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DESIGN, SYNTHESIS AND DETERMINATION OF IN-SILICO ANTIBACTERIAL ACTIVITY OF VARIOUS DERIVATIVES OF PHENYL BENZOATE

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ABSTRACT

The emergence of antibiotic-resistant bacteria necessitates the development of novel antibacterial agents. Phenyl benzoate is a white amorphous powder and it is used as a preservative. This study focuses on the design, synthesis, and in-silico evaluation of the antibacterial activity of different derivatives of phenyl benzoate. The design phase involved computational modeling and molecular docking studies to explore the structure-activity relationship of phenyl benzoate derivatives. Using established software tools, modifications were made to specific functional groups to create a library of derivatives. The synthetic routes employed were optimized for the synthesis of these compounds, ensuring high purity and yield. Following synthesis, the compounds were characterized using various spectroscopic techniques. Fourier Transform Infrared (FT-IR) spectroscopy was utilized to examine the functional groups and confirm the presence of desired chemical bonds. Carbon Nuclear Magnetic Resonance (13C-NMR) spectroscopy was employed to analyze the chemical environments of Carbon atoms, aiding in structural elucidation. Additionally, techniques such as Mass Spectrometry (MS) and Ultraviolet-Visible (UV-Vis) spectroscopywere employed to determine molecular weight and assess the presence of conjugated systems, respectively. The synthesized compounds were subjected to in-silico antibacterial activity evaluation through molecular docking simulations against clinically relevant bacterial targets. The binding affinities, interaction energies, and potential binding sites were investigated to assess the potential of the derivatives as antibacterial agents. The results of the spectroscopic analysis confirmed the successful synthesis and characterization

of the phenyl benzoate derivatives. The FT-IR, 13C-NMR, MS, and UV-Vis spectra provided crucial information regarding the functional groups, chemical environments, molecular weight, and conjugation of the compounds. The molecular docking simulations revealed promising interactions between the derivatives and bacterial targets, suggesting potential antibacterial activity. The computational evaluation, combined with the spectroscopic characterization, provides a solid foundation for further experimental investigations and optimization of lead compounds.

KEYWORDS: Phenyl benzoate derivatives, in-silico evaluation, antibacterial activity, spectroscopic techniques, nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, mass spectrometry, molecular docking.

1.0 INTRODUCTION

1.1 Microbes or microorganism

Microbes are extremely little living entities that are all around us but are too small to beseen with the unaided eye. These are aquatic, terrestrial, and avian species. Millions of these microbes, also known as microorganisms, reside in the human body. While certain microorganisms make us ill, others are vital to our wellbeing. The most prevalent kinds include fungus, viruses, and bacteria. Protozoa are a class of microorganisms as well. They are tiny living creatures that cause toxoplasmosis and malaria, among other illnesses (https://www.ncbi.nlm.nih.gov/books/NBK279387/) 10-05-2023; 08:36pm.

1.2 History of Microorganism

Between 1665 until around 1678, two extraordinary geniuses named Antoni van Leeuwenhoek and Robert Hooke made the first discovery of microorganisms. Their lives as scientists were brought together by a complicated set of circumstances despite the fact that theyoriginated from completely different backgrounds. Their potential interactions could not have been anticipated or even envisioned, but serendipity was at play. Making and employing microscopes, which they tackled in various ways, was the common factor that led to their discoveries of microbes. Leeuwenhoek is commonly referred to as the "first of the microbe hunters" in historical records, and his renowned letters from 9 October 1676 are frequently cited as providing the first conclusive observations of bacteria. The fact that Robert Hooke first identified microscopic fungus in 1665 is sometimes forgotten. Hooke made important contributions to the development of microscopy, the foundation of microbiology, and was the first to validate observations made by Leeuwenhoek that were regarded as questionable by

many of his colleagues. Hooke and Leeuwenhoek were both key players in the discovery of themicrobiological cosmos, according to a reexamination of The Royal Society's records and publications from 1665 to 1678 (H. Gest; 2004).

1.3 *Escherichia coli (E. coli)*

The majority of research has been done on and the most is known about *Escherichia coli*. German pediatrician Theodor Escherichia initially identified it in 1885. He recognized both its significant presence in healthy people's gut microbiota and its capacity to spread disease when directly injected into extraintestinal areas.1 Unsurprisingly, the knowledge that E. coli might also spread disease in the gastrointestinal system, where it normally lives, took much longer to become clear. Only two types of enterotoxins, heat-labile enterotoxin (LT) and heat- stable enterotoxin (ST) are secreted by ETEC, in contrast to the wide variety of colonization factors they use. In terms of biology and antigens, heat labile enterotoxin and cholera toxin are closely linked. Similar subunit structures can be seen in both poisons. consists of a single A subunit linked to five identical B subunits, which when pentameric zed allow the toxin to bindto the Gm1 ganglioside receptor on host cells. The A1 component of the attached toxin is internalized and processed, allowing it to be liberated from the holotoxin and released into thecytoplasm of the host cell. It does this by catalyzing the alteration of a regulatory component of membrane-associated adenylate cyclase, which results in the irreversible activation of the latter. The ensuing buildup of intracytoplasmic cAMP ultimately alters the way that enterocytestransport electrolytes, most notably by causing the crypt cells to secrete more chloride ions andthe villous cells to absorb less sodium and chloride ions. Both of these activities cause an electrolyte buildup in the luminal space, which draws water into the intestine along the resulting gradient in osmosis. The excess fluid is expelled as watery diarrhea, which is indicative of an infection with ETEC and *Vibrio cholerae*, if the volume of accumulated intraluminal fluid exceeds the large intestine's normal absorption capacity. In addition to affecting adenylate cyclase activity, cholera toxin and, presumably, LT also cause diarrhea by affecting prostaglandin metabolism and activating neurotransmitters in the enteric nervous system (R.M.Robins-Browne *et al*; 2002).

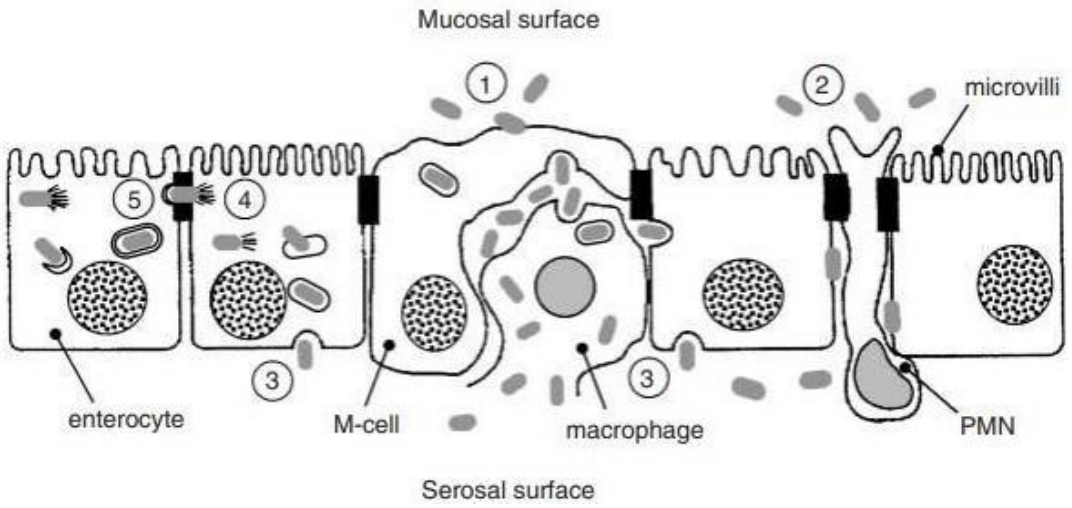

Figure 1.1: The process by which enteroinvasive *Escherichia coli* or Shigella species penetrate the intestinal barrier is depicted diagrammatically. Bacteria are discharged into the submucosa after entering the intestinal epithelium via M cells (1). The production of interleukin-1 and the subsequent inflammatory response that results in tissue obliteration are caused by macrophages that have undergone bacterial-induced apoptosis. In response to early signs of inflammation, polymorphonuclear leukocytes (PMN) are drawn to the site of infection. Tight connections allow the PMN to transmigrate to the apical side and create a passageway for the bacteria (2). Following one of these methods (1 + 2) for bacterial entry into the epithelium, they infect the enterocytes from the basolateral side (3). Bacteria that have been endocytosed can move intracellularly while being driven by actin tails (4), cell connections allow them to spread to nearby cells, lyse the double membrane created when protrusions form (5), and continue their intraepithelial cell-to-cell transit (P. J. Sansonetti;1992).

1.4 *Bacillus subtilis*

An endospore-forming, gram-positive, aerobic, rod-shaped bacteria called *Bacillus subtilis* is typically found in water, soil, air, and decomposing materials (M. Alexender; 1997). Gupta & Vyas (1989) initially documented *B. subtilis's* ability to kill mosquito larvae of the An. culicifacies species. A few years later, *B. subtilis* strains DM-03 and DM-04 werediscovered to be mosquitocidal by Das and Mukherjee (2006). This bacterium produces cyclic lipopeptides (CLPs), which are known to have larvicidal properties (K. Das *et al*; 2006). A strain of *Bacillus subtilis* discovered in 2007 at the VCRC was able to eradicate all mosquito immature stages (I. Geetha *et al*; 2007). Based on the sequencing of the gyrA gene, it was determined to be *Bacillus subtilis* subsp. subtilis (VCRC B471). According to Geetha &

Manonmani (2008), the CLP of the *B. subtilis* strain (VCRC B-471) is what causes the mosquitocidal action. The pupae are more susceptible to the CLP this strain produces than the larvae. The fall order of susceptibility is comparable to that of P. fluorescens (I. Geetha *et al*;2008). The CLPs can withstand a wide range of temperatures, pH levels, salinities, UV rays, and even prolonged exposure to sunshine (I. Geetha *et al*; 2010) and are mammals and aquatic life safe (K. Das *et al*;2006, A.M. Manonmani *et al*; 2011, J. L. Rodríguez-Chávez *et al*;2019). This mosquitocidal CLP has been identified as a surfactant and is a potent biosurfactant (I. Geetha *et al*;2010). Thisis the first account of a spore-forming bacteria that is effective against all stages of the mosquito life cycle, but particularly the pupal stage (A.M. Manonmani *et al*;2011, S. Bhuvaneswari *et al*;2015). Its mechanism of action on mosquitoes is still unknown.*B. subtilis* can be kept as spores for prolonged periods of time because it is a gramme positive, non-pathogenic species (K. S. Blackwood *et al*;2004). *B. subtilis* is the genetic model organism and the release of its genome sequence of the 4,214,810 base pairs is an important landmark (F. Kunst *et al*;1997).

INTRODUCTION

1.5 History of Antibacterial Agent

An Antibacterial agent is a "naturally occurring, semi-synthetic, or synthetic substance that exhibits Antibacterial activity (kills or inhibits the growth of microorganisms) at in vivo concentrations"(Nataliya Roth *et al*;2018). Bartolomeo Gosio (1863-1944), an Italian medical scientist, described the first antibiotic. In 1893, Gosio investigated why many underprivileged individuals in Southern Europe and the southern United States contracted pellagra. This social class's food was primarily comprised of maize, which was considered to be fungal infected. Theintake of this contaminated maize was thought to produce pellagra (Sydenstricker 1958; Bentley2000; Nord 2010). Photosensitive dermatitis, diarrhea, dementia, and mortality are all clinical symptoms of pellagra. Gosio isolated and cultured Penicillium breve-compactum from maize, yielding a crystalline product in the form of a filtrate. He discovered that this chemical had antibiotic efficacy against *Bacillus anthracis*, the anthrax causing agent. As a result, Gosio wasthe first (K.I. Mohr *et al*;2016).

1.6 Resistance to Antibacterial agents

Antibiotics have appropriately been dubbed "miracle drugs," yet sixty years of antibioticusage and misuse has resulted in rising resistance rates for most antibiotic-and-bacteria combinations. Indeed, bacterial adaptation evolution has been so successful that many

bacterial illnesses are virtually untreatable with antibiotics. Any resistance mechanism alters the bacterium's ability to endure the antibiotic, as well as its interaction with the host and environment. Thus, understanding the resistance mechanisms, the rate at which resistant variations evolve, and how antibiotic resistance affects the entire bacterial lifecycle is required to implement reasonable methods to minimize resistance development (D.I. Andersson *et al*; 2003).

Escherichia coli is a bacterium with a unique role in the microbiological world since it may cause serious illnesses in humans and animals and contributes significantly to the autochthonous microbiota of many hosts. The potential for the transmission of virulent and/or resistant *E. coli* from animals to people via a variety of routes, including direct touch, coming into contact with animal excrement, or through the food chain, is extremely concerning. Furthermore, *E. coli* is a significant source of resistance genes that may be to blame for failed medical interventions in both humans and animals. Over the last few decades, a rising number of resistance genes have been discovered in *E. coli* isolates, and many of these resistance genes were acquired through horizontal gene transfer. *E. coli* participates in the enterobacterial gene pool as both a donor and a recipient of resistance genes, enabling it to both take up and transmitresistance genes to other bacteria. Antibiotic resistance in *E. coli* is generally regarded as one of the biggest problems in both people and animals on a global scale and should be taken into account as a true public health concern. In *E. coli*, several genes of human and animal ancestrygive resistance to β-lactams. Even though some of them, such blaTEM-1, are common in E. coli from animals, they only encode for narrow-spectrum β-lactamases that can inactivate penicillin’s and aminopenicillins. However, ESBL/AmpC genes have recently started to appear in *E. coli* that came from humans and other animals. Recently, it has been discovered that *E. coli* with animal origins occasionally have genes that code for carbapenemases. The next subsections go into greater detail on ESBLs, AmpCs, and carbapenemases due to the importance of these last two types of β-lactamases (L. Poirel *et al*; 2018).

Important antibacterial medicines, quinolones and fluoroquinolones are used to treat a variety of infections in both humans and animals. It is known that they are bactericidal to almost all bacteria. However, other mechanisms such as decreased permeability of the outer membrane, protection of the target structures, or upregulated efflux pumps may also play a role.Resistance to these Antibacterial agents is typically caused by mutations in the drug targets, namely the

genes for DNA gyrase and topoisomerase IV (K.L. Hopkins *et al*;2005).

Aminoglycosides are medications with natural origins whose producers are found in thegenera Streptomyces (J. Devies *et al*;1997, Y. Doi *et al*;2016) and Micromonospora. They are frequently used in conjunction with another Antibacterial (typically a -lactam) to take advantage of their rapid bactericidal action for treating complicated infections in both humans and animals, including companion animals and animals used for food production. Neomycin and streptomycin derivatives are the most often utilized compounds in veterinary medicine. Additional antibiotics utilized include gentamicin, kanamycin, and paromomycin. Only infections in horses and pets should be treated with amikacin (L. Poirel *et al*;2018).

Aminoglycosides interfere with translation in both Gram-negative and Gram-positive bacterial species, having an impact on a wide range of pathogens (D. Fourmy *et al*;1998). The therapeutic efficacy of these crucial molecules may be restricted by two main problems, the first of which is related to their toxicity. On the basis of current developments in the knowledge of aminoglycoside pharmacodynamics, however, this problem is controlled by appropriate therapy regimens. The second problem is the global spread of bacterial resistance associated with the use of aminoglycosides. is only used to treat infections in horses and pets, and the subsections that follow give an overview of the mechanisms behind resistance toaminoglycosides and their epidemiology in *E. coli* of animal origin (L.Poirel *et al*;2018).

