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In silico studies of benzimidazole derivatives as sustainable inhibitors against Methicillin-resistant *Staphylococcus aureus*

T A Ogunnupebi [1, 2], G O Oduselu [1], O F Elebiju[1, 2], O O Ajani [1, 2]* and E Adebiyi [1, 3]

[1] Covenant University Bio-informatics Research Cluster (CUBRe), Covenant University, P.M.B. 1023, Ota, Ogun State, Nigeria
[2] Department of Chemistry, Covenant University, P.M.B. 1023, Ota, Ogun State, Nigeria
[3] Division of Applied Bioinformatics, German Cancer Research Center (DKFZ), Heidelberg, Germany

Temitope A. Ogunnupebi - temitope.ogunnupebipgs@stu.cu.edu.ng (0000-0002-0744-7812)
Gbolahan O. Oduselu - gbolahan.oduselu@covenantuniversity.edu.ng (0000-0003-0136-1732)
Oluwadunni F. Elebiju - oluwadunni.elebijupgs@stu.cu.edu.ng (0000-0002-3005-7003)
Olayinka O. Ajani - ola.ajani@covenantuniversity.edu.ng (0000-0002-3422-3478)
Ezekiel Adebiyi - ezekiel.adebiyi@covenantuniversity.edu.ng (0000-0002-1390-2359)

*Corresponding email: ola.ajani@covenantuniversity.edu.ng

Abstract. Antimicrobial resistance is becoming more rampant in our world today, and different measures are being taken to combat this challenge. Benzimidazoles are classified as heterocyclic compounds with notable pharmacological properties. As a result, benzimidazole has been combined with other compounds that have remarkable actions to create a more potent molecule. Exploring these substances to combat antibacterial resistance would therefore aid in achieving good health and wellbeing and promote sustainable development. Predicting the effectiveness of the compounds before manufacturing and clinical testing has made drug design easy. This study employs in silico methods like molecular docking to investigate alternate antibacterial agents from a library of benzimidazole derivatives. A library of compounds with a benzimidazole template was screened against the three-dimensional (3D) structure of peptidoglycan transpeptidase (PPB2A) of Staphylococcus aureus. Two binding sites were identified in the protein: the main site and the allosteric site. Molecular docking was done on the main and allosteric sites to obtain free binding energy ranging from -7.3 to -5.8 and -4.9 to -4.5 kcal/mol, respectively. The predictive Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) experiments were done on the compounds to ascertain their safety. The results were compared to those of known antibiotics, and the compounds performed effectively. The benzimidazole derivative can be adopted as a prospective antibacterial agent with an alternative pathway for combating resistance issues and enhancing the quality of health and well-being globally.

Keywords: Antibiotics, ADMET Properties, Heterocyclic Compounds, Infection, Molecular Docking, Sustainable Development.

1. Introduction

Antimicrobial resistance is one of the top 10 difficulties the public health sector faces, among many others. Due to the growing rate of resistance of microorganisms such as bacteria, fungi, parasites, and viruses to drugs and drug-like compounds, effective substitutes must be discovered [1–3]. According to global estimates, antimicrobial resistance was responsible for approximately 2.4 million deaths in 2019 [4]. Some of the common bacteria that cause some chronic diseases include *Mycobacterium tuberculosis* associated with tuberculosis [5], *Escherichia coli* associated with intestinal infections, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* [6,7]. *S. aureus* is one of the most common gram (+) bacteria, it is responsible for a wide range of clinical infections including skin and soft tissue infections that can develop into bone infections or severe muscle pain [8]. It can also affect the lungs and heart valves if not treated. They are significant contributors to food-borne illness [8].

Studies show that the bacterial cell wall of *S. aureus* contains peptidoglycans which are the main building blocks and thus influence its survival during cell division and growth [8,9]. In *S. aureus*, resistance is initiated by the Penicillin-binding protein (PBP2A), a peptidoglycan transpeptidase in conjunction with the PBP2 transglycosylase domain when β-lactam antibiotics are introduced [9]. As a result, researchers are looking for compounds that can inhibit the activity of these enzymes. Organic compounds, particularly heterocycles, have attracted attention due to their powerful pharmacological properties [10]. This group includes benzimidazole derivatives, which are composed of an imidazole fused with a benzene ring [11,12]. They have found numerous applications as antimicrobials [13], anti-cancer [14], anti-tubercular [15], anti-oxidant [16], antimalarial [17], etc. Some commercially available benzimidazole derivatives used in the treatment of various diseases are shown in Fig. 1. In order to shorten the 12 to 15-year process of drug design and development, Computer-aided Drug Design (CADD) has offered sustainable solutions to overcome the hurdles involved in the initial stage. There are several tools available for designing compounds, such as *in silico* ADMET predictors, molecular docking, and molecular dynamic simulation, which allows us to test the compounds on proteins responsible for an activity in the biological system [18]. This study seeks to investigate alternative drug-like substances from benzimidazole derivatives against the 3D structure of peptidoglycan transpeptidase (PBP2A) in *Staphylococcus aureus* as a sustainable treatment option for multi-drug resistant strains.

