SYNTHESIS AND EVALUATION OF SELECTED BENZIMIDAZOLE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

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SYNTHESIS AND EVALUATION OF SELECTED BENZIMIDAZOLE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

An investigation into the synthesis of substituted benzimidazoles and their evaluation *in vitro* for antimicrobial activity

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Keywords: benzimidazole, heterocycle, antibacterial activity, antifungal activity, antimicrobial activity.

Abstract

Microbe resistence is a serious issue, especially as they have become resistant to most well known drugs. Therefore this is considered as a global problem and is now dealt with at a poitical level. Since no new classes of antimicrobial agents have been discovered in the past three deacdes, the development of new drugs is extremely urgent. Therefore the aim of this project was to synthesise derivatives of benzimidazole, and then assesses their antimicrobial activities *in vitro* by using disc (well) diffusion and MICs tests.

A total of 69 benzimidazole derivatives, with substituents at positions 1, 2, and 5, were synthesised, characterised and tested against selected bacteria and fungi. In addition, six bezimidazole silver complexes were prepared and evaluated for their antimicrobial behavior.

The SAR showed that the antimicrobial activity of the compounds depended on the substituents attached to the bicyclic heterocycle. Some promising results were obtained. In particular, 5 compounds displayed antibacterial activity against two MRSA strains with MIC values corresponding to ciprofloxacin, which can be considered significant. The compounds have some common features; four possess 5-chloro or 5-bromo substituents; two are derivatives of *(S)*-2-ethanaminebenzimidazole and the others are derivative of one 2-(chloromethyl)-*1H*-

benzo[*d*]imidazole, (*1H*-benzo[*d*]imidazol-2-yl)methanethiol and 2-(methoxymethyl)-1-methyl-*1H*-benzo[*d*]imidazole.

The results from the antifungal screening were very interesting as there were 26 compounds, including two silver complexes, which were potent fungicides against the selected fungal species. They showed equivalent or greater potentency in their MIC values than amphotericin B. In particular, the 5-fluoro, 5-chloro and 5-bromo benzimidazole showed broad spectrum activity.

Materials from this thesis were presented at the following symposiums:

F. A. S. Alasmary,[1] N. Karodia,[1] and A. M. Snelling[2]. Synthesis and study of the antibacterial activity of some new benzimidazole derivatives. 2nd Bradford Royal Society of Chemistry Postgraduate Symposium , University of Bradford, UK , 24th October 2012 (First prize poster winner).

F. A. S. Alasmary,[1] N. Karodia,[1] and A. M. Snelling[2]. Synthesis and study of the antibacterial activity of some new benzimidazole derivatives. SLS Research and Development Symposium, University of Bradford, UK, 20th March 2013.

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ABBREVIATIONS

ATP	Adenosine tri phosphate
AZT	Azidothymidine
BDCRB	Bromo homologue -(1-β-D-ribofuranosyl) benzimidazole
C. difficile	*Clostridium difficile*
CV	Crystal violet
d	doublet
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
Eq.	equivalent
HEPA	High Efficiency Particulate Air
HCMV	Human Cytomegalovirus-(1-β-D-ribofuranosyl) benzimidazole
HGT	Horizontal gene transmission
HOAc	Acetic acid
HPK	Histidine Protein Kinase
MDR	Multidrug-resistant
MIC	This is a term in microbiology which defines the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation
mRNA	messenger ribonucleic acid
MRSA	methicillin-resistant *Staphylococcus aureus*
m.p.	Melting point
MS	Mass spectrometry
NAD	Nicotinamide Adenine Dinucleotide
NMR	Nuclear Magnetic Resonance
PABA	Para-aminobenzoic acid
PBP	Penicillin-Binding Proteins
q	Quartet

RNA	Ribonucleic acid
RR	Response Regulator
tRNA	transfer ribonucleic acid
s	Singlet
t	Triplet
TCRB	2, 5, 6-trichloro-(1-β-D-ribofuranosyl) benzimidazole
TCS	Two-Component System
VRE	vancomycin-resistant *Enterococcus*

1. INTRODUCTION

1.1 Microorganisms

'Microorganism' is an umbrella term used to signify a large range of small, replicating biological entities. The term is applied to viruses, bacteria, fungi, algae and protozoa (Nicklin *et al.*, 1999). Microorganisms were first identified approximately 300 years ago by van Leeuwenhoek followed by important discoveries by Pasteur and Koch. The term covers both prokaryotic and eukaryotic organisms. The prokaryotic bacteria are divided into 2 biological domains, namely the *Archaea* and the *Bacteria,* of which the bacterial group is of significant importance to humans. They have numerous beneficial uses, including involvement in processes like nitrogen fixation, methanogenesis and waste degradation. They are also used in the production of wine, cheese, antibiotics, vaccines, in nanotechnology for therapeutic purposes and in sewage treatment (Mongillo, 2007).