The synthesis of peptidoglycans is aided by the inhibition of the MurA enzyme by fosfomycin. In veterinary medicine, fosfomycin is only used to treat infections brought on by a variety of Gram-positive and Gram-negative bacteria, including *E. coli*, primarily in piglets and broiler chickens (M.E.Falagas *et al*;2016). There have been two main mechanisms for fosfomycin resistance identified: (i) mutations in the glpT and uhpA/T genes, which encode proteins involved in the fosfomycin uptake system, and (ii) the acquisition of fosfomycin-modifying enzymes like the metalloenzymes FosA, FosB, and FosX or the kinases FomA and FomB (L.L. Silver *et al*;2017). Most fos-like genes are plasmid-borne, and plasmids carrying the fos genes frequently carry additional resistance genes (A.Lupo *et al*;2018, X.Wang *et al*;2018) that raise the possibility of coselection of fosfomycin resistance under selective pressure by further antibiotics.

Tetracyclines are widely used in veterinary medicine. A summary of sales data in the 25 European Union and European Economic Area countries revealed that tetracyclines

accounted for 37% of the total sales of veterinary Antibacterial agents, followed by penicillins (23%) (K.Grave *et al*;2014). As a consequence of the selective pressure imposed by the widespread use of tetracyclines, many bacteria—including E. coli—have developed tetracycline resistance. According to the tetracycline resistance gene nomenclature centre (Tetracycline and MLS Nomenclature (washington.edu), nine tetracycline efflux genes [tet(A), tet(B), tet(C), tet(D), tet(E), tet(G), tet(J), tet(L), and tet(Y), tetracycline resistance genes have been found in E. coli, including tet(M), tet(W), and tet(X), which code for ribosome protectingproteins and an oxidoreductase that inactivates tetracyclines, respectively. Tetracycline resistance is primarily caused by two processes in *E. coli* of animal origin: (i) active efflux by proteins of the main facilitator superfamily, and (ii) ribosome protection. These 12 tet genes are not all present in *E. coli* from animal origins, as was discovered by a PubMed search for tetracycline resistance genes in *E. coli* of animal origin. The distribution of tet genes in *E. coli*from different animal sources is illustrated in the examples that follow.

Different processes in the production of folic acid are inhibited by sulfonamides and trimethoprim, two synthetic Antibacterials. Each of these drugs has bacteriostatic effects, but when combined with trimethoprim, sulfonamides have synergistic bactericidal effects on susceptible organisms; as a result, the drug combination is known as a "potentiated" sulfonamide. Both humans and animals have long utilized sulfonamides and trimethoprim.

Acquired resistance mechanisms have been discovered frequently, mostly as a result of (i) mutational changes in the target enzymes' genes, dihydropteroate synthase or dihydrofolate reductase, respectively, or (ii) the acquisition of sul genes for dihydropteroate synthases that are resistant to sulfonamides or dfr genes for dihydrofolate reductases that are resistant to trimethoprim (E. V. Duijkeren *et al*;2018).

1.7 Phenyl Benzoate

An organic compound called phenyl benzoate, which is white and powdery, belongs to the large class of substances known as esters. At normal temperature, the compound is solid; but, when brought down to a low enough temperature, it can become an oily liquid. Numerous polyesters, including those used in heavy industries and garments, can be made with phenyl benzoate. The creation of liquid crystal displays is one application for which the electrical properties of phenyl benzoate are utilized. Particularly at low temperatures, phenyl benzoate-based liquid crystals exhibit outstanding compatibility features with other substances used in

liquid crystal displays, such as biphenyl, phenyl cyclohexane, bicyclohexane, and fluorine kinds. Phenyl benzoate, which is recognized as an appropriate starting material for optical components, is used in particular to manufacture high-quality lenses for still and moving picturecameras (https://drugs.ncats.io/drug/B8A3WVZ590) 11-05-2023; 7:32pm.

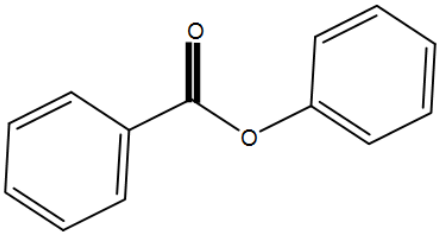

Figure 1.2: Structure of Phenyl Benzoate.

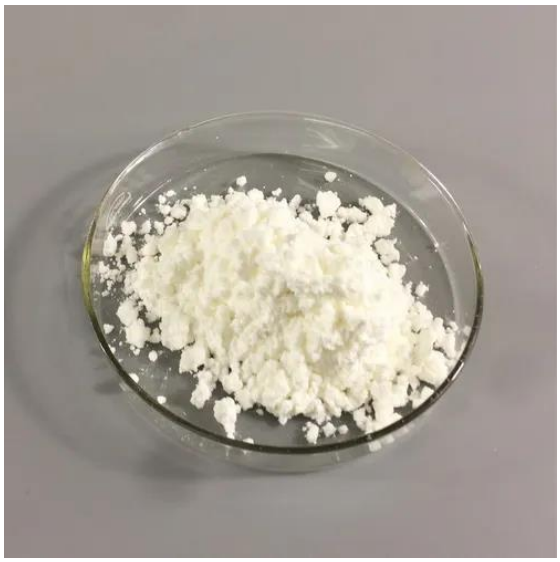

Figure 1.3: Phenyl Benzoate powder.

According to the Schotten-Baumann method of benzoylation, phenyl benzoate is made by reacting phenols with benzoyl chloride while agitating the reaction mixture. This is done in the presence of aqueous sodium hydroxide. Because it is water-insoluble, the solid benzoyl compound separates off under these conditions and benzoylation occurs without issue. The sodium phenoxide that is created when phenols are subjected to Schotten- Baumann benzoylation first dissolves in sodium hydroxide and is then benzoylated to form phenyl benzoate. Phenyl benzoate is insoluble in water but soluble in organic solvents like ethanol andchloroform (M.M. Thawkar *et al*;2022).

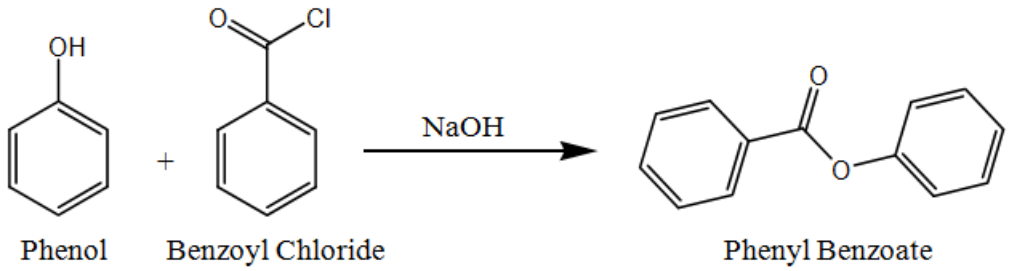

Figure 1.4: Reaction for the synthesis of phenyl benzoate.

In this study, we concentrated on the synthesised Phenyl benzoate derivative medication and came to a conclusion about its antibacterial efficacy. Both humans and animals can contract infections and diseases from a variety of species, including bacteria, fungus, and viruses for the Antibacterial activity against *Bacillus subtilis* and *Escherichia coli* (*B. subtilis, E. coli*), it contains both gramme positive and gramme negative microorganisms.

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2 LITERATURE REVIEW

2.0 Literature review

Manthan M. Thawkar *et al*;2022 reported that in this study, sodium hydroxide is utilized to benzoylate phenol and benzoyl chloride into phenyl benzoate using the Schotten-Baumann method. This phenyl benzoate molecule was described using a variety of qualitative and quantitative techniques. Many asthmatic and whooping cough treatments contain phenyl benzoate, which has spasmolytic and vasodilator effects. Benzyl benzoate is also used as a topical scabicide, acaricide, and pediculicide in veterinary hospitals. The antibacterial effectiveness of the substances against E. coli and S. aureus was on par with butanol and chloroform at the same concentration. The cup-and-plate method was used to ascertain the antibacterial activity and synthesis in the study.

Robert Hooke;1665 said that in order to discover microscopic life, Hooke and Leeuwenhoek employed microscopes to magnify items by a factor of 25–250 between 1665 and 1683 for this endeavor. Since then, the study of bacteria's role in infectious diseases and chemical recycling has centered on microscopy.

Escherichia coli is the most well-known gastrointestinal pathogen and a typical memberof the microbiota of the human stomach. Its four primary pathotypes are enterotoxigenic, enteroinvasive, enteropathogenic, and enterohemorrhagic. Our expanding awareness of the harmful mechanisms held by these bacteria has allowed us to design logical techniques for the treatment and prevention of E. coli-induced diarrhoea. The history and evolution of bacterial pathogens in general are being better understood because to research on the virulence of E. coli(Roy M Robins-Browne *et al*;2002).

Molecular and cellular biology was covered. Shigellosis is an invasive condition that affects people's intestines, especially in tropical areas where Shigella flexneri causes the endemic form and Shigella dyseriteriae 1 causes deadly outbreaks. The disease, which spreads by the fecal-oral route in places with poor hygiene and sanitation, most frequently affects children (P. J. Sensonetti;1992).

This study assessed the larvicidal efficacy of cyclic lipopeptides (CLPs) against third instar Culex quinquefasciatus larvae released by two strains of *Bacillus subtilis*. The larvicidal effectiveness of these CLPs was rarely affected by physico-chemical variables as pH, incubation temperature, heating, and sunlight exposure. These characteristics can be used to create a safer biopesticide that effectively controls mosquito larvae (K. Das *et al*;2006).

Described a bacillus that kills mosquitoes. 460 samples of soil, leaf, and water were taken from the Indian island group of Andaman-Nicobar in order to isolate mosquitocidal bacteria. The mosquitocidal efficacy of three Bacillus strains, B469, B471 and B474, was quite promising. The identification of B469 and B471 was verified by molecular characterization and flagellar serotyping, respectively. Anopheles stephensi, Culex quinquefasciatus, and Aedes aegypti larvae and pupae were resistant to the mosquitocidal action of *B. subtilis* strains when tested in a bioassay using culture supernatants (I. Geetha *et al*;2007).

By using a partial gyrA sequence, a strain of *Bacillus subtilis* that was active againstmosquito larvae and pupae was recognised as *B. subtilis* subsp. subtilis. The presentation covered a

study on the relationship between growth and sporulation in the generation of the mosquitocidal toxin and the susceptibility status of several mosquito species. The Crude Mosquitocidal Toxin (CMT) was found to be more toxic to pupae of Anopheles stephensi, Culex quinquefasciatus, and Aedes aegypti than to larvae, with A. stephensi being the most toxic species. The highest level of biomass production (15.46 g/l) was reached after 48 hours, whereas the highest level of CMT production (1.12 g/l) was seen after 24 hours. Production of the mosquitocidal toxin was discovered to be related to vegetative growth rather than sporulation of the organism. In mosquito control programmes that use bacterial biop, the mosquitocidal toxins of *B. subtilis* could be a potential substitute (A. M. Manonmani *et al*;2007).

In this study, the biosurfactant surfactin was examined to ascertain its effectiveness against abiotic conditions, and the rpoB gene of *Bacillus subtilis* (VCRC B471) was amplified to confirm its subspecies (I. Geetha *et al*;2010).

Reported on cyclic lipopeptide from *Bacillus subtilis* that kills mosquitoes. Surfactin, acyclic lipopeptide produced by the *Bacillus subtilis* subsp. subtilis strain VCRC B471, was found to be effective against both mosquito larval and pupal stages. This study tried to boost the synthesis of the mosquitocidal metabolite by changing the standard medium. The LC50 values for the CS generated in the improved medium were 5.57 and 0.71 l/ml, respectively. TheCMM yield doubled in the improved medium. By using MALDI-TOF analysis, the CMM wasdetermined to be surfactin (I. Geetha *et al*;2011).

J. L. Rodríguez-Chávez *et al*;2019 reported that the crude lipopeptide from *Bacillus subtilis* Q11 was tested in this study for its ability to inhibit the germination of conidia and lessen the blue mould rot caused by Penicillium expansum in apple (Malus domestica Borkh) fruit. It was also tested in vitro and in vivo. In antagonistic tests, Rhizoctonia solani, Sclerotiumrolfsii, Penicillium expansum, Fusarium stilboides, Colletotrichum gleosporides, and Botrytis cinerea's mycelial growth were all able to be inhibited by the lipopeptide component of Q11, with stronger inhibition at higher lipopeptide doses. The treatment with the lipopeptide fractionin the fruit assay decreased the severity of the rot lesion, with the largest results at 80 g, which resulted in a size reduction of the lesion of more than 60%. The development of products for biological control of postharvest blue mould disease in apples may benefit from these substances.

S. Bhuvaneswari *et al*;2015 reported that a strain of *Bacillus subtilis* subsp. subtilis (VCRC B471) was reported to produce a cyclic lipopeptide (CLP) surfactin with mosquitocidal action. This study used inexpensive materials to raise the level of surfactin.

K. S. Blackwood *et al*;2004 published a report on Bacillus species identification was made. There is a need for an effective method of species differentiation for the Bacillus genus because it is a sizable diverse group. They perform PCR and sequencing on a 500-bp product that encompasses the V1 to V3 sections of the 16S rRNA gene using 65 of the 83 type strains of the genus to assess the validity of a sequence-based method for identification. The rpoB gene,which differs just by four nucleotides in B. cereus and B. anthracis, turned out to be the most effective secondary target. The 16S rRNA gene sequences produced in this study have been sent to be added to its database, which is available to the general public.

As per F. Kunst *et al*;1997, the most well-known gram-positive bacterium, *Bacillus subtilis*, has a 4,214,810 base pair genome that contains 4,100 protein-coding genes. A quarter of the genome relates to numerous gene families that have been significantly increased through gene duplication, but 53% of these genes are only present once. A significant percentage of the genetic capacity is also devoted to using carbon sources, such as molecules derived from plants. Given that Bacillus strains can produce significant amounts of enzymes used in industry, the discovery of five signal peptidase genes and a number of genes for the secretion machinery is significant. The presence of at least ten bacteriophages in the genome suggests that horizontal gene transfer has played a significant evolutionary role in bacteriophage infection.

The purpose of this review was to determine the kind and quantity of antibiotics used in chicken production as well as the degree of antibiotic resistance in *Escherichia coli* isolated from broilers. From national monitoring programmes and research projects carried out in significant poultry-producing regions, isolated data was gathered. The survey's findings demonstrated the lack of a unified strategy for tracking antibiotic use by animal species and evaluating resistance using the same technique. In all of the nations that were considered, tetracyclines, aminoglycosides, sulfonamides, and penicillins are approved for use in poultry. Except for ampicillin in the US, all nations have resistance rates in E. coli to various antibiotic families that are more than 40% on average (N. Roth *et al*;2019).

According D. I. Andersson;2003, bacteria have successfully adapted to antibiotics, which has

led to health issues. According to the available data, a number of processes, including compensatory evolution, free resistances, and genetic linkage, will contribute to the long-term preservation of resistant bacteria. To enable the development of new drugs to catch up with bacterial resistance, it is critical to slow the rate at which resistant germs arise and spread.

