College of Health Sciences

School of Pharmacy

Department of Pharmaceutical Chemistry and Pharmacognosy

Synthesis, *In vitro* Antimicrobial and *In silico* Studies of Betti Base Derivatives of 2-Naphthol

By: Frehiwot Beyene(B.Pharm)

February,2024

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By: Frehiwot Beyene (B. Pharm.)

A Thesis Submitted to the Department of Pharmaceutical Chemistry and Pharmacognosy Presented in Partial fulfilment of the Requirements for the Degree of Master of Sciences in Medicinal Chemistry.

Advisors: Dr. Daniel Bisrat (PhD.)

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Addis Ababa, Ethiopia

Addis Ababa University

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This is to certify that the thesis prepared by Frehiwot Beyene, entitled: "***Synthesis, In vitro Antimicrobial and In silico Studies of Betti Base Derivatives of 2-Naphthol***" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Medicinal Chemistry complies with the regulations of the university and meets the accepted standards concerning originality and quality.

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Prof. Kaleab Asres (PhD.) Signature_____Date_____

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Chair of the Department

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List of Abbreviations

^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	Carbon Thirteen Nuclear Magnetic Resonance
ADMET	Absorption Distribution Metabolism Excretion Toxicity
AIDS	Acquired Immunodeficiency Syndrome
AMR	Antimicrobial Resistance
ATCC	American Type Culture Collection
ATP	Adenosine Triphosphate
BBB	Blood Brain Barrier
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
$CHCl_3$	Chloroform
CYP450	Cytochrome P450
DMSO	Dimethyl sulfoxide
DEPT	Distortion less Enhancement by Polarization Transfer
DNA	Deoxyribonucleic Acid
EAS	Electrophilic Aromatic Substitution
FAS	Fatty acid Synthase
GI	Gastrointestinal
H1N1	Influenza A Virus subtype
hERG	human ether-a-go-go related gene
HIV	Human Immunodeficiency Virus
MDR	multidrug-resistant
MHB	Mueller Hinton Broth
MHz	Megahertz
MIC	Minimum Inhibitory Concentration
NA	Nutrient Agar
NMR	nuclear magnetic resonance
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
PBPs	penicillin-binding protein
PDB	Protein Data Base

PH	Power of Hydrogen
p-gp	p-glycoprotein
PPM	Parts per Million
SBDD	Structure Based Drug Discovery
SDA	Sabouraud Dextrose Agar
SDB	Sabouraud Dextrose Broth
SDF	structure-data format
SMILES	Simplified Molecular-Input Line-Entry System
TLC	Thin layer Chromatography
UNAIDS	United Nations AIDS program
USA	United states of America
UV	Ultraviolet
WHO	World Health Organization
ZOI	Zone of Inhibition

Abstract

Synthesis, *In vitro* Antimicrobial and *In silico* Studies of Betti Base Derivatives of 2-Naphthol.

Frehiwot Beyene
Addis Ababa University, 2024

The development of novel antimicrobial agents is necessary due to the rising prevalence of multidrug resistant pathogens. In medicinal chemistry, 2-naphthol is a crucial starting compound that has attracted significant attention in the development of various biologically active compounds. This study aimed to synthesize novel compounds from 2-naphthol through Betti reaction and asses their antibacterial and anti-fungal activities. Accordingly, 2 compounds, namely, 1-(dimethyl amino) methyl) naphthalen-2-ol and 1-(piperidin-1-ylmethyl) naphthalen-2-ol, were synthesized. These two compounds were assessed for their antibacterial and antifungal activity against different strains by using disc diffusion and broth dilution assay. 1-(piperidin-1-ylmethyl) naphthalen-2-ol exhibited the highest antibacterial activity against *Pseudomonas aeruginosa* MDR1.The compound also demonstrated higher zone of inhibition against *Staphylococcus aureus* MDR 1. Furthermore, 1-(piperidin-1-ylmethyl) naphthalen-2-ol demonstrated antifungal activity against *Candida albicans* and *Aspergillus niger.* Based on preliminary docking analysis and existing literature, FabF (PDB ID: 3HO2) from *E. coli* was chosen for predicting ligand-protein interactions with antibacterial properties. From the two compounds, 1-(piperidin-1-ylmethyl) naphthalen-2-ol had a docking score of -6. 432.The study suggests that the synthesis and evaluation of Betti base derivatives of 2-naphthols is promising in contributing to the development of novel antimicrobial drugs against infectious diseases and combating antimicrobial resistance.

Keywords: 2-naphthol, antimicrobial, antimicrobial resistance, antibacterial, antifungal, Betti base, *in-silico* studies

1.Introduction

1.1. Overview of Infectious Diseases

Infectious diseases include those diseases caused by live parasites (helminths or protozoa), fungi, bacteria, inanimate viruses, prions, or a combination of these (Cole and Kramer, 2016; Sehgal *et al*., 2020). These diseases are substantial burdens on healthcare worldwide. They constitute a considerable amount of global health problems (Snowden, 2008).

The infectious diseases are responsible for around 7.7 million deaths worldwide, each year (Ikuta *et al*., 2022). From diagnosis point of view, over 1.3 million new HIV patients identified worldwide in 2022, where half of the new HIV patients were documented in Africa (UNAIDS, 2023). On the other hand, an estimated number of 10.6 million people contracted tuberculosis (TB) worldwide, and 1.6 million people died from it in 2021(Goletti *et al*., 2023). Because of the serious impacts of infectious diseases on the healthcare, they have been a prime target for the research therapeutics for the past several decades.

Developing countries are bearing most of the health care burdens (Snowden, 2008). For the case of Africa, HIV/AIDS, malaria, TB, and diarrhoea are the leading causes of death (Boutayeb, 2010).Among other diseases, around 2.5 million people were diagnosed with TB (WHO, 2023) in 2022.Narrowing down to Ethiopia, back in 2019, diarrhoea was the leading cause of early mortality, followed by lower respiratory infections, tuberculosis, and HIV/AIDS(Misganaw *et al*., 2022).

1.1.1. Bacterial Infections

Bacteria are single-celled microorganisms found in most vertebrates. Many of them are beneficial. However, they are also the source of major sicknesses (McCutcheon, 2021). Among the infectious diseases, those caused by bacterial infections are the deadliest and they

are the second leading reasons of death, only surpassed by ischemic heart disease (Vos *et al*., 2020).

From the list of bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Klebsiella pneumoniae* are the most common pathogenic in humans (Sharma *et al*., 2023). The most common pathogen in urinary tract infections is *Escherichia coli*, while *Staphylococcus aureus* causes nosocomial sepsis (Phule and Manwar, 2021). On the other hand, the most common pathogens in lower respiratory tract infections are *Haemophilus influenzae* and *Streptococcus pneumoniae*. Furthermore, *S. aureus*, *P. aeruginosa*, *E. coli* are the main nosocomial pathogens (Sikora and Zahra, 2020).

1.1.2. Fungal Infections

Fungal infections can vary from frequent and minor infections to major illnesses that could be fatal (Kumar *et al*., 2019). So far, more than 300 fungus species are known to cause diseases in humans, primarily in immuno-compromised and immune-competent populations, particularly those with underlying co-morbidities and chronic respiratory conditions as well as those living in poverty (Bongomin *et al*., 2017). Most cases of significant fungal diseases are caused by endemic dimorphic fungi such as Mucormycetes, Candida, and Cryptococcus species (Moore *et al*., 2020).

At a global scale, an equivalent number of fungal illnesses and bacterial diseases of occur annually, with various degrees of severity (Alastruey, 2022). For example, fungal pathogens cause at least 13 million illnesses and 1.5 million deaths, mostly in patients with weakened immune systems (Bongomin *et al*., 2017). Particularly, immunosuppressant medical conditions such as asthma, AIDS, cancer, organ transplantation, and corticosteroid therapy can lead to serious fungal infections. Because of the association of fungal infections with

those medical conditions, the global linked death rate to fungal disease is over 1.6 million, which is three times higher than that of malaria and comparable to TB (Denning, 2016).

According to recent worldwide estimates, there are approximately 700,000 cases of invasive candidiasis, over 10,000,000 cases of fungal asthma, and 1,000,000 cases of fungal keratitis annually (Singh *et al*., 2023). Despite these worries and many more critical health conditions causedby fungal infections, the research funding for them is still significantly lower than other pathogens with comparable mortality rate. Aside insufficient attention to this prevailing issue, majority of deaths from fungal disease are preventable (Rodrigues and Chuk, 2020).

1.1.3. Antimicrobial Resistance

The ability of bacteria and other microorganisms to resist the effects of antimicrobial agents is known as antimicrobial resistance (AMR). These resistant microorganisms can survive despite the delivery of antimicrobial medications, which hinders the ability to effectively prevent and cure a variety of infectious diseases (Abushaheen *et al*., 2020; WHO, 2021). As a result, the event of AMR has become one of the key global health challenges of the twenty-first century (Torres *et al*., 2019). It is believed to be responsible for about 700,000 deaths annually (Gajdács and Albericio, 2019) and it is estimated that 10 million individuals may pass away from AMR by the year 2050 (O'Neill, 2016).

The problem of AMR is particularly urgent when it comes to bacterial drug resistance. Over many years, several bacteria species that cause mild or severe illnesses have progressively developed resistance to every new antibiotic introduced to the market. Because of this, robust action must be taken to stop a global health care crisis from developing further (Prestinaci *et al*., 2015). As an example, in 2019, the bacterial AMR was linked to 4.95 million deaths worldwide (Murray *et al*., 2022). In addition to a demand for those actions, the issue is

becoming more urgent due to the unregulated use of antibiotics (Byarugaba 2004; Laxminarayan *et al*., 2013).

Specifically, multidrug-resistant (MDR) bacterial infections are being a rapidly spreading worldwide ailment and a major global health problem (Roca *et al*., 2015). Multidrug-resistant tuberculosis (MDR-TB) affects 500,000 individuals annually and significantly increases morbidity and death (Zumla and Hui, 2019).

Based on data from 2019, Western Sub-Saharan Africa had the largest number of AMR-related deaths. Approximately 142.1 deaths per 100,000 people in the area were either directly or indirectly caused by AMR. Furthermore, the highest rate of AMR-related all-age mortality was observed in Sub-Saharan Africa. Mainly, *Streptococcus pneumoniae* and *Klebsiella pneumoniae* have been linked to the greatest count of AMR-related deaths in this region (Kariuki *et al*., 2022).

1.2. Statement of the Problem

The rise of drug-resistant pathogens threatens the efficacy of current antimicrobial medications, posing a severe risk to global public health, particularly in resource-poor settings (Hajipour *et al.*, 2012). In 2019, 1.27 million deaths globally were attributed to drug-resistant infections, impacting medical treatment, and placing a significant burden on healthcare systems, especially in sub-Saharan Africa, where *S. pneumoniae* and *K. pneumoniae* contribute to high mortality rates (WHO 2021; Murray *et al.*, 2022). In the absence of effective antimicrobial drugs, essential medical procedures such as major surgeries, cancer chemotherapy, organ transplants, and diabetes management poses problem (Roberts *et al.*, 2017).

Additionally, aside from antimicrobial resistance issues, currently available antibiotic drugs pose a broad spectrum of side effects, covering from minor allergic reactions to severe adverse responses (Niu and Li, 2019).

On top of the antimicrobial resistance and drug side effect problems, there is a shortage of newly introduced antibiotics to the market. For example, in 2019, the World Health Organization (WHO) recognized 32 antibiotics in the clinical stage, aimed at priority pathogens, but only six of them were deemed novel (WHO, 2021).

The challenge is even exacerbated for antifungals, as research coverage has not been as extensive as for antibacterial. Unlike the numerous antibiotics for bacterial infections, there has been limited progress in discovering new antifungal agents in recent years. Even approved medications in the past decade belong to the same class as existing agents, without introducing compounds from a different class (Vitiello *et al*., 2023). Consequently, there is an urgent need to address the design and discovery of new antimicrobial agents.

1.3. Significance of the Study

Undoubtedly, antimicrobial resistance has become a global public health concern demanding urgent solutions (Akram *et al.*, 2022). This study is particularly important as it reveals that Betti base derivatives of 2-naphthols are promising classes of compounds demonstrating strong antibacterial activity against multi-drug resistant bacteria such as *S. aureus* MDR.

Since early 20th century, studies have indicated the antimicrobial properties of 2-naphthol and its derivatives (Baichwal et al., 1957). Despite this, their exploration has been limited, and this investigation into the antimicrobial activity of 2-naphthol derivatives aims to uncover new antimicrobial agents effective against a broader spectrum of bacteria and fungi.

This research also adds to the existing body of knowledge by assessing the antimicrobial effectiveness of the synthesized compounds. Furthermore, the *in silico* analysis performed on these compounds lays the groundwork for future studies.

1.4. Objectives

1.4.1. General objective

To synthesize Betti base derivatives of 2-naphthol, evaluate their *in vitro* antimicrobial activity along with conducting *in silico* analysis.

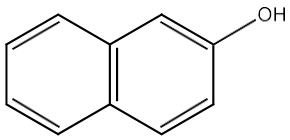
1.4.2. Specific objectives

- To synthesize, purify and characterize Betti base derivatives of 2-naphthol.
- To evaluate *in vitro* antibacterial activity of Betti base derivatives of 2-naphthol on different bacterial strains.
- To evaluate *in vitro* antifungal activity of Betti base derivatives of 2-naphthol on selected fungal species.
- To conduct *In-silico* study of Betti base derivatives of 2-naphthol.

2.Literature Review

2.1. Antimicrobial Activity of 2-naphthol Derivatives

The 2-naphthol, with a hydroxyl group at the 2-position, is phenol's naphthalene homologue. It is more reactive than phenols, like the isomer 1-Naphthol. Other names for 2-Naphthol include β-naphthol, 2-naphthalenol, naphth-2-ol, naphthalen-2-ol, and 2-hydroxynaphthalene. It has molecular formula of $C_{10}H_8O$, molecular weight 144.16, boiling point 295°C, and melting point 123 °C (Booth, 2000).



2-naphthol(**1**)

Figure 1: the structure of 2-naphthol.

Several compounds can be synthesized from the 2-naphthol derivative such as xanthenes, chromenes, oxazines, furans, naphthopyrans, and other heterocyclic compounds in organic synthesis, which has garnered significant interest as a useful precursor (Chaudhary, 2021). This is because three nucleophilic sites are accessible: the C-1 position, the phenolic oxygen, and the (much lesser) C-3 position. For medicinal chemists, 2-naphthol is an intriguing option due to its distinct reactivity, ease of handling, moisture stability, and inexpensive cost (Surya *et al*., 2021). However, it also has its disadvantages like low solubility, sensitivity to air and light.

So far, various medications containing the 2-naphthol have been released into the market for different conditions (Makar *et al*., 2019). One of them is naphyrone (**2**), classified as a norepinephrine-dopamine reuptake inhibitor (NDRI) for depression prevention (Iversen et al., 2013). Additionally, tolnaftate **(3**), a synthetic derivative of thiocarbamate, has been approved

as an antifungal (Walby and Albertson, 1988). Moreover, an imidazole compound nafimidone (**4**), exhibits anticonvulsant properties (Kupferberg and Kapetianovic, 1984). Nabumetone (**5**) falls under the category of Nonsteroidal Anti-inflammatory Drugs (NSAIDs) and is commonly prescribed for the treatment of arthritic pain (Dahl, 1993). Like nabumetone, naproxen (**6**), another NSAID, proves effective in managing gout, rheumatoid arthritis, and menstrual cramps (Brutzkus et al., 2018).

Apart from that, several 2-naphthol derivatives such as 6-Bromo 2-naphthol derivatives(**13)** (Chopde *et al*., 2010), 1-amido alkyl-2-naphthols derivatives (**14**) (Ghorbani and Pourmousavi, 2022), another amido alkyl 2-naphthol derivatives from azo compounds(**15**) (Bananezhad *et al*., 2019), spironaphthopyran derivatives (**16**) (Asadi *et al*., 2015), and pyrimidine-2-hydroxy-4-one Derivatives(**17**) (Devi, 2021) have shown antibacterial and antifungal activities.

In addition, certain 2-naphthol derivatives like 2-aminobenzothiazolomethyl Naphthol derivatives (**18**) were equipotent as ciprofloxacin against E. coli (1.62 µg/mL), *S. aureus*, and *S. typhi* (3.25 *µg/mL), P. aeruginosa* and *B. cereus* (1.25 *µg/mL*) and one compound has shown activity against the Salmonella species, *i.e*., 31.3mm, which is higher than standard 19.1mm zone of inhibition(ZOI) (Sahu *et al*., 2015).

The other synthesized derivatives, the 2,5-disubstituted indole-3-carboxaldehydes (**19**) have shown antibacterial activity against *E. coli*, *S. aureus, K. pneumonia,* and *P. aeruginosa*. Their antifungal activity results revealed that one compound exhibited excellent activity with MIC 08 *µ*g/mL against *A. oryzae, A. niger*, and *A. terreus* and against *A. flavus* with MIC 16 *µ*g/mL (Raghunath and Mathada, 2014).

