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Research Article

Benzotriazole Derivatives: Design, In Silico Studies, And Biological Evaluation As Antiarthritic Agents

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ABSTRACT

In the current work, a series of benzotriazoles is produced by slightly altering the azole ring, and triazole derivatives are found to have similar or better activity in addition to fewer side effects. With two nitrogen atoms in its ring, benzotriazole is an organic heterocyclic molecule with a variety of biological activities, including anti-tubercular, anti-cancer, and anti-microbial effects. The rigid docking technique was used to determine the affinity between the protein and ligand. The voltage-gated sodium channel complex inhibitor protein (PDB ID:4DCK) has a three-dimensional (3D) crystal structure that can be downloaded from the protein database. Using Chem Draw, the chosen ligand molecules are produced. The method used to determine the binding affinities between ligands and proteins is rigid docking. The antiarthritic protein was used in docking experiments for 25 benzotriazole derivatives, as well as the crystal structure of the voltage-gated sodium channel C-terminus in complex (PDB ID:4DCK) inhibitors, using the molecular docking tool PyRx and some other resources. Comparing the docking scores of these compounds D1, D2, D3, D4, D5, D7, and D8 to the reference compound Indomethacin (-9.8 Kcal/mol), the results were -9.40, -10.00, -10.3, -11.1, -10.80, -11.1, and -11.1 Kcal/mol respectively. The antiarthritic drugs' results were confirmed by molecular docking and biological assessment investigations, indicating that these derivatives can function as complex (PDB ID:4DCK) inhibitors by forming the crystal structure of the voltage-gated sodium channel C-terminus. Therefore, these molecules can be further altered to create novel arthritic and anti-inflammatory drugs. According to this study, the majority of the compounds that were synthesized may be attractive therapeutic candidates with a promising pharmacological profile. Additionally, the majority of these derivatives may be useful for the continued development of better antiarthritic activity. Out of 25 substances, biological assessment

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studies have demonstrated the in vitro antiarthritic effectiveness of D5. When the effect was compared to a typical medication, it was discovered that the percentage inhibition was 66.13% (Diclofenac sodium).

INTRODUCTION

Azoles are widely used as antifungal agents and belong to the most prominent class of heterocycles [1]. Drug discovery and development have benefited greatly from 1,2,3-triazole-containing pharmacophores, which act as a bio isostere for the production of novel lead compounds, such as benzotriazole [2]. According to the literature review, benzotriazole-containing compounds have demonstrated a wide range of pharmacological activities [3], including anti-inflammatory [4], antitumor [5], anti-cancer [6], antimycotic [7], antioxidant [8], antiprotozoal [9], anthelmintic [10], antitubercular [11], antiviral [12], antiproliferative [13], enzyme inhibitors, and many more. Particularly potent inhibitors of specific enzymes, agonists, antagonists, and ligands are 1,2,3-triazole derivatives in studies of receptor-ligand interaction for drug development [14].

MATERIALS AND METHODS:

All of the chemicals used were obtained from laboratory chemical suppliers such as Sigma Aldrich, Merck, and CDH. Thin-layer chromatography and melting or boiling point measurements were used to confirm the purity of the starting ingredients utilised in the reactions.

Software required:

For the computational parameters of the molecular docking investigation, an AMD RyzenTM 5 Hexa Core 5500 APU @ 2.1GHz with turbo boost up to 4GHz Processor version 5500U, 16.00 GB RAM, and 64-bit Windows-11 operating system were utilised. The system also contained visualizer tools for PyRx, Swiss Dock, and Biovia Discovery Studio.

Preparation of target protein:

The three-dimensional model of the protein C terminus voltage-gated sodium channel complex inhibitor protein (PDB ID:4DCK) is available from the RCSB protein data bank (https://www.rcsb.org/) [15]. The C terminus voltage-gated sodium channel complex inhibitor can be imported into the Discovery studio client as shown in Figure 1. The protein-receptor binding domain's structure as a crystal, along with bound ligand and crystallographic water, are shown in a three-dimensional window. Water molecules have been removed from the hierarchy view. Now that the protein structure has been purified, hydrogen atoms are inserted. The linked ligand has been eliminated, and by predicting the ligand's binding site based on its current position, the file can be saved in PDB format.