L. Poirel *et al*;2018 reported on Escherichia coli's antibiotic resistance. *Escherichia coli* multidrug resistance is a concerning problem that is increasingly seen in both human and veterinary medicine across the globe. The acquisition of extended-spectrum -lactamases, carbapenemases, 16S rRNA methylases, plasmid-mediated quinolone resistance (PMQR) genes, and mcr genes are the most troublesome methods. The widespread use of Antibacterialsin animal medicine contributes to coselection and persistence of resistances to cruciallyimportant Antibacterials in human treatment.

K. L. Hopkins *et al*;2005 displayed the citation Broad-spectrum antibiotics known as fluoroquinolones are efficient in treating a number of illnesses. Point mutations, decreased expression of outer membrane porins, or overexpression of multidrug efflux pumps can contribute to the spontaneous emergence of resistance. The recent identification of plasmid-mediated quinolone resistance may lead to horizontal resistance transfer between bacteria. To stop the development of resistant zoonotic and non-zoonotic bacterial infections, caution mustbe exercised to avoid overusing this significant class of antibiotics.

According to J. Davies *et al*;1997, bacterial resistance to drugs known as aminoglycosides. The superfamily of aminoglycoside-modifying enzymes has been implicated in the development of resistance to the aminoglycoside antibiotics, which are frequently used to treat bacterial infections. The mechanisms of resistance are now of renewed interest as a result of this.

Y. Doi *et al*;2016 said that gram-negative bacteria, where strains are forming that are resistant to several or almost all of the existing antibiotics, Antibacterial resistance is a serious public health and socioeconomic problem. In the 1990s, ESBL-producing Enterobacteriaceae were followed by the appearance and quick spread of carbapenemase-producing pathogens.

D. Fourmy *et al*;1998 reported that antibiotics called aminoglycosides can lead to incorrect reading of the genetic code and prevent translocation if they attach to ribosomal RNA at the aminoacyl-tRNA site (A-site). In this study, the structure of the A-site RNA in free formwas

characterised and compared to the structure of the paromomycin-RNA complex. Two generally conserved residues of the A site of the 16 S rRNA, A1492 and A1493, are shifted towards the minor groove of the RNA helix in the presence of antibiotic, according to a comparison of the free and bound conformations of the RNA. This points to a method via whichaminoglycosides affect translation.

Gram-negative infection was mentioned. Because of the fast rise in carbapenem- resistant Enterobacteriaceae (CRE), gram-negative resistance has become a global crisis. Utilising Colistin to fight CRE infections has resulted in CCRE infections, further jeopardisingour final line of protection. They assess the potential of intravenous fosfomycin for treating severe systemic infections brought on by Enterobacteriaceae that are multidrug resistant. To make intravenous fosfomycin the last line of defence against serious Gram-negative infections,more research is required (P. V. saiprasad *et al*;2016).

Given this study's goal to investigate the genetic epidemiology of carbapenem-resistantGram-negative bacterial (GNB) isolates from both community and hospital settings. Few Antibacterial substances are effective against bacteria that are resistant to carbapenem (A. Garg*et al*;2019).

As per A. Lupo *et al*;2018, the second-most frequent ESBL-encoding gene in Asian nations is blaCTX-M-55. It co-localizes with the fosA and rmtB genes on occasional epidemicplasmids outside of Asia. The dissemination of blaCTX-M-55 and atypical resistance genes invarious animal species was supported by a wide variety of E. coli clones and plasmid types, according to a global assessment of ESBLs in animals conducted in France between 2010 and 2013. Public health is a problem with this topic. the plasmids cfr-containing. 370 E. coli isolateswere found in Northeast China between June 2015 and April 2016 in pigs, chickens, and dairy cows. A total of 111 of them, including 109 isolates with the floR gene and 6 positives for cfr, were florfenicol resistant. High similarity was discovered between the IncX4-type pEC14cfr plasmid and two other cfr-harboring plasmids, pSD11 and pGXEC6, detected in swine E. coli isolates from southern China, according to a complete sequencing study of two cfr-carrying plasmids. In five provinces in southern and northern China, pEC14cfr-like plasmids have beendiscovered, indicating that pEC14cfr-like.

In this paper, researchers looked at the frequency of the cfr gene in *Escherichia coli* isolates from domestic animals in Northeast China and described plasmids that may beindicative of a

widespread cfr epidemic (X. Wang *et al*;2018).

Package-level data on sales of veterinary Antibacterial drugs from 25 EU member states and EEA countries for 2011 was gathered. The information was calculated to reflect sales of each package's active ingredient in metric tonnes. The animal biomass that might have been treated with Antibacterial drugs was represented by a population correction unit (PCU), and the indicator used to express sales was milligrammes of active substance per PCU (K. Grave *et al*;2014).

A study on bacteria's susceptibility to antibiotics was published. The mechanisms of resistance to almost all Antibacterial agents in bacteria of animal origin are covered in this chapter. Enzymatic inactivation, decreased intracellular accumulation, and adjustments at the cellular target locations are the key processes. This is an updated version of the relevant chapter from the 2006 publication Antibacterial Resistance in Bacteria of Animal Origin. To reflect the developments in understanding since 2006, new sections have been included for oxazolidinones, polypeptides, mupirocin, ansamycins, fosfomycin, fusidic acid, and streptomycins. The chapters for the remaining classes of Antibacterial drugs have also been entirely revised (E. V. Duijkeren *et al*;2018).

M. M. Thawkar *et al*;2022 reported on phenyl benzoate's antibacterial properties. In this study, the Schotten-Baumann benzoylation process was used to create phenyl benzoate from phenol and benzoyl chloride with the use of sodium hydroxide. When used against E. coli and S. aureus, the products had antibacterial activity that was comparable to that of butanol and chloroform at the same concentration. Many asthmatic and whooping cough medications contain it because of its vasodilating and spasmolytic properties. In veterinary hospitals, it is also applied topically as a scabicide, acaricide, and pediculicide.

As per R. Chintakunta *et al*;2020, for research of synthetic derivatives, online software tools like Molinspiration and ChemDraw have been used. *B. subtilis* and P. aeruginosa minimum inhibitory concentration (MIC) values were reported, and Molinspiration scores and benzimidazole ligands were created. Different derivatives' bioactivity scores were recorded, along with physical parameters like solubility and melting point. The MIC technique (aerobic)was used to carry out the antibacterial activity.

The coronavirus responsible for the 2019 coronavirus illness (COVID-19) is the severe acute

respiratory syndrome coronavirus-2 (SARS-CoV-2). The primary protease (Mpro) of COVID-19 may be inhibited by the unique natural metabolites ursolic acid, carvacrol, and oleanolic acid, according to this study. Three ligands bound to the protease during 50 ns of molecular dynamic (MD) simulations, which included molecular docking and MD simulations. Ursolic acid, carvacrol, and oleanolic acid, the three molecules, all passed the ADME property and Lipinski's rule of five. The three phytochemicals may act as possible inhibitors in regulatingthe Mpro protein's function and preventing viral replication, according to this study's primary premise (A. Kumar *et al*;2021).

D. Joshi *et al*;2014 reported on antibacterial agent derivatives. To create bioactive compounds with strong antibacterial properties, electron-withdrawing and electron-donating substituents were added to benzimidazole analogues throughout the synthetic process. The compounds' capabilities against Gram-positive and Gram-negative bacteria as well as fungus were tested, and they were validated by spectrum characterization. According to the results of the SAR analysis, derivatives with electron-withdrawing functional groups were more biologically active than those with electron-donating functional groups.

As per R. Chintakunta *et al*;2020, the o-phenylenediamine is a flexible precursor for a number of chemicals. It was created using the amino acids glycine, alanine, aspartic acid, and L-proline, and at concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.6, 0.8, 0.4, and 0.2 g/ml, it had potential antibacterial activity against *Bacillus subtilis* and Pseudomonas aeruginosa. Ciprofloxacin was the go-to medication. The procedure of recrystallization with ethanol was used to carry out the synthesis of benzimidazole derivatives and purify them. Melting point, TLC, and spectroscopic techniques were used to describe substituted derivatives.

S. B. Bai *et al*;2022 reported on antibacterial activity. Drugs called antibiotics are usedto stop or treat bacterial infections. Antibiotic resistance is escalating on a global scale, yet it can be overcome using resistance-busting drugs or stronger antibiotics. Enzymatic breakdown of antibacterial medicines, alterations to bacterial proteins, and modifications to membrane permeability to antibiotics are three pathways of Antibacterial resistance. The most crucial components of the nucleic acids employed in the chemotherapy for AIDS are pyrrimidines, which must be synthesised into novel medications.

A report on antibacterial activity conducted in vitro. Barbituric acid, aromatic aldehydes, active methylene compounds, and pyrano[2,3-d] pyrimidine/pyrano[2,3-d] uracil derivatives

were synthesised in the presence of a dibutylamine (DBA) catalyst in ethanol. According to antibacterial testing, the inclusion of heteroaryl, cyano, and amino groups on the uracil skeleton boosts the product's biological vigour and penetrating strength on bacterial cellwalls. The findings on antibacterial activity revealed some intriguing information regarding the structure-activity relationship (SAR) of manufactured compounds (A. R. Bhat;2018).

According to N. Kapoor *Et al*;2006, the nucleoside reverse transcriptase inhibitors lamivudine, stavudine, and nevirapine in pharmaceutical fixed dose combinations are simultaneously determined quantitatively using an accurate, sensitive, and specific reversed phase high performance liquid chromatographic method (RP-HPLC) described in this paper. Gradient elution was used to carry out the procedure on a C-18 column using two mobile phasecomponents: 80% of a 10 mM acetate buffer, pH-3.5 glacial acetic acid, 20% methanol, and 50% acetonitrile, 50% isopropyl alcohol. Lamivudine, stavudine, and nevirapine had average retention durations of 5.9, 8.8, and 14.2 minutes, respectively. For each analyte, the calibration curves were linear throughout the range. The procedure is exact and accurate, yielding recoveries of lamivudine in the range of 98.7-100.4%, stavudine in the range of 99.2-100.6%, and nevirapine in the range of 98.3-100.3%.

S. Aggarwal *et al*;2010 displayed the citation Poorly soluble pharmaceuticals have beenmade more soluble and bioavailable using solid dispersion methods, but their commercial usagehas been constrained by manufacturing challenges and stability issues. Surfactants have recently been added to formulations to stabilise them, and new production procedures have beencreated to lessen the shortcomings of the original method. This review highlights recent revivaland the preeminent method to increase solubility or the rate of dissolution.

Chi-Yuan Wu *et al*;2005 demonstrated the research on the Biopharmaceutics Classification System (BCS), which was created to forecast the pharmacokinetic performance of pharmaceuticals in vivo. This study raises the possibility that a modified version of the system could be helpful in predicting overall drug disposition, including routes of drug elimination, effects of efflux and absorptive transporters on oral drug absorption, when transporter-enzyme interactions will result in clinically significant effects, the direction, mechanism, and significance of food effects, and transporter effects on postabsorption systemic drug concentrations after oral and intravenous dosing. Studies conducted in our lab lend validityto these assumptions.

In this investigation, six isolates of Fusarium were used to determine the antifungal effects of ZnO and ZnO-EO (Zataria multiflora Boiss essential oil loaded on ZnO) materials.

Z. multiflora essential oil (EO)'s chemical makeup was investigated using GC-MS, and the physiochemical characteristics of artificial materials were investigated using SEM, BET, FT-IR, TGA, EDX, XRD, and DLS studies. In accordance with the findings, ZnO-EO nanocomposite exhibited fungistatic activity against all investigated fungi with the exception of F. oxysporum f.sp. lentis and fungicidal activity against F. graminearum at a concentration of 1000 ppm. The MGI of the ZnO-EO nanocomposite increased by 66.33% when compared to Z. multiflora EO and by 42.70% when compared to pure ZnO. The ZnO-EO nanocomposite can be regarded as a bio-rational efficient in light of the most recent studies (S. Enayati *et al*;2021).

H. Awad *et al*;2015 reported on ionisation in mass spectrometry. The most widely used mass spectrometry (MS) techniques are matrix-assisted laser desorption ionisation (MALDI) and electrospray ionisation (ESI), which are essential tools in the life sciences. The atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photo ionisation (APPI) processes are more appropriate for these jobs because ESI is unable to effectively ionise nonpolar substances. In 2004, ambient MS, in which ionisation takes place at the sample in its natural form, was developed. An overview of the primary ionisation techniques and ion production mechanisms is given in this article.

S. Chandrasekaran *et al*;2005 displayed the novel's references from 3-aryl-1-thien- 2ylprop-2-en-1-ones and guanidine hydrochloride, 2-amino-6-aryl-4- (2-thienyl) pyrimidines were created. There in vitro antibacterial effectiveness was assessed. Contrary to ciprofloxacin and norfloxacin, which were used as references, compounds 5a-e were almost completely ineffective against Gram-negative bacteria. The most effective compounds against Gram-positive bacteria were compounds 5c and e.

Y. Traoré *et al*;2018 demonstrated the analgesic activity using the QSAR reference approach. A model that connects analgesic action as represented by logAA, energy, dipole moment, and lipophilic coefficient ACD/logP was discovered through a QSAR investigation of 20 tri-substituted pyrimidine derivatives. Statistical indicators demonstrated internal performance and stability, and it was demonstrated that exterior prediction was accurate brand-new tri-substituted pyrimidine derivatives outperformed the research compounds in terms of

analgesic efficacy.

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AIM AND OBJECTIVE

3.0 Aim and objective

3.1 Aim: To design, synthesize and determination of in-silico antibacterial activity of various derivatives of phenyl benzoate.

3.2 Objective

Following are the main objectives of present study:

- To design a library of compounds for In-silico screening.
- To carry out the In-silico screening of phenyl benzoate derivatives.
- To select the compounds from the In-silico screening.
- To carry out the optimization and synthesis of selected phenyl benzoate derivatives.
- Characterization and purification of synthesized phenyl benzoate derivatives.
- To study the in-silico antibacterial activity of synthesized phenyl benzoate derivatives.