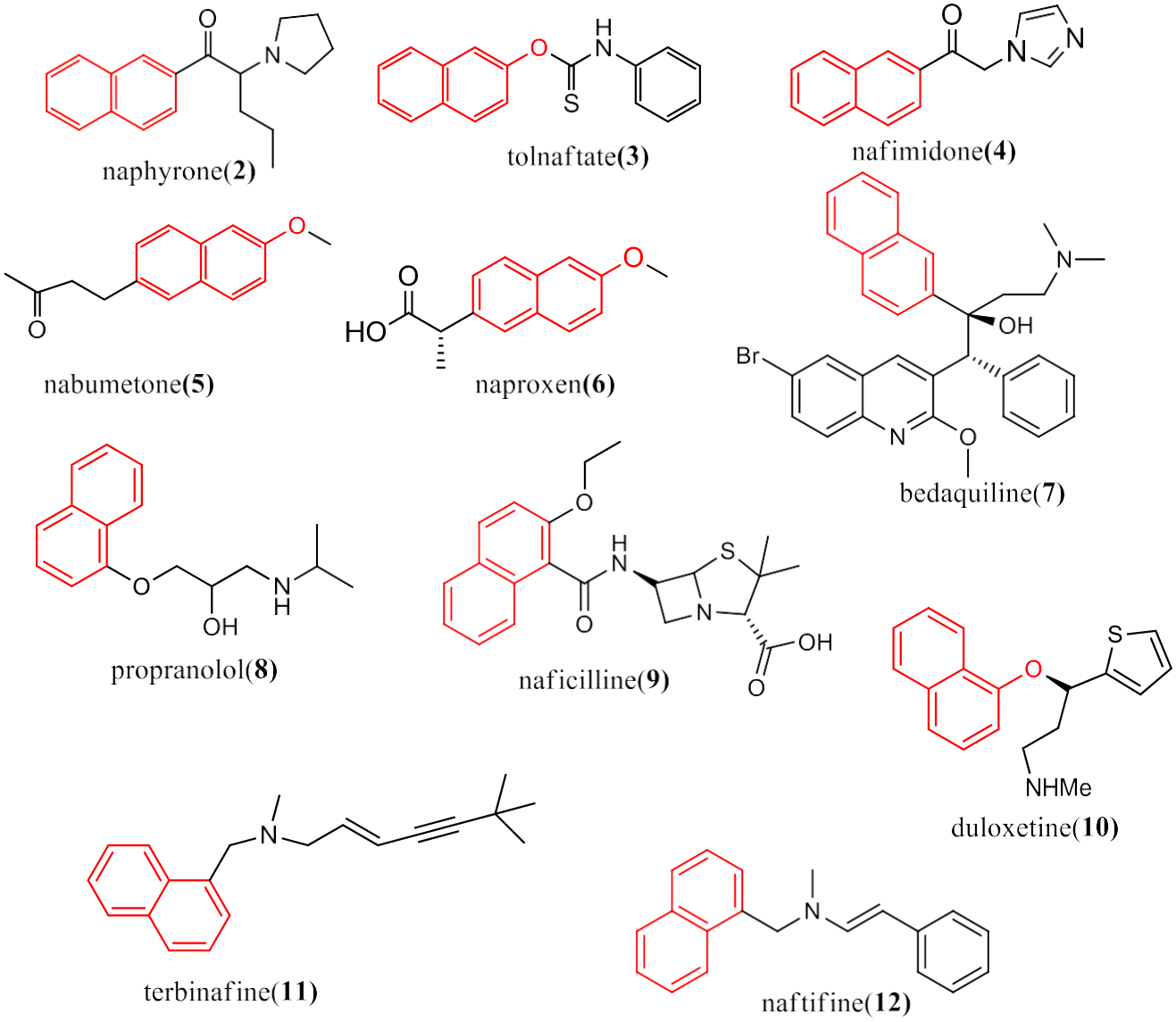


Figure 2: The 2-naphthol containing drugs in the market.

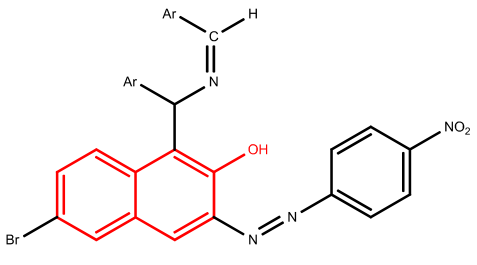


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Figure 3: The 6-bromo 2-naphthol derivatives.

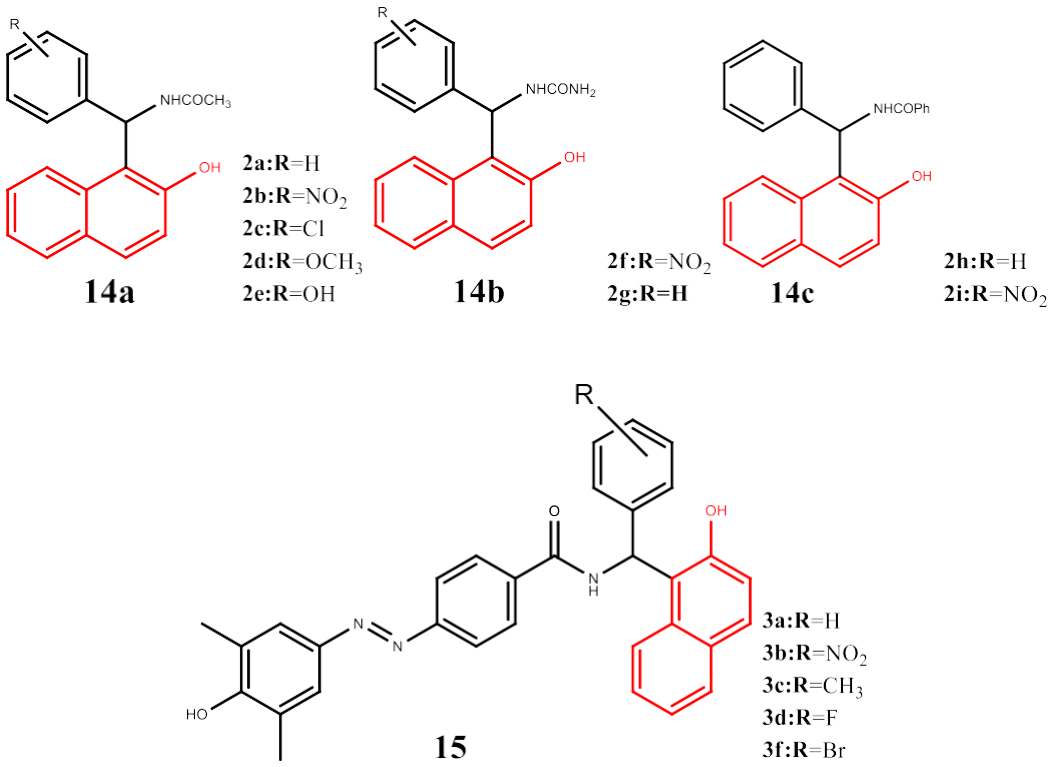


Figure 4: A 1-amidoalkyl 2-naphthol derivatives with antimicrobial activities.

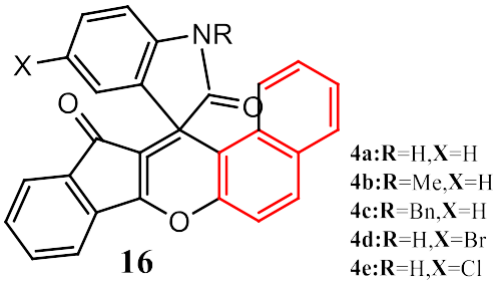


Figure 5: Some spironaphthopyran derivatives with antimicrobial activities.

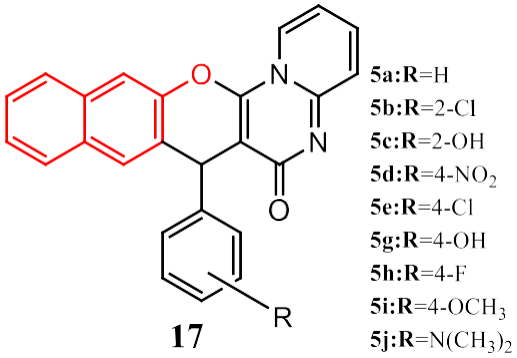


Figure 6: pyrimidine-2-hydroxy-4-one Derivatives.

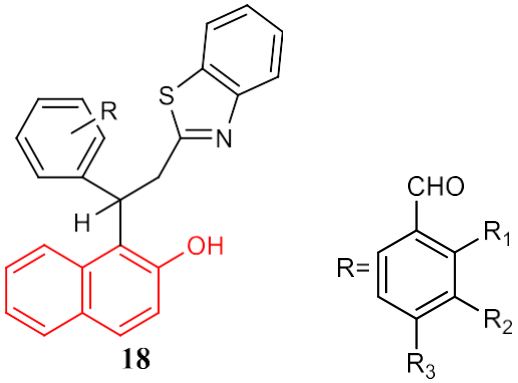
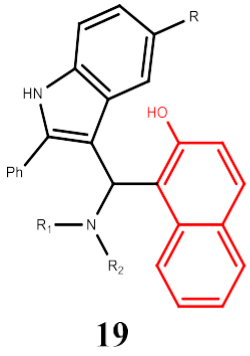


Figure 7: The 2-aminobenzothiazolomethyl Naphthol derivatives.



6a:R_1,R_2=pipieridine, **R**=H
6b:R_1,R_2=piperidine, **R**=Cl
6c:R_1,R_2=piperidine, **R**=CH_3
6d:R_1,R_2=Diethylamine,**R**=H
6f;R_1,R_2=Diethylamine,**R**=CH_3
6g:R_1,R_2=Diethylamine,**R**=Cl
6h:R_1,R_2=Dimethylamine,**R**=H
6i:R_1,R_2=Dimethylamine,**R**=CH_3
6j:R_1,R_2=Dimethylamine,**R**=Cl

Figure 8: The 2,5-disubstituted indole-3-carboxaldehyde.

2.2. Potential mechanism of action of 2-naphthol derivatives

Though the mechanism of action of 2-naphthol and its derivatives is not fully explained, it is hypothesized to damage bacterial cell wall and enhance cell membrane permeability by disrupting the lipopolysaccharide layer. In the market there are two types of cell wall synthesis inhibitors; the beta-lactams and the glycopeptides antibiotics (Sarkar *et al*., 2017).

Penicillins, Cephalosporins, Carbapenems, Monobactams, and beta-lactamase Inhibitors are among the beta-lactams (Cascella and Pandey, 2019) while complestatin, vancomycin, and teicoplanin are categorized under glycopeptide antibiotics. The first groups, the beta-lactams, work by attaching to penicillin-binding proteins (PBPs) and preventing the formation of peptidoglycan., while the glycopeptide antibiotics impede the formation of peptidoglycans by

blocking the production of amino acids. Up to recent years, vancomycin was reserved as a last resort the drug-resistant Gram-positive bacteria (Sarkar *et al*., 2017). yet, due to antimicrobial drug resistance, the scientific community is searching for new class of compounds.

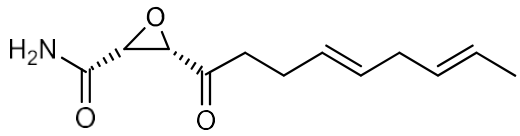
Currently, the fatty acid synthesis process is one of the promising areas in the development of antimicrobial compounds. The lipids found in plants, animals, and microorganisms are largely composed of fatty acids. These lipids are biologically involved in regulating gene expression, intracellular signalling pathways, transcription factor activity, membrane structure and function, and the synthesis of bioactive lipid mediators (Zhang *et al*., 2022). From the process perspective, the rationale for the antimicrobials' selectivity lies in the differences between fatty acid production in humans and bacteria; and it won't quickly develop bacterial resistance (Radka and Rock, 2022).

All living things use highly conserved chemistry to produce fatty acids. For its production, some inputs, mainly the Acetyl-CoA, is used as the starting point for fatty acid biosynthesis. Carboxylation creates the malonyl-CoA building blocks, which are then condensed and reduced repeatedly until the fatty acid chain reaches maturity for cell use. There are two ways to stimulate this cycle. Mammals contain fatty acid synthase I (FAS I), whereas bacteria, plants, and parasites contain FAS II. The ending of the repeated elongation indicates the variation in the production of fatty acids (Beld *et al*., 2015).

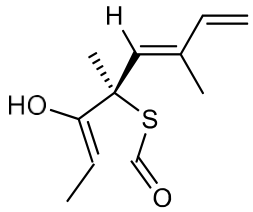
An initiation condensing enzyme, called FabH, and an elongation condensing enzyme, called FabF, are shown to be critical in this biosynthetic pathway and are substantially conserved among important pathogens (Choi *et al*., 2000).

Despite the lack of many pharmaceuticals that specifically target condensing enzymes, two natural products; thiolactomycin (an antibiotic) and cerulenin (an antifungal), are employed

in clinical settings and they specifically inhibit the condensation enzymes FabF and FabH respectively (Kremer *et al*., 2000; Straub *et al*., 2002).



Cerulenin (**20**)

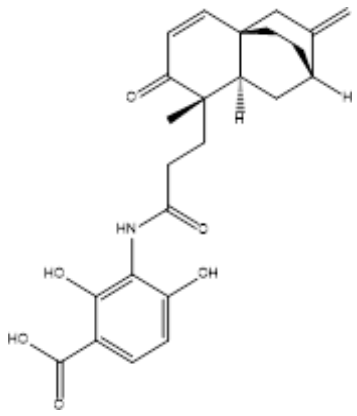


Thiolactomycin (**21**)

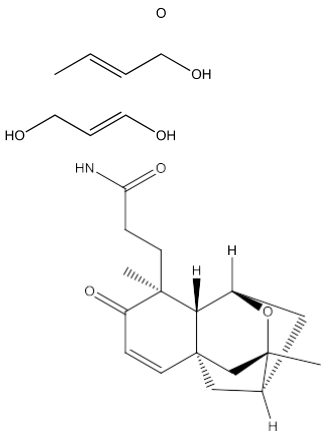
Figure 9: FabH inhibitor cerulenin and FabF inhibitor thiolactomycin.

The other novel compounds from this class of antibiotics are Platencin and Platensimycin. They are secondary metabolites from *Streptomyces platensis* that were discovered by Merck in 2006. Platencin is an inhibitor of both FabH and FabF while the mechanism of action of Platensimycin, is the selective inhibition of FabF. Both bind with the malonyl binding site of the catalytic triad of acyl enzyme intermediate (Wang *et al*., 2006).

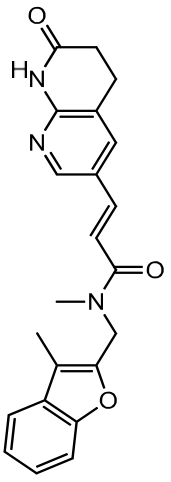
The final stage of the FAS pathway which is performed by the enoyl-acyl carrier protein (ACP) reductase (FabI) enzyme can also be targeted and it is inhibited by AFN-1252 (Karlowsky, *et al.*,2009).



Platencin (**22**)



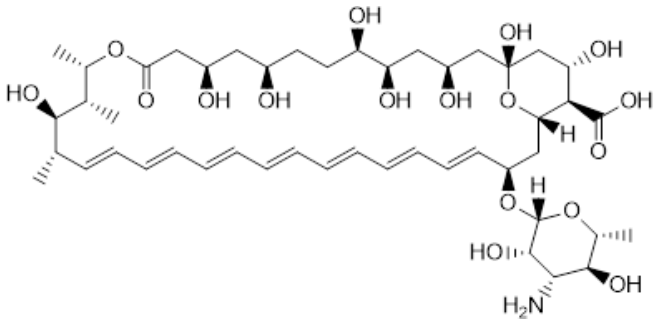
Platensimycin(**23**)



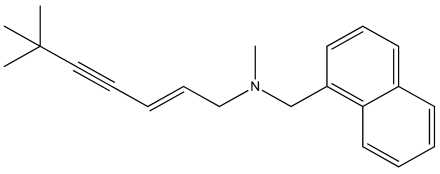
AFN-1252(**24**)

Figure 10: Platencin, Platensimycin, and AFN-1252.

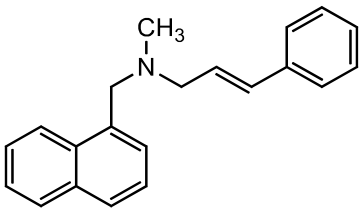
When we come to antifungals, the majority of these drugs target ergosterol synthesis or function, which is a crucial part of the fungal cell membrane (Rodrigues, 2018). For example, the polyene antifungal agent Amphotericin B, binds physically to ergosterol within the membrane, thus creating a polar pore in fungal membranes (Grela et al., 2018). Allylamines, like terbinafine and naftifine, inhibit squalene epoxidation, a reaction catalysed by squalene epoxidase, leading to the accumulation of the sterol precursor squalene and the absence of any other sterol intermediate (Petranyi *et al*., 1984). In contrast, azoles like fluconazole and ketoconazole inhibit 14α-demethylase enzymes which leads to inhibiting the conversion of lanosterol to ergosterol (Elewski, 1993).



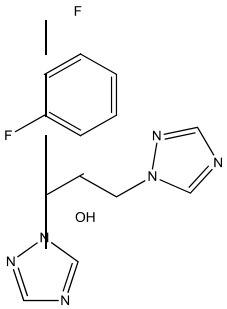
Amphotericin B (**25**)



terbinafine (**26**)



naftifine (**27**)



fluconazole (**28**)

Figure 11: The different ergosterol synthesis inhibitor anti-fungal agents.

3. Materials and Methods

3.1. Materials

3.1.1. Chemicals and reagents

The chemicals and reagents that were used for the synthesis process are: hexane, ethyl acetate, methanol, ethanol (99%), formaldehyde (37%), chloroform (99.8%), dimethyl amine, and piperidine (all from Sigma-Aldrich Co., MO, USA). All the solvents and reagents are of analytical grade.

3.1.2. Instruments

For the analysis and purification of the compounds, analytical silica gel TLC plates (60 F254, 0.2 mm thick, Merck KGaA, Darmstadt, Germany) and column silica gel (60 F254, 70-240 mesh, Merck KGaA, Darmstadt, Germany) were used. In addition, to remove the solvents, Rotary evaporator (BUCHI Rotavapor™ R-300, Switzerland) was used.

For a purpose antimicrobial test, incubator (Modell 100-800 memmert), autoclave, biosafety cabinet (biostarmodell50/60), vortex (Fisher Brand), water bath, inoculating loops, electrical weighing balance, magnetic stirrer, cavate, test tube, pipette tips, pipette filler, micro-pipette (10-200µl), micro-titter plates, capillary tube, and measuring cylinder were used. To visualize the TLC chromatogram plates, UV-visible Spectrophotometer (Evolution 60S) was used. The ^{1}H and ^{13}C-NMR spectra were recorded using a JEOL 400 MHz NMR (Japan), tetramethyl silane (TMS) as internal standard, where chloroform was used as solvent for the NMR spectroscopy.

3.1.3. Media, microbial strains, and standard drugs

For the media preparation, the nutrient agar (NA), Mueller-Hinton broth (MHB), Sabouraud dextrose agar (SDA) and Sabouraud dextrose broth (SDB) were used. In addition, Ciprofloxacin was used as a positive control for antibacterial test while 1% Dimethyl

sulfoxide (DMSO) was used as a negative control. For the case of antifungal test, Griseofulvin was used as a reference standard.

In vitro antibacterial assays were performed on the following Gram-positive bacterial strains: *Bacillus pumilus* 82, *B. subtilis* ATCC 6633, *Staphylococcus aureus* ML 267, *S.aureus* MDR 1,and*S.aureus* MDR 2. The Gram-negative bacterial strains used were: *E. coli* 3:37C, *E. coli* 7360, *E. coli* 872, *E. coli* CD/99/1, *E. coli* K 88, *E. coli* T 37, *E. coli* ROW 7/12, *E. coli* 5933,*E.coli* HB101, *E.coli* C600, *Salmonella enterica* TD 01, *Salmonella typhi* Ty2, *Shigella boydii* D13629, *Salmonella dysentery* 8, *Shigella flexneri* Type 6, *Shigella sonnei*1, *Vibrio cholerae* NCTC 5596, *V. cholerae* NCTC 10732, *Vibrio cholerae* NCTC 11501, *Vibrio cholerae* NCTC 4693, and *P. aeruginosa* MDR 1. All the bacterial strains were procured from the Department of Technology, Jadavpur University, Central Drugs Laboratory, Kolkata and Institute of Microbial Technology, Chandigarh, India. Before sensitivity tests were performed on the test samples, the purity of the strains was checked in accordance with the standard microbiological, cultural, and biochemical tests.