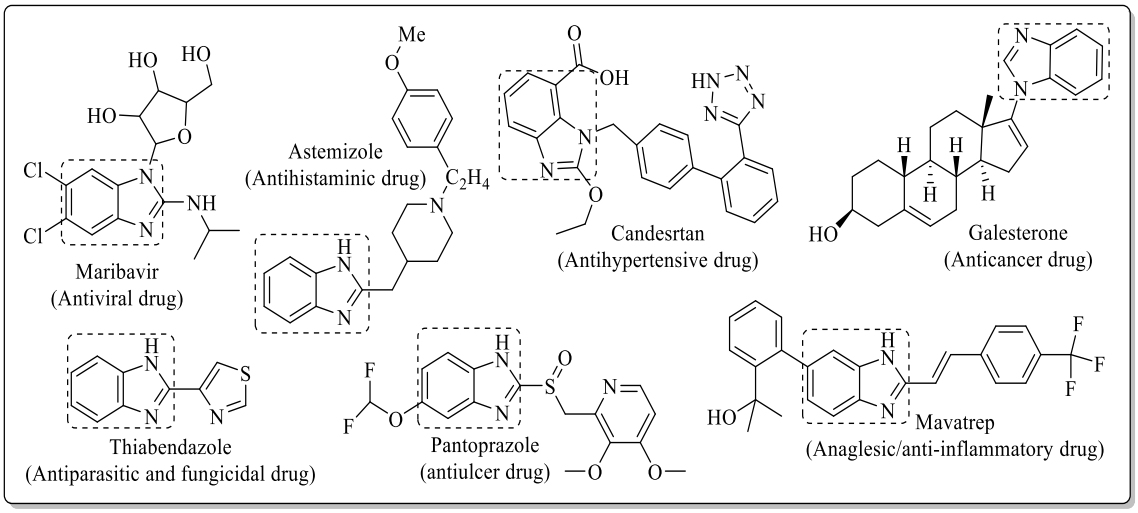

Figure 1: Structures of some commercially available benzimidazole derivatives

2. Material and Methods

2.1 Protein preparation

The experimentally validated 3D structure of PBP2A of Methicillin-resistant *Staphylococcus aureus* (MRSA) was downloaded from the Protein data bank (PDB) using the PDB ID: 4CJN as reported by [19]. The protein was then prepared using the chimera software by removing non-amino residues, minimizing the structure, and adding Gasteiger charges. The protein was taken further for the docking and simulation studies.

2.2 Ligand preparation

The benzimidazole template was utilized for the compound search on the PubChem database [20]. The compounds were screened using Lipinski's rule of five (LO5), and a total of about 1510 compounds were downloaded alongside four (4) recognized antibiotics in Structure-Data Files (SDF) format. The Open Babel in PyRx was then used to convert these compounds into Autodock docking formats (pdbqt).

2.3 Virtual screening and post-docking analysis

The prepared ligands and known antibiotics (gentamicin, penicillin G, streptomycin, ampicillin) were screened against PBP2A using Autodock vina [21]. The active site for the main and allosteric sites was set using the amino acid residues identified by [22]. Using Discovery Studio, the binding interactions between the top five hits, and four antibiotics were depicted.

2.4 In silico toxicity prediction and drug-likeness

The *in silico* toxicity and drug-likeness were analyzed using OSIRIS Property Explorer and ADMETlab webserver [23]. The prediction took Lipinski's rule of five (LO5) into account when assessing the pharmacokinetic and toxicological profiling of the compounds and known antibiotics [24].

3. Results and Discussion

3.1 3D Structure of Protein

The protein has two chains with three domains. The domains are the PBP2A domain (containing 2-114 amino acids sequence), N-terminal transpeptidase domain (122-283 amino acid sequence), and transpeptidase domain (320-631 amino acids sequence) (Fig. 2). The transpeptidase domain containing the Ser403 amino acid responsible for resistance in MRSA was selected as the main active site. The protein had an allosteric site which serves as a regulatory system that affects the activity at the main binding site [8,9,25].