3.3 Plan of work

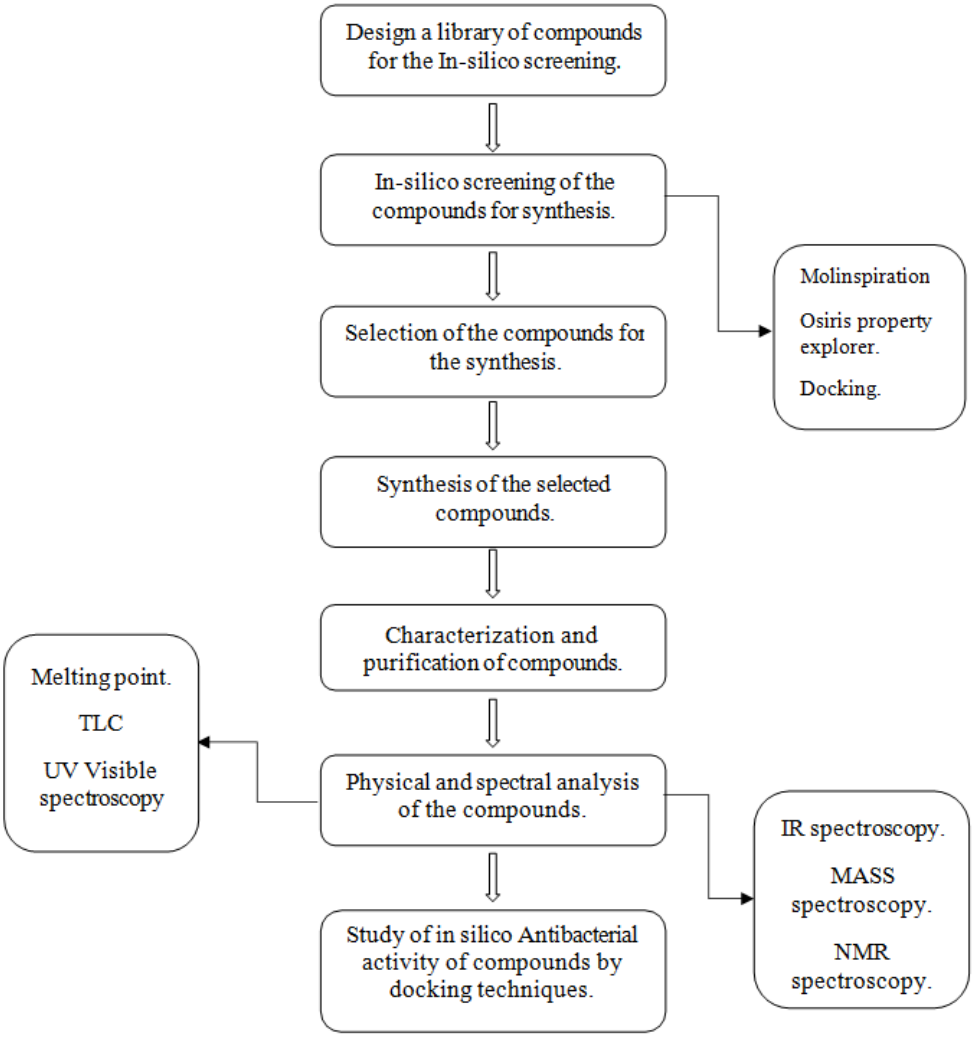

Figure 3.1 – Plan of work.

4.0 In-Silico Screening

The field of bioinformatics is the fusion of biology, computer science, and information science. It uses cutting-edge computing methods to handle and evaluate biological data. "Performed on the computer or through computer simulation" is what the phrase "in silico" means. Pedro Miramontes coined the phrase "in silico" in the first instance. He conducted biological research primarily using a computer. There is an increasing need for computational tools that can locate and analyze the active sites and provide potential drug molecules that can specifically bind to these sites as structural analogues of more protein targets are presented through bioinformatics, NMR, and crystallography methods. Everyone's contributions are crucial in the fight against diseases like HIV, TB, and malaria that are deadly. The time and money needed to develop a new medicine are excessive and unacceptably high. Computer-based modelling called "in silico" uses cutting-edge technology to help identify therapeutic

targets and create new drugs (R. Chintakunta *et al*;2020, K. Anuj *et al*;2020 and D. Joshi *et al*;2013).

4.1 Molinspiration

The sum of the fragment-based aids and correction factors is measured by Molinspiration Log P. All organic and organometallic compounds are processed using this technique. The total of all contributions from polar fragments with an O or N center is known as the topological polar surface area (TPSA). Drug absorption, intestinal absorption, bioavailability, and blood-brain barrier penetration are all included in this useful description. Based on group contributions, molecular volume is calculated. mostly compounds that are similar to drugs. A measure of molecular flexibility is the ability of some bonds to rotate. It is an excellent way to describe the oral bioavailability of medications. A non-terminal heavy atomis the only type of single non-ring bond that may be considered a rotatable bond. According tothe Lipinski rule of five, most drug-like compounds have log P values greater than or equal to 5, molecular weight greater than or equal to 500, more hydrogen bond acceptors than or equal to 10, and fewer hydrogen bond donors than or equal to 5. If a molecule violates more than oneof these guidelines, bioavailability issues may have arisen. The five-point guideline is referred to as the Lipinski guideline. The java software called Molinspiration is a toolkit for computing molecular properties that is used in batch processing of large numbers of molecules. It can process data at a rate of about 10,000 molecules per minute and connects to the internet via aweb interface (A. R. Bhat; 2018, R. Chintakunta *et al*;2020 and S. B. Bai *et al*;2022).

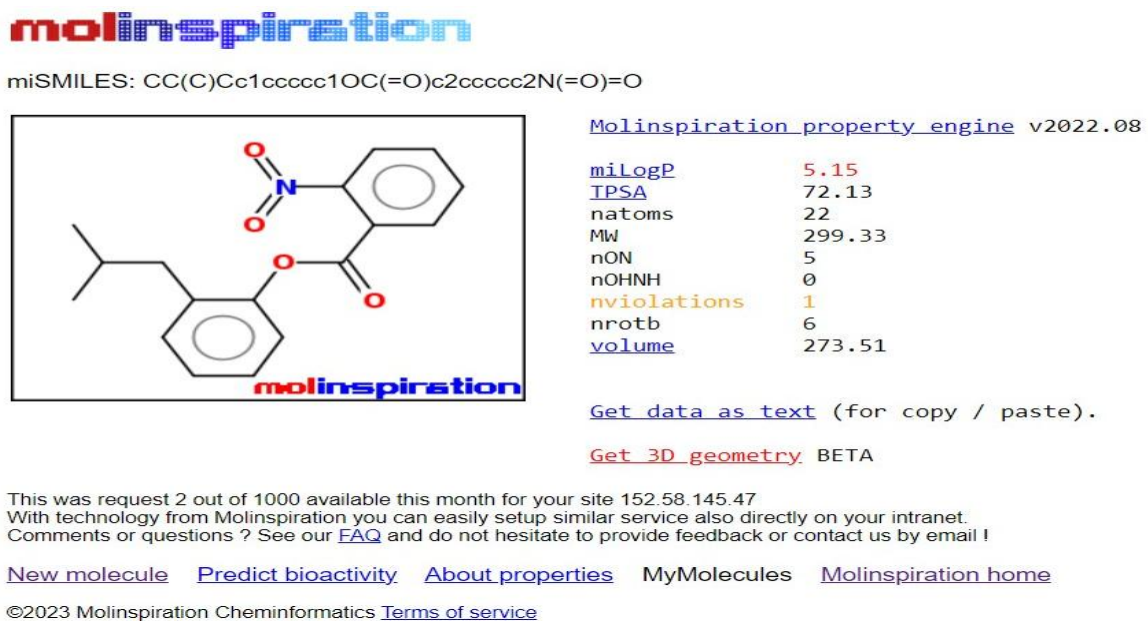

Figure 4.1: Molinspiration of a compound.

Table 4.1: Calculated physicochemical properties of molecules.

	MOLINSPIRATION							
sl no.	COMPOUNDS	miLogP	TPSA	natoms	MW	nON	nOHNH	nviolations
1	A.a.B.α	4.3	72.13	19	257.25	5	0	0
2	A.b.B.α	4.76	72.13	20	271.27	5	0	0
3	A.c.B.α	5.15	72.13	21	285.3	5	0	1
4	A.d.B.α	5.71	72.13	22	299.33	5	0	1
5	A.e.B.α	5.15	72.13	22	299.33	5	0	1
6	A.f.B.α	4.42	72.13	20	291.69	5	0	0
7	A.g.B.α	4.63	72.13	21	305.72	5	0	0
8	A.h.B.α	5.15	72.13	22	319.74	5	0	1
9	A.i.B.α	5.42	72.13	23	333.77	5	0	1
10	A.a.B.β	4.21	26.3	16	212.25	2	0	0
11	A.b.B.β	4.67	26.3	17	226.28	2	0	0
12	A.c.B.β	5.06	26.3	18	240.3	2	0	1
13	A.d.B.β	5.62	26.3	19	254.33	2	0	1
14	A.e.B.β	5.06	26.3	19	254.33	2	0	1
15	A.f.B.β	4.43	26.3	16	232.67	2	0	0
16	A.g.B.β	4.33	26.3	17	246.69	2	0	0
17	A.h.B.β	5.06	26.3	19	274.75	2	0	1
18	A.i.B.β	5.33	26.3	20	288.77	2	0	1
19	A.1.a.B.α	3.71	84.16	20	272.26	6	1	0
20	A.1.b.B.α	4.08	84.16	21	286.29	6	1	0
21	A.1.c.B.α	4.58	84.16	22	300.31	6	1	0
22	A.1.d.B.α	5.14	84.16	23	314.34	6	1	1
23	A.1.e.B.α	4.83	84.16	23	314.34	6	1	0
24	A.1.f.B.α	4.3	84.16	21	306.7	6	1	0
25	A.1.g.B.α	4.31	84.16	22	320.73	6	1	0
26	A.1.h.B.α	4.58	84.16	23	334.76	6	1	0
27	A.1.i.B.α	4.85	84.16	24	348.79	6	1	0
28	A.1.a.B.β	3.62	38.33	17	227.26	3	1	0
29	A.1.b.B.β	3.99	38.33	18	241.29	3	1	0
30	A.1.c.B.β	4.49	38.33	19	255.32	3	1	0
31	A.1.d.B.β	5.05	38.33	20	269.34	3	1	1
32	A.1.e.B.β	4.74	38.33	20	269.34	3	1	0
33	A.1.f.B.β	4.21	38.33	18	261.71	3	1	0
34	A.1.g.B.β	4.22	38.33	19	275.74	3	1	0
35	A.1.h.B.β	4.49	38.33	20	289.76	3	1	0
36	A.1.i.B.β	4.76	38.33	21	303.79	3	1	0
37	A.I.B.α	4.66	72.13	19	322.11	5	0	0
38	A.II.B.α	3.81	118	21	288.21	8	0	0
39	A.III.B.α	0.84	126.5	22	323.28	8	1	0
40	A.IV.B.α	3.64	89.2	20	271.23	6	0	0
41	A.V.B.α	3.84	109.4	21	287.23	7	1	0
42	A.VI.B.α	4.53	72.13	19	277.66	5	0	0
43	A.VII.B.α	3.75	89.2	21	285.25	6	0	0
44	A.VIII.B.α	3.19	92.38	20	273.24	6	1	0
45	A.IX.B.α	3.44	95.98	20	270.24	6	1	0

46	A.X.B.α	0.35	112.3	21	286.22	7	0	0
47	A.XI.B.α	3.2	92.36	19	259.22	6	1	0
48	A.I.B.β	4.57	26.3	16	277.12	2	0	0
49	A.II.B.β	3.72	72.13	18	243.22	5	0	0
50	A.III.B.β	0.75	80.67	19	278.29	5	1	0
51	A.IV.B.β	3.55	43.39	17	226.23	3	0	0
52	A.V.B.β	3.75	63.6	18	242.23	4	1	0
53	A.VI.B.β	4.43	26.3	16	232.67	2	0	0
54	A.VII.B.β	3.66	43.38	18	240.26	3	0	0
55	A.VIII.B.β	3.1	46.53	17	228.25	3	1	0
56	A.IX.B.β	3.35	50.16	17	225.25	3	1	0
57	A.X.B.β	0.26	66.43	18	241.22	4	0	0
58	A.XI.B.β	3.11	46.53	16	214.22	3	1	0
59	A.XII.B.β	3.3	46.53	16	214.22	3	1	0
60	A.XIII.B.β	4.99	26.3	19	248.28	2	0	0

4.2 Osiris Property Explorer

Although structure-based design is now fairly common, many potential drugs never make it to the clinic due to ADME-Tox liabilities. The cytochromes P450 enzyme class is a significant one and is in charge of many ADMET issues. Many negative medication reactions can be caused by these inhibitors or by the generation of undesirable metabolites. Online access to Osiris, the most significant programme, is already available. With the recent publications of the drug design combination of various pharmacophore sites, using Spiroheterocyclic structure, it is now possible to predict activity and/or inhibition with increasing success in various targets (bacteria/virus or bacteria/fungus or virus/fungus). An example of this combined electronic/structure docking process will be provided. It is possible to measure the function performed by various organic groups in facilitating or impeding a drug's ability to link with DNA by looking at the very well-behaved mutagenicity of numerous synthetic compounds categorized in the data base of the Swiss company CELERON (A. R. Bhat;2018).

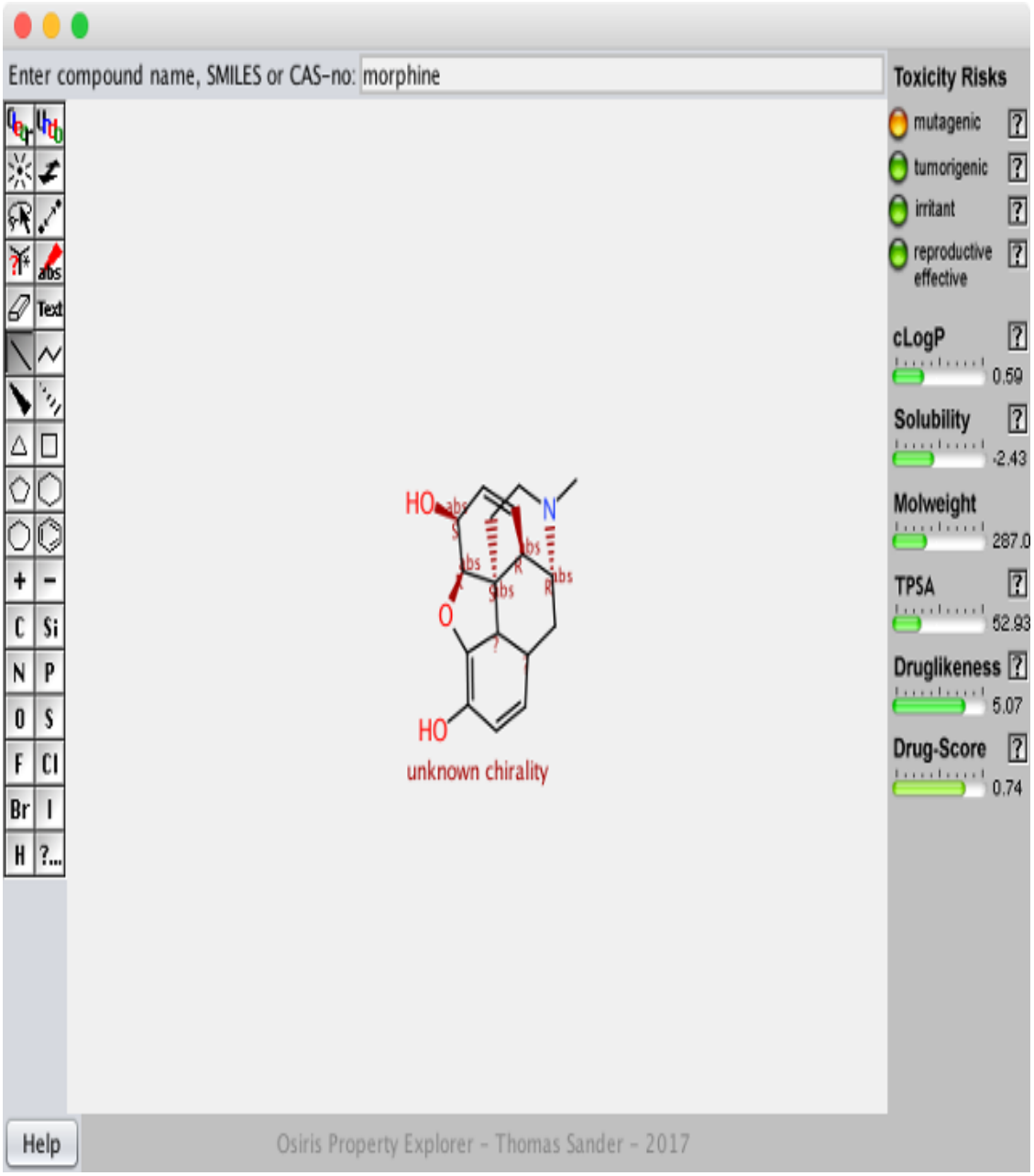

Figure 4.2: Osiris property of a compound.