On the other hand, the antifungal activity testing was performed on: *A. niger* ATCC 6275, *C. albicans* ATCC 10231, *Penicillium funiclosum* NCTC 287, and *P. notatum* ATCC 11625. All the fungal species were provided from Central Drugs Laboratory, Kolkata Institute of Microbial Technology, Chandigarh, India.

3.2. Methods

3.2.1. Synthesis of 2-naphthol derivatives (29 and 30)

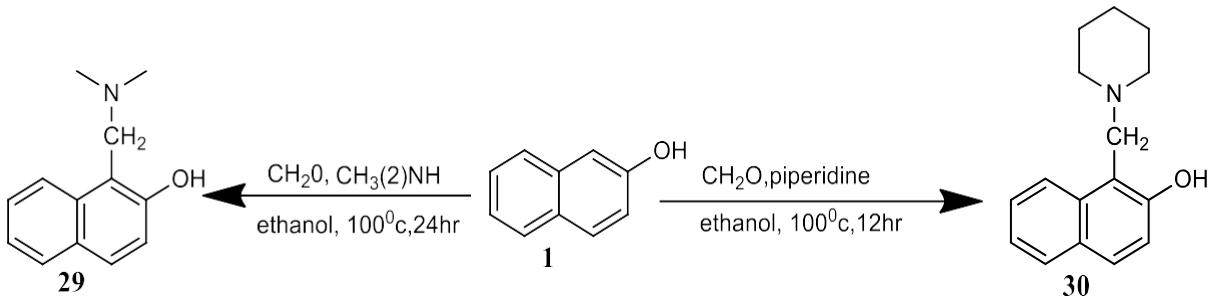
The synthesis of two Betti base derivatives of 2-naphthol involved refluxing 2-naphthol, formaldehyde, and amines in the presence of an acid catalyst, following the procedure outlined in Scheme 1 (Marinescu *et al*., 2020).

3.2.1.1-(Dimethyl amino)methyl) naphthalen-2-ol (29)

Compound **29** was synthesized through a Betti base reaction. A solution containing 1 ml (0.011 mol) of 37% aqueous formaldehyde in 10 ml of ethanol, with five drops of acetic acid as a catalyst, was prepared. Dimethylamine (0.5 ml, 0.011 mol) was added dropwise to formaldehyde-acetic acid solution form an iminium ion, which was then added dropwise to a solution of 2-naphthol (1.5 g, 0.011 mol) in ethanol. The mixture was stirred and refluxed for 24 hrs at 100 ºC. The reaction progress was monitored by TLC. After completion, the solvent was removed using a Rota evaporator, and the reaction mixture was purified by silica gel column chromatography with an increasing gradient of chloroform in methanol as the eluting solvent.

3.2.1.2-(piperidin-1-ylmethyl) naphthalen-2-ol (30)

Compound **30** was synthesized in a manner like the synthesis described for compound **29** via the Betti base reaction. A solution was prepared in a 50 ml beaker, consisting of 0.5 ml (0.011 mol) of 37% aqueous formaldehyde in 10 ml of ethanol, with five drops of acetic acid as a catalyst. 1 ml (0.011 mol) of piperidine was added dropwise to the formaldehyde and acetic acid solution. This mixture was then added dropwise to a solution of 1.5 g (0.011 mol) of 2-naphthol in ethanol. The mixture was refluxed with stirring in a 250 ml Erlenmeyer flask for 12 hrs at 100ºC. The reaction progress was monitored using analytical TLC with a hexane/Ethyl acetate (3:2) solvent system. After completion, solvent removal was done using a Rotary Evaporator, and the reaction mixture was purified by silica gel column chromatography with an increasing ethyl acetate gradient in petroleum ether as the eluting solvent.



Scheme 1: Synthesis of 2-naphthol derivatives (**29** and **30**)

3.2.2. Structural elucidations of the synthesized compounds

The structures of compounds **29** and **30** were identified by analysing their ^{1}H and ^{13}C-NMR spectra data. Chemical shifts are denoted in *ppm* and coupling constants (*J*) are expressed in *Hz*. Signal multiplicities are indicated as follows: *s* = singlet; *d* = doublet; *t* = triplet; *ddd* = doublet of doublet of doublets; and *m* = multiplet.

3.2.3. *In vitro* antibacterial and antifungal activity assay

3.2.3.1. Disk diffusion method

By comparing the zones of inhibition produced by the test samples with those of ciprofloxacin, the disc diffusion method was used to screen the *in vitro* antibacterial assay (Mitchell and Carter, 2000). To investigate the synthesized compounds' antibacterial and antifungal activities, a stock solution containing 1 mg/ml of the 2-naphthol derivatives was made in 1% DMSO. By diluting the stock solution in the appropriate quantities of water, concentrations ranging from 5 to 800 µg/mL for antibacterial activity tests and 50 to 2000 µg/mL for antifungal activity tests were used. After that, sterile Petri dishes were filled with molten media to create serial nutrient agar plates, which were then incubated for 24 hours at 37 °C to check for contamination. After creating serial nutrients, the inoculums were evenly swabbed and given five minutes to dry. Next, the test samples were impregnated onto 6 mm filter paper discs (Whatman no. 1), which were then placed on the medium's surface. After that, the Petri dishes were incubated for 24 hours at a temperature of 37 °C, and the diameter

of the inhibitory zone was measured in millimetres. The procedure was repeated for Ciprofloxacin and ZOI was compared appropriately. A negative control of 1% DMSO was used for the experiment (Shihabudeen *et al*., 2010; Pellizzoni *et al*., 2012)

Using similar technique, the test samples' antifungal potential was assessed against the fungal pathogens on Saborauds dextrose media. After three days of incubation at a room temperature, the diameter of the ZOI in the Petri dishes was determined in millimetres. Here, the Griseofulvin served as the standard for comparison.

3.2.3.2. Broth dilution method

By using this broth dilution method, the minimum inhibitory concentration (MIC) of the synthesized compounds was ascertained, following the instructions provided by Jean B. et al. (2015). For bacterial and fungal growth, the Mueller–Hinton broth and Saborauds dextrose broth were utilized respectively. Concentrations of 5, 10, 25, 50, 100, 200, 400 and 800 μg/mL for antibacterial activity test and 50, 100, 200,400, 800, 1000, 1500 and 2000 μg/mL for antifungal activity test of the synthesized compounds dissolved in 1% DMSO were used. Additionally, a sterility control (growth control with nutritional broth and DMSO, free of antimicrobials) was performed. Every test and growth control well were incubated for three days at temperature of 25 °C for fungi and for 24 hours at temperature of 37 °C for bacteria. Absence of turbidity in the test tubes marked the MIC (Pandey *et al*., 2010; Pellizzoni *et al*., 2012)

3.2.4. *In silico* studies

3.2.4.1. Molecular docking study

Using Maestro V.13.5 software by Schrodinger 2023-1 Suite, molecular docking of the synthesized compounds was carried out on the FabF-platencin co-crystallized enzyme of *E. coli* (PDB ID: 3HO2) as potential target for antibacterial activity. The molecular docking

process comprised four steps: protein preparation, ligand preparation, grid receptor generation, and molecular docking, utilizing default values.

3.2.4.1.1. Protein preparation

The protein structure of E. coli FabF (PDB ID: 3HO2) chain A; with a resolution 2.00 Å complexed with platencin was downloaded from the protein data bank. Using Schrödinger 2023-1 Protein Preparation Workflow module, the 3D structure of protein in complex with platencin (PDB ID: 3HO2, chain A) was prepared by adding hydrogens, correcting errors such as absent side chains, allocating appropriate bond ordering correcting charges, bond orders, and atom types, removing water molecules, and filling in missing side chains and loops. The Optimized Potentials for Liquid Simulations 4(OPLS4) force field was applied to optimize and remove steric hindrance (Madhavi Sastry *et al*., 2013).

3.2.4.1.2. Ligand preparation

After the preparation of the protein, the compounds drawn in ChemDraw 22.0.0SD format were imported and prepared using the Ligprep module. the Ligprep module performed hydrogen addition, 2D to 3D conversion, ionization, and tautomeric state generation (via Epik) at the physiological pH 7.0± 2.0, and also produced ring configurations via the standard settings.

3.2.4.1.3. Receptor grid generation

A receptor grid generating panel was then performed in order to form a grid box surrounding the co-crystallized ligand with the receptor protein to allow docking into the active region. Using the advanced setup option of the site, the protein structure was selected on the workspace to define the ligand-binding site, which has a radius of 6.0 Å. The van der Waals radii of the receptor atoms was set with partial atomic charge scaling factor of 0.8 and partial cut-off of 0.15 to soften the receptor’s nonpolar parts.

3.2.4.1.4. Ligand docking

The docking was conducted on the synthesized compounds under extra precision (XP) mode. Redocking method was employed to validate the molecular docking protocol, involving the docking of the co-crystal native ligand platencin back into 3HO2.

3.2.4.2. Pharmacokinetics and drug-likeness properties

The physicochemical, pharmacokinetic, and toxicity profiles of the synthesized compounds were predicted using the online software tools provided by ADMET lab 2.0, as outlined by Xiong *et al*. in 2021. To facilitate this analysis, the compounds' structures were transformed into SDF format using ChemDraw 22.0.0 software. The resulting SDF format for each compound was then submitted as input to the web server.

4. Results and Discussion

4.1. Synthesis

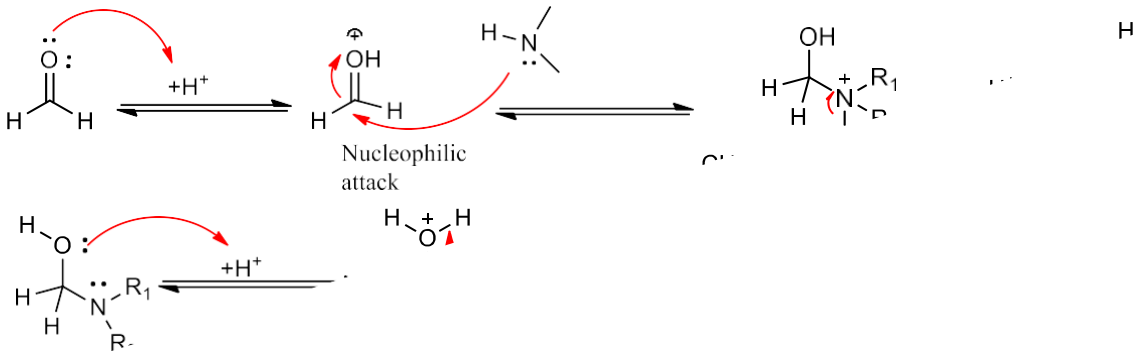
In this study, we synthesized two Betti base derivatives of 2-naphthol. This synthesis was driven by the diverse biological activities associated with 2-naphthol derivatives, particularly their antimicrobial properties, as highlighted in previous studies by Bedair *et al*. (2001) and Kaur *et al*. (2019).

Table 1: Physical properties of the synthesised compounds **29** and **30**

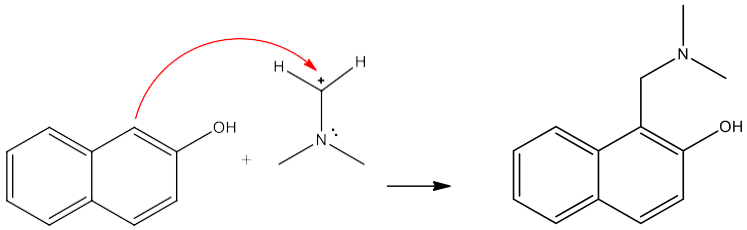
Compound	Molecular Formula	Predicted MW(g/mol)	Physical State	Color	% Yield	R_f value
29	$C_{13}H_{15}NO$	201.26	Crystal	Colorless	24	0.8
30	$C_{16}H_{19}NO$	241.33	Crystal	Colorless	66	0.3

4.1.1. Synthesis of ((dimethyl amino) methyl) naphthalen-2-ol (29)

Compound **29** was synthesized through a one-pot Betti base reaction, constituting a three-component process. This synthesis involved the condensation of formaldehyde, dimethylamine, and 2-naphthol in the presence of an acid catalyst. The reaction follows a sequence of steps. Initially, acetic acid enhances the electrophilicity of the carbonyl carbon in formaldehyde. Following this, dimethylamine engages in a nucleophilic addition reaction with the carbonyl group of formaldehyde, causing water loss and forming an iminium ion intermediate. Subsequently, the iminium ion undergoes in electrophilic aromatic substitution with 2-naphthol, resulting in the ultimate formation of compound **29**. The reaction mechanism is depicted in Scheme 2 and 3.



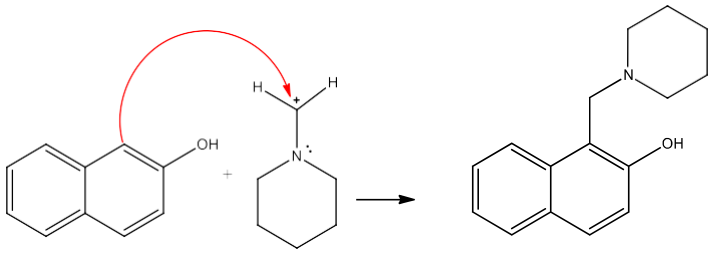
Scheme 2: Iminium formation mechanism of the Betti base derivatives.



Scheme 3: Electrophilic aromatic substitution of compound **29**.

4.1.2. Synthesis of 1-(piperidin-1ylmethyl) naphthalen-2-ol (30)

Following the same synthesis approach outlined for compound **29**, compound **30** was synthesized using the Betti base synthesis procedure, but with the substitution of dimethylamine by piperidine. This substitution led to the formation of compound **30**. The reaction mechanism for compound **30** closely resembles that of compound **29**, and it is illustrated in Scheme 2 and 4.



Scheme 4: Electrophilic aromatic substitution of compound **30**.

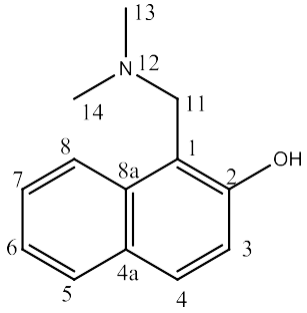
4.2. Structural elucidation of compounds 29 and 30

Compounds **29** and **30** were characterized as ((dimethyl amino) methyl) naphthalen-2-ol and 1-(piperidin-1ylmethyl) naphthalen-2-ol, respectively, through the analysis of both ^{1}H and ^{13}C-NMR spectral data.

4.2.1. Structural elucidation of ((dimethyl amino) methyl) naphthalen-2-ol (29)

Compound **29** was successfully synthesised as a colourless crystal (2.4mmol., 24%w/w, Mwt 201.26) with an Rf value of 0.8 on TLC using $CHCl_3$/MeOH (8:1) as the mobile phase (Appendix 2).

Table 2: ^{1}H and ^{13}C-NMR spectral data of compound **29**

Position	Compound **29** 	
	δ_H (ppm)	**δ_C (ppm)**
1		111.48
2		156.90
3	7.12(1H, *d*, J=8.9 Hz)	119.36
4	7.69(1H, *d*, J=8.9Hz)	128.99
5	7.76(1H, *d*, J=8.1Hz)	128.52
6	7.44(1H, *ddd*, J=8.4, 6.8, 1.4 Hz)	122.47
7	7.29(1H, *ddd*, J=8.0, 6.8, 1.1 Hz)	126.40
8	7.81(1H, *d*, J=7.7Hz)	121.04
8a	-	129.31
4a	-	132.64
11	4.10(2H, *s*)	57.94
13,14	2.42(6H, *s*)	44.78

The ^{1}H-NMR spectrum of compound **29** exhibited the presence of six aromatic protons, specifically assigned to H-3 (δ 7.12, 1H, *d, J*=8.9 Hz), H-4 (δ 7.69, 1H, *d, J*=8.9 Hz), H-5 (δ 7.76, 1H, *m*), H-6 (δ 7.44, 1H, *ddd*, J=8.4, 6.8, 1.4 Hz), H-7 (δ 7.29, 1H, *ddd*, J=8.0, 6.8, 1.1

Hz) and H-8 (δ 7.81, 1H, *d*, J=7.7 Hz). This confirmed the replacement of one aromatic proton from 2-naphthol with an alkyl group. Additionally, the presence of a methylene group attached to the nitrogen group was indicated by a signal at δ 4.10 (2H, *s*), suggesting the formation of an amino alkyl group in compound **29(**Appendix 1).

The ^{13}C-NMR, along with the DEPT-135 spectrum demonstrated the presence of a total of 13 carbon atoms, including two identical methyl (2xCH_3), six methines (6xCH), one methylene (CH_2), and four quaternary carbons in compound **29**. Compound **29** was identified as 1-((dimethyl amino) methyl) naphthalen-2-ol based on the ^{1}H and ^{13}C-NMR spectral data. The chemical shifts of all other protons and carbons in compound **29** are detailed in Table 2.

4.2.2. Structural elucidation of 1-(piperidin-1ylmethyl) naphthalen-2-ol (30)

Compound **30** was obtained as a colourless crystal (4.13mmol, 66%; w/w, Mwt 241.33) with an Rf value of 0.3 on TLC hexane/Ethyl acetate (3:2) as a mobile phase. (Appendix 2)

Analysis of the ^{1}H-NMR spectrum of compound **30** revealed the presence of six aromatic protons. This observation indicated the substitution of one aromatic proton in 2-naphthol with an alkyl group, confirming the reaction occurrence at the 2-naphthol ring. A singlet at δ 3.99, integrating for two protons, was identified, corresponding to a CH_2 group at the 7' position, supporting the formation of an amino alkyl group. The protons at 2’ and 6’ are equivalent and appeared as multiplet at δ of 2. 66ppm.The equivalent protons at 3’ and 5’ showed a multiplet signal at δ of 1.58. The 4’ protons resonated upfield with δ of 1.47ppm (Appendix 1)

Table 3: ^{1}H and ^{13}C-NMR spectral data of compound **30**

Position	Compound **30**	
	δ_H (ppm)	**δ_C (ppm)**
1		111.48
2		156.90
3	6.79(1H, *d*,J=8.8 Hz)	119.36
4	7.79(1H, n.r.)	128.99
5	7.85(1H, *m*)	128.52
6	7.48(1H, *ddd*,J=8.1Hz,7.2,1.5)	122.47
7	7.42(1H, *ddd*,J=7.2,1. 3Hz)	126.40
8	7.79(1H, n.r.)	121.04
8a		129.31
4a		132.64
2', 6'	2.66(4H, *m*)	54.28
3', 5'	1.58(4H, *m*)	25.95
4'	1.47(2H, *m*)	24.07
7'	3.99(2H, *s*)	57.37

n. r.= not resolved

The ^{13}C-NMR and DEPT-135 spectral data of compound **30** demonstrated the existence of a total of 16 carbon atoms, including four quaternary carbons, six methines (CH), and four methylene groups (CH_2). Therefore, both ^{1}H-NMR and ^{13}C-NMR analyses provided confirmation of the structure of compound **30**. Detailed chemical shifts for all other protons and carbons in compound **30** are presented in Table 3.