Ligand and macromolecule preparation:

The freeware programme Chemsketch 2021 was employed to generate and refine the chemical structures of the ligands used in this study. Then, using Open Bable-2.3.2 software—which was required to execute the PyRx program—the ligands were saved in mol format and later transformed into PDB format. The macromolecule, or target enzyme, was prepared prior to starting the molecular docking procedure. The target enzyme's native ligand and water molecules had to be eliminated in order to accomplish this. Next, the files were saved as ligand pdbqt, the ligand was loaded, and torsions and rotatable bonds were assigned to the hydrogen atoms that needed to be studied.

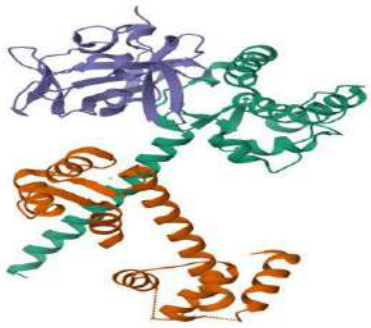
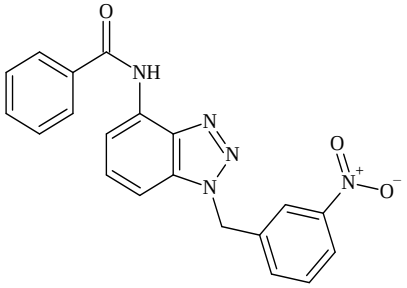
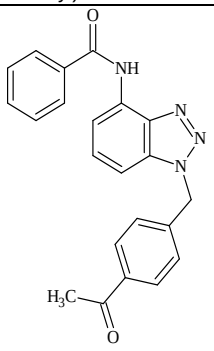
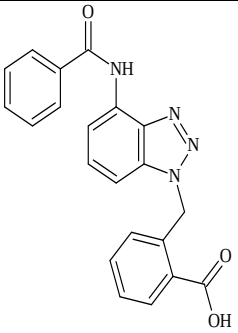
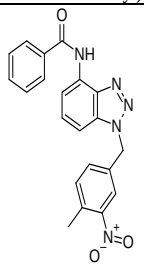
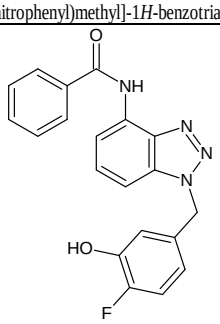
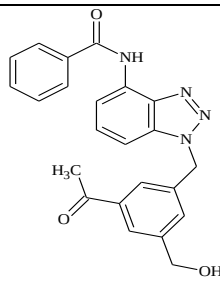


Figure.1. The voltage-gated sodium channel's C-terminus crystal structure in combination with FGF13 and CaM

Table no: 1. Benzotriazole analogues

D1	 *N*-[1-(3-nitrobenzyl)-1*H*-benzotriazol-4-yl]benzamide
D2	 *N*-{1-[(4-acetylphenyl)methyl]-1*H*-benzotriazol-4-yl}benzamide
D3	 2-[(4-benzamido-1*H*-benzotriazol-1-yl)methyl]benzoic acid
D4	 *N*-{1-[(4-methyl-3-nitrophenyl)methyl]-1*H*-benzotriazol-4-yl}benzamide
D5	 *N*-{1-[(4-fluoro-3-hydroxyphenyl)methyl]-1*H*-benzotriazol-4-yl}benzamide

D6	 *N*-(1-{[3-acetyl-5-(hydroxymethyl)phenyl]methyl}-1*H*-benzotriazol-4-yl)benzamide

RESULTS:

Predicting pharmacological activity involves using a docking score, which is a representation of the binding energy required to form a connection between the ligand and the receptor. It also contributes to strengthening the ligand-receptor indomethacin. Derivative D4, in comparison to other Benzotriazole derivatives, has a higher interaction. Table 2 shows the binding energy value of benzotriazoles. The approximate docking scores of eleven benzotriazole compounds range from -9.4 to -11.1 kcal/mol. The docking scores of all twenty-five benzotriazole derivatives were greater than those of the reference drug,
binding energy due to its lowest docking score of -11.1 kcal/mol.