Table 4.2: Osiris properties of molecule.

		Osiris property			
Sl. No.	**Compounds**	**Mutagenic**	**Tumorigenic**	**Irritant**	**Reproductive effect**
1	**A.a.B.α**	-	-	+	-
2	**A.b.B.α**	-	-	-	+++
3	**A.c.B.α**				
4	**A.d.B.α**				
5	**A.e.B.α**				
6	**A.f.B.α**	+++	+++	-	+++
7	**A.g.B.α**	++	+++	-	+++
8	**A.h.B.α**				

9	A.i.B.α				
10	A.a.B.β	-	-	+++	-
11	A.b.B.β	-	-	-	+++
12	A.c.B.β				
13	A.d.B.β				
14	A.e.B.β				
15	A.f.B.β	+++	++	-	+++
16	A.g.B.β	++	+++	-	+++
17	A.h.B.β				
18	A.i.B.β				
19	A.1.a.B.α	-	-	+++	-
20	A.1.b.B.α	-	-	-	-
21	A.1.c.B.α	-	-	-	-
22	A.1.d.B.α				
23	A.1.e.B.α	-	-	-	-
24	A.1.f.B.α	-	-	-	-
25	A.1.g.B.α	+++	+++	-	+++
26	A.1.h.B.α	++	+++	-	+++
27	A.1.i.B.α	++	+++	-	+++
28	A.1.a.B.β	-	-	-	-
29	A.1.b.B.β	-	-	-	-
30	A.1.c.B.β	-	-	-	-
31	A.1.d.B.β				
32	A.1.e.B.β	-	-	-	-
33	A.1.f.B.β	-	-	-	-
34	A.1.g.B.β	+++	+++	-	+++
35	A.1.h.B.β	++	+++	-	+++
36	A.1.i.B.β	++	+++	-	+++
37	A.I.B.α	-	-	-	++
38	A.II.B.α	-	-	-	-
39	A.III.B.α	-	-	-	-
40	A.IV.B.α	-	-	++	+++
41	A.V.B.α	-	-	-	-
42	A.VI.B.α	-	-	++	-
43	A.VII.B.α	-	-	-	-
43	A.VII.B.α	-	-	-	-
44	A.VIII.B.α	-	-	-	-
45	A.IX.B.α	-	-	-	-
46	A.X.B.α	-	-	-	-
47	A.XI.B.α	-	-	-	+++
48	A.I.B.β	-	-	-	-
49	A.II.B.β	-	-	-	-
50	A.III.B.β	-	-	-	-
51	A.IV.B.β	-	-	++	+++
52	A.V.B.β	-	-	-	-
53	A.VI.B.β	-	-	++	-
54	A.VII.B.β	-	-	-	-

55	**A.VIII.B.β**	**-**	**-**	**-**	**-**
56	**A.IX.B.β**	**-**	**-**	**-**	**-**
57	**A.X.B.β**	**-**	**-**	**-**	**-**
58	**A.XI.B.β**	**-**	**-**	**-**	**+++**
59	**A.XII.B.β**	**-**	**-**	**+**	**-**
60	**A.XIII.B.β**	**-**	**-**	**-**	**-**
60	**A.XIII.B.β**	**-**	**-**	**-**	**-**

4.3 REFERENCES

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5.0 MATERIALS AND METHODS

5.1 Chemicals and equipment

Table 5.1: List of chemicals used.

Chemicals used	**Source**
Phenol	Oxford
Benzoyl chloride	Oxford
Sodium hydroxide	Oxford
Resorcinol	Oxford
2-napthol	Oxford

Sulfuric Acid	Oxford
Nitric Acid	Oxford
Carbon Disulfide	Oxford
Aniline	Oxford
Methanol	Oxford
Ethanol	Oxford
Salicylic Acid	Oxford
Hydrochloric Acid	Oxford
Chloroform	Oxford
Cresol	Oxford
Benzoic Acid	Oxford
Benzene	Oxford
Diethyl ether	Oxford
Formaldehyde	Oxford
Bromine	Oxford
Acetone	Oxford

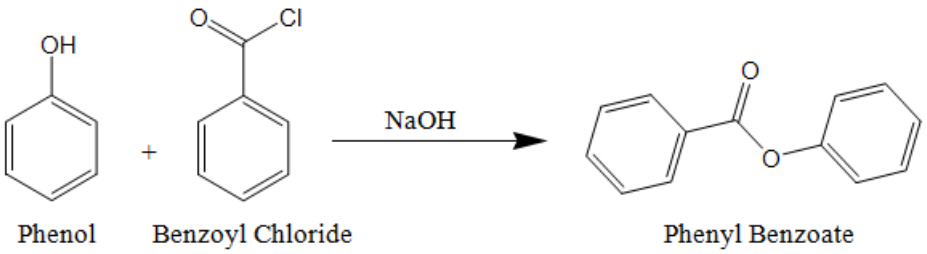
Table 5.2: List of instrument and equipment used.

Instrument and equipment	Model and make
Beaker	Borosilicate glass
Conical Flask	Borosilicate glass
Iodine Flask	Borosilicate glass
Measuring cylinder	Borosilicate glass
Vacuum filter	-
Melting point apparatus	-
UV-visible spectrophotometer	-
Weighing balance	-
FT-IR Spectrophotometer	Thermo nicolet iS10 FT-IR
NMR Spectrophotometer	Bruker
Mass Spectrophotometer	-
Micro Pipette	Borosilicate glass

5.2 Synthesis

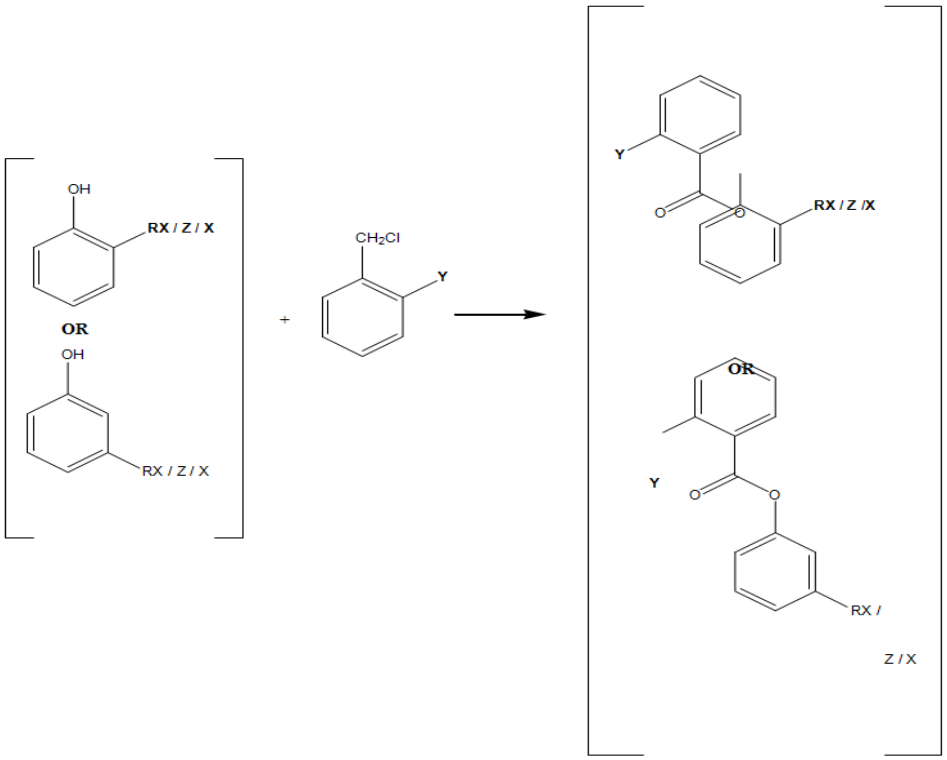
5.2.1 General Procedure

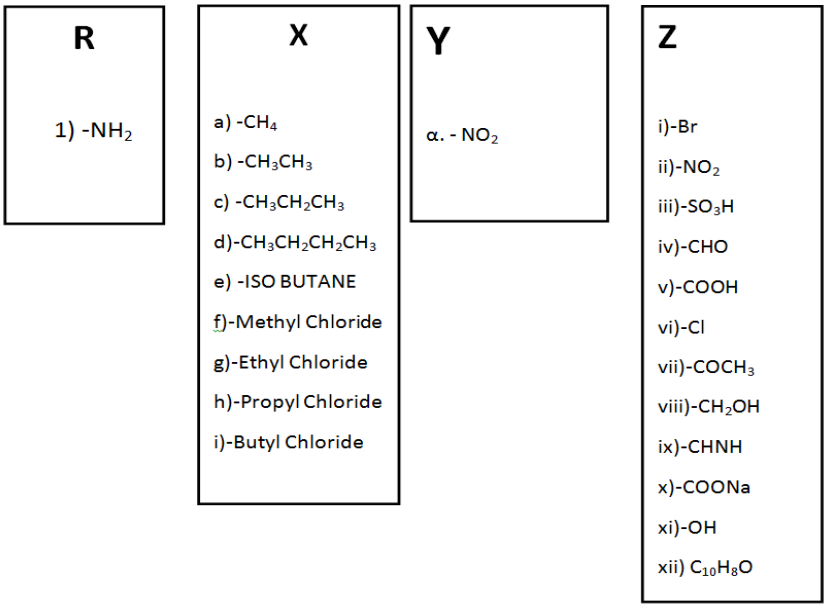
According to the Schotten-Baumann method of benzoylation, phenyl benzoate is made by reacting phenols with benzoyl chloride while agitating the reaction mixture. This is done in the presence of aqueous sodium hydroxide. Because it is water-insoluble, the solid benzoyl compound separates off under these conditions and benzoylation occurs without issue. The sodium phenoxide that is created when phenols are subjected to Schotten- Baumann benzoylation first dissolves in sodium hydroxide and is then benzoylated to form phenyl benzoate. Phenyl benzoate is insoluble in water but soluble in organic solvents like ethanol andchloroform (M.M. Thawkar *et al*;2022).



Scheme 1: Synthesis of Phenyl benzoate.

5.2.2 Compound Library





5.2.3 Chemistry and physicochemical characterization

5.2.3.1 Solubility

The preferred method of taking the dose form is orally. The bioavailability of the active ingredient upon oral delivery is the main issue. The term "solubility" refers to the greatest amount of solute that can dissolve in a given amount of solvent or solution at a certain temperature. Bioavailability increases as solubility does (Y. Qui *et al*;2011). The solubility defined as:

Table 5.3: Definition of solubility (Indian Pharmacopoeia; 1996).

Definition	Parts of solvent required for one part of solid
Very Soluble	< 1
Freely Soluble	1 – 10
Soluble	10 – 30
Sparingly Soluble	30 – 100
Slightly Soluble	100 – 1000
Very Slightly Soluble	1000 – 10,000
Insoluble	> 10,000

The BCS (Biopharmaceutics classification system) divides drugs into four types based on their solubility and permeability. Solubility challenges are faced in the Class II and Class IVof the BCS system (where dissolution becomes the rate limiting step for the absorption of drug) which comprises of newer generation of NSAIDs like Zaltoprofen, Aceclofenac, Flurbiprofen,their older congeners like Indomethacin, Ibuprofen, Ketoprofen and Diclofenac; anti-diabetics Gliclazide, Glipizide; newer calcium channel blockers (CCBs) like Nimodipine, Felodipine. Amidon *et al*. first proposed the BCS in 1995 (S. Agarwal *et*

al;2010).

Table 5.4: BCS Classification of Drug (C.Y. Wu *et al*; 2005).

Class	Permeability	Solubility	Examples
I	High	High	Metoprolol
II	High	Low	Neteglinide
III	Low	High	Cimetidine
IV	Low	Low	Hydrochlorothiazide

5.2.3.2 Thin layer chromatography (TLC)

The separation of a combination of unidentified substances has been accomplished using thin layer chromatography (TLC). TLC has been extensively utilised to optimise the reaction conditions, by which their target compounds are created effectively, particularly in organic synthesis laboratories. TLC's ability to identify unidentified chemicals is, however, limited (K.Matsumoto *et al*;1999). It was desirable to scale up to a preparative approach that could separate volumes ranging from 100 to 500 mg because TLC is effective with small amounts. The TLC adsorbents were not successfully used in packed columns. It took a long time to remove the substance from the column due to the resistance to flow through it. For horizontal column chromatography in a cellophane tube, a preparative method has been described. Regular 20 X 20 cm plates used for preliminary TLC were described. The capacity was inadequate for complex mixes. Using big plates of 10 by 15 inches and coated in an adsorbent that is 1 mm thick, the scale-up is completed. Approximately 55 grammes of silica gel or 120 grammes of aluminium oxide are used to surface a plate of this size (B. P. Korzun *etal*;1963).

5.2.3.3 Melting Point (MP)

Thomas Hoover Electronic Apparatus of melting point measurements were hand-me- down for determination of the (melting points) stated by the following work.

5.2.3.4 UV-Visible Spectroscopy

It is based on the Beer-Lambert law, which states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and path length. Ultraviolet (UV) spectroscopy is a physical method of optical spectroscopy that uses light in the visible, ultraviolet, and near-infrared ranges. Consequently, it can be used to calculate the concentration of the absorber in a solution for a specific path length. It is critical to understand how quickly the absorbance varies with concentration. UV-VIS spectroscopy has

been widely used for the past 37 years and during this time has emerged as the most crucial analytical tool in the modern laboratory. There are many applications where different methodscould be used, but none compare to UV-VIS spectroscopy's ease of use, adaptability, precision,speed, and cost-effectiveness (G. Varma *et al*;2018).

5.2.3.5 Infrared (IR) Spectroscopy

One of the most popular spectroscopic methods used by organic and inorganic chemists is infrared (IR) spectroscopy. It is simply the measurement of distinct IR frequency absorption by a sample that is placed in the path of an IR beam. Finding the sample's chemical functional groups is the major objective of IR spectroscopic analysis. Different functional groups absorb IR radiation at particular frequencies. IR spectrometers can accept a variety of sample types, including gases, liquids, and solids, using various sampling accessories. Consequently, IR spectroscopy is a crucial and well-liked instrument for understanding structure and identifyingcompounds. Wavenumbers (ν) or wavelengths (λ) are the two most common ways to represent IR absorption positions. The quantity of waves per length is known as wavenumber. As a result, wavenumbers and the IR absorption energy are both linearly proportional to frequency. Modern IR instruments that are linear in the cm^{-1} scale increasingly frequently employ the wavenumber unit (cm^{-1}, reciprocal centimetre). On the other hand, wavelengths are inversely related to frequencies and the energy they carry. Currently, the wavelength is measured in micrometres (μm), while some older literature still refers to wavelengths in nanometres (nm) (C.P. ShermanHsu;1997).