1.1.1 Bacteria

Some bacterial genera can infect humans and cause disease. Bacteria are classified into two groups based on the type of nutrition, autotrophic and heterotrophic bacteria, and are also classified based on their morphology, habitat, or their need for oxygen (aerobic or anaerobic bacteria). The current classification of bacteria into 24 different phyla is based on cell morphology (e.g. spherical cocci, rod-shaped bacilli, spiral spirochetes, curved rods called vibrios), habitat, DNA, physiological and biochemical characteristics. Another factor that is used to classify bacteria is their motility. Modern sub-classification is based on analysis of the sequence of the 16SrRNA gene (Nicklin *et al.*, 1999, Madigan *et al.*, 2000).

Bacteria can be divided into two main groups based on cell wall structure. The cell membrane is protected by the rigid cell wall. The cell wall of Gram +ve bacteria has

a very thick layer composed of peptidoglycan while the peptidoglycan layer of Gram -ve bacteria is thinner. Also, Gram -ve bacteria possess another outer membrane which functions as a permeability barrier. The section between the inner and outer membranes is referred to as the periplasmic space, which Gram +ve bacteria lack. Gram -ve bacteria store enzymes in the periplasmic space that carry out extra-cellular digestion which is necessary in order to move big molecules across the outer membrane or cell membrane (Figure 1.1).

The two types of cell wall can be distinguished by a Gram stain. When a Gram stain is carried out, Gram +ve bacteria retain the color of crystal violet when washed, while Gram -ve ones do not and then accept a counter-stain. This difference is observed because of basic structural differences in the cell wall of both types (Figure 1.1). Bacterial cell membranes are the site for oxidative phosphorylation as bacteria possess no mitochondria. Crystal violet separates into CV^+ and chloride (Cl^-) ions in solutions. These ions cross the cell wall and cell membrane of both types of bacteria and when the iodine is added, it reacts with CV^+ and forms large molecules in the cell wall. The alcohol applied next reacts with the lipids in the cell membrane. In Gram -ve bacteria, the outer membrane as well as the peptidoglycan layer is exposed, and the CV-I molecules are lost with the outer membrane. In Gram +ve bacteria there is dehydration and the CV-I molecules are retained by the peptidoglycan. Thus Gram +ve bacteria retain the purple color which Gram -ve ones lose (Guilfoile, 2006, Bauman, 2010).