Table no: 2. Molecular Docking ratings of particular compounds with a C crystal structure-terminus of complex inhibitors of the voltage-gated sodium channel (PDB ID:4DCK)

Ligand	Binding Affinity
D1	-9.40
D2	-10.00
D3	-10.3
D4	-11.1
D5	-10.80
D6	-9.60
D7	-11.1
D8	-11.1
D9	-10.5
D10	-10.3
D11	-9.5
D12	-10
D13	-11.3
D14	-9.9
D15	-9.8
D16	-11.1
D17	-9.7
D18	-9.7
D19	-9.9
D20	-10.8
D21	-9.5
D22	-10.3
D23	-10
D24	-10.4
D25	-9.6
Indomethacin	-9.8

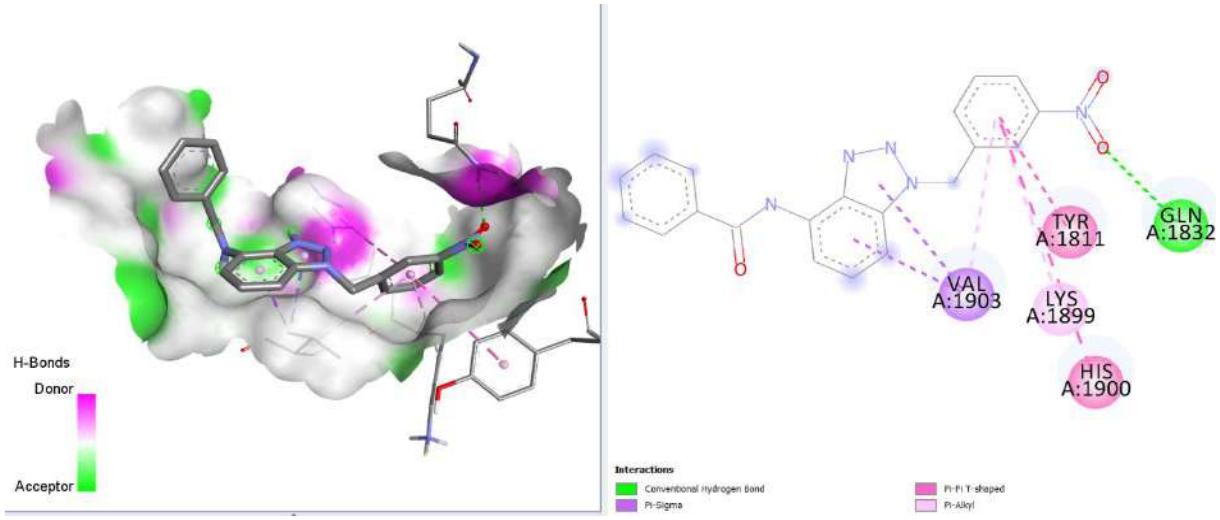


Figure No 2. Interactions between D1 and the complex's 3D and 2D crystal structure of the voltage-gated sodium channel's C-terminus (PDB ID:4DCK)

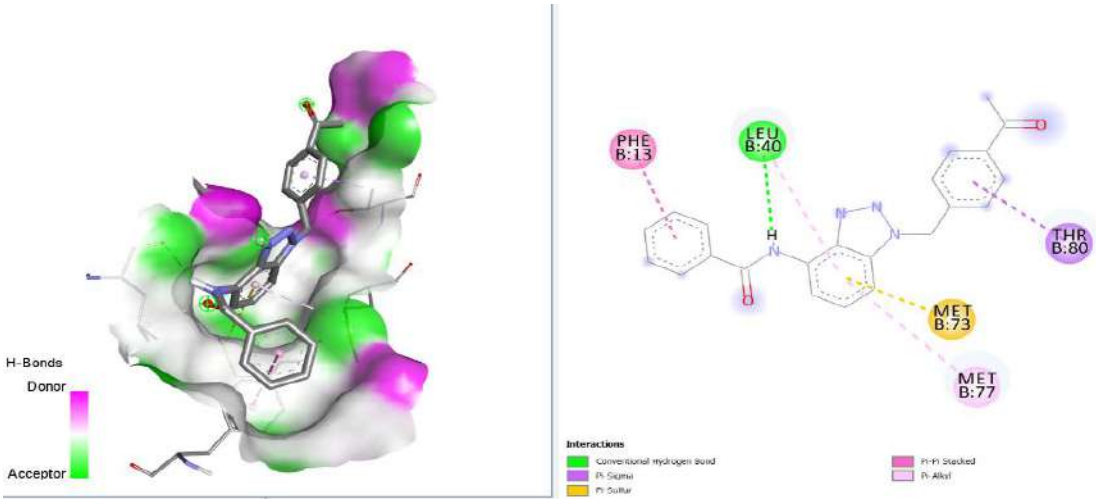


Figure No 3. Interactions between D2 and the complex's 3D and 2D crystal structure of the voltage-gated sodium channel's C-terminus (PDB ID:4DCK)