5.2.3.6 Nuclear Magnetic Resonance (NMR) Spectroscopy

The analysis method of choice for exploring molecule level structure and dynamics is nuclear magnetic resonance (NMR) spectroscopy (F. Bloch *et al*;1946 and E. M. Purcell *et al*;1946). Nuclei that have a non-vanishing spin and a non-zero nuclear magnetic moment are used in NMR investigations (A. Abragam;1961 and C. P. Slichter;1996). In an NMR experiment, the sample being investigated is surrounded by a sizable external magnetic field that has a high degree of spatial and temporal homogeneity. The sample's nuclear spins are then briefly exposed to a radiofrequency (RF) range resonant magnetic field oscillation. Nuclear magnetization is tipped into a plane perpendicular to the static magnetic field as a result of thisshort radiation, known as an RF pulse. Nuclear magnetization precesses around the static magnetic field after the RF pulse is turned off, causing a voltage to be induced in the same coilthat was used for excitation. The NMR signal is made up of this recorded current. The

NMR spectrum is created by Fourier transforming the NMR signal (R. R. Ernst *et al*;1966). In order to obtain information on molecular structure and dynamics, an NMR spectrum's appearance depends on the sample's spin interactions and, consequently, on the environment of theexperiment's nuclei (R. R. Ernst *et al*;1987 and M. Goldman;1988).

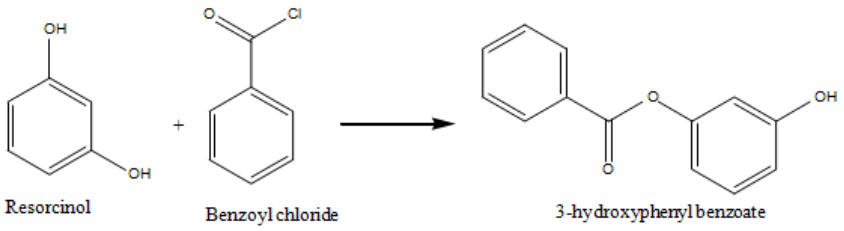
5.2.3.7 Mass Spectroscopy

The structure and gas-phase rearrangements of various molecules can be clarified using the sophisticated analytical technique known as mass spectrometry, which can also be used to quantify known and unknown chemicals within a sample. The entire procedure entails converting molecules into gaseous ions, with or without rearrangement and fragmentation, andthen measuring the mass-to-charge ratios (m/z) and relative abundances of those gaseous ions.The gas phase's molecular ions undergo chemical processes that result in the creation of ionic and neutral species. From the sample under study, a mass spectrometer produces molecule and fragment ions, separates them according on their m/z, and logs the relative abundance of each ion. A mass spectrum is displayed as the outcome. Electron-impact ionisation is the process by which a molecular molecule forms a molecular ion when a high-energy electron collides with it. The dissociation of the molecular ion into fragment ions and neutral species is brought on bythe excess energy that the impact imparts to the molecule. The fragments of the radical cation, which is the molecular ion, can be odd electron ions or even electron ions (H. Awad et al;2014).

5.2.4 Synthesis of selected molecules

5.2.4.1 Synthesis of 3-hydroxyphenyl benzoate

3-hydroxyphenyl benzoate is made by reacting phenols with benzoyl chloride while agitating the reaction mixture. This is done in the presence of aqueous sodium hydroxide. Because it is water-insoluble, the solid benzoyl compound separates off under these conditions and benzoylation occurs without issue. 3-hydroxyphenyl benzoate is insoluble in water but soluble in organic solvents like ethanol and chloroform.



Scheme 2: Synthesis of 3-hydroxyphenyl benzoate.

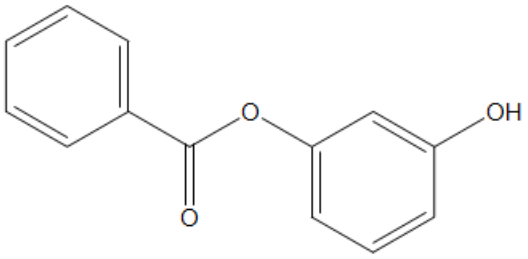
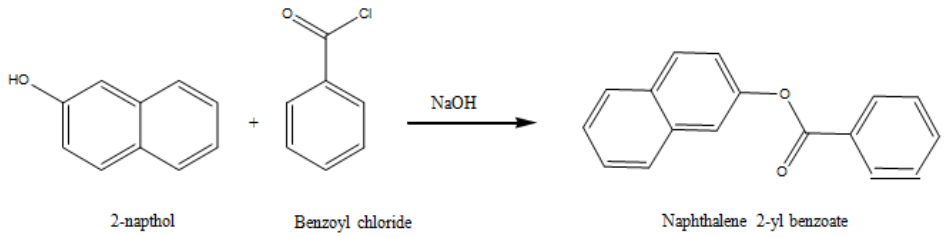


Figure 5.1 – Compound A.XII.B.β

Physical state: brown crystal **% yield:** 85; **M.P.:** 77°C; **R_f (silica gel G):** 0.13 (n-Hexane : Ethyl acetate 4:1); **Solubility:** Chloroform; **FTIR (cm^{-1}):** 3061 (C-H, Str.), 1731(C=O, Str.), 1450 (C=C, Str.), 1312 (O-H, Str.), 1058 (C-O, Str); **MS(ES+):** 214 m/z; **13C NMR:** δ 115.9, 128.6, 130.2, 133.8, 151.4, 164.9.

5.2.4.2 Synthesis of Naphthalene 2-yl benzoate

Naphthalene 2-yl benzoate is made by reacting phenols with benzoyl chloride while agitating the reaction mixture. This is done in the presence of aqueous sodium hydroxide. Because it is water-insoluble, the solid benzoyl compound separates off under these conditions and benzoylation occurs without issue. Naphthalene 2-yl benzoate is insoluble in water but soluble in organic solvents like ethanol and chloroform.



Scheme 3: Synthesis of Naphthalene 2-yl benzoate.

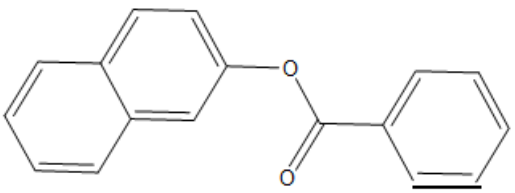


Figure 5.2: Compound A.XIII.B.β.

Physical state: white crystal; **% yield:** 85; **M.P.:** 75^{o}C; **R_f (silica gel G):** 0.11 (n-Hexane : Ethyl acetate 4:1); **Solubility:** Chloroform; **FTIR (cm^{-1}):** 3055 (C-H, Str.), 1682 (C=O, Str.),1451 (C=C, Str.), 1023 (C-O, Str.); **MS(ES+):** 248 m/z; **13C NMR:** δ 125.7, 126.6, 128.6. 130.3, 133.7, 165.4.

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6.0 In-silico antibacterial study

6.1 Selection and preparation of the protein

The RCSB PDB (Protein Data Bank) (https://www.rcsb.org) has been used to retrieve the three-dimensional protein structure. The protein was chosen based on the reactive chemicals bound to the particular protein and the microorganisms from which it was obtained. The PDB ID: (4DX5, 1I6W) From Bacteria.

6.2 Synthesized compounds docking with the marker protein

6.2.1 Ligand preparation

The chosen compounds were synthesized, and as is customary, Gentamycin was obtained from the PubChem database (https://www.pubchem.ncbi.nih.gov) in 3D.sdf format and translated to.pdb using the Open Babel GUI (Graphical User Interface).

6.2.2 Protein preparation

The chosen target protein, which was in complex with the ligand molecules, was obtained from the RCSB PDB (Protein Data Bank) (https://www.rcsb.org/) with the PDB ID: 4DX5, 1I6W. To avoid docking interference, the water molecules were further taken out and polar

hydrogen was supplied using BIOVIA Discovery Studio 2021, and the data was stored in.pdb format.

6.2.3 Ligand protein docking

Using the PyRx and AutoDock Vina, the chemicals were docked with the chosen protein. In BIOVIA Discovery Studio 2021, where 2D and 3D structural pictures of the binding interaction between the ligand protein were generated and visualised, the optimal posture of the ligand on the binding energy (Kcal/mol) was selected after docking (G. M. Morris *et al*:2009, O. Trott *et al*:2021).

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7.0 RESULT AND DISCUSSION

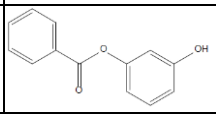
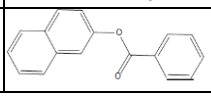
7.1 Synthesis

60 new substituted phenyl benzoate compounds were developed and assessed using in silico methods. They were investigated and evaluated for their toxicological and physico- chemical characteristics. Two compounds were chosen in the end. One phase of synthesis was used to produce each finished good. using the nucleophilic substitution method.

The compounds were assessed utilising various physico-chemical and spectroscopic techniques. The melting points, maximum wavelengths of absorption, FTIR, mass spectra, and13C NMR data of all the compounds were documented and analysed.

All of the compound spectra were shown in the appendix. The results of the mass spectra, FTIR, and 13C NMR studies provide support for the information comparing the synthesised product with planned or designed compounds.

Table 7.1: Synthesized compounds.

Compounds	Structure	IUPAC name	
A.XII.B.β		3-hydroxyphenyl benzoate	
A.XIII.B.β		Naphthalene benzoate	2-yl

7.2 In-silico antibacterial study

Protein-ligand docking may help identify potential compounds at an early stage of the drug development process. Molecular docking was used to validate the proposed technique because the autodocking programme concentrates on protein-ligand binding interactions. The docking results were examined and rendered in the BIOVIA Discovery Studio 2021. The figures show that the substances have a strong binding connection to the proteins and display a positive binding interaction. The following table lists the binding statistics and protein-bondeddata.

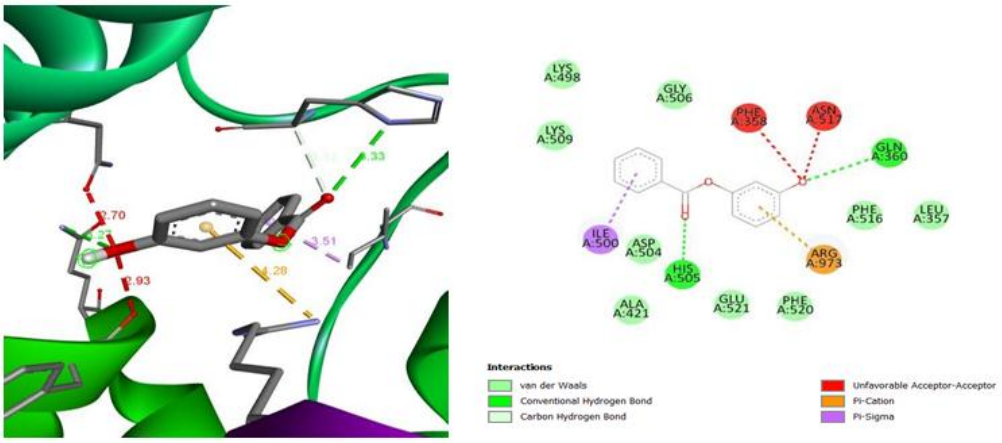


Figure 7.1: 3-hydroxyphenyl benzoate – 4DX5.

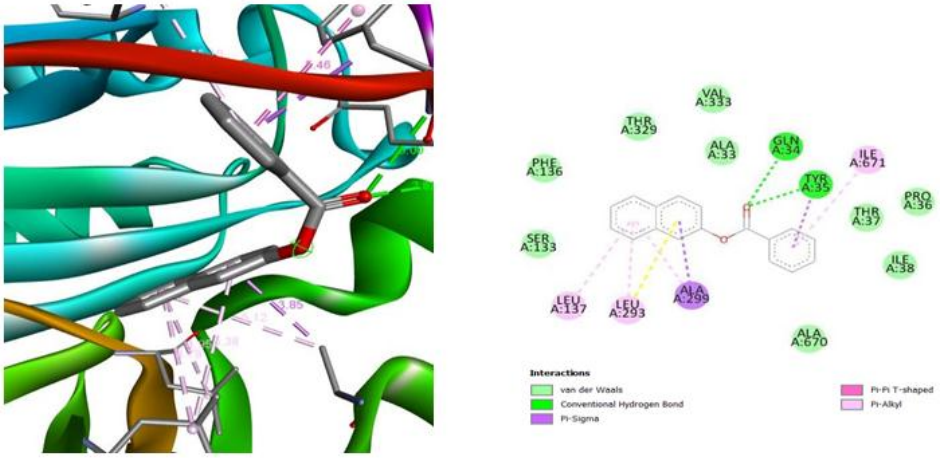


Figure 7.2: Naphthalene 2-yl benzoate - 4DX5

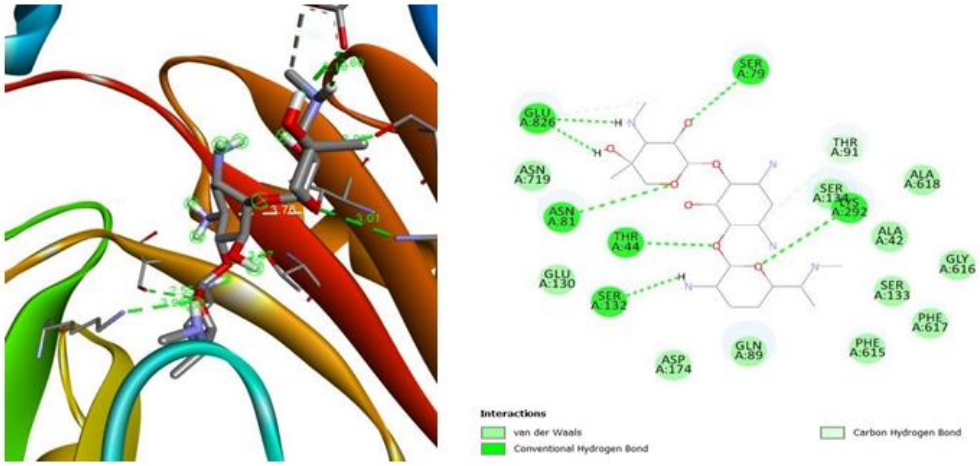


Figure 7.3: Gentamycin (Standard) – 4DX5.

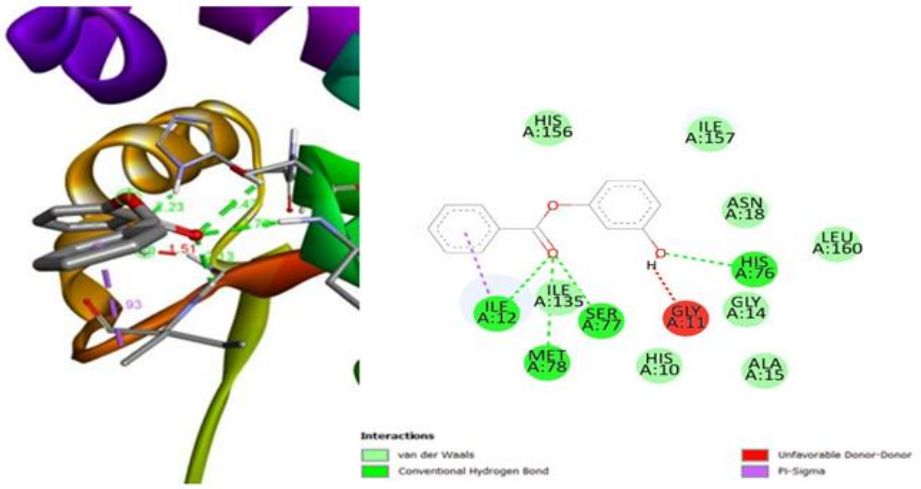


Figure 7.4: 3-hydroxyphenyl benzoate – 1I6W.

