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Design, Synthesis, Characterization and Biological Evaluation Of 2, 4, 5-Trisubstituted Imidazole Derivatives As Potent Antimicrobial Agents

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ABSTRACT

Imidazole derivatives are thought to be promising chemical substances that may have therapeutic efficacy against a number of dangerous pathogenic microorganisms. Molecular docking is an effective method in modern drug design to have greater comprehension of computational binding. New imidazole derivatives were designed for this investigation. Out of the designed compounds two derivatives, N1 (naphthalen-1-yl)-4,5-diphenyl-1H-imidazole) & N2 (2-(2-methylenaphthalen-1-yl)-4,5-diphenyl-1H-imidazole), exhibit good affinity for the active pocket of glucosamine-6-phosphate synthase (ID2VF5), according to the molecular docking studies. The compounds with a high binding affinity were synthesized by using a condensation reaction in the presence of a catalyst. IR and 1H NMR were used to confirm the synthesized compound's structure. By employing the cup & plate procedure, it was found that the synthesized imidazole derivatives exhibited antimicrobial activity toward both the Gram-positive organism *Staphylococcus aureus* and the Gram-negative organism *Escherichia coli.*

Keywords: *Molecular docking, 2,4,5 -triphenyleimidazole, Antimicrobial activity*

1. INTRODUCTION

In recent years, medicinal chemists have focused mostly on developing medications with improved duration of action and efficacy, as well as reducing side effects and toxicities and developing new medications through molecular modification. The pharmaceutical sector has remained committed to this drug development, particularly medicinal chemists. Both natural and synthetic sources can be found in organic therapeutic compounds. Synthetic medications are made either by pure synthesis or by altering the structures of natural drugs. As novel medication therapy has advanced over time, it has gotten more complicated, time-consuming, and expensive. In order to improve the efficiency of their job in drug development, professional medicinal chemists have also been flooded with new instruments and techniques **(Verma et al., 2017).** The in silico docking optimization challenge aims to estimate the structure of the between-molecules complex that forms between multiple molecules by describing the expected binding mode for a ligand to bind to a particular protein that is relevant. Because of its uses in medicine, the protein-ligand interaction is the most intriguing example. A small molecule known as a ligand interacts with protein binding sites. Protein regions known to be active in compound formation are called binding sites. There are a number of potential mutual configurations where binding could take place. These are frequently referred to as binding modes **(Sharma et al., 2009).**

Molecular docking has become common in present-day drug design to investigate drug-receptor interactions. Molecular docking is often used to forecast the direction in which small molecule therapy candidates will bind to their protein targets, hence predicting the small molecule's affinity and activity. It also offers valuable information on drug receptor interactions. For many years, humans have been continuously exposed to germs. Globally, invasive microbial infections are a serious issue, particularly for people with weakened immune systems. The urgent need for novel antimicrobial drugs to treat these potentially fatal invasive diseases has led to the recent growth of antimicrobial medication research. The development in antimicrobial resistance during the last one century has created a demand for novel antimicrobial medicines that are more effective, selective, and no more hazardous than the medications now utilized in therapeutic intervention. It has been discovered that hetero cycles with an azole-based ring structure contain a variety of biological characteristics, such as

antifungal and antibecterial properties. Nowadays, imidazole and its related compounds are highly significant because of its many physiological functions and applications in synthetic chemical research. Imidazole and it's derived compounds have a number of pharmacological propertiesor bioactivities. Because they function as coordination system ligands, donors of protons and/or acceptors, and the foundation of charge-transfer processes throughout biological systems—especially in enzymes—imidazole and its derived compounds are extremely significant **(Vijesh A. M. et al., 2013).** In several domains, including pharmaceutical chemistry **(Ai, J. et. at., 2023)** and the science of materials for nonlinear optical purposes **(Abd-Alaziz, A.M. et al., 2024)**. imidazole is essential. In commercial use, numerous imidazole compounds act as catalysts **(Sallal A. H. et al., 2024).**