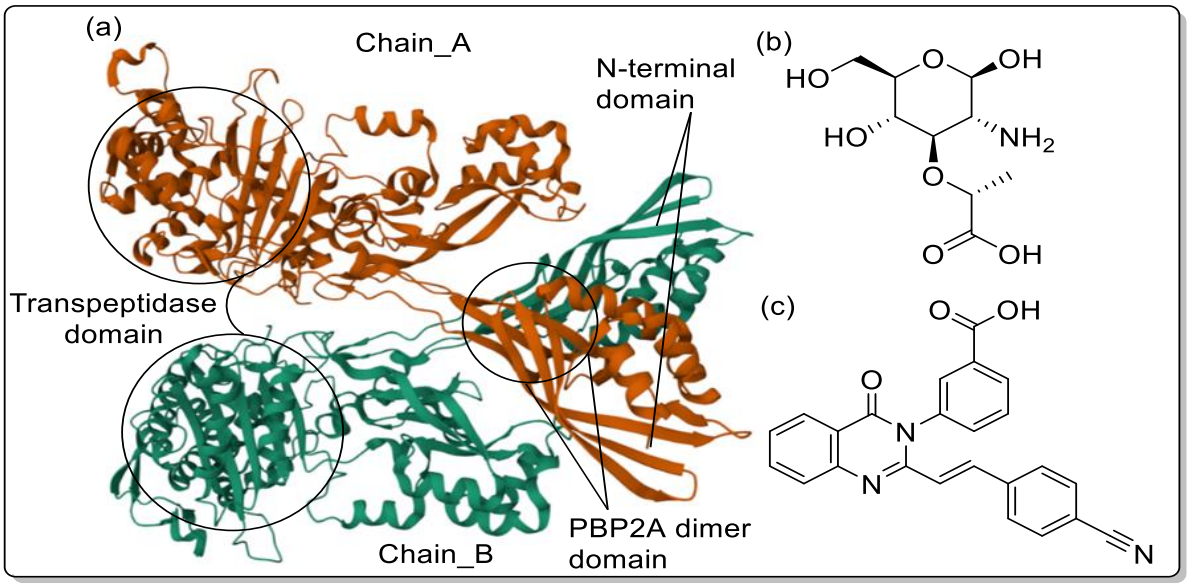

Figure 2: (a) 3D structure of PBP2A, the co-crystallized ligands (b) QNZ and (c) MUR [19,22]

3.2 Virtual screening and post-docking analysis

A total of 1510 compounds were docked against the main site and allosteric site of PBP2A. The 5 top hits compounds in the main site were reported to have binding energy ranging from -7.3 to -7.1 kcal/mol whereas that of the allosteric site ranged from -5.4 to 5.0 kcal/mol (Table 1). With the help of BIOVIA Discovery studio, the 2D and 3D interaction between the amino acid residues of PBP2A and the atoms of ligands was observed (Fig. 3). The ligand with the highest binding affinity, **1023408**, interacted with Ser 461, Ser 462, Asn 464, Thr 600, and Glu 602. The NH linker carrying the imidazole and methylene groups that were joined to the benzimidazole template helped to make the ligand more active. Similarly, for ligand **3079203**, the contact between atoms and Tyr 529, Thr 444, and Glu 602 was facilitated by the NH present in the benzimidazole molecule and the azanecarboximidamide fragment. Ligand **137054718** formed a hydrogen bond with the NH on the benzimidazole using Tyr 441 while at the allosteric site, the ligand **137054718** was also the third best. The ligands functioned comparably to the well-known antibiotics, despite streptomycin and ligand **137054718** having identical binding affinities whereas, at the allosteric site, all the ligands performed better than the known especially Pencillin G which is a known β-lactam antibiotic.

Table 1: Docking score of top 5 best hits and known antibiotics in PBP2A.

PubChem ID	Binding energy in the main site (kcal/mol)	PubChem ID	Binding energy in the allosteric site (kcal/mol)
10243408	-7.3	10243408	-4.7
3079203	-7.3	3079203	-4.9
137054718	-7.2	137054718	-4.8
91296309	-7.1	91296309	-4.8
93993728	-7.1	93993728	-4.8
Streptomycin	-7.2	Streptomycin	-4.6
Penicillin G	-6.9	Penicillin G	-4.9
Ampicillin	-6.5	Ampicillin	-4.9
Gentamicin	-5.8	Gentamicin	-4.5