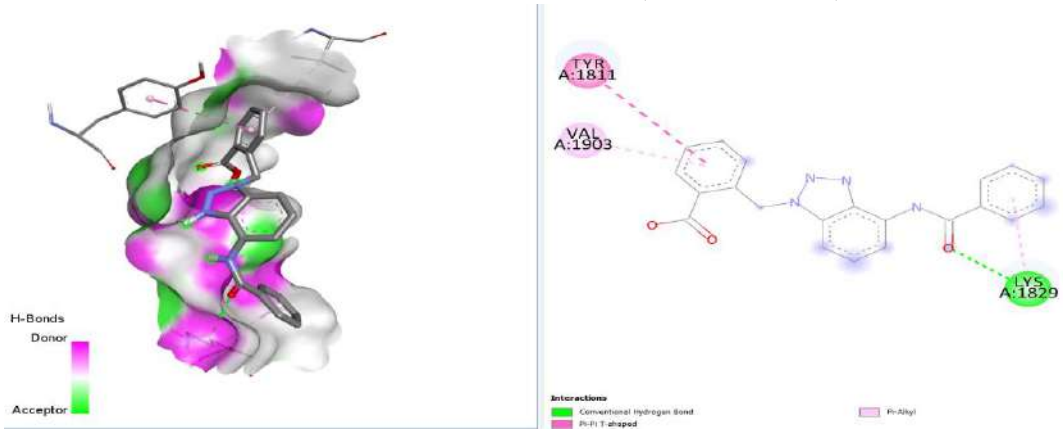


Figure No 4. Interactions between D4 and the complex's 3D and 2D crystal structure of the voltage-gated sodium channel's C-terminus (PDB ID:4DCK)

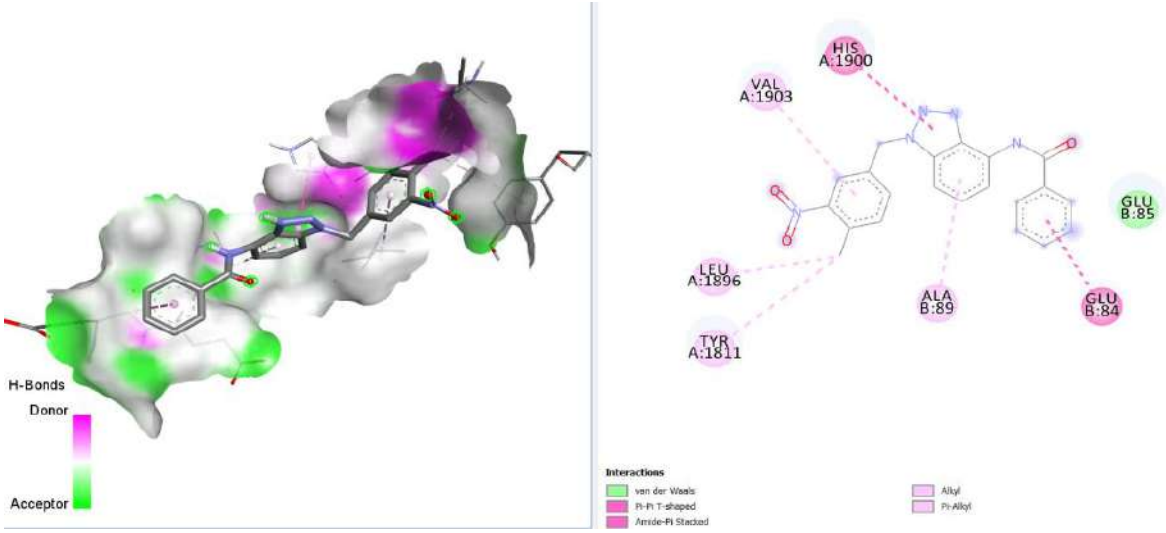


Figure No 5. Interactions between D6 and the complex's 3D and 2D crystal structure of the voltage-gated sodium channel's C-terminus (PDB ID:4DCK)

However, the study showed that all the compounds synthesized are potential for the anticancer activity and can be selected based on further in-vitro and in-vivo activity studies.