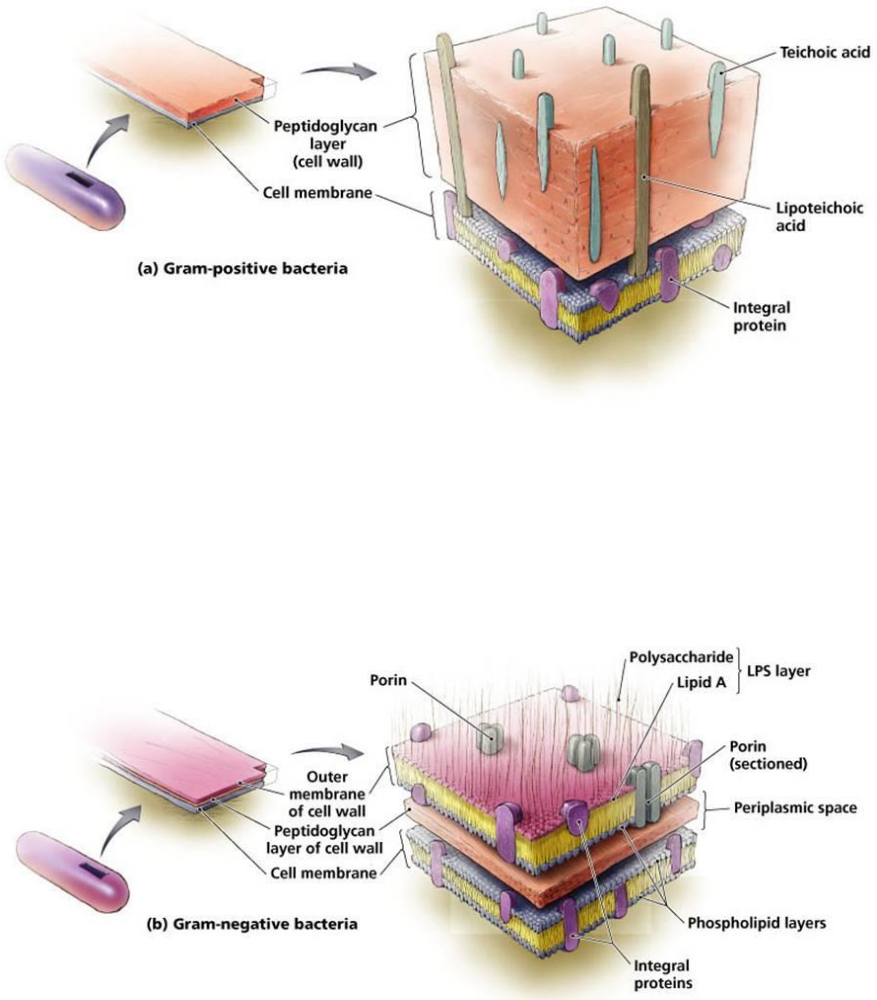


Figure 1.1 Differences in the cell wall of a) Gram +ve and b) Gram -ve bacteria. *Source*: (Bauman, 2010).

1.1.2 Fungi

Fungi such as molds, mushrooms, and yeast are like bacteria in that they have cell walls, unlike protozoa. Fungi are different from animals for having cell walls, and different from plants as well for not performing photosynthesis. The study of fungi is called mycology. In contrast to bacteria, fungi are eukaryotic cells. The main target for the antifungal drugs is the cell wall, which is composed of about 90% of the carbohydrates glucan, mannan, and chitin. Chitin is a strong, flexible, nitrogenous polysaccharide.

The thallus is the vegetative body of the fungus, and its morphology is different between the molds and yeasts. The thallus of molds is large and composed of long, branched, and tubular filaments called hyphae. In contrast to molds, the thallus of yeasts is small, globular, and composed of a single cell. The environmental conditions such as temperature or carbon dioxide concentration, affect the fungi which can produce two types of thallus, this phenomena is called dimorphic (which means “two shaped”). Many pathogenic fungi are dimorphic (Figure 1.2) such as *Histoplasma capsulatum,* which causes histoplasmosis, a respiratory disease (Müller and Polak, 2003, Bauman, 2010).

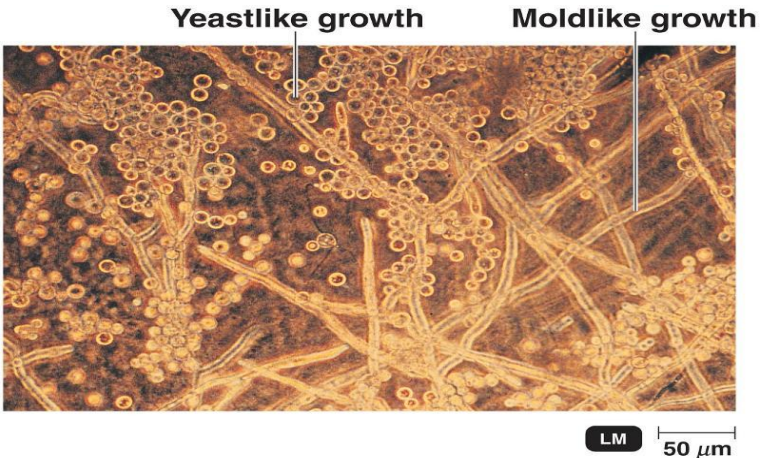


Figure 1.2 Illustration of fungal morphology. *Source*: (Bauman, 2010)

1.2 Inhibition of growth of microorganisms

The inhibition of microorganisms is carried out by several different methods. In order to carry out efficient control of microorganisms in various sensitive situations such as hospital, laboratory and industrial environments, inhibition is essential and has to be effective. Food also has to be kept preserved in a manner which inhibits the growth of microorganisms, so as to prevent spoilage and infections in humans. The methods used to inhibit microorganisms include chemical procedures, utilisation of various physical techniques as well as the use of chemotherapeutic agents. The main aim of inhibition of microorganisms is to achieve either sanitisation, disinfection, a sterile environment or sterilization of equipment or the preservation of food (Block, 2001).

Disinfectants like liquid germicides, ethylene oxide gas, formaldehyde infused steam, hydrogen peroxide and gases such as chlorine dioxide and ozone are all used in hospitals to achieve sterilization. Many of these chemicals are used as disinfectants for sensitive hospital equipment which cannot be sterilized by using harsh physical agents like heat. Disinfectants should only be used on non-living objects since they are usually harmful to human beings. Antiseptics are used to disinfect living tissue such as human skin before surgery or the administration of

therapeutic agents that need to be given directly through subcutaneous injection or intravenous routes as well as for cleaning hands. Chemical disinfectants and antiseptics can reduce microbial populations by a significant proportion but they mostly lack any sporicidal action. Thus, disinfectants and antiseptics are unable to carry out true chemical sterilization (Nicklin *et al.*, 1999, Block, 2001, Bauman, 2010).