4.3. Antimicrobial activity of compound 29 and 30

4.3.1. Antibacterial activity

In this study, the synthesized compounds **29** and **30** were tested against 26 bacterial strains and both compounds have comparable antibacterial activity with the range of MIC from 10-400 µg/ml. The best performance was observed by compound **30** against *Pseudomonas aeruginosa* MDR 1, an opportunistic gram-negative bacterium, at 10 µg/ml. This result is

particularly significant, since this bacterium is a major cause of nosocomial infections and the drug resistant strains of *P. aeruginosa* are becoming difficult to treat (Al-Orphaly *et al.,* 2021).

Both of synthesized compounds have shown equal and favourable MIC value for all gram-negative *E. coli* strains (25 µg/ml). Worth to note that the is *E. coli* is the most common gram-negative bacterium that causes extraintestinal diseases in humans. It causes meningitis, pneumonia, bacteremia, urinary tract infections, pelvic and abdominal infections, to mention some (Santos *et al.*, 2020).

The least activity of both synthesized compounds was for *Bacillus pumilus* (400 µg/ml). Similarly, compound **29** has shown lower activity for *Bacillus subtilis* ATCC 6633, *S.aureus* MDR 1, and *S.aureus* MDR 2. It is to be noted that the least activity is observed on the gram-positive bacteria. From the gram-positive bacteria, compound **30** has shown higher activity for *S.aureus* MDR 1 and *S.aureus* MDR 2 (100 µg/ml). This *Staphylococcus aureus* is a commensal as well as an opportunistic bacterium and itis a major contributor to a variety of infections from mild skin infections to serious illnesses (Wang *et al*., 2017). Interestingly, compound **30** has shown a higher ZOI than ciprofloxacin against the *S. aureus* MDR 1, *i.e.*, 13.5 mm. Besides that, compound **29** showed a closer ZOI for *E.coli* LT37 and *E.coli* C600 to ciprofloxacin while compound **30** showed closer ZOI for *E.coli* HB101, *S. aureus* MDR 2, and *Pseudomonas aeruginosa* MDR 1.

Table 4: Zone of inhibition and minimum inhibitory concentration of the synthesized compounds against the tested bacterial strains

Bacteria	Zone of inhibition in mm (200 µg/ml)			MIC (µg/ml)	
	Compound **29**	Compound **30**	Ciprofloxacin	Compound **29**	Compound **30**
E.coli NCTC 5933	14.0	14.0	16.0	25	25
E.coli K88	14.0	14.5	17.0	25	25
E.coli NCTC 7360	14.5	14.5	17.0	25	25
E.coli LT37	15.0	13.5	16.0	25	25
E.coli 872	14.5	14.0	16.0	25	25
E.coli ROW 7/12	13.0	14.5	16.5	25	25
E.coli 3:37C	13.5	14.0	16.5	25	25
E.coli CD/99/1	15.0	15.0	17.0	25	25
E.coli HB101	12.0	13.5	14.0	25	25
E.coli C600	12.5	11.5	13.5	25	25
Salmonella typhi Ty2	14.0	13.0	16.0	100	100
Salmonella enterica TD 01	14.5	13.5	19.0	100	100
Shigella dysentery 8	14.0	13.5	20.0	50	100
Shigella soneii 1	14.0	13.0	19.5	50	100
Shigella boydii D13629	12.5	14.0	20.0	50	100
Shigella flexneri Type 6	12.5	14.0	20.5	50	100
Staphylococcus aureus ML 267	15.0	16.0	18.0	50	50
*S.aureus*MDR 1	10.0	13.5	11.5	400	100
S.aureus MDR 2	10.0	11.0	12.0	400	100
Bacillus pumilus 82	7.5	8.0	19.0	400	400
Bacillus subtilis ATCC 6633	7.5	8.0	18.0	400	200
Vibrio cholerae NCTC 4693	12.0	15.0	17.5	50	50
Vibrio cholerae NCTC5596	12.5	14.0	18.5	50	50
Vibrio cholerae NCTC 10732	13.0	14.5	19.0	50	50
Vibrio cholerae NCTC 11501	12.0	14.0	18.5	50	50
Pseudomonas aeruginosa MDR 1	11.0	12.0	12.5	100	10

4.3.2. Antifungal Activity

The 2-naphthol derivatives were tested on four fungal species and they showed moderate activity against the species. Despite this, compound **29** has shown a greater ZOI than Griseofulvin for *Penicillium notatum*(13.5mm) and *P. funiculusum* (13mm). The MIC value of the synthesized compounds range from 400-1500µg/ml. For compound **29**, the highest activity is observed on *P. funiculusum* NCTC 287 with MIC value of 400µg/ml. In contrast, the highest activity of compound **30,** was observed on *Candida albicans* ATCC 1023 and *A. niger* ATCC 6275 (400 µg/ml).

Table 5: Zone of inhibition and minimum inhibitory concentration of compound **29** and **30** against the tested fungal species.

Fungi	Zone of inhibition in mm(200µg/ml)			MIC(µg/ml)	
	Compound **29**	Compound **30**	Griseofulvin	Compound **29**	Compound **30**
Candida albicans ATCC 10231	11.0	12.0	15.0	1500	400
Aspergillus niger ATCC 6275	12.5	13.5	14.5	800	400
Penicillium notatum ATCC 11625	13.5	10.5	12.0	800	1000
Penicillium funiculosum NCTC 287	13.0	10.0	11.0	400	1000

4.4. *In silico* Studies

4.4.1. Molecular docking

Following preliminary docking and literature review, the *E. coli* FabF protein (PDB ID: 3HO2) (Singh *et al*., 2009) was selected to assess the binding affinity of synthesized compounds within the enzyme's active site.

The synthesized compounds in this study exhibited satisfactory binding to the *E. coli* FabF enzyme, as indicated in Table 6. Specifically, compound **29** demonstrated a docking score of -6.432 kcal/mol within the active site of the *E. coli* FabF enzyme, forming two hydrogen bonds with HIE340 and PHE398 via its hydroxyl group, and a pi-cation bond with PHE400 via its amine group. On the other hand, compound **30** exhibited lower affinity binding (-5.248 kcal/mol), forming a single hydrogen bond with THR305 via its hydroxyl group and engaging in pi-pi stacking with HIE305 through its naphthalene ring.

Table 6: Docking scores of the 2-naphthol derivatives with 3HO2 according to Schrodinger 2023 suite docking software.

Ligand	**Docking score (kcal/mol) within 3HO2**	**Interaction with amino acid residues**	
		H-bonds	**Non-H-bonds**
29	**-6.432**	HIE340 and PHE398	PHE400
30	**-5.248**	THR305	HIE305
Platencin	**-7.650**	THR240, THR307 and HIE340	PHE400

The docking protocol underwent validation using the native ligands platencin for antibacterial target. When platencin was redocked into the active site of the E. coli FabF enzyme, it yielded a docking score of -7.650 kcal/mol, demonstrating a relatively comparable affinity to compound **30**(-6.432 kcal/mol).

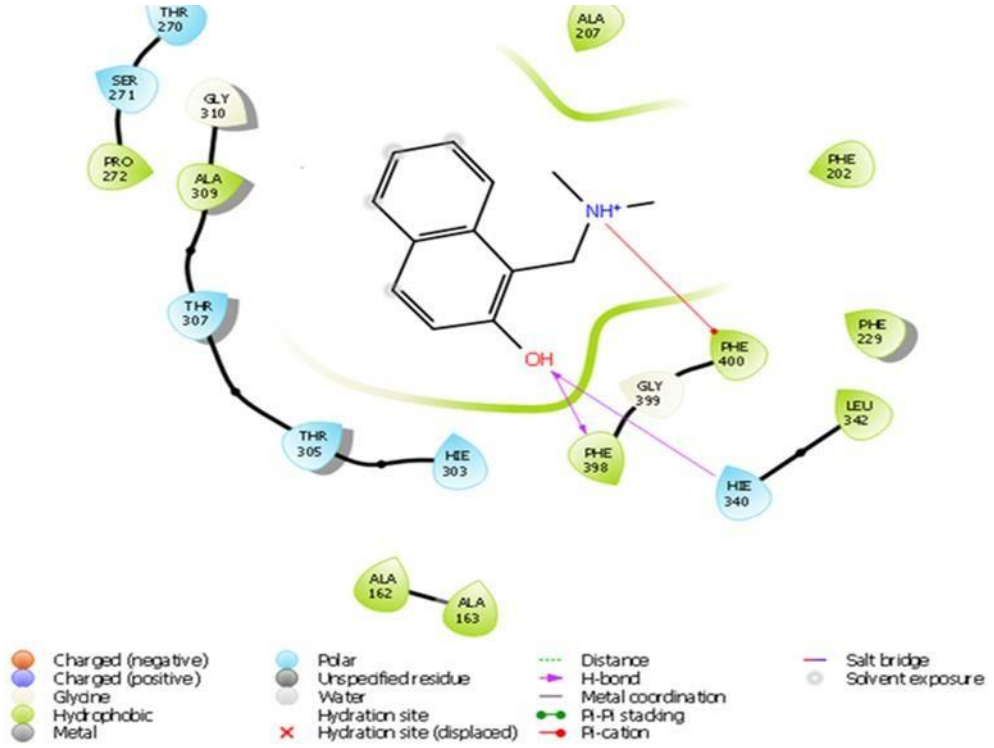


Figure 12:2D interaction between compound **29** and E. coli FabF (PDB ID: 3HO2)

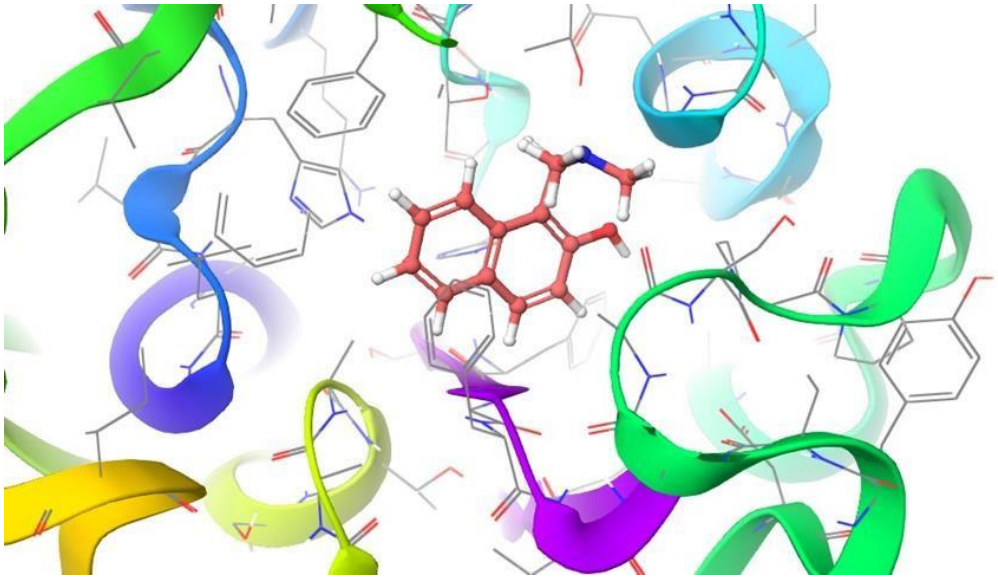


Figure 13: 3D interaction between compound **29** and E. coli FabF (PDB ID: 3HO2)

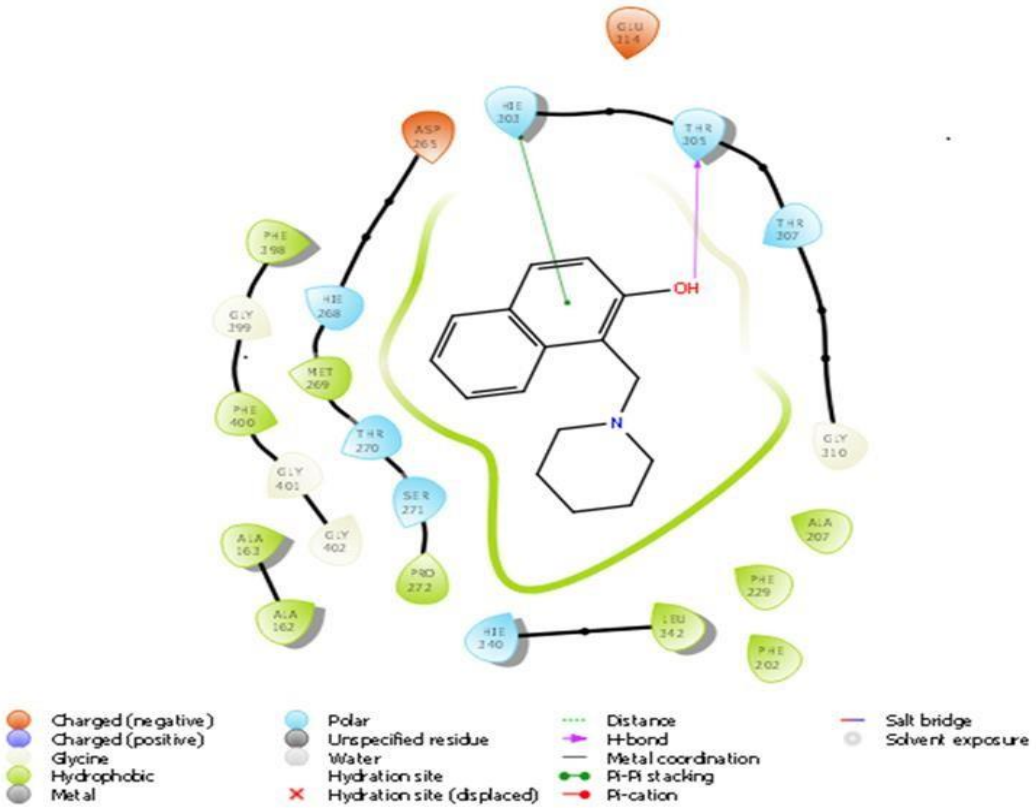


Figure 14: 2D interaction between compound **30** and E. coli FabF (PDB ID: 3HO2)

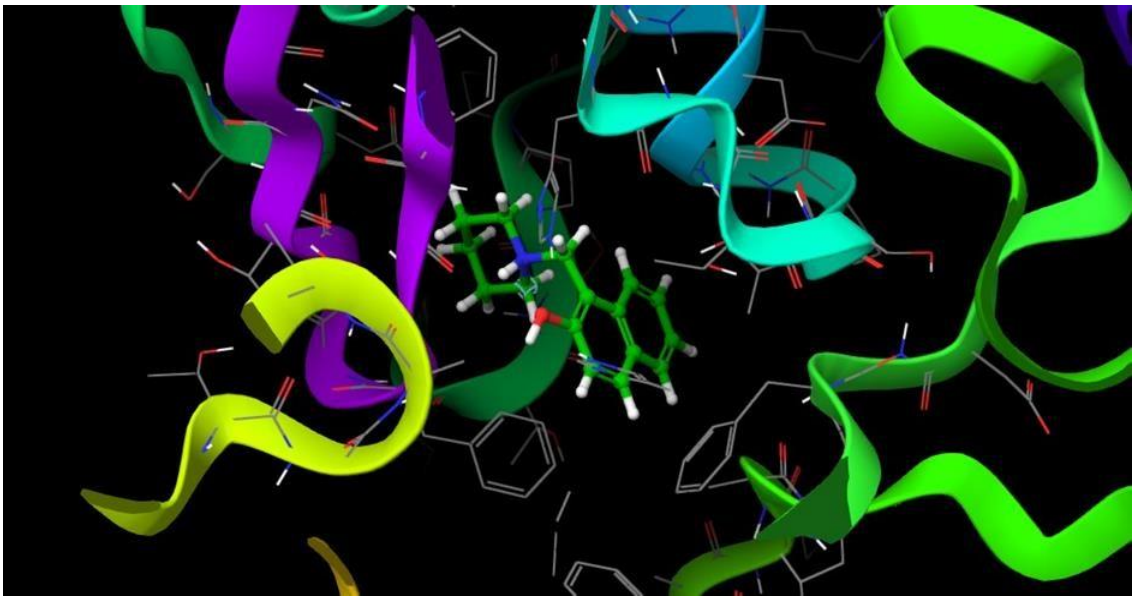


Figure 15: 3D interaction between compound **30** and E. coli FabF (PDB ID: 3HO2)

4.4.2. Pharmacokinetics and drug-likeness properties

In drug design and optimization, *in silico* ADMET prediction models have been utilized to aid medicinal chemists in guiding the design and optimization of lead compounds, as well as evaluating crucial pharmacokinetic and physical-chemical properties associated with these compounds. They play a role in the early stages of drug design by helping to filter out undesirable compounds and providing rapid input on ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) information for lead optimization (Xiong *et al*., 2021).

This study utilized the ADMET 2.0 online server to evaluate the absorption, transport, and metabolism of substances into non-toxic, water-soluble metabolites for excretion. The tool also provides insights into how substances cross the blood-brain barrier, are absorbed in the human gastrointestinal system, and interact with p-gp (p-glycoprotein) substrates and CYP450 enzymes (Xiong *et al*., 2021).

The present findings revealed that both compounds exhibited excellent absorption but had low blood-brain barrier (BBB) profiles, making them less suitable for meningococcal infections. Compound **30** has a high Plasma Protein Binding (PPB) of 90%, which may impact its effectiveness (Fasano *et al*., 2005). Both compounds **29** and **30** act as inhibitors for CYP1A2 and CYP2D6, potentially interacting with the substrates of these enzymes. They are not substrates for p-gp, indicating they cannot be effluxed from the cell. The compounds demonstrated good clearance. However, compound **30** poses a risk of hERG (human ether-a-go-go related gene) toxicity, potentially leading to cardiac arrhythmia (Garrido *et al*., 2020).

Table 7: Pharmacokinetics and toxicity profile prediction of compounds **29** and **30** using ADMETlab 2.0 software.

