 4-pyridyl ring. The 3,5-dinitro group on the phenyl ring in chemical 6b showed very little action. Contrarily, compound 6e, which has an aryl ring substituted with a 4-chloro group and an ethyl functionality, had good activity in four different cell lines (MCF-7: 1.98 0.60 M, A549: 0.79 0.02 M, Colo-205: 2.12 1.76 M, and A2780: 1.84 0.63 M). Additionally, compound 6f, which had a methyl group and an electron-withdrawing group at the third position of the phenyl moiety, was more active than compound 6e (MCF-7 =1.02 0.34 M, A549 =1.18 0.45 M, Colo-205 =0.57 0.068 M, and A2780 = 0.19 0.55 M). Similar to 6e and 6f, when 6p was combined with MCF-7, A549, Colo-205, and A2780, particularly two cell lines (A549, A2780), the electron withdrawing group (3-chloro), (R = ethyl) shown loss of activity (MCF-7= 1.59 0.73M, A549= 2.44 1.84M, Colo-205= 1.88 0.63M, and A2780= 2. Surprisingly, compound 6j demonstrated the second-highest anticancer activity (MCF-7 = 0.076 0.002 M, A549 = 0.23 0.045 M, Colo-205 = 0.64 0.031 M, and A2780 = 0.75 0.063 M). On the phenyl ring, it had a 4-nitro group, while the methylene carbon had a 4-fluorophenyl group. According to acceptable activities, the compounds 6g (Ar = 4-nitrophenyl and R = 3-chlorophenyl), 6h (Ar = 3-chlorophenyl and R = 4-chlorophenyl), 6i (AR = 4-chloro and R = 4-N, N'- dimethylamino), 6l (Ar = 4-nitrophenyl and R = H), and 6o (Ar = 3-chlorophenyl and R = H) attached.

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**Table 5.** In vitro cytotoxicity of newly compounds **6a-p** with $IC_{50}$ in µM.

| Compound | [c]MCF-7 | [d]A549 | [e]Colo-205 | [f]A2780 |
|---|---|---|---|---|
| **6a** | 0.012±0.001 | 0.18±0.076 | 0.34±0.083 | 0.07±0.006 |
| **6b** | - | 16.4±5.88 | 7.89±6.78 | 13.5±4.18 |
| **6c** | 1.65±0.18 | 0.72±0.044 | 1.27±0.39 | 2.08±1.82 |
| **6e** | 1.98±0.60 | 0.79±0.029 | 2.12±1.76 | 1.84±0.63 |
| **6f** | 1.02±0.34 | 1.18±0.45 | 0.57±0.068 | 0.19±0.55 |
| **6g** | 3.98±2.48 | 7.12±5.87 | 12.6±7.54 | 9.34±6.88 |
| **6h** | 3.77±2.36 | 3.28±2.68 | - | 10.5±1.67 |
| **6i** | 2.77±1.57 | - | 3.19±1.98 | 3.22±2.12 |
| **6j** | 0.076±0.002 | 0.23±0.045 | 0.64±0.031 | 0.75±0.063 |
| **6k** | 18.5±6.43 | 22.9±9.11 | 3.90±4.92 | 6.27±3.76 |
| **6l** | 4.85±2.71 | 7.53±3.68 | 3.88±2.17 | - |
| **6m** | 9.56±4.53 | - | - | 15.7±6.46 |
| **6n** | 0.97±0.062 | 1.87±0.51 | 2.06±0.33 | 2.33±1.64 |
| **6o** | 3.47±1.97 | 8.55±4.32 | - | - |
| **6p** | 1.59±0.73 | 2.44±1.84 | 1.88±0.63 | 2.27±1.49 |
| **Etoposide** | 2.11 ± 0.024 | 3.08 ± 0.135 | 0.13 ± 0.017 | 1.31 ± 0.27 |

"-" = Not active.

Each piece of data is represented by its mean and standard deviation values from separate trials that were carried out in triplicate on human breast cancer cell line [bc]MCF-7, human lung cancer cell line [d]A549, human colon cancer cell line [e]Colo-205, human ovarian cancer cell line [f]A2780.

## 4. Conclusion

The main amino acid residues in the binding site that interact with the test drugs through chemical interactions are the same as those in XP docking. The IFD results, which agree with those of XP docking tests, thereby confirm that the test compounds bind at the binding site of legumain. Compounds' anticipated ADMET characteristics are within acceptable bounds. As none of the chemicals were expected to exhibit CNS action from this in silico investigation, they do not have any CNS effects. In order to analyse the newly synthesised benzotriazole (6a–p) mannich base derivatives, 1HNMR, 13CNMR, and mass spectrum data were collected. Using the MTT technique, these compounds (6a-p) were examined for their anticancer effects on four distinct human cancer cell lines. Etoposide is the clinical medication utilised as a positive control. As compared to the control medication, the majority of the compounds showed excellent to moderate anticancer activity. Positive control values ranged from 0.13 ±0.017 to 3.08 ±0.135 M, and the drugs' IC50 values ranged from

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0.012 ±0.001 to 22.9 ±9.11 M. These 6a, 6c, 6e, 6f, 6j, 6n, and 6p were shown to have more powerful anticancer properties than etoposide among synthetic chemicals. Additionally, the structure-activity relationship of each compound was investigated. The results showed that compound 6a, which has an electron-donor (3,5-dimethoxy) group on the phenyl ring, had the strongest anticancer effects on the four cancer cell lines tested (MCF-7: 0.012 ±0.001M, A549: 0.18 ±0.076M, Colo-205: 0.34 ±0.083M, and A2780: 0.07 ±0.006M, respectively).

**Human and Animal Rights**

None of the human participants or animals were involved in the study.

**Conflict of Interest**

No conflict of interest is associated with this study.

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