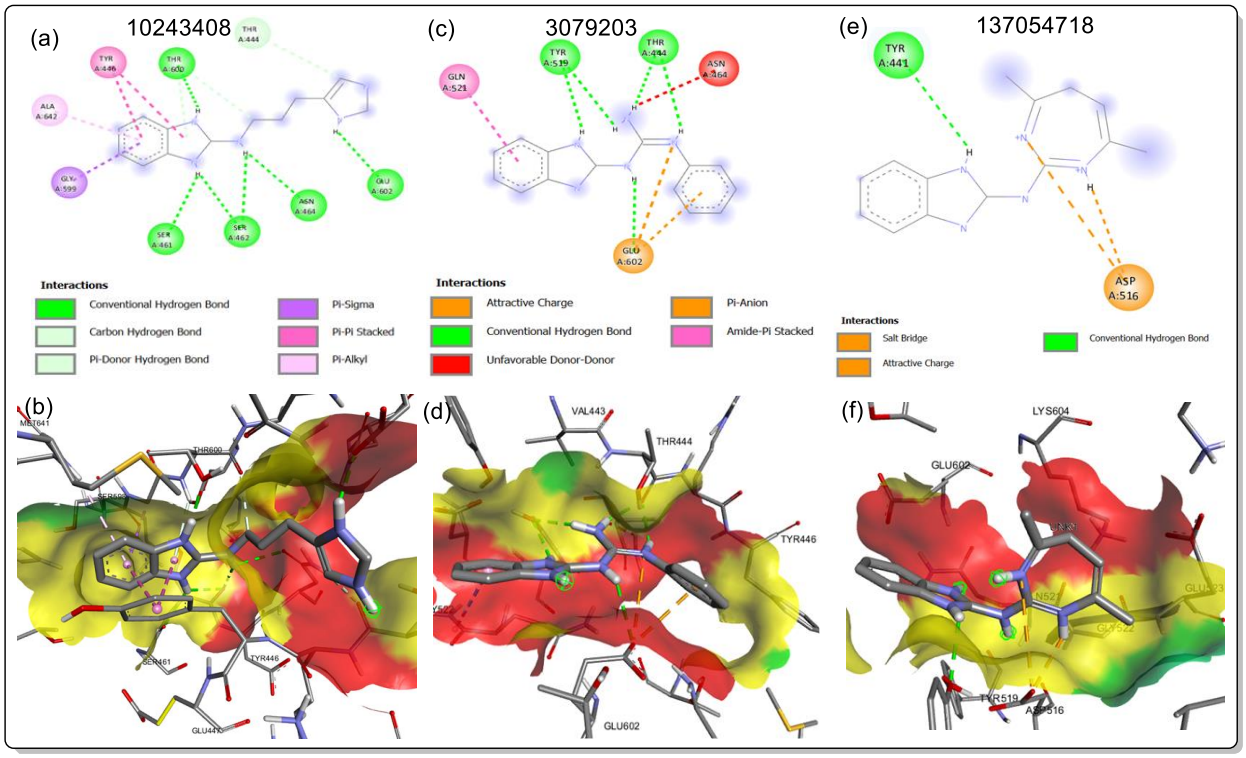

Figure 3: 2D and 3D interaction between PBP2A and the top-hits at the transpeptidase domain (a) & (b) 2D and 3D interaction between **10243408** and PBP2A; (c) & (d) 2D and 3D interaction between **3079203** and PBP2A; (e) & (f) 2D and 3D interaction between **137054718** and PBP2A

3.3 In silico toxicity prediction and drug-likeness

According to OSIRIS Property Explorer's predictions for in silico toxicity and drug-likeness, all the compounds have a variety of drug-relevant properties that fall within acceptable ranges (molecular weight [MW] ⩽ 500 g/mol; TPSA ⩽ 160 Å; clogP ⩽ 5; logS > -4 mol/dm3) as shown in Table 2. The molecular weight depicts how heavy a molecule is, streptomycin went over the allowable limit of 500 g/mol. When considering drug bioavailability, the topological polar surface area is crucial. When the value exceeds 160 Å, it will have an impact on the hydrogen bonding of the compounds. All the screened compounds fall within this range of the partition coefficient (clogP), which indicates that they have good absorption properties. Similarly, the solubility prediction is associated with absorption and distribution properties and all the screened compounds within the range. More specifically, toxicity characteristics such as impact on the reproductive system, irritability, and tumorigenicity are assessed. The top 5 hits of benzimidazoles didn't pose any toxicity danger, the structures are shown in Fig. 4. Penicillin G is the only mutagenic or tumorigenic substance that exhibits high risk. None of the substances, except for streptomycin, demonstrated a high risk to the reproductive system. Toxicology and other physicochemical properties add up to create the drug score. A ligand becomes a possible drug candidate if its drug score is greater.