Antibacterial drugs have long been thought to be interested in the enzymes involved in the biosynthesis of peptidoglycan. The majority of recognized, well-established "cell-wall-acting" drugs obstruct the peptide portion of bacterial peptidoglycan's production, assembly, and cross-linking **(Chmara et al., 1994).** One potential new target for antifungals is glucosamine-6-phosphate (GlmS) synthase **(Chmara et al., 1984)**. GlcN-6-P synthase catalyzes the first stage of hexosamine biosynthesis through the conversion of fructose 6-phosphate (Fru6P) to glucosamine 6-phosphate (GlcN6P) when glutamine is present. GlmS catalyzes a reaction that is considered a committed step because it cannot be undone. The pathway's final product, N-acetyl glucosamine, is a crucial component of fungal and bacterial cell walls. Prokaryotic and human enzymes differ structurally, which might be used to create particular inhibitors that could be used as the basis for antifungal and antibacterial medication prototypes **(Vijesh A. M. et al., 2013).** Because mammalian cells stay alive for longer, GlcN-6-P synthase possesses an extended half-life and the gene that codes for the enzyme is produced more quickly in mammals. Mammals are not killed by temporary destruction of the collection of amino sugar, but fungal cells are killed by even a brief suppression of the enzyme. **(Milewski et al., 1986).**

It is commonly known that minor structural changes to the targets are changing both their physiochemical and biological characteristics. A thorough analysis of published studies on the microbial properties of different kinds of compounds showed that boosting effect requires the existence of some kind of bioactive scaffold, for instance azoles derivatives (imidazole & prazole) **(Vijesh A. M. et al., 2013).** Substituted imidazole derivatives show a wide range of therapeutic effects, analgesic and antifungal **(Gaba, M., Mohan, C 2016),** anti-depressant agents **(Murali A. & Shaji A. 2019),** anti-cancer agents **(Ali et al., 2017)**, antimalarial agents **(Kondaparla S. Et al., 2018)** and antimicrobial activities etc. **(Verma et al., 2017).** In order to better understand the computational binding, I have performed molecular docking experiments on imidazole derivatives.This may aid in the investigation and creation of imidazole compounds with strong antibacterial properties in the future.

2. MATERIALS AND METHOD

2.1 MOLECULAR DOCKING STUDIES

ChemDraw Pro 1.2. software was used to draw the ligands' two-dimensional structures. Energy minimization of compounds was done with the help of software ChemDraw 3D. All the compounds were saved in PDB form. Autodockvina 4.2 software was used to prepare ligands for the docking process. In this software, the PDB forms of compound structures were opened, hydrogen was added, charges were computed, and AD4 types of atoms were assigned. The files were saved in the pdbqt. The receptor ID2VF5 was downloaded in PDB format. The receptor that was downloaded was visualized in PyMOL, the ligand attached to the receptor was separated, and the water molecules were also removed. After hydrogen was added, charges were computed (add Kollman charges), and AD4 type atoms were assigned, the protein was then saved in pdbqt format. A grid box and AutoGrid from AutoDock Tools were used to construct the grid map. Following grid calculation, a conf file containing the grid parameter file was stored for docking. PDB with ID 2VF5 was used to select binding site. This grid needs to surround the macromolecule's active site, or region of interest. The area surrounding the active site that contains all 12 amino acid residues (Ala602, Val399, Ala400, Gly301, Thr302, Ser303, Cys300, Gln348, Ser349, Thr352, Ser347, and Lys603). Final docking of all the ligands was done by using the command prompt program using AutoDock Vina. After the final docking process, we got binding energies of all possible orientations of ligand with receptor. After this whole docking process, the Discovery Studio Visualizer 2024 Client was used. By opening the structure in it, this gave us the binding site of the structure and also amino acids that surround this structure.