In vitro antiarthritic activity

Table no: 3. In-vitro antiarthiritc Activity of derivatives of n-(1h-benzotriazol-6-yl)-benzamide

Comp Code	% Inhibition		
	250µgm	500 µgm	Average
D1	24.15	45.79	35.41
D2	28.41	56.28	44.02
D3	30.40	59.75	45.10
D4	55.68	76.95	67.13
D5	30.77	58.50	44.20
D6	42.42	66.75	54.63
Diclofenac sodium	78.68	91.46	83.69

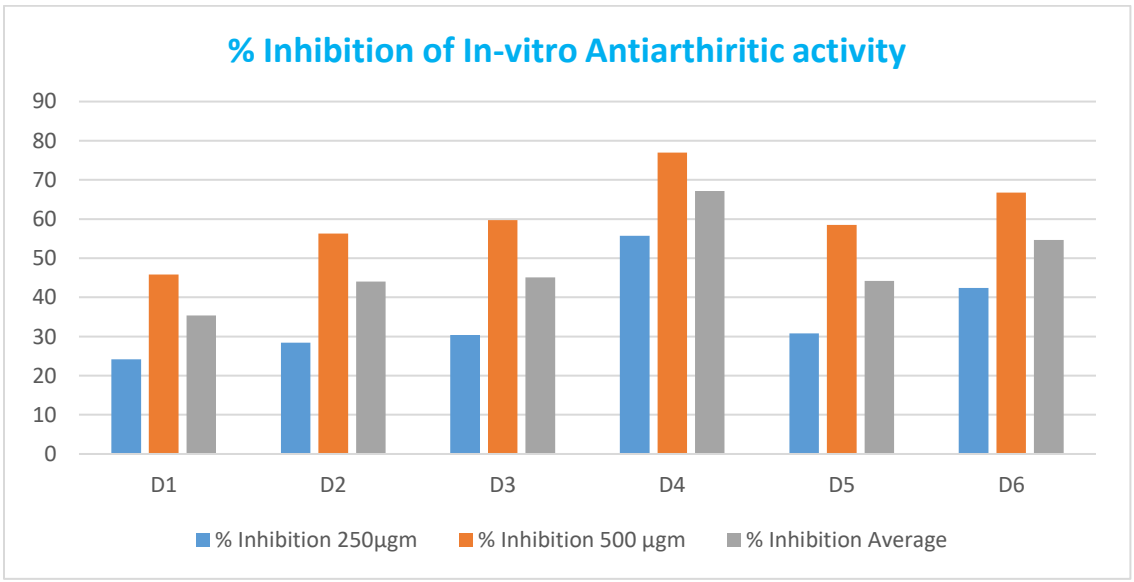


Figure No. 6: In-vitro anti-arthiritc Activity of derivatives of n-(1h-benzotriazol-6-yl)-benzamide

According to the in vitro antiarthritic activity protein denaturation method, denaturation of the protein may be the cause of autoantigen formation in some arthritic disorders. When the effect was compared to a typical medication, it was discovered that the percentage inhibition was 66.13% (Diclofenac sodium).

DISCUSSION:

The antiarthritic protein was utilized in the molecular docking program PyRx along with various additional resources to perform docking studies for 25 benzotriazole derivatives and the crystal structure of the voltage-gated sodium channel's C-terminus in complex (PDB ID:4DCK) inhibitors. In contrast to the reference compound Indomethacin (-9.8 Kcal/mol), the docking scores of these compounds, D1, D2, D3, D4, D5, D7, and D8, were, respectively, -9.40, -10.00, -10.3, -11.1, -10.80, -11.1, and -11.1 Kcal/mol. By establishing the crystal structure of the voltage-gated sodium channel's C-terminus, these derivatives can operate as complex (PDB ID:4DCK) inhibitors. The outcomes of molecular docking and biological evaluation investigations supporting the antiarthritic medications were validated. To produce brand-new anti-inflammatory and arthritic medications, these molecules can therefore be further modified. As per the findings of this investigation, most of the synthesized compounds have intriguing pharmacological profiles and could be appealing therapeutic options. Better antiarthritic activity may also be developed in the future with the help of most of these compounds.

CONCLUSION:

Compounds D1, D2, D3, D4, D5, and D6 showed a very good docking score with the complex (PDB ID:4DCK) antiarthritic drugs' crystal structure of the voltage-gated sodium channel's C-terminus. In vitro antiarthritic action Protein Denaturation Method: Denaturation of proteins may be the cause of autoantigen formation in some arthritic conditions. When the effect was compared to a typical medication, the percentage inhibition was determined to be 66.13% (Diclofenac sodium)

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CONFLICT OF INTERESTS:

None

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