Compound	Caco-2	Pgp-S	HIA	PPB (%)	BBB	VD	CYP1 A2-I	CYP 2C9 - I	CYP 2D6-I	CL	T½	hERG	Ames	SR-p53
29	-4.508	0.002	0.008	78.10	0.988	2.508	0.739	0.011	0.899	15.676	0.613	0.241	0.214	0.126
30	-4.732	0.005	0.007	90.96	0.993	2.576	0.685	0.017	0.974	15.139	0.32	0.839	0.475	0.577
Recommended values	>-5.15	0-0.3	0-0.3	≤ 90%	0-0.3	0.04-20	Category 0-1	Category 0-1	Category 0-1	≥ 5	0-0.3 excellent 0.3-0.7 medium	0-0.3 excellent 0.3-0.7 medium	0-0.3 excellent 0.3-0.7 medium	0-0.3 excellent 0.3-0.7 medium

Caco-2 = human colon adenocarcinoma cell lines, >-5.15; Pgp-sub = P-glycoprotein substrate; HIA=Human intestinal absorption; PPB=plasma protein binding; BBB = blood–brain barrier, VD = Volume of distribution; CYP450=cytochrome P450, category 0 - non inhibitor category 1-inhibitor ; CL = clearance of a drug;T½= half-life; hERG = human ether-a-go-go related gene,; AMES = a test for mutagenicity; SR-p53=a tumour suppressor protein p53

Table 8: Prediction of physicochemical and medicinal chemistry friendliness of compounds **29** and **30** using ADMETlab 2.0 software

Compound	nHA	nHD	nRot	TPSA	LogS (log mol/l)	LogP	LogD	LR-5	QED	SAscore
29	2	1	2	23.47	-1.871	2.596	2.426	Accepted	0.807	1.837
30	2	1	2	23.47	-2.865	3.814	3.285	Accepted	0.869	1.789
Recommended values	0-12	0-7	0-11	0-140	-4-0.5	0-3	1-3		> 0.67	≤ 6

nHA= number of hydrogen bond acceptors, nHD= number of hydrogen bond donors, nRot= number of rotatable bonds, TPSA=topological polar surface area, Log P = partition coefficient, Log S = aqueous solubility,Log D=n-octanol/water distribution coefficients at pH=7.4, LR-5=Lipinski rule of 5, QED=measure of drug-likeness, SA score=synthetic accessibility score.

In drug research and discovery, predicting the physicochemical and medicinal chemistry compatibility profiles of compounds is crucial. Early parameter prediction, such as utilizing Lipinski's rule of five, enhances the success rate of compounds progressing to lead optimization (Takács *et al*., 2021). We used the ADMET 2.0 online tool to assess the physicochemical and medicinal chemistry of drug-likeness behaviour of compounds **29** and **30**.

Results of this analysis indicated that compound **29** exhibits excellent physicochemical and medicinal chemistry friendliness. In contrast, compound **30** has higher log P and log D, indicating slightly increased lipophilicity. Nevertheless, both compounds adhere to Lipinski's rule of five, suggesting a high probability of oral availability.

Global health is still threatened by antimicrobial resistance, prompting researchers to develop various strategies to combat this issue. One approach involves synthesizing a diverse class of compounds and assessing their antimicrobial activity.

In this study, we successfully synthesized two amino alkyl derivatives of 2-naphthol (**29** and **30**), commonly referred to as Betti bases. Both these compounds exhibited strong antimicrobial properties. Betti bases play a crucial role as precursors for 1,3-amino oxygenated compounds, which are integral components of biologically significant natural products and potent medications like certain nucleoside antibiotics and HIV protease inhibitors (Mannhold, and Kubinyi, 2006). These bases are recognized for their antibacterial, hypotensive, and bradycardic effects, as highlighted by Knapp in 1995. Furthermore, recent research by Mokhtary and Torabi (2017) has emphasized the potential utility of Betti bases as essential building blocks.

Interestingly, both synthesized compounds demonstrated potent antibacterial activity against multidrug-resistant bacterial strains, with compound **30** exhibiting strong efficacy against *P. aeruginosa* MDR 1 (MIC = 10 µg/ml). Multidrug resistance (MDR) significantly hampers the effectiveness of antimicrobial drugs, contributing to elevated mortality rates and increased medical

expenses. This resistance amplifies the risk of spreading resistant microorganisms, undermining disease control, diminishing treatment efficacy, and prolonging the duration of infection in patients. In 2019, an estimated 4.95 million deaths worldwide were attributed to AMR (Murray *et al*., 2022). The escalating treatment costs result from microorganisms developing resistance to commercially available medications, necessitating the use of more expensive therapies. If the current trajectory persists, the projected cost of AMR to the global economy by 2050 is estimated to reach 100 trillion US dollars (O'Neill, 2016).

Additionally, the compounds demonstrated potent antibacterial effects against Gram-negative bacteria, which are challenging to treat with antibiotics due to the additional layer in their cell structure (Miller, 2016). For instance, both compounds exhibited strong antibacterial activity against for *E. coli* strains (MIC=25 µg/ml).

Given the promising antimicrobial effects observed in both compounds **29** and **30**, understanding their mechanism of action becomes crucial. In this context, there is an interest in exploring the fatty acid synthesis pathway for the development of novel antibacterial compounds. This pathway, regulated by distinct enzymes in bacteria, presents a promising target. Of particular significance are the enzymes FabF and FabB, which, due to their overlapping substrate specificities, contribute to the elongation process in fatty acid synthesis. The catalytic site of these enzymes, characterized by a CYS/HIS/HIS triad, plays a central role, as highlighted by Huang *et al*. in 1998.

Compounds **29** and **30** exhibited favorable binding interactions within the active site of the *E. coli* FabF enzyme. They achieved docking scores of -6.432 kcal/mol and -5.248 kcal/mol, respectively, through hydrogen bonds, pi-cation bonds, and pi-pi stacking interactions. Particularly, compound **29** (Figure II & III in Appendices 7.3) displayed a higher docking score compared to compound **30**. Despite this difference, both compounds yielded comparable docking results, involving distinct

amino acid residues. The difference in their docking scores could be attributed to the formation of fewer hydrogen bonds in compound **30**.

In the FabF complex with platencin, strong hydrogen bonds are formed between the amide carbonyl groups of THR270 and THR307, as well as the hydroxyl group of histidine HIE340 in FabF, and the ligand. Additionally, the gatekeeper phenylalanine PHE400 is implicated in establishing a bond with platencin. Previous research has shown that platencin targets FabF/B by binding to the malonyl binding site, inhibiting acyl intermediates (Singh *et al*., 2009). Based on these findings, we hypothesize that the synthesized compounds likely function as FabF/B inhibitors. Overall, the results suggest that further modifications to the compounds could enhance their potential as antimicrobial agents.

5. Conclusion

In this study, we successfully synthesized two Betti base derivatives of 2-naphthol, both of which exhibited strong antimicrobial activity. Particularly, compound **30** displayed the highest antibacterial activity, notably against multi-drug resistant *P. aeruginosa* MDR1 (MIC= 10 µg/ml) and *S. aureus* MDR 1 (ZOI=13.5 mm and MIC=100µg/ml). Moreover, both synthesized compounds demonstrated favourable interactions within the active site of the *E. coli* FabF enzyme, a key player in bacterial fatty acid biosynthesis and crucial for fatty acid elongation in bacteria.

In conclusion, these aminoalkyl-2-naphthols exhibit promising attributes as lead compounds for developing new antimicrobial agents. Their effectiveness against various bacterial strains, including multi-drug resistant ones, positions them as potential candidates to address the escalating challenges of infectious diseases and antimicrobial resistance.

6. Recommendation

The following recommendations are proposed from the present study:

- Conduct experimental validation of the molecular docking results.
- Synthesize more 2-naphthol derivatives and evaluate their potential antibacterial effects.
- Explore other potential biological activities, including antioxidant, anti-inflammatory, anticancer, and antiviral activity, for the two synthesized aminoalkyl-2-naphthol compounds.
- Perform comprehensive toxicity studies to assess the safety profile of the synthesized compounds.

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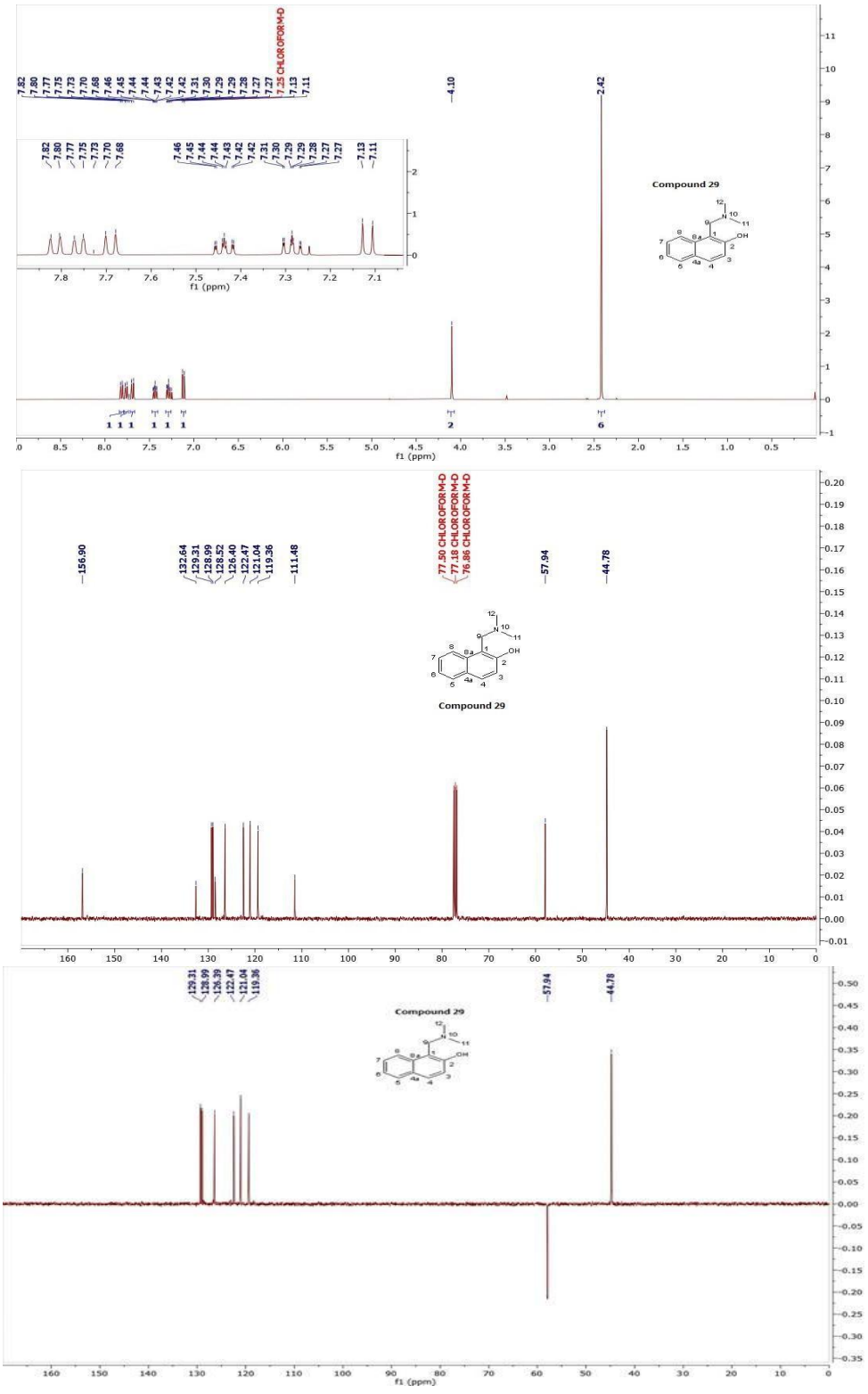
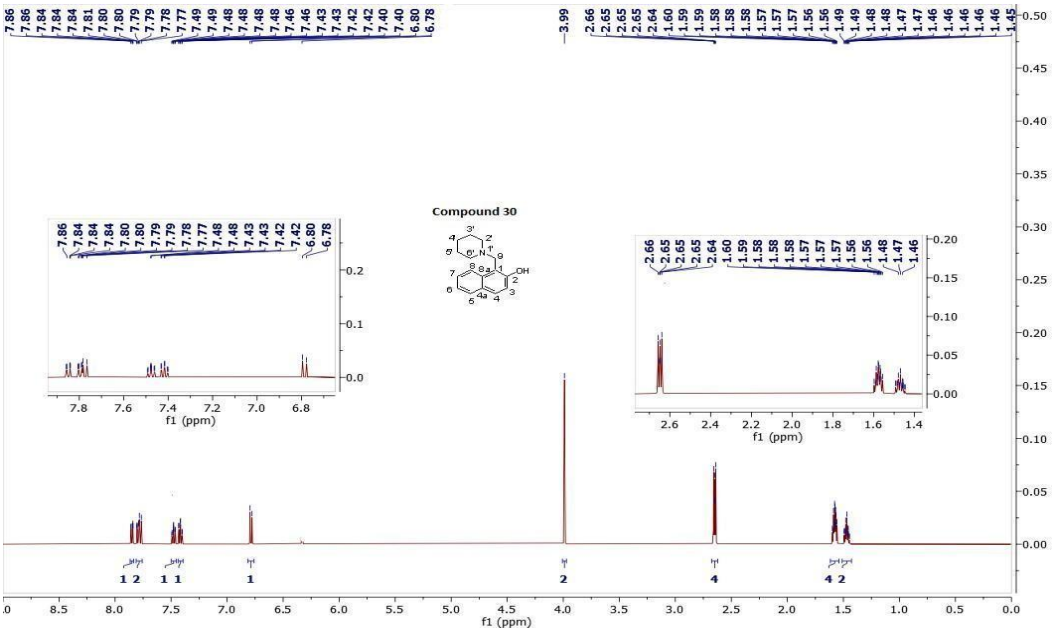
Appendices

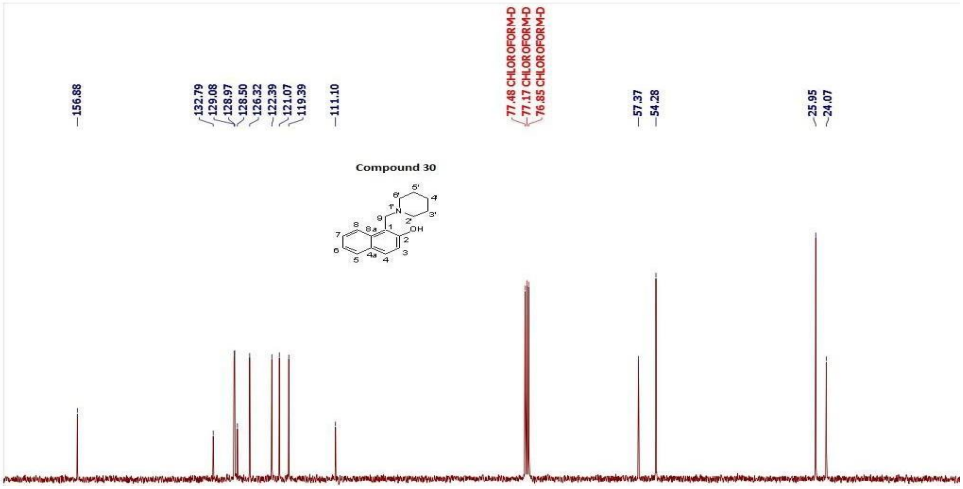
Appendix 1. The ^{1}H,^{13}C, DEPT 135 and DEPT-90 NMR spectra of the synthesized compounds

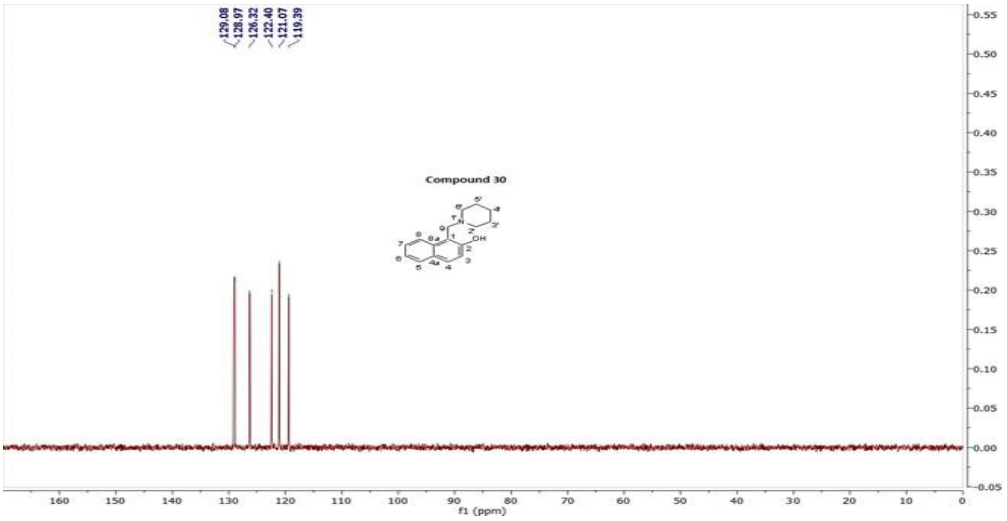
^{1}H,^{13}C and DEPT-135 NMR spectra of compound **29**