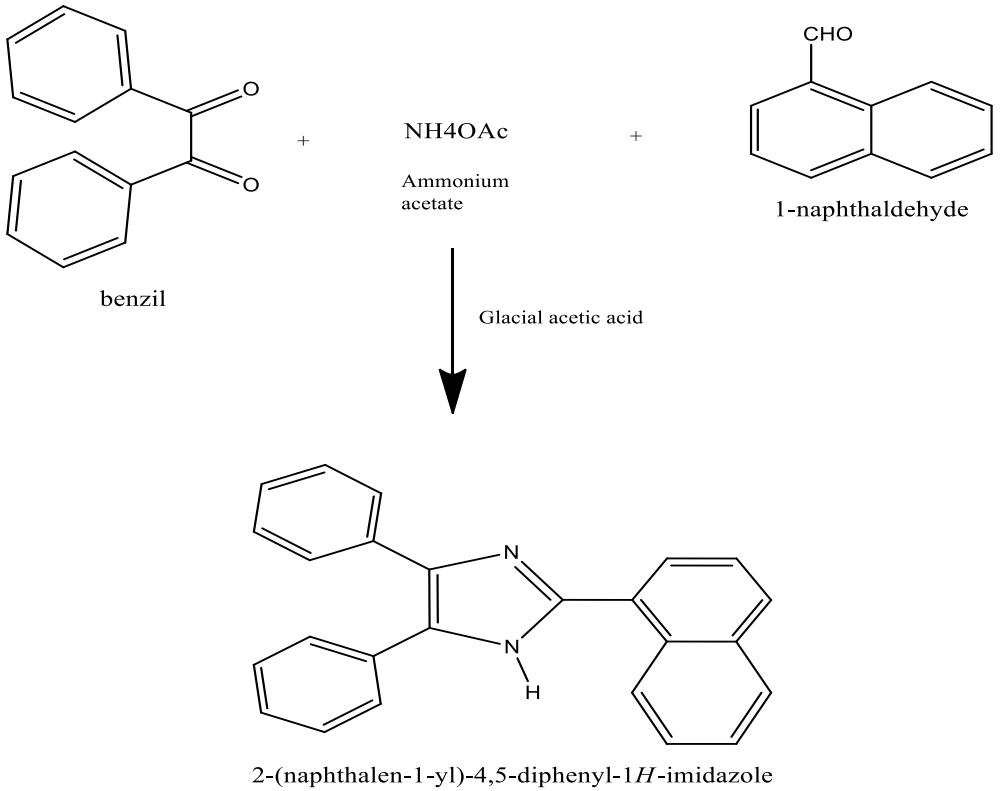
2.2 SYNTHESIS OF SELECTED COMPOUNDS

2.2.1 GENERAL PROCEDURE

2.2.1.1 Synthesis of compound N1 (2-(naphthalen-1-yl)-4,5-diphenyl-1H-imidazole)

1 gram of benzil, 2 ml of 1-naphthaldehyde, 1 gram of ammonium acetate, and 4 ml of glacial acetic acid were taken in RBF, and then, for 3 hours at 50-60°C temperature, the mixture was subjected to reflux.Then the mixture's deep orange color, which the TLC verified, signaled the reaction's completion. After then, the resultant mixture was left to stand till it came to room temperature.Following the addition of adding of water (150ml), neutralize the solution by adding 10 ml of ammonium hydroxide and examine using litmus paper. After then, the solid was filtered. The solid underwent recrystallization with the

ethanol.