Physical agents used in inhibition of microorganisms include the use of high temperatures in the form of both moist and dry heat. Moist heat is far more efficient at destroying microorganisms and inhibiting their growth than dry heat. This is because the mechanism of action for moist heat is the denaturation as well as coagulation of vital cell enzymes and proteins, leading to a faster destruction of microorganisms. Dry heat kills microorganisms by using the mechanism of oxidation of several components. As a result dry heat takes far longer to destroy microorganisms. An autoclave is used to reach a higher temperature than boiling water for sterilization purposes since this is able to kill both spores and vegetative cells. Incineration is used for the disposal of extremely hazardous waste material (Block, 2001, Pelczar *et al.*, 2009, Bauman, 2010).

Radiation in the form of electron beams, gamma rays and x-rays are used to sterilize packaging materials and products enclosed inside them. Ultraviolet radiation is also used to kill and inhibit the growth of microorganisms. Filtration can be used to sterilize liquids and air. Membrane filters are used to sterilize heat-sensitive liquid reagents in the laboratory. High efficiency particulate air (HEPA) filters are used in safety equipment especially when handling infectious and hazardous materials. If vegetative cells of microorganisms are subjected to desiccation, their growth is usually arrested and they are inhibited. This process is used in the preservation of several kinds of food items. Food items which contain high concentrations of either sugar or salt also have a desiccating effect on microorganisms. The high concentrations of solutes result in the removal of water

from the microbial cells through the process of osmosis thus inhibiting their growth. Refrigeration is also used as a physical agent to inhibit the growth of microorganisms in food items. Many genera of Gram -ve cocci such as *Neisseria* are extremely susceptible to desiccation. *Staphylococcus* and most other species of the Gram +ve bacteria are quite resistant to desiccation. A process known as lysophylization in which the microorganisms are desiccated and quickly frozen under a vacuum is utilized to preserve cultures of microorganisms in laboratories (Pelczar *et al.*, 2009, Bauman, 2010).

1.3 Antimicrobial chemotherapy

1.3.1 Use of natural products

There is evidence that many natural products were used as remedies for ailments in the pre-antibiotic period. Ancient Chinese herbalists made use of a very powerful anti-malaria medicine, qinghaosu (artemisinin). Qinghaosu was made from the *Artemisia* plants and utilized as a cure for many ailments by the Chinese for thousands of years (Aminov, 2010). Antimicrobial action is exhibited by several other Chinese traditional remedies (Aminov, 2010). Natural quinine found in the bark of the *Cinchona* tree was utilized in Europe and South America to cure malaria. Another good example of the use of natural products as antimicrobial agents is the use of red soil in Jordan which has been known throughout time to possess antimicrobial activity. In past times and even at present they are used to treat skin diseases. Research carried out on this resulted in the identification of several bacterial species which produce antibiotics and give this gives red soil its antimicrobial capacity (Rahman, 1998, Aminov, 2010). With the advent of increasing microbial resistance to antibiotics, there is always need for new antimicrobial agents. The identification of new active antimicrobial compounds from these

traditional remedies may be very useful in combating antibiotic resistance in microorganisms, which is a big issue in present day medicine (Rahman, 1998, Aminov, 2010).

1.3.2 Semi-synthetic compounds

Semi-synthetic compounds have been produced to make natural compounds more potent, decrease the side effects, and increase the antibacterial spectrum and to cope with the ever increasing problem of resistance development by bacteria. The antibiotic era began with Fleming's discovery of natural penicillin produced by the mould *Penicillium notatum* (Ligon, 2004, Greenwood *et al.*, 2007). The antimicrobial chemical which is active in natural penicillins is 6-amino-penicillianic acid, which is modified to improve such attributes as activity, acid stability, toxicity and half-life in the body. Many semi-synthetic types of penicillin have been made by researchers by attaching different chemical side chains to the 6-amino-penicillianic acid core. Examples of such semi-synthetic compounds include phenthicillin, methicillin, clavulanic acid, sulbactam, cephalosporins, carbapenems, ampicillin and amoxicillin. Aminoglycosides are produced by actinomycetes that were first isolated from soil microbes and are further modified to produce several semi-synthetic antimicrobial compounds that inhibit protein synthesis. Some examples of semi-synthetic derivatives made from aminoglycosides include the antibiotics kanamycin, amikacin and dibekacin. Tetracyclines are also produced by actinomycetes and are in turn used to produce semi-synthetic compounds such as chlortetracycline, oxytetracycline and doxycycline. Macrolides obtained from actinomycetes are used in making semi-synthetic drugs like erythromycin (Dax, 1997, Finch *et al.*, 2003, Hooper and Rubinstein, 2003, Bryskier, 2005, Lorian, 2005, Greenwood *et al.*, 2007, Aminov, 2010).