Table 2: Physicochemical properties and toxicity risks of top 5 hits in comparison to known antibiotics.

PubChem ID	Physicochemical properties						Toxicity risks			
	cLogP	MW	TPSA	logS	Drug likeness	Drug-score	Mutagenic	Tumorigenic	Irritant	Reproductive effect
10243408	1.79	241.29	69.39	-2.7	2.22	0.87	None	None	None	None
3079203	2.15	251.29	79.09	-3.92	-0.15	0.61	None	None	None	None
137054718	2.32	253.3	65.43	-3.99	1.82	0.76	None	None	None	None
91296309	0.25	264.29	91.45	-3.53	2.0	0.83	None	None	None	None
93993728	1.77	230.31	52.74	-2.98	0.97	0.78	None	None	None	None
Gentamicin	-4.21	477.6	199.7	-1.18	4.88	0.77	None	None	None	None
Penicillin G	1.54	334.39	112	-2.04	11.28	0.33	High	High	None	None
Streptomycin	-7.86	581.57	336.44	-0.95	0.83	0.32	None	None	High	None
Ampicillin	-1.66	349.4	138	-1.57	10.72	0.91	None	None	None	None

Molecular weight [MW], partition co-efficient [clogP], Topology polar surface area [TPSA], solubility prediction [logS]

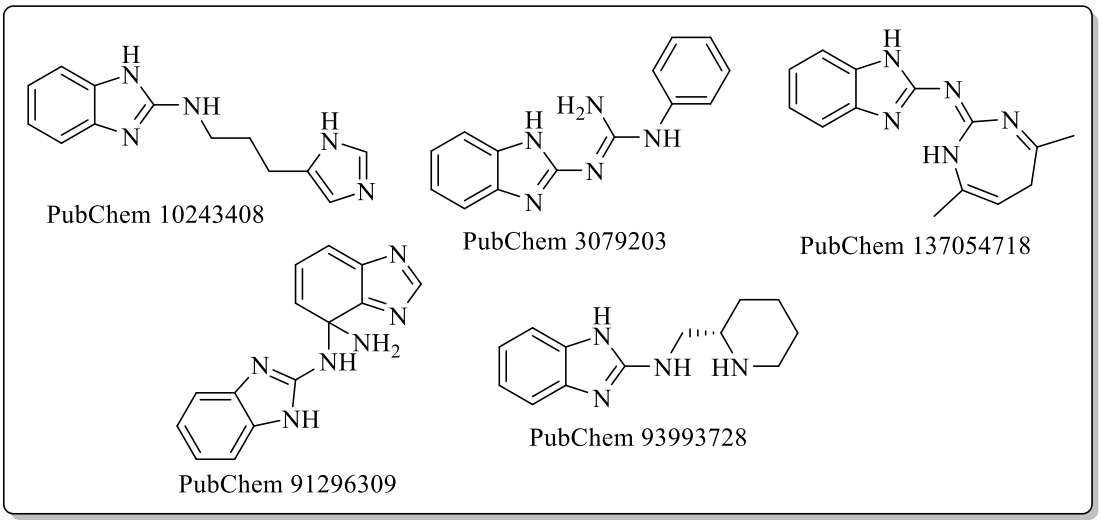

Figure 4: Chemical Structures of the Best Five (5) Hits with their PubChem ID

4. Conclusion and Recommendation

This work detailed *in silico* analyses of possible antibiotics from a library of benzimidazole compounds that already exist against 3D structure of PBP2A of MRSA. In this way, the process of drug research and development can be sped up by repurposing active compounds which is a sustainable strategy to addressing antimicrobial resistance. Amongst the 1510 benzimidazole derivative, compound **10244308**: (N-(3-(1H-Imidazol-5-yl)propyl)-1H-benzo[d]imidazol-2-amine), compound **3079203**: 2-(1H-benzimidazol-2-yl)-1-phenylguanidine and compound **137054718**: N-(1H-benzimidazol-2-yl)-4,7-dimethyl-1,5-dihydro-1,3-diazepin-2-imine had binding affinities of -7.3, -7.3, and -7.2 kcal/mol. Also, the proposed compounds showed favourable *in silico* ADMET properties, although several antibiotics have been known to cause side effects such as diarrhoea, vomiting, and weight loss [8]. The benzimidazole derivatives explored in the docking studies are potential transpeptidase domain antagonists to be considered for further optimization and development into effective inhibitors.

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