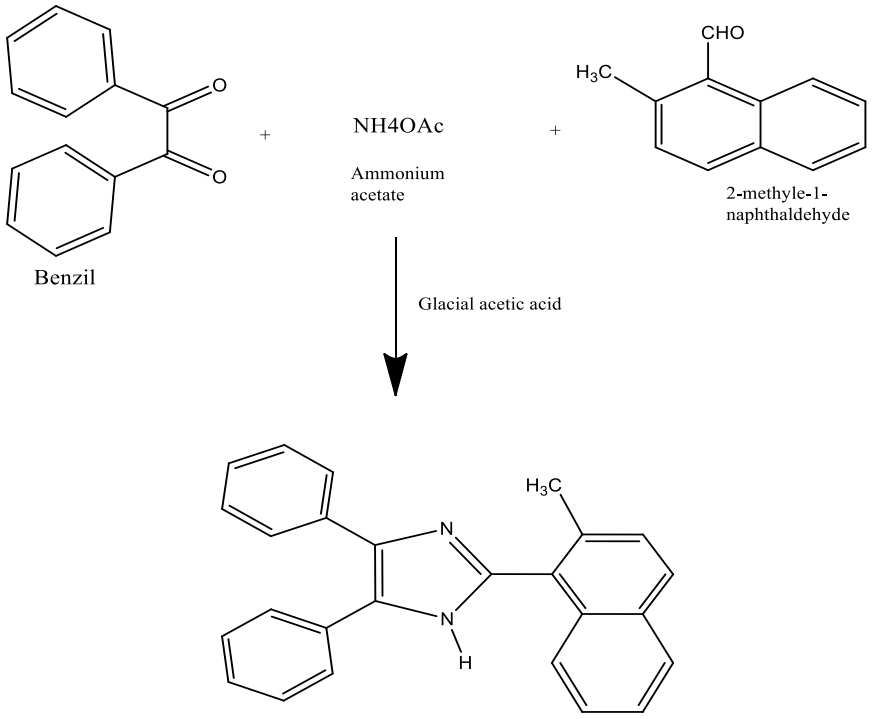


2-(naphthalen-1-yl)-4,5-diphenyl-1*H*-imidazole

SCHEME 1: Synthesis of N1 (2-(naphthalen-1-yl)-4,5-diphenyl-1H-imidazole)

2.2.1.2 Synthesis of compound N2 (2-(2-methylenaphthalen-1-yl)-4,5-diphenyl-1H- imidazole)

1 gram of benzil, 2 ml of 2-methyle-1-naphthaldehyde, 1 gram of ammonium acetate, and 4 ml of glacial acetic acid were taken in RBF, and then, for 3 hours at 50-60°C temperature, the mixture was subjected to reflux Then the mixture's deep orange color, which the TLC verified, signaled the reaction's completion. After then, the resultant mixture was left to stand till it came to room temperature. Following the addition of adding of water (150ml), neutralize the solution by adding 10 ml of ammonium hydroxide and examine using litmus paper. After then, the solid was filtered. The solid underwent recrystallization with the ethanol.



2-(2-methylnaphthalen-1-yl)-4,5-diphenyl-1*H*-imidazole

SCHEME 2: Synthesis of N2 (2-(2-methylenaphthalen-1-yl)-4, 5-diphenyl-1H-imidazole)

1.2.2. ANTIBICROBIAL ACTIVITY

These two microorganisms were used to assess the produced compounds' antimicrobial properties.

1. *Staphylococcus aureus* (Gram-positive bacteria)

2. *Escherichia coli* (Gram-negative bacteria)

Cup plate method: The sterile melted agar (30 ml) was allowed to cool to 40°C. After thoroughly mixing the sterile molten nutritional agar with 1 ml of inoculum, the mixture was transferred into sterile petri dishes and let to harden. In petri plates, At 37°C, the culture of bacteria were cultured for a whole day(24 hours).Following the addition of 1 milliliter of the chemical solution to each well. The antibacterial investigations were conducted at concentration level: 250µg/ml. At one dose of each drug, the zones of inhibition against each bacterium were assessed. A comparison was made between the standard (250µg/ml) and the zones of inhibition **(Burungale D.S. et al., 2013).**

3. RESULT & DISCUSSION

3.1. MOLECULAR DOCKING RESULT

Table 3.1. Results of Molecular Docking representing, binding energy (kcal/mol), hydrogen bonding and pi-pi interaction.