1.3.3 Entirely synthetic compounds

The synthetic compound sold under the brand name of Salvarsan was used extensively to treat syphilis before the discovery of penicillins and other antibiotics (Lloyd *et al.*, 2005). Sulfonamides and their derivatives have long been used to treat several diseases. Some of the other groups of synthetic compounds which are prevalent in clinical practice include synthetic quinolones such as nalidixic acid which are used as antibacterials. Fluoroquinolones like ciprofloxacin and oxazolidinones such as linezoild are also widely used. Many other synthetic compounds find use against bacterial infections and these include nitrofurans and isoniazids. Alkaloids are another group of synthetic compounds that are used against some kinds of bacterial infections. Alkaloids exhibit antibacterial action against several Gram +ve and Gram -ve bacterial infections. Alkaloids are also frequently used in treating infections caused by oral bacteria and certain fungal as well as protozoan infections also. Because the treatment of viral infections with conventional antibiotics does not work, that has led to the development of synthetic compounds such as vidarabine, acyclovir and AZT in antiviral therapy. Protozoan infections are able to thrive in humans because of the ability of the parasite to exist in different forms at various stages of its life cycle and also because of the similarities of the organism to human cells as both are eukaryotic. As a result it is difficult to use traditional antimicrobial agents against protozoan infections. Several synthetic compounds such as quinacrine, diiodohydroxyquin, pentamidine and metronidazole are used in anti-protozoan therapy. The same problem exists in the treatment of infections caused by fungi and helminths. Synthetic compounds are thus used in chemotherapy against fungi and helminth infections also (Saxton, 1971, Clitherow *et al.*, 2003, Cordell, 2008, Perry, 2008).

1.4 Modes of action of antibacterial agents

Antibiotics are antimicrobial agents which are used in clinical care for many kinds of infections caused by microorganisms. They are generally classified into groups based on either their chemical structure or their modes of action. Antibiotics are known as “bactericidal” if they kill the target bacteria and as “bacteriostatic” if the mode of action is to reversibly inhibit the growth and proliferation of bacteria. The range of bacteria against which an antibiotic shows activity is known as “its spectrum.” The following sections review the main modes of action of antibiotics which are summarised in Figure 1.3:

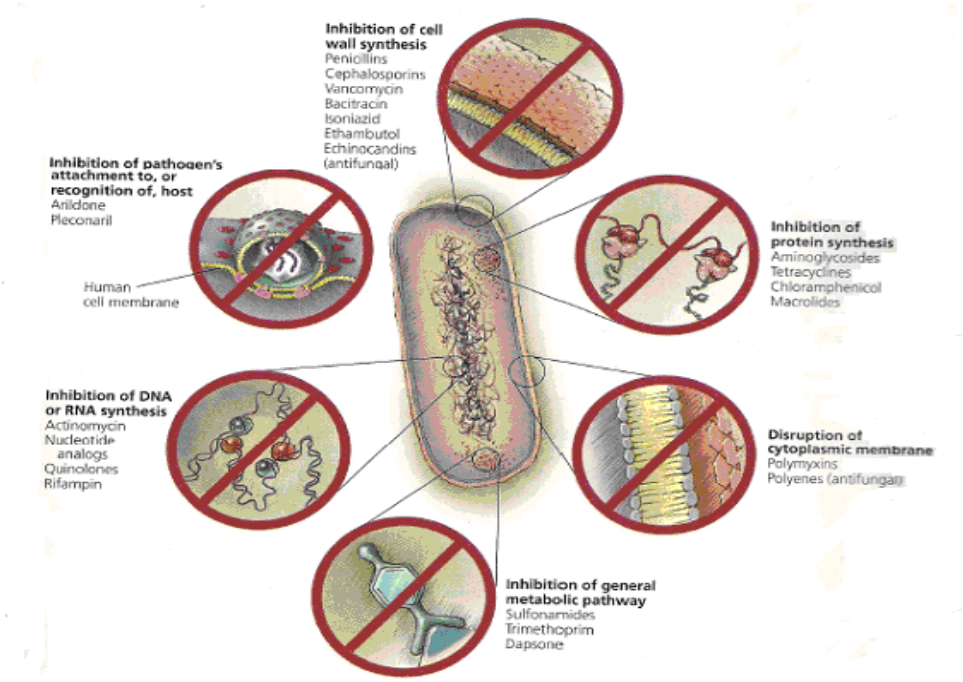


Figure 1.3 Main cellular targets for inhibiting bacteria. Source: (Bauman, 2010)