^{1}H, ^{13}C and DEPT-90 NMR spectra of compound **30**







Appendix 2. TLC chromatogram of synthesized compounds

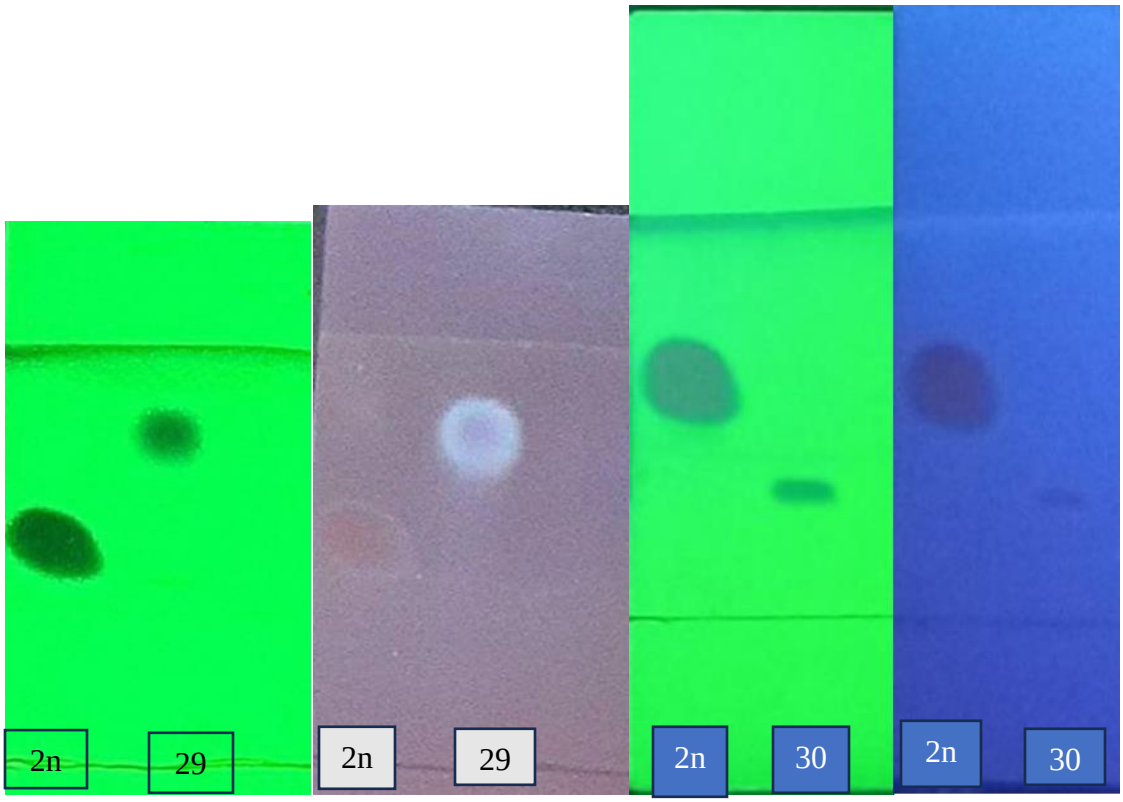
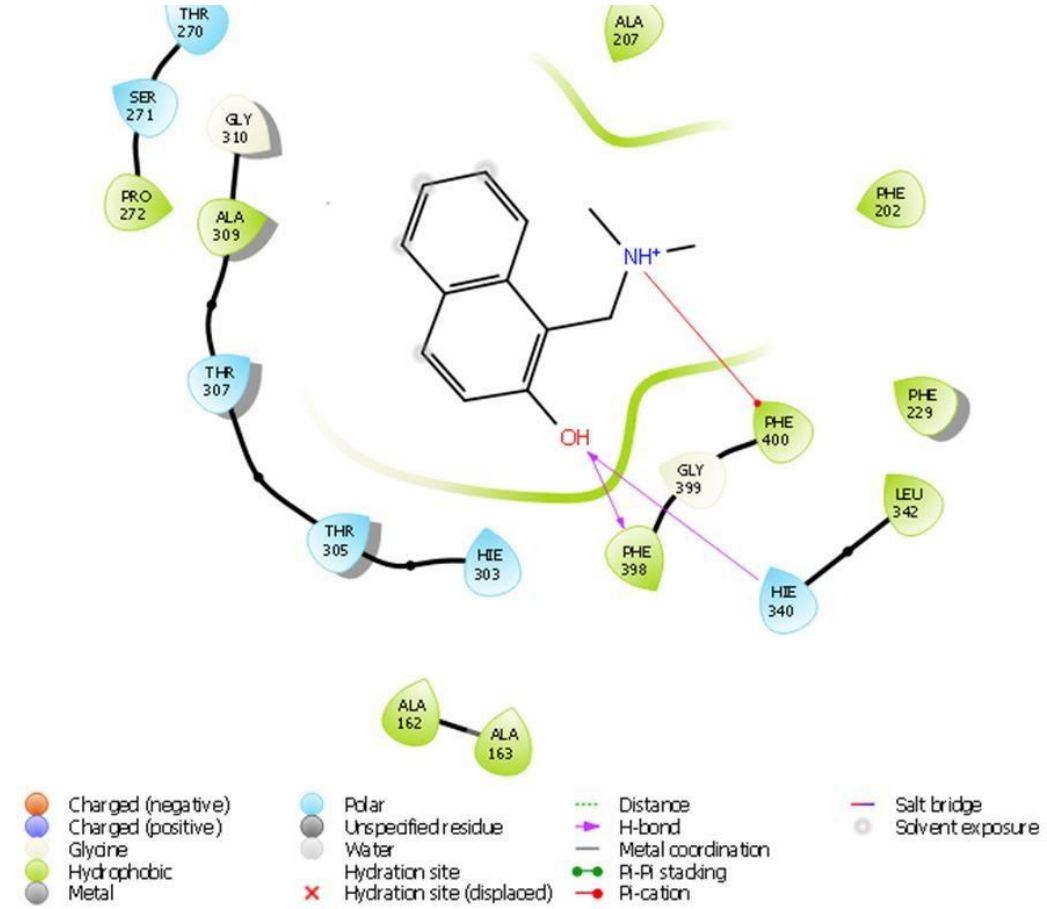


Figure I: Normal Phase TLC chromatograms of compound 29 viewed under UV 254(A) and UV 366(B) using solvent system chloroform: methanol (8:1); compound 30 viewed under UV 254(C) and UV 366(D) using solvent system hexane: ethyl acetate (3:2).

Appendix 3. Binding interactions of the synthesized platencin, compounds **29** and **30**.



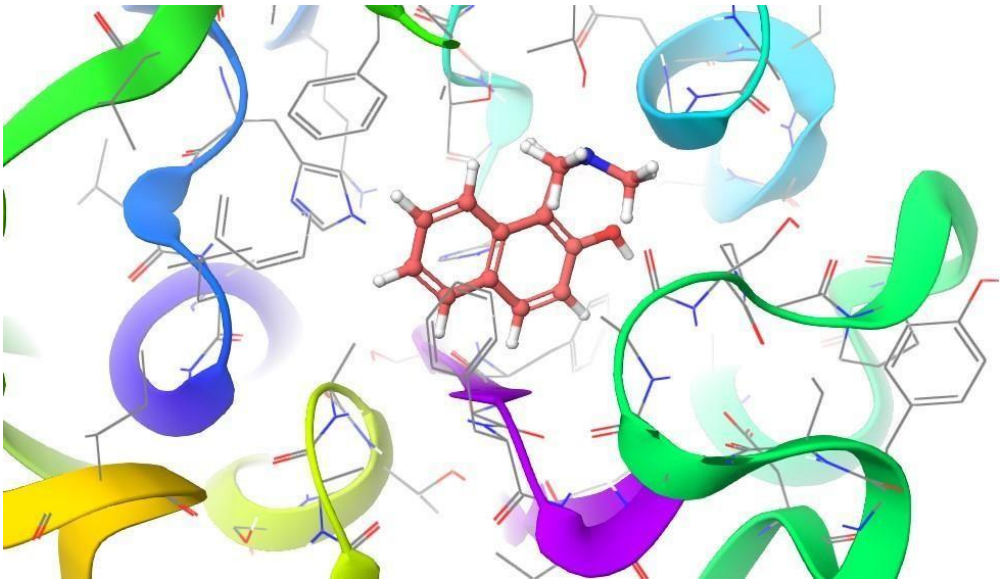
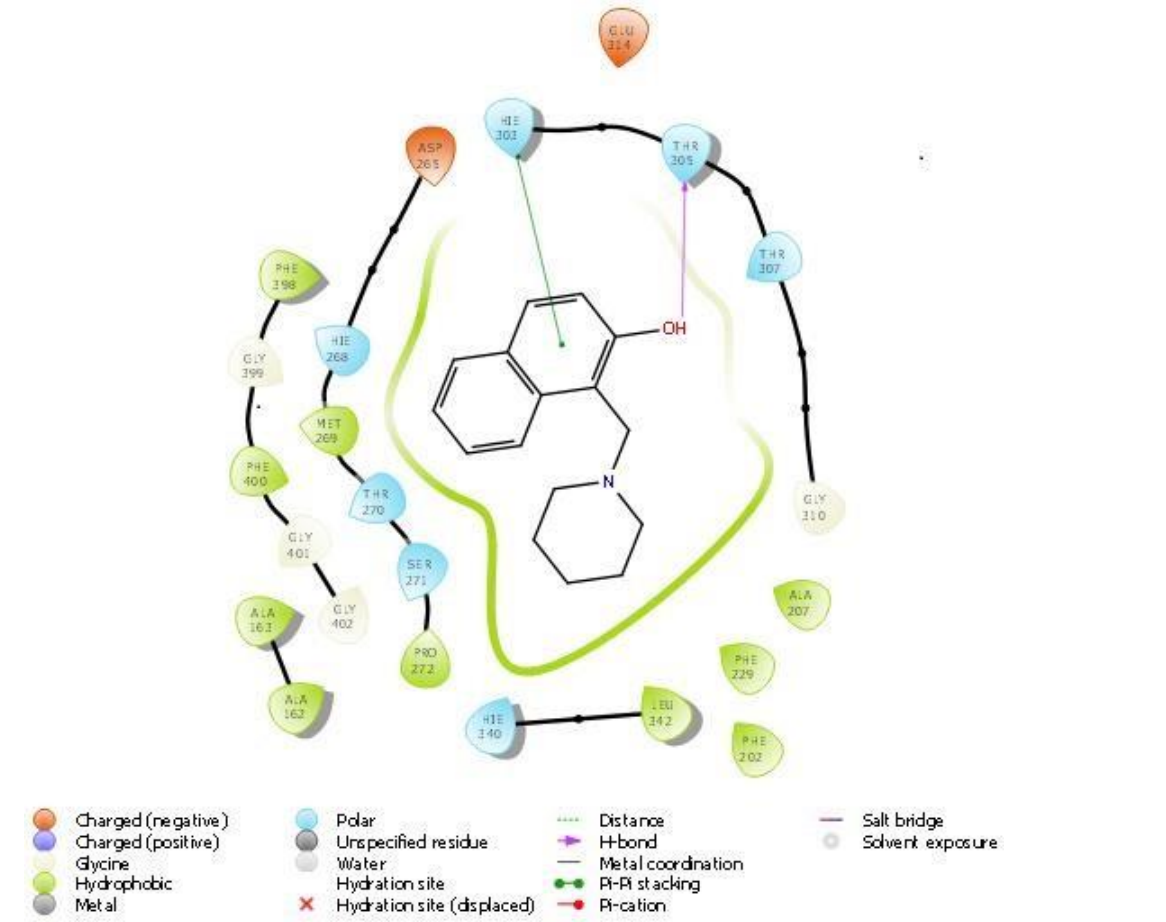


Figure II: 2D and 3D binding interaction of compound **29** with E. coli FabF (3HO2.pdb).



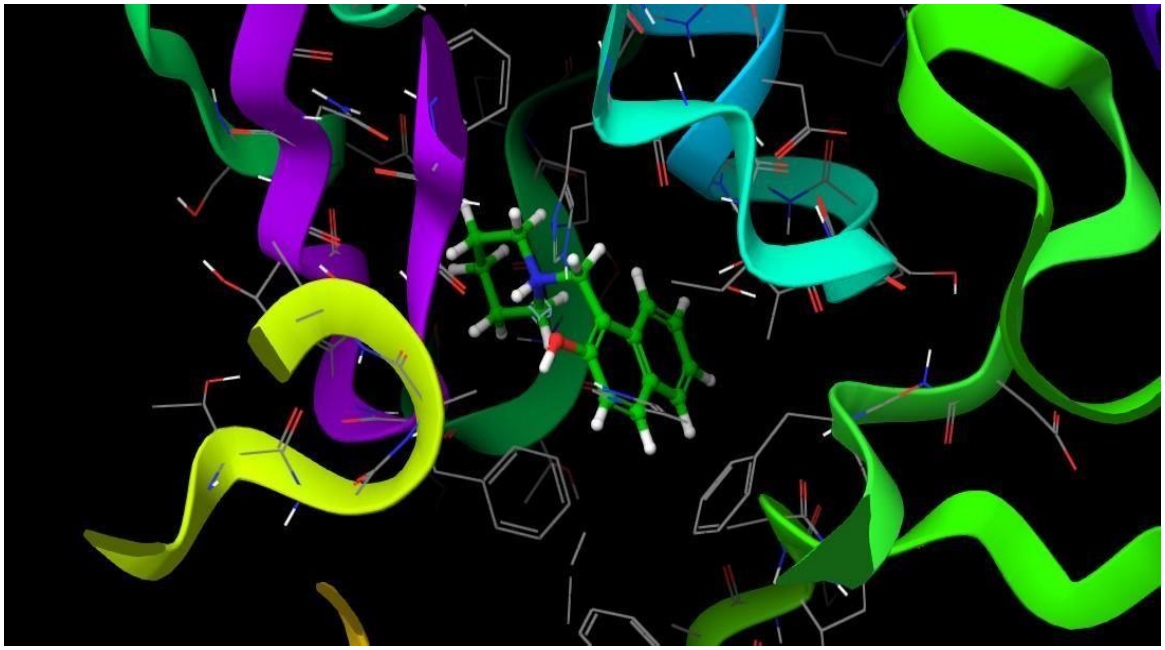


Figure III**:** 2D and 3D binding interaction of Compound **30** with *E. Coli* FabF (3HO2.pdb)

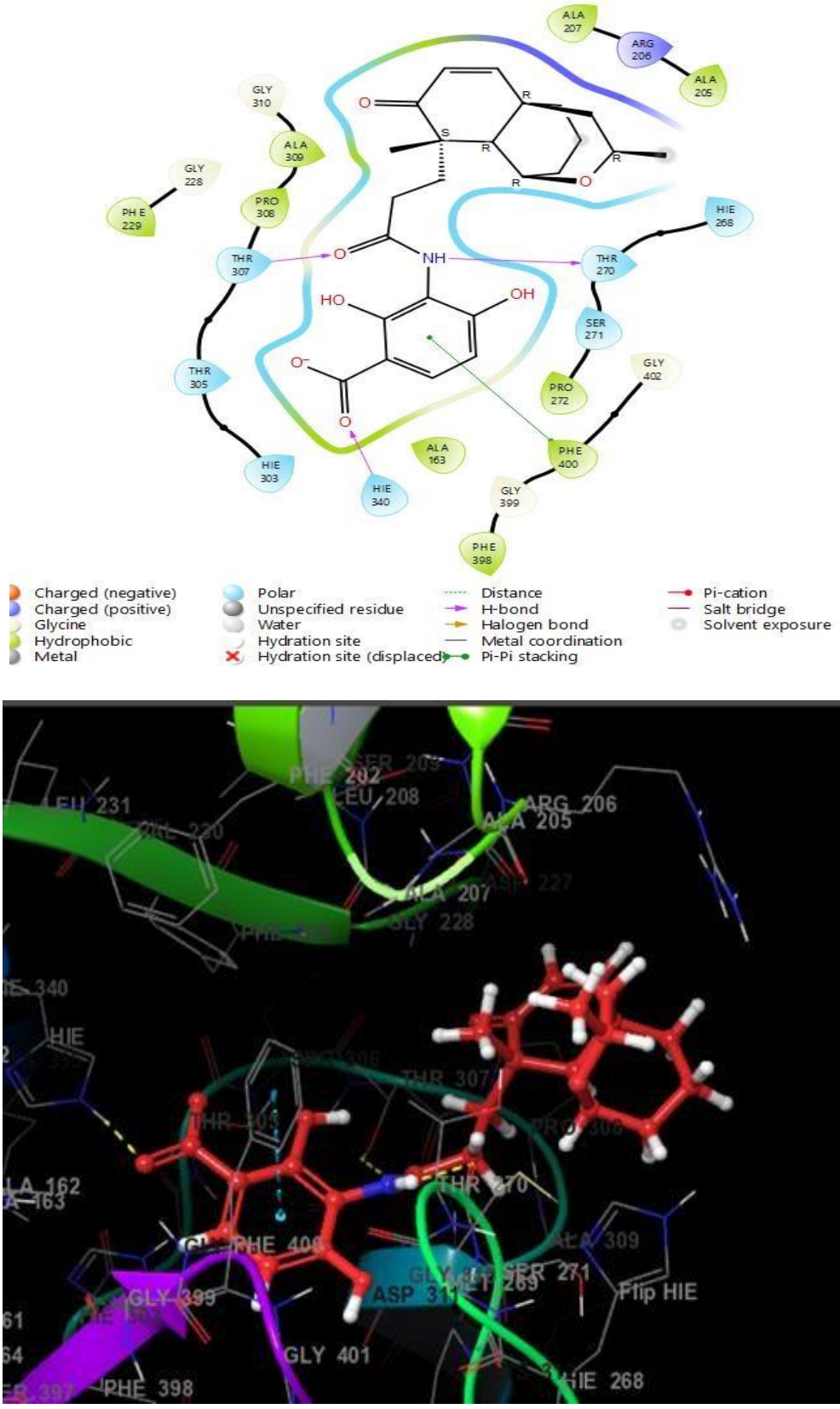


Figure IV: 2D and 3D binding interaction of platencin with E. Coli FabF (3HO2.pdb)

Draft Manuscript from the thesis

Synthesis, In vitro Antimicrobial and In silico Studies of Betti Base Derivatives of 2-Naphtol

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Abstract

Synthesis, *In vitro* Antimicrobial and *In silico* Studies of the Betti Base Derivatives of 2-Naphtol.

Frehiwot Beyene

The development of novel antimicrobial agents is necessary due to the rising prevalence of multidrug resistance pathogens to currently available drugs. In medicinal chemistry, 2-naphthol is a crucial starting compound that has attracted significant attention in the development of various biologically active compounds because of its ease of handling and cost-effectiveness. Considering this synthesis, the study aimed to synthesize 2-naphtol derivatives via the Betti reaction and assess their antimicrobial activity against twenty-six bacterial and four fungal strains, using the disk diffusion and broth dilution methods. As a result, Compounds **29** and **30** were synthesized through a Betti reaction involving 2-naphthol, formaldehyde, and a secondary amine. Compounds **29** and **30** were characterized by analysing their ^{1}H, ^{13}C-NMR, and DEPT-135 spectral data, and their chemical structures were identified as 1-((dimethyl amino) methyl) naphthalen-2-ol and 1-(piperidin-1-ylmethyl) naphthalen-2-ol, respectively. Among the synthesized compounds, compound **30** exhibited the highest antibacterial activity against *P. aeruginosa* MDR1, displaying a zone of inhibition (ZOI) of 12.0 mm at 200 µg/ml and a minimum inhibition concentration (MIC) value of 10 µg/ml. We also noted that compound **30** demonstrated superior antibacterial efficacy with a ZOI of 13.5 mm compared to the standard ciprofloxacin (ZOI=11.5 mm) against *S. aureus*

MDR 1. Furthermore, compound **30** demonstrated antifungal activity against *C. albicans* and *A. niger*, each with a MIC value of 400 µg/ml. The *P. funiculosum* also exhibited high sensitivity to compound **29**, showing a ZOI value of 13.0 mm at 1500 µg/ml and a MIC value of 400 µg/ml.

Based on preliminary docking analysis and existing literature, FabF (PDB ID: 3HO2) from *E. coli* was chosen for predicting ligand-protein interactions with antibacterial and antifungal properties. Subsequently, molecular docking of compounds **29** and **30** in the active pocket site of FabF *E.coli* enzyme resulted in docking scores of -6.08 and -5.62, respectively, forming hydrogen, and pi-cation bond interactions.. Overall, these findings indicate that the synthesis and evaluation of Betti base derivatives of 2-naphthols are promising in contributing to the development of novel antimicrobial drugs against infectious diseases and combating antimicrobial resistance.