S. no	Compound	Binding energy (Kcal/Mol)	Hydrogen binding Site	Pi-Pi\Pi Sigma	Pi-Alkyl
N1	1-naphthaldehyde	-8.3	ALA 483 CYS 300 GLY 301 LEU 480 LEU 484	LEU 484 GLY 301	LEU 601 ALA 483 LEU 480 CYS 300

			LEU 601		
N2	2-methyl-1-naphthaldehyde	-8.9	CYS 300 TYR 304 ILE 326 LEU 480 ALA 483 LEU 484 LYS 487	LEU 484	LYS487 LEU 480 ILE 326 TYR 304
Standard Drug	Streptomycin	-6.9	VAL399 CYS300 ASP354 ALA602	----------	----------

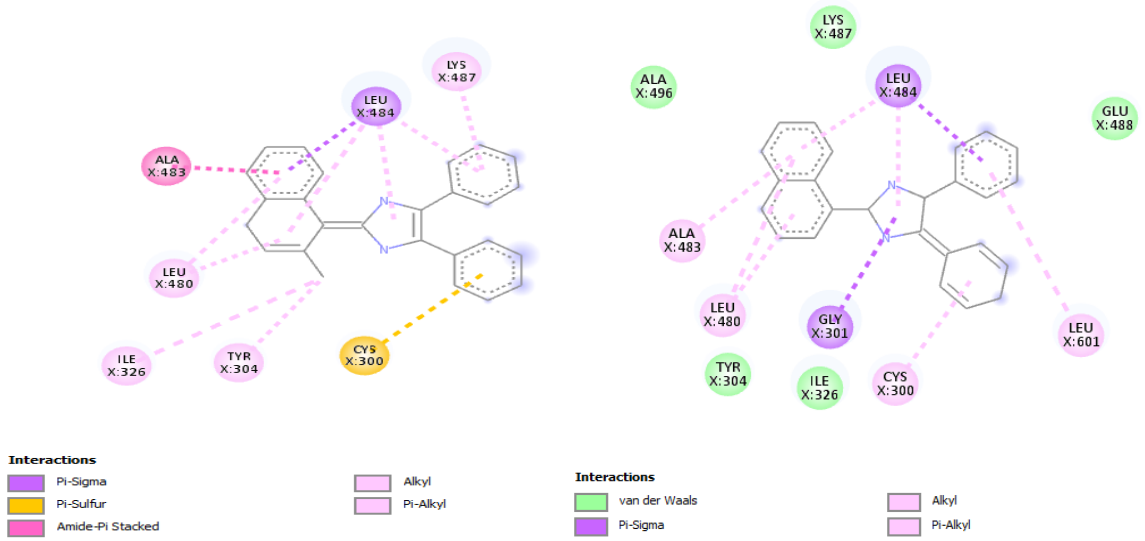


Figure 3.1. 2D Structure of N1 & N2 Compounds

3.2. CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS

Rf Value, Spectral interpretation of IR and ^{1}H-NNMR

The solvent system chloroform : acetone has been used for TLC. The TLC results demonstrated the purity of the produced compounds and evaluated the reactions' progress. The compound's structures were characterized from the IR spectra, which showed the characteristic absorption bands of the functional groups contained in the compound, and H-NMR spectra, which also showed the distinctive peaks of the compound's protons and carbons.

N1: (2-(naphthalen-1-yl)-4, 5-diphenyl-1H-imidazole)

Yellow solid, Yield: 67.7%, M.F: $C_{25}H_{18}N_2$, **Rf value** – 0.72 ; **IR (KBr, ν_{max} cm 1)** ; 3241 (N-H-str), 2783 (C-H-str), 1683 (C=O-str), 1611(C=N-str), 1468 (C=C-str), 1178(C-C-str); **^{1}H NMR (700 MHz, $CDCl_3$)** δ 13.05 (s,1H), 8.08 (d, *J* = 81.8 Hz, 2H), 7.58 – 7.35 (m, 2H), 7.25 (s, 1H), 7.20 (s, 1H).