1.4.1 Antibiotics which inhibit cell wall synthesis

The cell wall of bacteria is made up of a layer of peptidoglycan. Several antibiotics work by using the action of the β-lactam ring. This ring inhibits the process of

peptidoglycan formation by irreversibly binding to the enzymes that cross-link peptidoglycan subunits. The particular enzymes are referred to as penicillin-binding proteins (PBPs) and are usually anchored to the cell wall or membrane. When the β-lactam ring binds to PBPs, the process of cell wall synthesis is inhibited. Antibiotics which use this mechanism of action have a broad spectrum and can be used against both Gram +ve as well as Gram -ve bacterial infections. Examples of antibiotics belonging to this group are the β-lactam antibiotics (such as penicillin, ampicillin, cefazolin, cefuroxime, cefotetan, cefotaxime, ceftriaxone, ceftazidime and cefepime) and monobactams like aztreonam, plus carbapenems such as imipenem and meropenem (Greenwood *et al.*, 2007).

1.4.2 Antibiotics which function as inhibitors of cell membrane functions

A good example of such an antibiotic is daptomycin. It acts by binding and disrupting the cell membrane of Gram +ve cocci like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). However it is not able to act on Gram -ve bacilli since it cannot penetrate the outer membrane and as such is not effective against them. Polymyxins disrupt cell membranes and are more active against Gram -ve bacteria, with very little activity against Gram +ve bacteria. They are no longer widely used in medicine, although sometimes they are employed against strains that are resistant to more modern drugs (Lorian, 2005, Karaiskos *et al.*, 2013, Rogers *et al.*, 2013).

1.4.3 Antibiotics which inhibit protein synthesis

These antibiotics target the process of bacterial protein synthesis and seriously damage the metabolism of the cell. The bacterial cell uses ribosomes to translate

information from messenger RNA templates into proteins. Many antimicrobial agents exploit differences between prokaryotic and eukaryotic ribosomes in structure and size, to selectively target bacterial protein translation. Bacterial ribosomes are 70S and composed of 30S and 50S subunits, while the eukaryotic ribosomes are 80S with 60S and 40S subunits. 30S and 50S subunits of ribosomes play a role in:

- Initiation of protein synthesis.
- Codon recognition.
- Docking of tRNA-amino acid complexes.
- 50S subunit contains the enzymatic portion that actually forms peptide bonds between amino acids.

Aminoglycosides and tetracyclines are types of antimicrobial agent which use the 30S ribosomal subunit as a target for inhibition of protein synthesis. Aminoglycosides such as streptomycin and gentamicin change the shape of the 30S subunit, and then it is impossible for the ribosomes to read the codons of mRNA correctly. Chloramphenicol blocks the active site of the 50S subunit which prevents translation. Some other drugs block ribosomal subunits from attaching to mRNA; such as fomiversen (Figure 1.4).

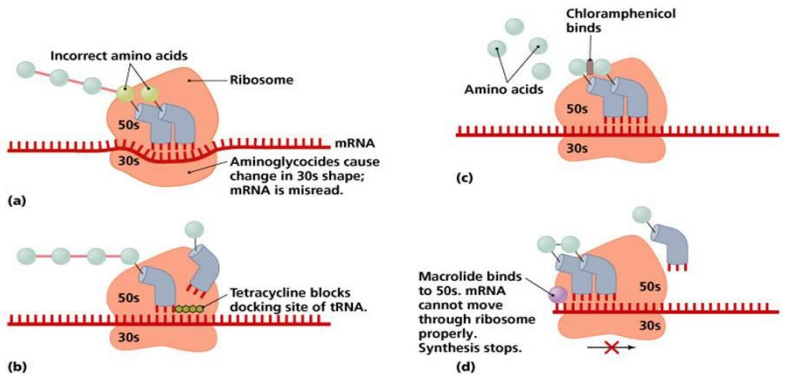


Figure 1.4 The summary of mechanisms by which antimicrobials inhibit protein synthesis by targeting prokaryotic ribosomes. *Source*:(Bauman, 2010)**.**

1.4.4 Antibiotics that inhibit the synthesis of nucleic acids

The nucleic acids DNA and RNA are essential for life and are built from purine and pyrimidine nucleotides. Many drugs, such as actinomycin, can block either replication of DNA or its transcription into RNA. Nucleotide analogues are drugs which interfere with the function of nucleic acid. Other drugs like rifampicin function by binding to and inhibiting the action of RNA polymerases during the synthesis of RNA from a DNA template. Fluoroquinolones are one of the broad-spectrum antibiotics which inhibit bacteria by binding to one of the two essential enzymes for the replication of DNA; DNA gyrase or DNA topoisomerase IV (Drlica, 1999).