Keywords: 2-naphtol, antimicrobial, antimicrobial resistance, Betti base, , *in-silico* studies

1. Introduction

Infectious diseases include those diseases caused by live parasites (helminths or protozoa), fungi, bacteria, inanimate viruses, prions, or a combination of these (Cole and Kramer, 2016; Sehgal *et al*., 2020). These diseases are substantial burdens on healthcare worldwide. They constitute a considerable amount of global health problems (Snowden, 2008).

Developing countries are bearing most of the health care burdens (Snowden, 2008). For the case of Africa, HIV/AIDS, malaria, TB, and diarrhoea are the leading causes of death (Boutayeb, 2010). Among other diseases, around 2.5 million people were diagnosed with TB (WHO, 2023) in 2022. Narrowing down to Ethiopia, back in 2019, Diarrhoea was the leading cause of early mortality, followed by lower respiratory infections, tuberculosis, and HIV/AIDS (Misganaw *et al*., 2022).

Among the infectious diseases, those caused by bacterial infections are the deadliest and they are the second leading reasons of death, only surpassed by ischemic heart disease (Vos *et al*., 2020). At a global scale, an equivalent number of fungal illnesses and bacterial diseases of occur annually, with various degrees of severity (Alastuey-Izquierdo, 2022). For example, fungal pathogens cause at least 13 million illnesses and 1.5 million deaths, mostly in patients with weakened immune systems (Bongomin *et al*., 2017).

Nowadays, the event of AMR has become one of the key global health challenges of the twenty-first century (Torres *et al*., 2019). It is believed to be responsible for about 700,000 deaths annually (Gajdács and Albericio, 2019) and it is estimated that 10 million individuals may pass away from AMR by the year 2050 (O'Neill, 2016).

The 2-naphthol, with a hydroxyl group at the 2-position, is phenol's naphthalene homologue. It is more reactive than phenols, like the isomer 1-Naphthol. Other names for 2-Naphthol include β-naphthol, 2-naphthalenol, naphth-2-ol, naphthalen-2-ol, and 2-hydroxynaphthalene. It has molecular formula of $C_{10}H_8O$, molecular weight 144.16, boiling point 295°C, and melting point 123 °C (Booth, 2000).

Several compounds can be synthesized from the 2-naphtol derivative such as xanthenes, chromenes, oxazines, furans, naphthopyrans, and other heterocyclic compounds in organic synthesis, which has garnered significant interest as a useful precursor (Chaudhary, 2021). This is because three nucleophilic sites are accessible: the C-1 position, the phenolic oxygen, and the (much lesser) C-3 position. For organic chemists, 2-Naphthol is an intriguing option due to its distinct reactivity, ease of handling, moisture stability, and inexpensive cost (Surya *et al*., 2021).

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals and reagents

The chemicals and reagents that were used for the synthesis process are: hexane, ethyl acetate, methanol, ethanol (99%), formaldehyde (37%), chloroform (99.8%), dimethyl amine, and piperidine (all from Sigma-Aldrich Co., MO, USA). As a matter of the synthesis quality, all the solvents and reagents are of analytical grade.

2.1.2. Instruments

For the analysis and purification of the compounds, analytical silica gel TLC plates (60 F254, 0.2 mm thick, Merck KGaA, Darmstadt, Germany) and column silica gel (60 F254, 70-240 mesh, Merck KGaA, Darmstadt, Germany) were used. In addition, to remove the solvents, Rotary evaporator (BUCHI Rotavapor™ R-300, Switzerland) was used. For a purpose antimicrobial test,

incubator (Modell 100-800 memmert), autoclave, biosafety cabinet (biostarmodell50/60), vortex (Fisher Brand), water bath, inoculating loops, electrical weighing balance, magnetic stirrer, cavate, test tube, pipette tips, pipette filler, micro-pipette (10-200µl), micro-titter plates, capillary tube, and measuring cylinder were used. To visualize the TLC chromatogram plates, UV-visible Spectrophotometer (Evolution 60S) was used. The ^{1}H and ^{13}C-NMR spectra were recorded using a JEOL 400 MHz NMR (Japan), tetramethyl silane (TMS) as internal standard, where chloroform was used as solvent for the NMR spectroscopy.

2.1.3. Media, microbial strains, and standard drugs

For the media preparation, the nutrient agar (NA), Mueller-Hinton broth (MHB), Sabouraud dextrose agar (SDA) and Sabouraud dextrose broth (SDB) were used. In addition, Ciprofloxacin was used as a positive control for antibacterial test while 1% Dimethyl sulfoxide (DMSO) was used as a negative control. For the case of antifungal test, Griseofulvin was used as a reference standard.

In vitro antibacterial assays were performed on the following Gram-positive bacterial strains: *Bacillus pumilus* 82, *B. subtilis* ATCC 6633, *Staphylococcus aureus* ML 267, *S. aureus* MDR 1, and *S. aureus* MDR 2. The Gram-negative bacterial strains used were: *Escherichia coli* 3:37C, *E. coli* 7360, *E. coli* 872, *E. coli* CD/99/1, *E. coli* K 88, *E. coli* T 37, *E. coli* ROW 7/12, *E. coli* 5933, *E.coli* HB101, *E.coli* C600, *Salmonella enterica* TD 01, *Salmonella typhi* Ty2, *Shigella boydii* D13629, *Salmonella dysentery* 8, *Shigella flexneri* Type 6, *Shigella sonnei* 1, *Vibrio cholerae* NCTC 5596, *V. cholerae* NCTC 10732, *Vibrio cholerae* NCTC 11501, *Vibrio cholerae* NCTC 4693, and *Pseudomonas aeruginosa* MDR 1. All the bacterial strains were procured from the Department of Technology, Jadavpur University, Central Drugs Laboratory, Kolkata and Institute of Microbial Technology, Chandigarh, India. Before sensitivity tests were performed on the test samples, the purity of the strains was checked in accordance with the standard microbiological, cultural, and biochemical tests.

On the other hand, the antifungal activity testing was performed on: *Aspargillus niger* ATCC 6275, *Candida albicans* ATCC 10231, *Penicillium funiclosum* NCTC 287, and *P. notatum* ATCC 11625. All the fungal strains were provided from Central Drugs Laboratory, Kolkata Institute of Microbial Technology, Chandigarh, India.

2.2. Methods

2.2.1. Synthesis of 2-naphtol derivatives (29 and 30)

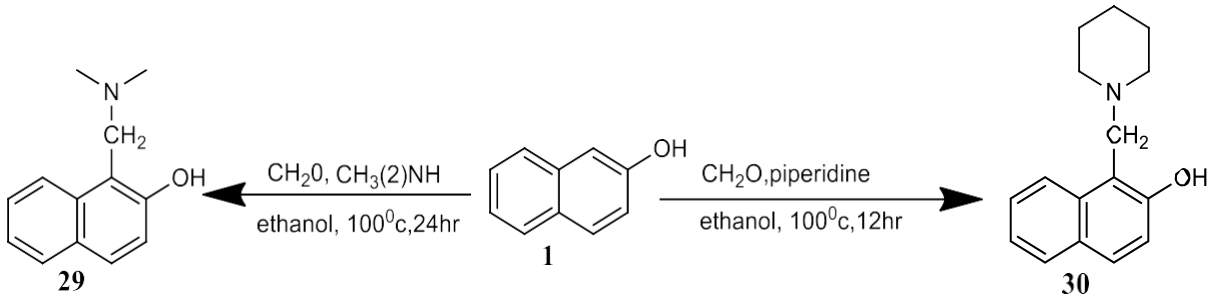
The synthesis of two Betti base derivatives of 2-naphthol (**29** and **30**) involved refluxing 2-naphthol, formaldehyde, and amines in the presence of an acid catalyst, following the procedure outlined in Scheme 1 (Marinescu et al., 2020).

2.2.1.1-((Dimethylamino) methyl) naphthalen-2-ol (29)

Compound **29** was synthesized through a Betti base base reaction. A solution containing 1 ml (0.011 mol) of 37% aqueous formaldehyde in 10 ml of ethanol, with five drops of acetic acid as a catalyst, was prepared. Dimethylamine (0.5 ml, 0.011 mol) was added dropwise to formaldehyde-acetic acid solution form an iminium ion, which was then added dropwise to a solution of 2-naphthol (1.5 g, 0.011 mol) in ethanol. The mixture was stirred and refluxed for 24 hrs at 100 ºC. The reaction progress was monitored by TLC. After completion, the solvent was removed using a Rota evaporator, and the reaction mixture was purified by silica gel column chromatography with an increasing gradient of ethyl acetate in petroleum ether as the eluting solvent, yielding compound **29** (0.5 g, 24%).

2.2.1.2-(piperidin-1-ylmethyl) naphthalen-2-ol (30)

Compound **30** was synthesized in a manner like the synthesis described for compound **29** via the Betti base reaction. A solution was prepared in a 50 ml beaker, consisting of 0.5 ml (0.011 mol) of 37% aqueous formaldehyde in 10 ml of ethanol, with five drops of acetic acid as a catalyst. 1 ml (0.011 mol) of piperidine was added dropwise to the formaldehyde and acetic acid solution. This mixture was then added dropwise to a solution of 1.5 g (0.011 mol) of 2-naphthol in ethanol. The mixture was refluxed with stirring in a 250 ml Erlenmeyer flask for 12 hrs at 100ºC. The reaction progress was monitored using analytical TLC with a hexane/Ethyl acetate (3:2) solvent system. After completion, solvent removal was done using a Rotary Evaporator, and the reaction mixture was purified by silica gel column chromatography with an increasing Ethyl acetate gradient in petroleum ether as the eluting solvent, resulting in compound **30** (1.0 g, 66%).



29 1 30

Scheme 1: Synthesis of 2-naphtol derivatives (29 and 30)

3.2.2. Structural elucidations of the synthesized compounds

The structures of compounds **29** and **30** were identified by analyzing their ^{1}H and ^{13}C-NMR spectra data. Chemical shifts are denoted in *ppm* and coupling constants (*J*) are expressed in *Hz*. Signal multiplicities are indicated as follows: *s* = singlet; *d* = doublet; *t* = triplet; *td* = triplet of doublet; *ddd* = doublet of doublet of doublets; and *m* = multiplet.

2.2.3. In vitro antimicrobial activity test

2.2.3.1. Disk diffusion method

By comparing the zones of inhibition produced by the test samples with those of ciprofloxacin, the disc diffusion method was used to screen the in vitro antibacterial assay (Mitchell and Carter, 2000). To investigate the synthesized compounds' antibacterial and antifungal activities, a stock solution containing 1 mg/ml of the 2-Naphtol derivatives was made in 1% DMSO. By diluting the stock solution in the appropriate quantities of water, concentrations ranging from 5 to 800 µg/mL for antibacterial activity tests and 50 to 2000 µg/mL for antifungal activity tests were used. After that, sterile Petri dishes were filled with molten media to create serial nutrient agar plates, which were then incubated for 24 hours at 37 °C to check for contamination.

After creating serial nutrients, the inoculums were evenly swabbed and given five minutes to dry. Next, the test samples were impregnated onto 6 mm filter paper discs (Whatman no. 1), which were then placed on the medium's surface. After that, the Petri dishes were incubated for 24 hours at a temperature of 37 °C, and the diameter of the inhibitory zone was measured in millimetres. The procedure was repeated for Ciprofloxacin and zone of inhibition was compared appropriately. A negative control of 1% DMSO was used for the experiment.

Using similar technique, the test samples' antifungal potential was assessed against the fungal pathogens on Saborauds dextrose media. After three days of incubation at a room temperature, the diameter of the zone of inhibition in the Petri dishes was determined in millimeters. Here, the Griseofulvin served as the standard for comparison.

2.2.3.2. Broth dilution method

By using this broth dilution method, the minimum inhibitory concentration (MIC) of the synthesized compounds was ascertained, following the instructions provided by Lalitha (2004) and Jean B. et al. (2015). For bacterial and fungal growth, the Mueller–Hinton broth and Saborauds dextrose broth were utilized respectively. Concentrations of 5, 10, 25, 50, 100, 200, 400 and 800 μg/mL for antibacterial activity test and 50, 100, 200,400, 800, 1000, 1500 and 2000 μg/mL for antifungal activity test of the synthesized compounds dissolved in DMSO were used. Additionally, a sterility control (growth control with nutritional broth and DMSO, free of antimicrobials) was performed. Every test and growth control well were incubated for three days at temperature of 25 °C for fungi and for 24 hours at temperature of 37 °C for bacteria.

2.2.4. *In silico* studies

2.2.4.1. Molecular docking study

Using Maestro V.13.5 software by Schrodinger 2023-1 Suite, molecular docking of the synthesized compounds was carried out on the FabF-platencin co-crystallized enzyme of *E. coli* (PDB ID: 3HO2) as potential target for antibacterial activity. The molecular docking process comprised four steps: protein preparation, ligand preparation, grid receptor generation, and molecular docking, utilizing default values.

2.2.4.2. Pharmacokinetics and drug-likeness properties

The physicochemical, pharmacokinetic, and toxicity profiles of the synthesized compounds were predicted using the online software tools provided by ADMET lab 2.0, as outlined by Xiong et al. in 2021. To facilitate this analysis, the compounds' structures were transformed into SDF format using ChemDraw 22.0.0 software. The resulting SDF format for each compound was then submitted as input to the web server.

3. Results and Discussion

3.1. Synthesis

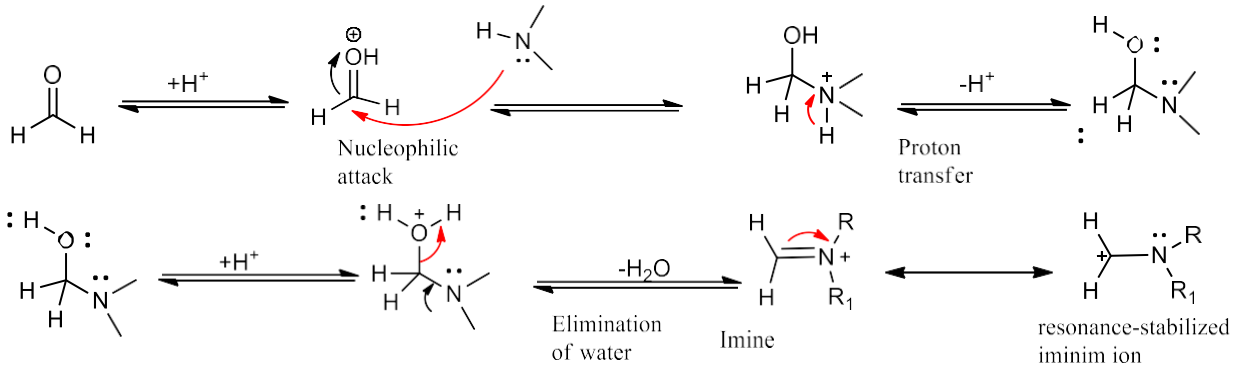
In this study, we synthesized two Betti base derivatives of 2-naphthol. This synthesis was driven by the diverse biological activities associated with 2-naphthol derivatives, particularly their antimicrobial properties, as highlighted in previous studies by Bedair *et al*. (2001) and Kaur *et al*. (2019).

Table 1: Physical properties of the synthesised compounds **29** and **30**

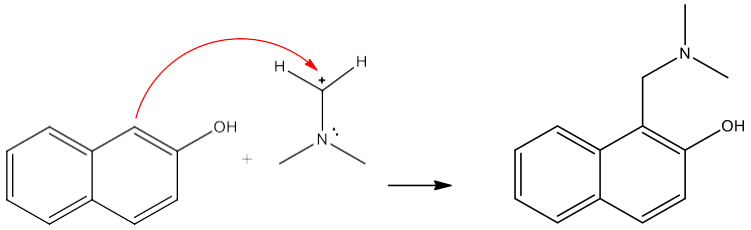
Compound	Molecular Formula	Predicted MW(g/mol)	Physical State	Color	% Yield	R_f value
29	$C_{13}H_{15}NO$	201.26	Crystal	Colorless	24	0.8
30	$C_{16}H_{19}NO$	241.33	Crystal	Colorless	66	0.3

3.1.1. Synthesis of ((dimethyl amino) methyl) naphthalen-2-ol (29)

Compound **29** was synthesized through a one-pot Betti base reaction, constituting a three-component process. This synthesis involved the condensation of formaldehyde, dimethylamine, and 2-naphthol in the presence of an acid catalyst. The reaction follows a sequence of steps. Initially, acetic acid enhances the electrophilicity of the carbonyl carbon in formaldehyde. Following this, dimethylamine engages in a nucleophilic addition reaction with the carbonyl group of formaldehyde, causing water loss and forming an iminium ion intermediate. Subsequently, the iminium ion undergoes in electrophilic aromatic substitution with 2-naphthol, resulting in the ultimate formation of compound **29**. The reaction mechanism is depicted in Scheme 1 and 2.



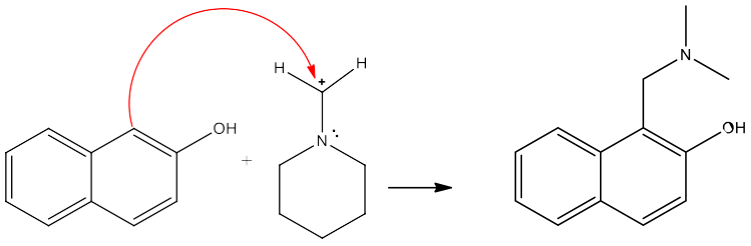
Scheme 1: Iminium formation mechanism of the Betti base derivatives.



Scheme 2: Electrophilic aromatic substitution of compound 29.

3.1.2. Synthesis of 1-(piperidin-1ylmethyl) naphthalen-2-ol (30)

Following the same synthesis approach outlined for compound **29**, compound **30** was synthesized using the Betti base synthesis procedure, but with the substitution of dimethylamine by piperidine. This substitution led to the formation of compound **30**. The reaction mechanism for compound **30** closely resembles that of compound **29**, and it is illustrated in Scheme 1and 3.



Scheme 3: Electrophilic aromatic substitution of compound 30.

3.2. Structural elucidation of compounds 29 and 30

Compounds **29** and **30** were characterized as ((dimethyl amino) methyl) naphthalen-2-ol and 1-(piperidin-1ylmethyl) naphthalen-2-ol, respectively, through the analysis of both ^{1}H and ^{13}C-NMR spectral data.

3.2.1. Structural elucidation of ((dimethyl amino) methyl) naphthalen-2-ol(29)

Compound **29** was successfully synthesised as a colourless crystal (2.4mmol., 24%w/w, Mwt 201.26) with an Rf value of 0.8 on TLC using MeOH/$CHCl_3$ (8:1) as the mobile phase.