N2:(2-(2-methylenaphthalen-1-yl)-4,5-diphenyl-1H-imidazole)

Light Yellow solid, Yield: 63.1%, M.F: $C_{26}H_{20}N_2$, **Rf value** – 0.69 ; **IR (KBr, ν_{max} cm 1)** ; 3121 (N-H-str), 2850 (C-H-str), 1665 (C=O-str),1598 (C=N-str), 1561 (C=C-str); **^{1}H NMR (700 MHz, $CDCl_3$)** δ 13.41 (s,1H), 8.08 (d, *J* = 81.8 Hz, 2H), 7.58 – 7.35 (m, 2H), 7.25 (s, 1H), 7.20 (s, 1H), δ 2.64 (s, 3H).

3.3. ANTIMICROBIAL ACTIVITY

Table 3.2 results of antimicrobial activity of synthesized compounds and with comparision of standard drug

Compound	Zone of inhibition (in mm) at 250 µg/ml	
	Bacterial species	
	S. Aureus	*E. coli*
N1	9.33 ± 1.52	9.66 ± 1.52
N2	7 ± 1	7.66 ± 1.52
Streptomycin	17.33 ± 2.08	16.66 ± 1.15

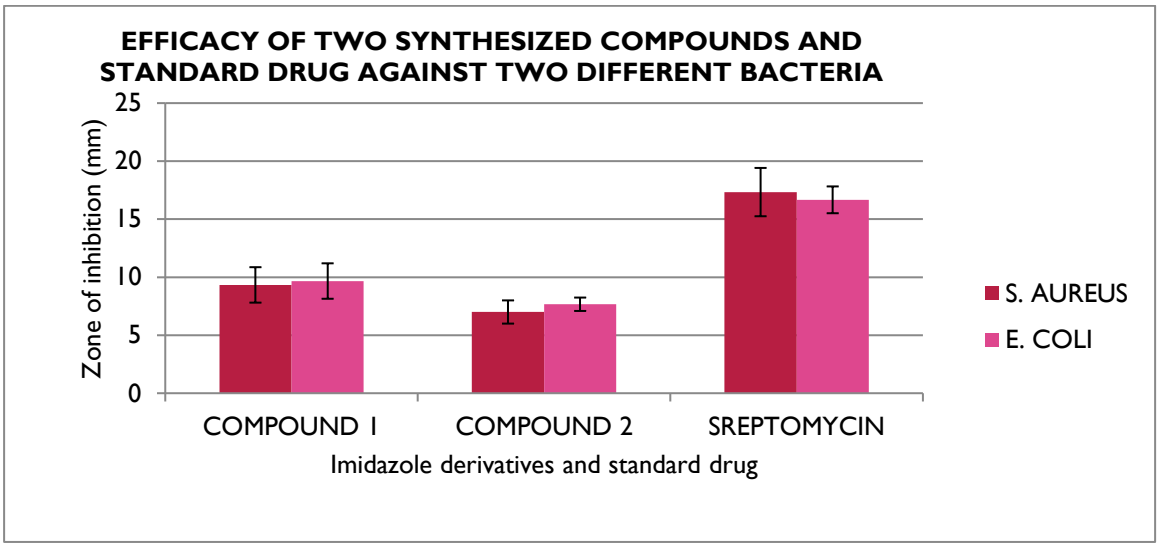


Figure 3.2 Graphical Representation of results of antimicrobial activity tests compound N1 & N2 with standard drug

4. CONCLUSION

In this investigation, molecular docking of newly designed compounds was performed and by using molecular docking results, two compounds were selected for synthesis and evaluation of antimicrobial activity. The two compounds exhibit a good affinity for binding when compared to marketed anti-microbial drug, according to the molecular docking studies. IR and 1H NMR were used to characterize the structures of these two synthesized molecules. The imidazole derivative N1 and N2 were evaluated for antibacterial capacity employing the cup & plate approach. A comparison was made between the zones of inhibition for the test compounds and standard antibacterial medications. The compounds' good activity was comparable to that of the conventional medications. The study showed that the activity of imidazole can be improved by designing new compounds. A certain group's introduction and removal can alter the antimicrobial activity of imidazole derivatives. The study concludes the identification of newer derivatives which can prove to be effective anti-microbial agents.

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