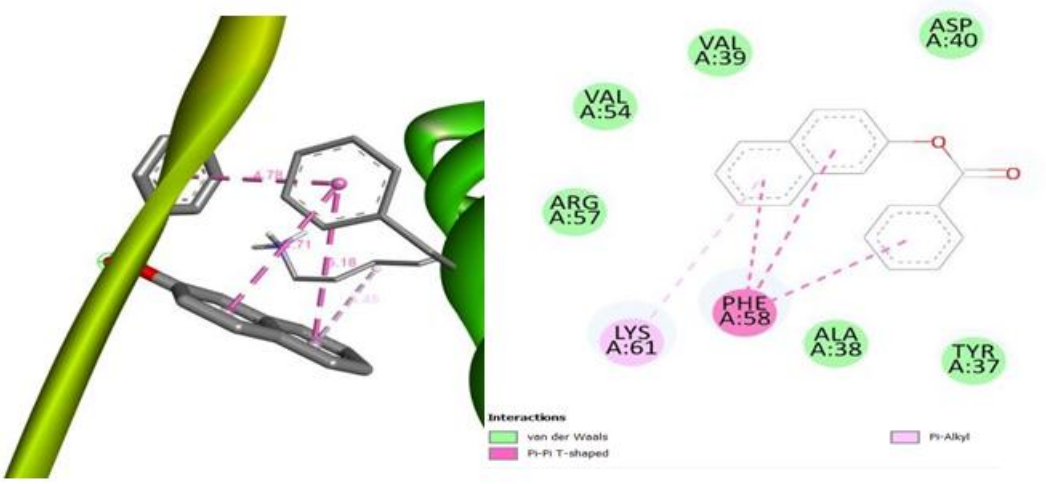


Figure 7.5: Naphthalene 2-yl benzoate – 1I6W

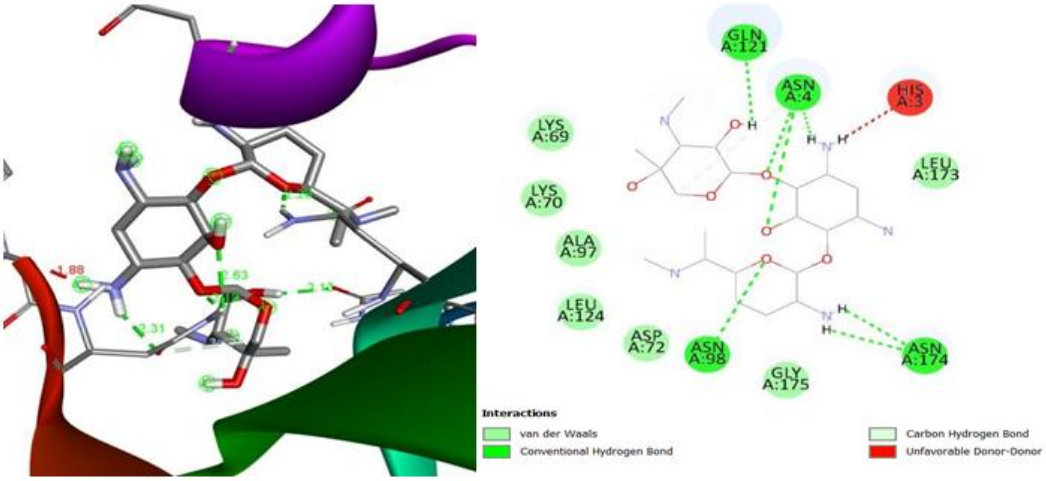


Figure 7.6: Gentamycin (Standard) – 1I6W.

For, *E. coli* (PDB ID: 4DX5)

3-hydroxyphenyl benzoate – 4DX5 – PHE A:358, ASN A:517, GLN A:360, ARGA:973, HIS A:505, ILE A:500.

Naphthalene 2-yl benzoate - 4DX5 – ILE A:671, ALA A:299, GLN A:34, TYR A:35,LEU A:293, LEU A:137.

Gentamycin (Standard) – 4DX5 – SER A:79, ASN A:719, GLU A:826, THR A: 44,SER A:132, LYS A:292.

For, *B. subtilis* (PDB ID: 1I6W)

3-hydroxyphenyl benzoate – 4DX5 – ILE A:12, MET A:78, SER A:77, GLY A:11, HISA:76.

Naphthalene 2-yl benzoate - 4DX5 – LYS A:61, PHE A:58.

Gentamycin (Standard) – 4DX5 – ASN A:98, ASN A:174, HIS A:3, ASN A:4, GLN A:121. When compared to other compounds that target the same proteins, these compounds' binding energies have the best anti-microbial action by having the lowest binding energies. It has been tabulated the binding energy (Kcal/mol).

Table 7.2 – Binding energy.

Compounds/Ligands	Protein	Binding energy
3-hydroxyphenyl benzoate	4DX5 1I6W	-7.5 -6.2
Naphthalene 2-yl benzoate	4DX5 1I6W	-7.6 -6.1
Gentamycin (Standard)	4DX5 1I6W	-7.5 -6.3

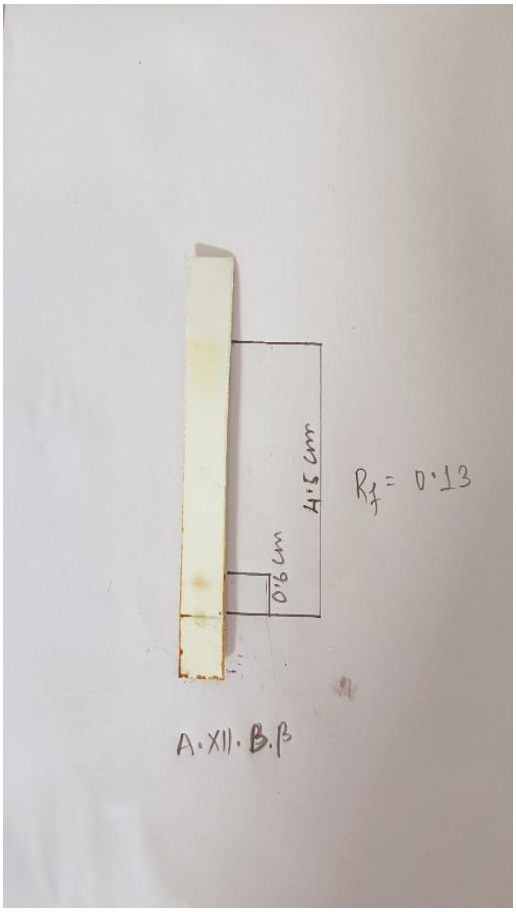


Figure 9.1: TLC of 3-hydroxyphenyl benzoate.

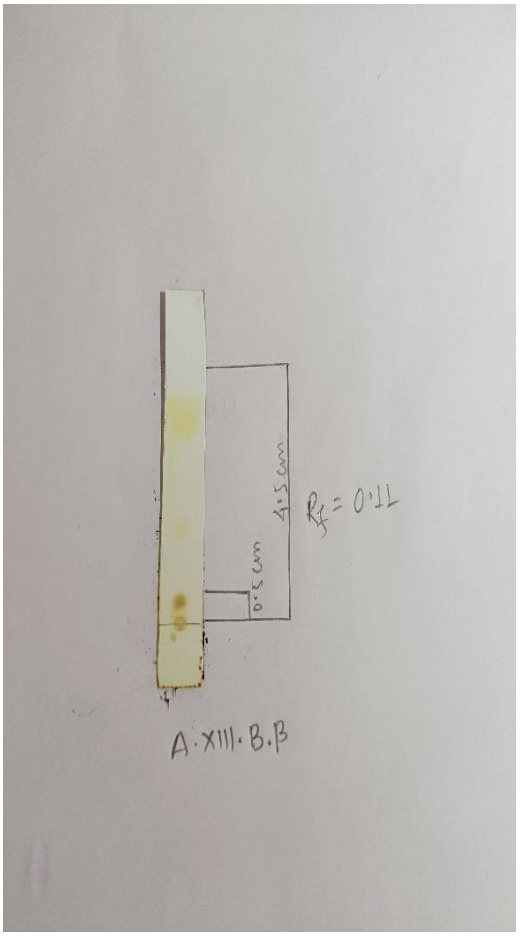


Figure 9.2: TLC of Naphthalene 2-yl benzoate.

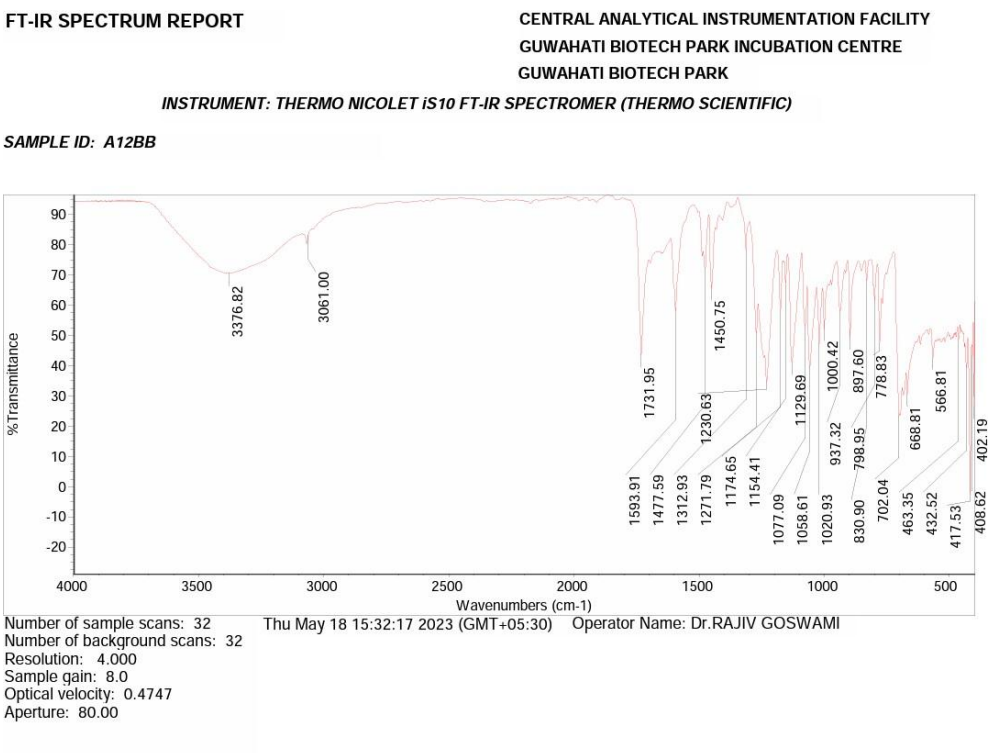


Figure 9.3: FTIR spectra of 3-hydroxyphenyl benzoate.

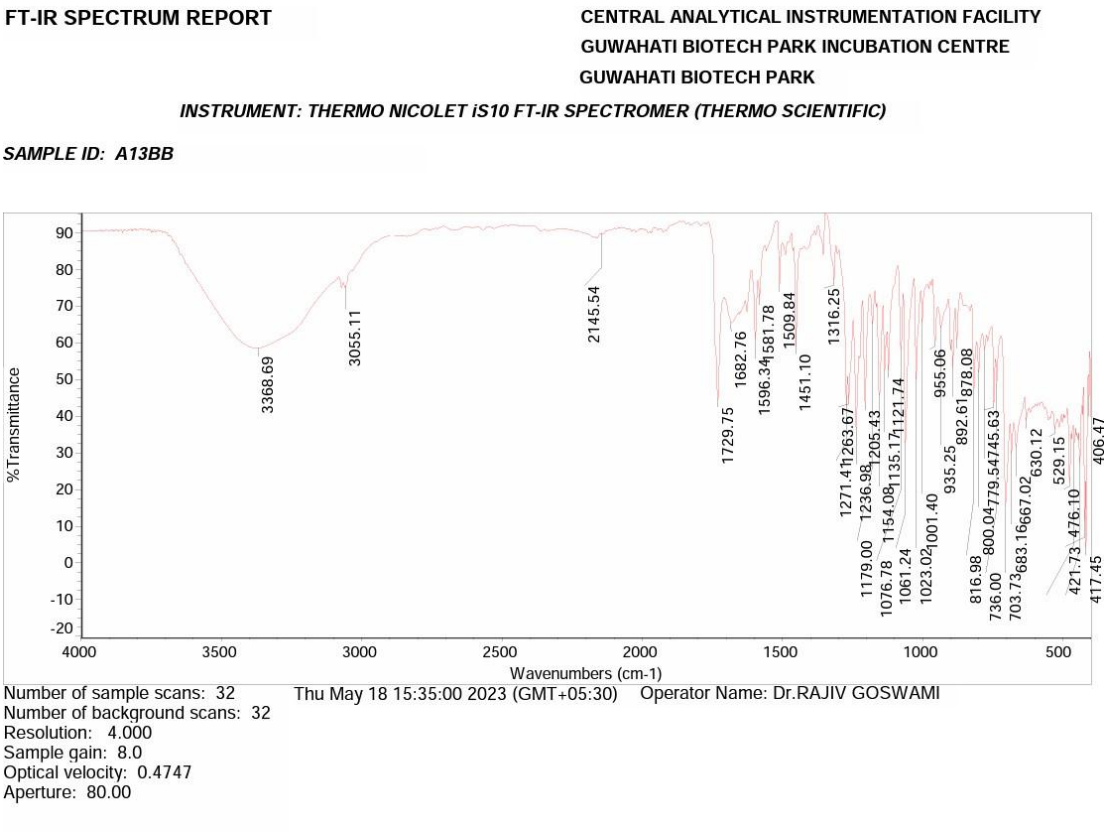


Figure 9.4: FTIR spectra of Naphthalene 2-yl benzoate.