1.4.5 Antibiotics that inhibit metabolic pathways

Sulfonamides act as anti-metabolic drugs and are structural analogues of para-aminobenzoic acid (PABA). PABA is essential for the synthesis of nucleotides required for DNA and RNA synthesis. As analogues of PABA, sulfonamides compete with PABA molecules for the active site of the enzyme involved in the production of dihydrofolic acid. This competition leads to a decrease in the production of tetrahydrofolic acid, and thus of DNA and RNA, which then leads to cell death. Some other drugs such as trimethoprim, interfere with nucleic acid synthesis, by binding to the enzyme that converts dihydrofolic acid to tetrahydrofolic acid (Hooper and Rubinstein, 2003, Lorian, 2005, Pelczar *et al.*, 2009, Bauman, 2010).

1.5 Modes of action of antifungal agents

Unlike bacteria, both fungi and human cells are eukaryotic. Fungi make β-glucan, but human cells do not, so any drugs that target β-glucan biosynthesis; might have low side-effects. Different classes of drugs target the plasma membrane, sterol biosynthesis, DNA biosynthesis, and β-glucan biosynthesis. Drugs known as fungistatic drugs are those that inhibit growth; whereas fungicidal drugs kill fungal pathogens (Graybill *et al.*, 1997). The following sections review the main modes of action of antifungal drugs and these are also summarised in Figure 1.5 (Kathiravan *et al.*, 2012):

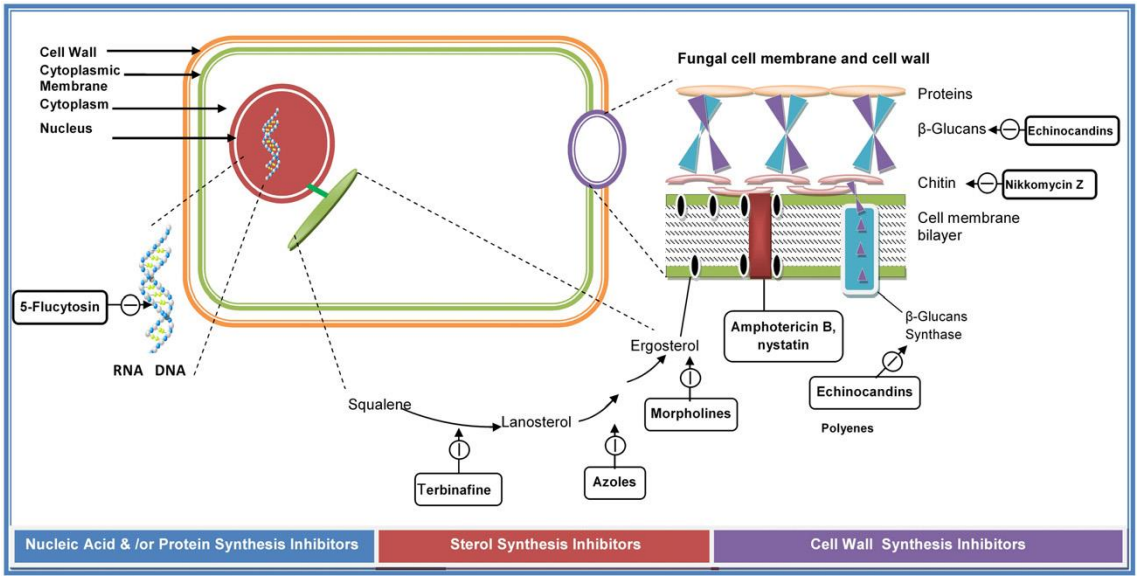


Figure 1.5 Targets for antifungal therapy. *Source*:(Kathiravan *et al.*, 2012).

1.5.1 Sterol synthesis inhibitors

1.5.1.1 Fungal ergosterol synthesis inhibitors

The major component of the fungal cell membrane is ergosterol which is a sterol. Fungicide drugs such as azole, ketoconazole, itraconazole, fluconazole, voriconazole, posoconazole and ravuconazole are demethylase inhibitors which inhibit the lanosterol 14 α-demethylase ((CYP51A1, P45014DM) which is a

cytochrome P450, an enzyme that converts lanosterol to cholesterol); which will result in decreased ergosterol synthesis (Kathiravan *et al.*, 2012, Rajput and Karuppayil, 2013).