The ^{1}H-NMR spectrum of compound **29** exhibited the presence of six aromatic protons, specifically assigned to H-3 (δ 7.12, 1H, *d*, *J*=8.9 Hz), H-4 (δ 7.69, 1H, *d*, *J*=8.9 Hz), H-5 (δ 7.76, 1H, *m*), H-6 (δ 7.29, 1H, *ddd*, J=8.4 Hz), H-7 (δ 7.44, 1H, *ddd*, J=8 Hz), and H-8 (δ 7.81, 1H, *dq*, J=7.7 Hz). This confirmed the replacement of one aromatic proton from 2-naphthol with an alkyl group. Additionally, the presence of a methylene group attached to the nitrogen group was indicated by a signal at δ 4.10 (2H, s), suggesting the formation of an aminoalkyl group in compound **29**.

The ^{13}C-NMR, along with the DEPT-135 spectrum demonstrated the presence of a total of 13 carbon atoms, including two identical methyl (2xCH_3), six methines (6xCH), one methylene (CH_2), and four quaternary carbons in compound **29**. Compound **29** was identified as 1-((dimethylamino)methyl)naphthalen-2-ol based on the ^{1}H and ^{13}C-NMR spectral data. The chemical shifts of all other protons and carbons in compound **29** are detailed in Table 2.

Table 2: ^{1}H and ^{13}C-NMR spectral data of compound **29**

Position	Compound **29**	
	δ_H (ppm)	**δ_C (ppm)**
1		111.48
2		156.90
3	7.12(1H,*d*,J=8.9 Hz)	119.36
4	7.69(1H,*d*,J=8.9Hz)	128.99
5	7.76(1H,*d*,J=8.1Hz)	128.52
6	7.44(1H,*ddd*,J=8.4 Hz)	122.47
7	7.28(1H,*m*,J=8.0Hz)	126.40
8	7.81(1H,*d*, J=7.7Hz)	121.04
8a		129.31
4a		132.64
11	4.10(2H,*s*)	57.94
13,14	2.42(6H,*s*)	44.78

3.2.2. Structural elucidation of 1-(piperidin-1ylmethyl) naphthalen-2-ol (30)

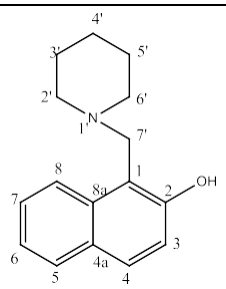
Compound **30** was obtained as a colorless crystal (4.13mmol, 66%; w/w, Mwt 241.33) with an Rf value of 0.3 on TLC hexane/Ethyl acetate (3:2) as a mobile phase.

Analysis of the ^{1}H-NMR spectrum of compound **30** revealed the presence of six aromatic protons. This observation indicated the substitution of one aromatic proton in 2-naphthol with an alkyl group, confirming the reaction occurrence at the 2-naphthol ring. A singlet at δ 3.99, integrating for two protons, was identified, corresponding to a CH_2 group at the 7' position, supporting the formation of an amino alkyl group.

The ^{13}C-NMR and DEPT-135 spectral data of compound 30 demonstrated the existence of a total of 14 carbon atoms, including two identical methyl (CH_3), six methines (CH), and one methylene

(CH_2). Therefore, both 1H-NMR and 13C-NMR analyses provided confirmation of the structure of compound 30. Detailed chemical shifts for all other protons and carbons in compound 30 are presented in Table 3.

Table 3: ^{1}H and ^{13}C-NMR spectral data of compound **30**

Position	Compound **30** 	
	δ_H (ppm)	**δ_C (ppm)**
1		111.48
2		156.90
3	6.79(1H,*d*,J=8.8 Hz)	119.36
4	7.79(1H,n.r.)	128.99
5	7.85(1H,*m*)	128.52
6	7.48(1H,*ddd*,J=8.1Hz,7.2,1.5)	122.47
7	7.42(1H,*td*,J=7.2,1. 3Hz)	126.40
8	7.79(1H ,n.r.)	121.04
8a		129.31
4a		132.64
2', 6'	2.66(4H, *m*)	54.28
3', 5'	1.58(4H,*m*)	25.95
4'	1.47(2H,*m*)	24.07
7'	3.99(2H,*s*)	57.37

n. r.= not resolved

3.3. Antimicrobial Activity

In this study, the synthesized compounds **29** and **30** were tested against 13 bacterial strains and both compounds have comparable activity with the range of the MIC from 10-400 µg/ml. The best performance was observed by compound **30** against *Pseudomonas aeruginosa* MDR1. The *Pseudomonas aeruginosa*, an opportunistic gram-negative bacterium, is a major cause of nosocomial infections and the drug resistant strains of *P. aeruginosa* are becoming difficult to treat (Al-Orphaly *et al.,* 2021).

Both of synthesized compounds have shown equal MIC value for all *E. coli* strains (25 µg/ml). Worth to note that the is *E. coli* is the most common gram-negative bacterium that causes extraintestinal diseases in humans. It causes meningitis, pneumonia, bacteremia, urinary tract infections, pelvic and abdominal infections, to mention some (Santos *et al.*, 2020).

The least activity of both synthesized compounds was for *Bacillus pumilus* (400 µg/ml). In addition, compound **29** has shown similar least activity for *Bacillus subtilis* ATCC 6633, *S. aureus* MDR 1, and *S. aureus* MDR2. It is to be noted that the least activity is observed on the gram-positive bacteria. The maximum activity for gram-positive bacteria was observed by compound **30** for *S. aureus* MDR 1 and *S. aureus* MDR 2 (100 µg/ml). Note that the *Staphylococcus aureus* is a commensal as well as an opportunistic bacterium, which is a major contributor to a variety of infections from mild skin infections to serious illnesses (Wang *et al*., 2017). From our experiment, compound **30** has shown a higher zone of inhibition than ciprofloxacin against the *S. aureus* MDR 1, *i.e.*, 13.5 mm.

Table 4: Zone of inhibition and minimum inhibitory concentration of the synthesized compounds against the tested bacterial strains

Bacteria	Zone of inhibition in mm (200 µg/ml)			MIC (µg/ml)	
	Compound **29**	Compound **30**	Ciprofloxacin	Compound **29**	Compound **30**
E.coli NCTC 5933	14.0	14.0	16.0	25	25
E.coli K88	14.0	14.5	17.0	25	25
E.coli NCTC 7360	14.5	14.5	17.0	25	25
E.coli LT37	15.0	13.5	16.0	25	25
E.coli 872	14.5	14.0	16.0	25	25
E.coli ROW 7/12	13.0	14.5	16.5	25	25
E.coli 3:37C	13.5	14.0	16.5	25	25
E.coli CD/99/1	15.0	15.0	17.0	25	25
E.coli HB101	12.0	13.5	14.0	25	25
E.coli C600	12.5	11.5	13.5	25	25
Salmonella typhi Ty2	14.0	13.0	16.0	100	100
Salmonella enterica TD 01	14.5	13.5	19.0	100	100
Shigella dysentery 8	14.0	13.5	20.0	50	100
Shigella soneii 1	14.0	13.0	19.5	50	100
Shigella boydii D13629	12.5	14.0	20.0	50	100
Shigella flexneri Type 6	12.5	14.0	20.5	50	100
Staphylococcus aureus ML 267	15.0	16.0	18.0	50	50
S. aureus MDR 1	10.0	13.5	11.5	400	100
S. aureus MDR 2	10.0	11.0	12.0	400	100
Bacillus pumilus 82	7.5	8.0	19.0	400	400
Bacillus subtilis ATCC 6633	7.5	8.0	18.0	400	200
Vibrio cholerae NCTC 4693	12.0	15.0	17.5	50	50
Vibrio cholerae NCTC5596	12.5	14.0	18.5	50	50
Vibrio cholerae NCTC 10732	13.0	14.5	19.0	50	50
Vibrio cholerae NCTC 11501	12.0	14.0	18.5	50	50
Pseudomonas aeruginosa MDR 1	11.0	12.0	12.5	100	10

3.4. Antifungal Activity

The 2-naphtol derivatives were tested on four fungal strains and they showed moderate activity against the strains. However, compound **29** has shown a greater zone of inhibition than Griseofulvin for *Penicillium notatum* and *Penicillium funiculusum*. The MIC value ranges from 400-1500µg/ml. For compound **29**, the highest activity is observed on *Penicillium funiculusum* NCTC 287 with MIC value of 400µg/ml. In contrast, the highest activity for compound **30,** was observed on *Candida albicans* ATCC 1023 and *Aspergillus niger* ATCC 6275 (400 µg/ml).

Table 5: Zone of inhibition and minimum inhibitory concentration of compound 29 and 30 against the tested fungal strains.

Fungi	Zone of inhibition in mm(200µg/ml)			MIC(µg/ml)	
	Compound **29**	Compound **30**	Griseofulvin	Compound **29**	Compound **30**
Candida albicans ATCC 10231	11.0	12.0	15.0	1500	400
Aspergillus niger ATCC 6275	12.5	13.5	14.5	800	400
Penicillium notatum ATCC 11625	13.5	10.5	12.0	800	1000
Penicillium funiculosum NCTC 287	13.0	10.0	11.0	400	1000

3.5. *In silico* Studies

3.5.1. Molecular docking

Following preliminary docking and literature review, the *E. coli* FabF protein (PDB ID: 3HO2) (Singh et al., 2009) was selected to assess the binding affinity of synthesized compounds within the enzyme's active site.

The synthesized compounds in this study exhibited satisfactory binding to the *E. coli* FabF enzyme, as indicated in Table 6. Specifically, compound **29** demonstrated a docking score of -6.432 kcal/mol within the active site of the *E. coli* FabF enzyme, forming two hydrogen bonds with HIE340 and PHE398 via its hydroxyl group, and a pi-cation bond with PHE400 via its amine group. On the other hand, compound **30** exhibited lower affinity binding (-5.248 kcal/mol), forming a single hydrogen bond with THR305 via its hydroxyl group and engaging in pi-pi stacking with HIE305 through its naphthalene ring.

Table 1: Docking scores of the 2-Naphtol derivatives with 3HO2 and 1IYL according to Schrodinger 2023 suite docking software.

Ligand	**Docking score (kcal/mol) within 3HO2**	**Interaction with amino acid residues**	
		H-bonds	**Non H-bonds**
29	**-6.432**	HIE340 and PHE398	PHE400
30	**-5.248**	THR305	HIE305
Platencin	**-7.650**	THR240,THR307 and HIE340	PHE400

The docking protocol underwent validation using the native ligands platencin for antibacterial target. When platencin was redocked into the active site of the E. coli FabF enzyme, it yielded a docking score of -7.650 kcal/mol, demonstrating a relatively comparable affinity to compound **30**(-6.432 kcal/mol).

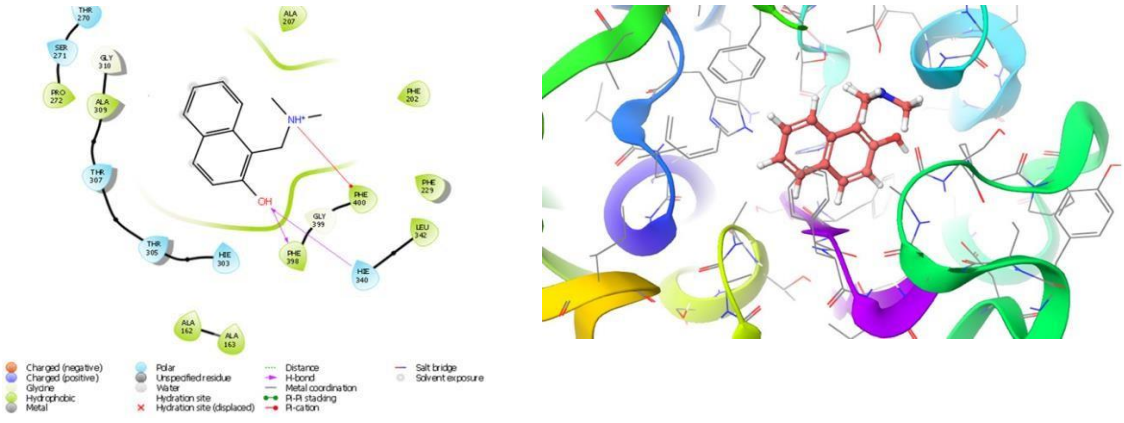


Figure 1: 2D and 3D docking of compound **29**(PDB; 3HO2)

3.5.2. **Pharmacokinetics** and **drug-likeness properties**

This study utilized the ADMET 2.0 online server to evaluate the absorption, transport, and metabolism of substances into non-toxic, water-soluble metabolites for excretion. The tool also provides insights into how substances cross the blood-brain barrier, are absorbed in the human gastrointestinal system, and interact with p-gp (p-glycoprotein) substrates and CYP450 enzymes (Xiong et al., 2021).

The present findings revealed that both compounds exhibited excellent absorption but had low blood-brain barrier (BBB) profiles, making them less suitable for meningococcal infections. Compound **30**, in particular, has a high Plasma Protein Binding (PPB) of 90%, which may impact its effectiveness (Fasano et al., 2005). Both compounds **29** and **30** act as inhibitors for CYP1A2 and CYP2D6, potentially interacting with the substrates of these enzymes. They are not substrates for p-gp, indicating they cannot be effluxed from the cell. The compounds demonstrated good clearance. However, compound **30** poses a risk of hERG (human ether-a-go-go related gene) toxicity, potentially leading to cardiac arrhythmia (Garrido et al., 2020).

Table 2: Drug likeness properties of synthesised compounds

	nHA	**nHD**	**nRot**	**Molecular weight**	**LogP**	LogD	**LR-5**
29	2	1	2	201.26	2.596	2.426	Accepted
30	2	1	2	241.33	3.814	3.285	Accepted
Recommended values	0-12	0-7	0-11	<500	0-3	1-3	

In drug research and discovery, predicting the physicochemical and medicinal chemistry compatibility profiles of compounds is crucial. Early parameter prediction, such as utilizing Lipinski's rule of five, enhances the success rate of compounds progressing to lead optimization (Takács et al., 2021). We used the ADMET 2.0 online tool to assess the physicochemical and medicinal chemistry of drug-likeness behaviour of compounds **29** and **30**.

Results of this analysis indicated that compound **29** exhibits excellent physicochemical and medicinal chemistry friendliness. In contrast, compound **30** has higher log P and log D, indicating

slightly increased lipophilicity. Nevertheless, both compounds adhere to Lipinski's rule of five, suggesting a high probability of oral availability.

Global health is still threatened by antimicrobial resistance, prompting researchers to develop various strategies to combat this issue. One approach involves synthesizing a diverse class of compounds and assessing their antimicrobial activity.

In this study, we successfully synthesized two aminoalkyl derivatives of 2-naphthol (**29** and **30**), commonly referred to as Betti bases. Both these compounds exhibited strong antimicrobial properties. Betti bases play a crucial role as precursors for 1,3-amino oxygenated compounds, which are integral components of biologically significant natural products and potent medications like certain nucleoside antibiotics and HIV protease inhibitors (Mannhold, and Kubinyi, H., 2006). These bases are recognized for their antibacterial, hypotensive, and bradycardiac effects, as highlighted by Knapp in 1995. Furthermore, recent research by Mokhtary and Torabi in 2017 has emphasized the potential utility of Betti bases as essential building blocks.

Interestingly, both synthesized compounds demonstrated potent antibacterial activity against multidrug-resistant bacterial strains, with compound **30**, in particular, exhibiting strong efficacy against *P. aeruginosa* MDR 1 (MIC = 10 µg/ml). Multidrug resistance (MDR) significantly hampers the effectiveness of antimicrobial drugs, contributing to elevated mortality rates and increased medical expenses. This resistance amplifies the risk of spreading resistant microorganisms, undermining disease control, diminishing treatment efficacy, and prolonging the duration of infection in patients. In 2019, an estimated 4.95 million deaths worldwide were attributed to AMR (Murray et al., 2022). The escalating treatment costs result from microorganisms developing resistance to commercially available medications, necessitating the use of more expensive therapies. If the current trajectory persists, the projected cost of AMR to the global economy by 2050 is estimated to reach 100 trillion US dollars (O'Neill, 2016).

Additionally, the compounds demonstrated potent antibacterial effects against Gram-negative bacteria, which are challenging to treat with antibiotics due to the additional layer in their cell structure (Miller, 2016). For instance, both compounds exhibited strong antibacterial activity against for *E.coli* strains (MIC=25 µg/ml).

Given the promising antimicrobial effects observed in both compounds **29** and **30**, understanding their mechanism of action becomes crucial. In this context, there is an interest in exploring the

fatty acid synthesis pathway for the development of novel antibacterial compounds. This pathway, regulated by distinct enzymes in bacteria, presents a promising target. Of particular significance are the enzymes FabF and FabB, which, due to their overlapping substrate specificities, contribute to the elongation process in fatty acid synthesis. The catalytic site of these enzymes, characterized by a CYS/HIS/HIS triad, plays a central role, as highlighted by Huang et al. in 1998.

Compounds **29** and **30** exhibited favorable binding interactions within the active site of the *E. coli* FabF enzyme. They achieved docking scores of -6.432 kcal/mol and -5.248 kcal/mol, respectively, through hydrogen bonds, pi-cation bonds, and pi-pi stacking interactions. Particularly, compound **29** (Figure II & III in Appendices 7.3) displayed a higher docking score compared to compound **30**. Despite this difference, both compounds yielded comparable docking results, involving distinct amino acid residues. The difference in their docking scores could be attributed to the formation of fewer hydrogen bonds in compound **30**.

In the FabF complex with platencin, strong hydrogen bonds are formed between the amide carbonyl groups of THR270 and THR307, as well as the hydroxyl group of histidine HIE340 in FabF, and the ligand. Additionally, the gatekeeper phenylalanine PHE400 is implicated in establishing a bond with platencin. Previous research has shown that platencin targets FabF/B by binding to the malonyl binding site, inhibiting acyl intermediates (Singh et al., 2009). Based on these findings, we hypothesize that the synthesized compounds likely function as FabF/B inhibitors. Overall, the results suggest that further modifications to the compounds could enhance their potential as antimicrobial agents.

4. Conclusion and Recommendation

In this study, we successfully synthesized two aminoalkyl derivatives of 2-naphthol, both of which exhibited strong antimicrobial activity. The main accomplishment in this study was the clear demonstration of potent antibacterial effectiveness exhibited by both compounds against Gram-negative and MDR bacteria strains. Particularly, compound **30** displayed the highest antibacterial activity, notably against multi-drug resistant *P. aeruginosa* MDR1 (MIC= 10 µg/ml) and *S. aureus* MDR 1 (ZOI=13.5 mm and MIC=100µg/ml). Moreover, both synthesized compounds demonstrated favorable interactions within the active site of the *E. coli* FabF enzyme, a key player in bacterial fatty acid biosynthesis and crucial for fatty acid elongation in bacteria. According to predictions from the ADMET 2.0 online tool, both compounds have shown potential safety for oral administration. In conclusion, these aminoalkyl-2-naphthols exhibit promising attributes as

lead compounds for developing new antimicrobial agents. Their effectiveness against various bacterial strains, including multi-drug resistant ones, positions them as potential candidates to address the escalating challenges of infectious diseases and antimicrobial resistance.

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