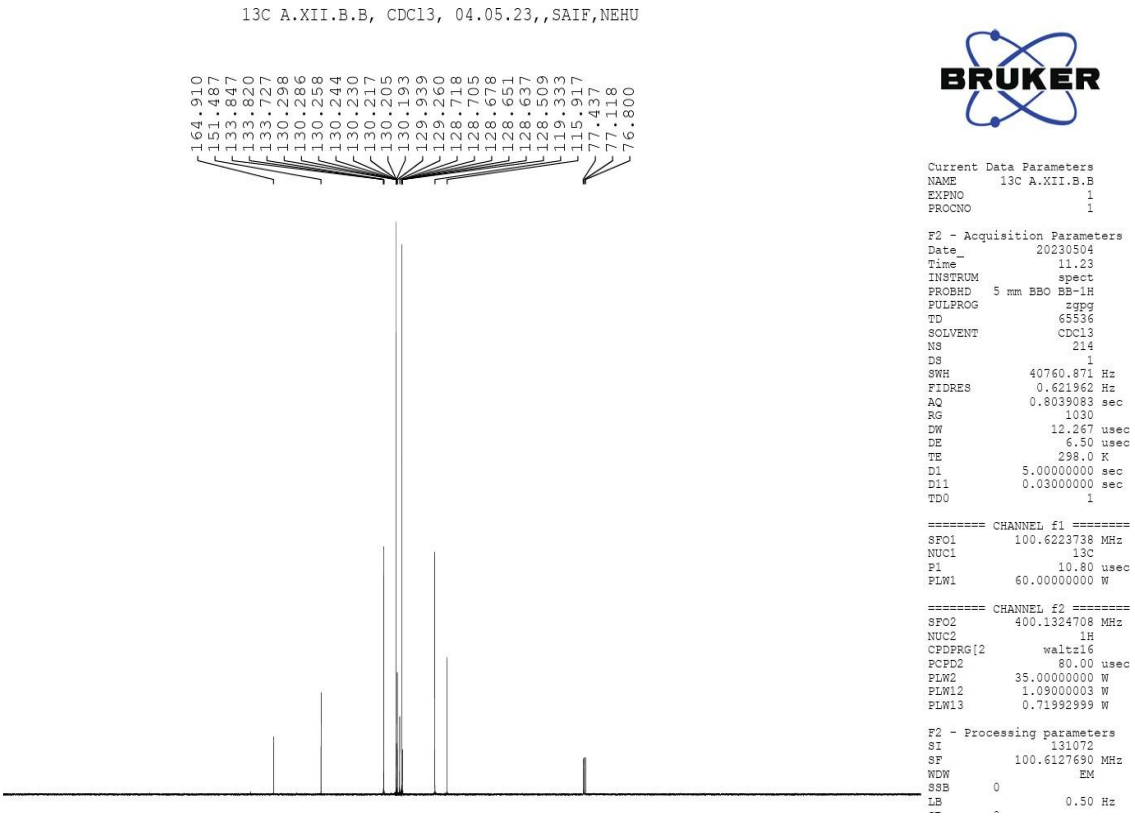


Figure 9.5: NMR spectra of 3-hydroxyphenyl benzoate.

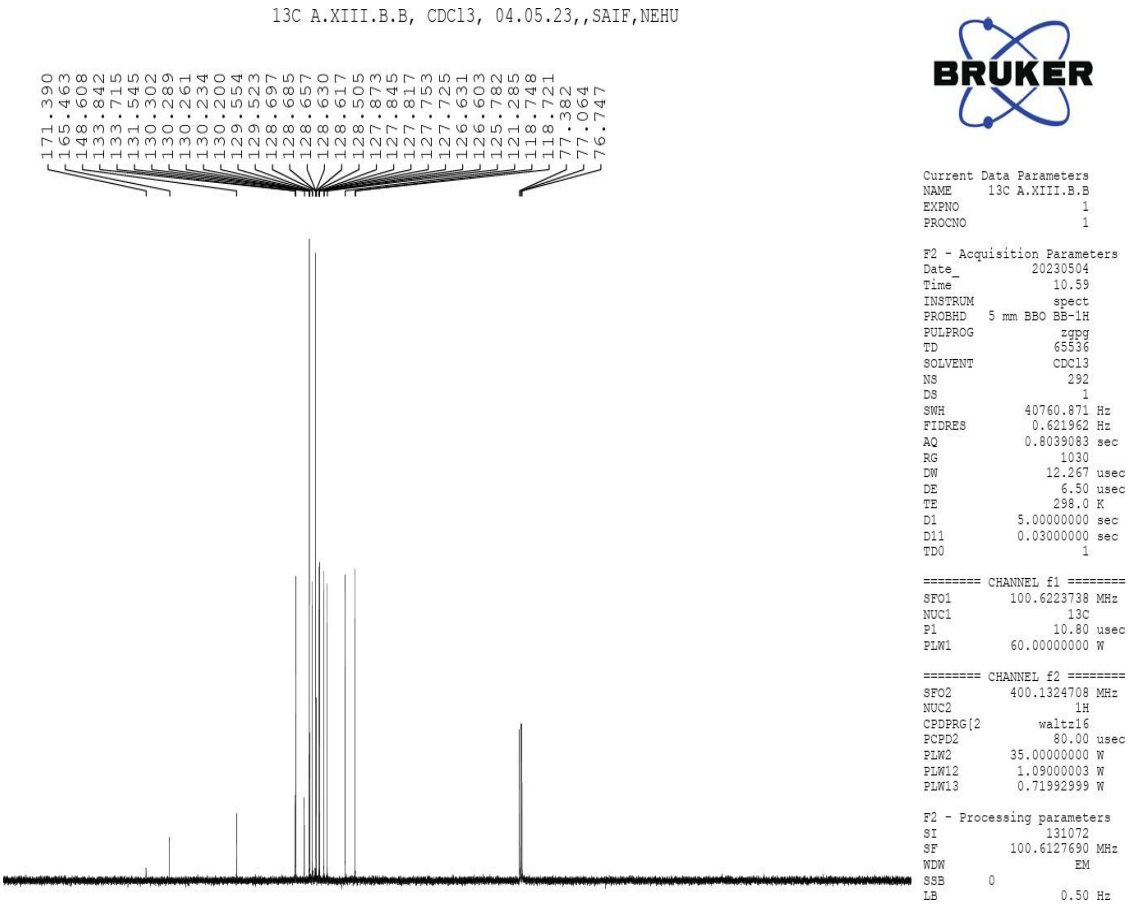


Figure 9.6: NMR spectra of Naphthalene 2-yl benzoate.

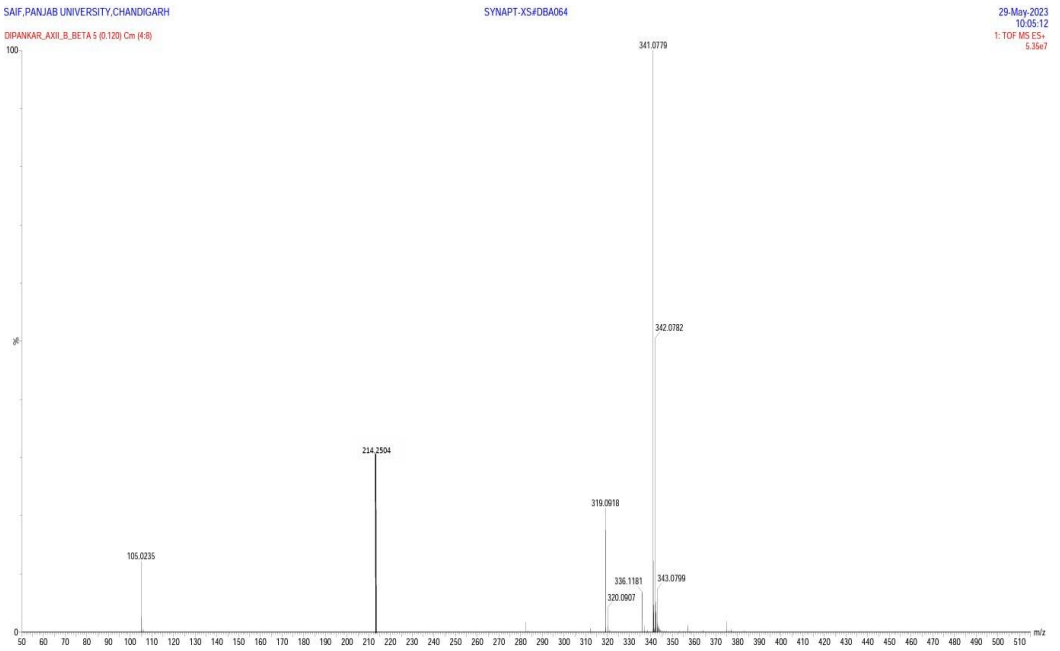


Figure 9.7: Mass spectra of 3-hydroxyphenyl benzoate.

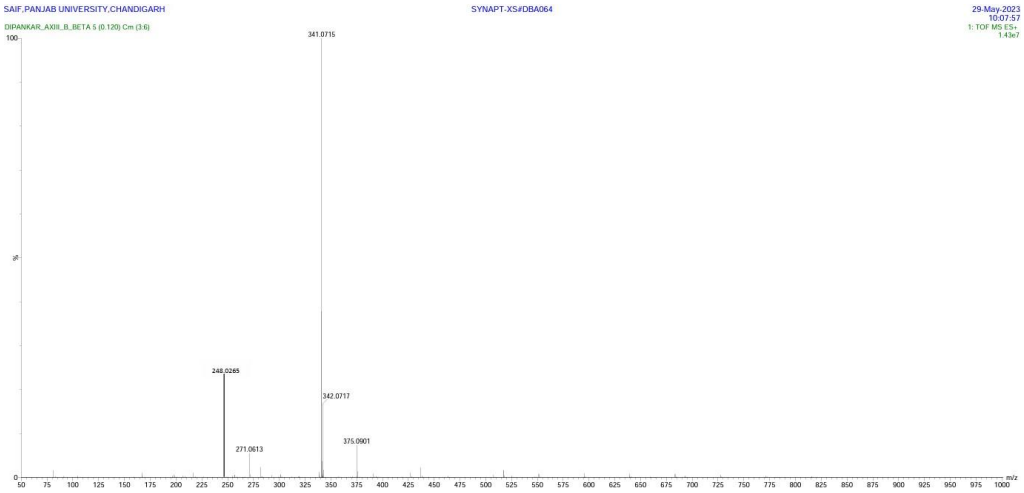


Figure 9.8: Mass spectra of Naphthalene 2-yl benzoate.

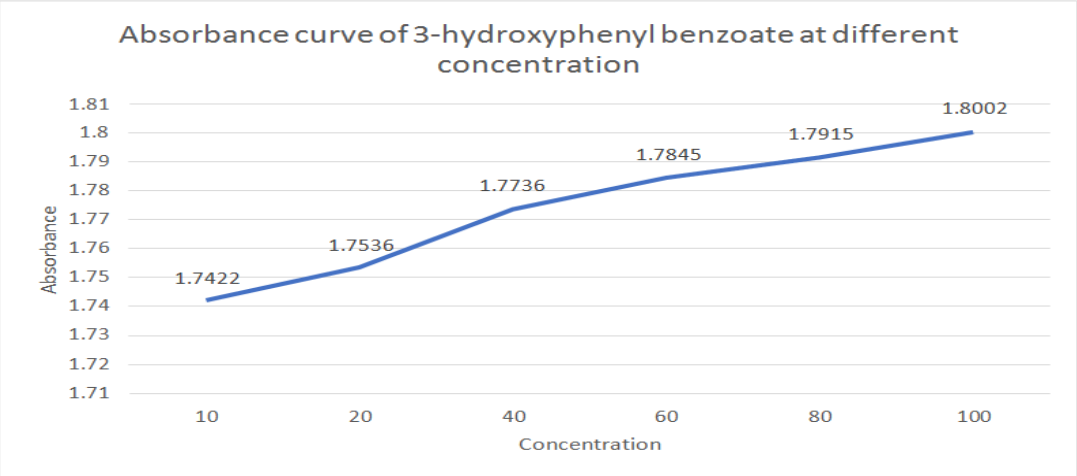


Figure 9.9: Absorbance curve of 3-hydroxyphenyl benzoate.

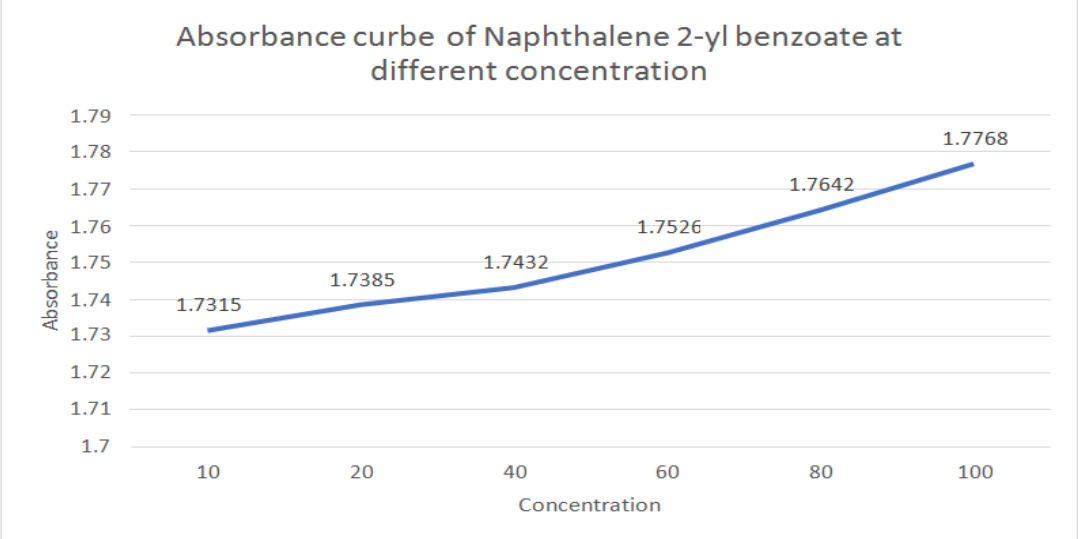


Figure 9.10: Absorbance curve of Naphthalene 2-yl benzoate.

8.0 SUMMARY AND CONCLUSION

In conclusion, this study aimed to design, synthesize, and evaluate the in-silico antibacterial activity of various derivatives of phenyl benzoate. The utilization of computational modelling and molecular docking simulations provided insights into the structure-activity relationship of these derivatives.

The synthesis of the compounds was successfully achieved using optimized synthetic methodologies, ensuring high purity and yield. Characterization of the synthesized compounds using spectroscopic techniques, including FT-IR, 13C-NMR, MS, and UV-Vis spectroscopy, confirmed their structural identification and provided valuable information about their chemicalproperties.

The in-silico evaluation of the derivatives using molecular docking simulations demonstrated promising interactions with bacterial targets, suggesting potential antibacterial activity. The Molinspiration and Osiris Property Explorer tools were employed to assess the drug-likeness and ADMET properties of the compounds. These analyses indicated that the derivatives possess favourable drug-like characteristics and are likely to have improved pharmacokinetic profiles.

While the in-silico results are promising, it is important to note that further experimental studies, including in vitro and in vivo assays, are required to validate the antibacterial activity and evaluate the safety and efficacy of the synthesized derivatives. The in-silico evaluation, combined with the spectroscopic characterization, provides a solid foundation for guiding further experimental investigations.

The Molinspiration and Osiris Property Explorer tools played a crucial role in assessing the drug-likeness and ADMET properties of the compounds. Molinspiration provided valuable insights into physicochemical properties, drug-likeness, and bioactivity predictions, aiding in compound selection. Osiris Property Explorer assisted in evaluating the compounds' ADMET properties, offering valuable information regarding absorption, distribution, metabolism, excretion, and toxicity.

In summary, the design, synthesis, and in-silico evaluation of various derivatives of phenyl benzoate presented promising results regarding their potential antibacterial activity. The utilization of spectroscopic techniques for compound characterization, along with the.

Molinspiration and Osiris Property Explorer tools, enhanced our understanding of the compounds' properties. This study sets the stage for further experimental investigations and optimization of lead compounds as potential antibacterial agent.