1.5.1.2 Squalene epoxidase inhibitors

Squalene epoxidase is a key flavin adenine dinucleotide (FAD)-dependent enzyme of ergosterol and cholesterol biosynthetic pathways (Nowosielski *et al.*, 2011), which together with squalene cyclase, converts squalene to lanosterol. It is an attractive potential target for drugs such as morpholine, amorolfine, and terbinafine used to inhibit the growth of pathogenic fungi or to lower cholesterol levels (Kathiravan *et al.*, 2012).

1.5.2 Cell wall synthesis inhibitors

1.5.2.1 Ergosterol disruptors

The polyenes such as amphotericin B, and nystatin are fungicidal drugs and have the broadest spectrum of antifungal activity, which disrupt the fungal plasma membrane. This results in increased membrane permeability, by forming a complex with ergosterol. This will lead to leakage of the cytoplasmic contents and end with death of the fungal cell (Kathiravan *et al.*, 2012).

1.5.2.2 Glucan synthesis inhibitors

One of the major components that strengthen the cell wall is glucan. D-Glucose monomers attached to each other by β-1,3-glucan or β-1,6-glucan linkages to form the glucan polysaccharide. The glucan synthesis inhibitors such as echinocandins, caspofungin, micafungin, and anidulafungin are lipopeptide class inhibitors that block fungal cell wall synthesis by inhibiting the enzyme β-1,3-glucan synthase via noncompetitive inhibition. The blockage of glucan synthase results in a decrease in the incorporation of glucose to the glucan, and leads to disruption of the structure of the cell wall, resulting in osmotic instability and death of the cells (Cabello *et al.*, 2001, Kathiravan *et al.*, 2012).

1.5.2.3 Chitin synthesis inhibitors

The insoluble polymer consisting of β-1,4-homopolymer of *N*-acetylglucosamine that folds in an anti-parallel manner forming intra-chain hydrogen bonds is called chitin, which is important in determining cell shape. Chitin chains are cross-linked covalently to β-1,3-glucan in order to strengthen the cell-wall, to form the inner skeleton of most fungi (Figure 1.6). Chitin synthase is an attractive target for the antifungal agents like polyoxins and nikkomycin, because it is essential for the fungi, and does not exist in human cells (Lenardon *et al.*, 2010, Kathiravan *et al.*, 2012).

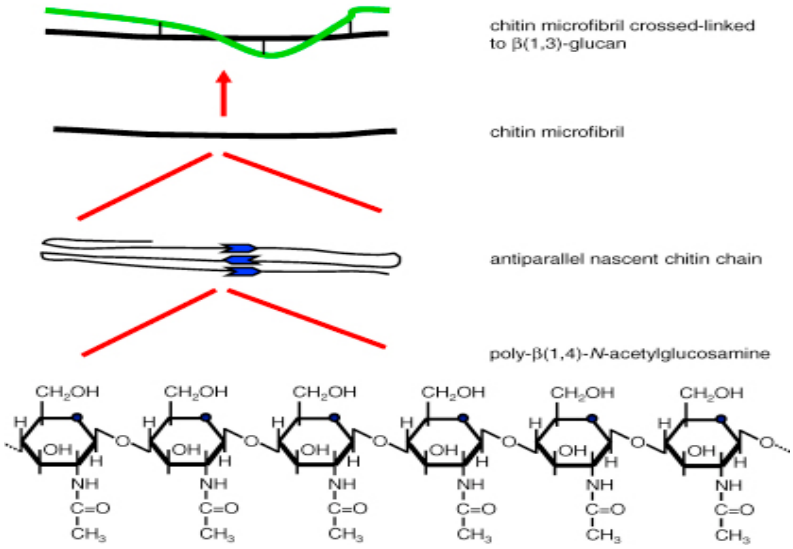


Figure 1.6 Chitin structure and diversity in fungi. *Source*: (Lenardon *et al.*, 2010).

1.5.3 Nuclic acid and /or protein synthesis inhibitors

1.5.3.1 Nucleic acid synthesis inhibitors

Flucytosine is an antifungal drug synthesised in the 1950's, which is transported into the fungal cell by a cytosine permease. This molecule is converted in the cytoplasm to 5-fluorouracil by a cytosine deaminase, and into 5-fluorouridylic acid by another enzyme (UMP pyrophosphorylase), which then disrupts the protein synthesis by

phosphorylation and interferes with RNA. Moreover, 5-fluorouracil inhibits thymidylate synthase, an enzyme involved in DNA synthesis and the nuclear division process; by converting to 5-fluorodeoxyuridine monophosphate (Vermes *et al.*, 2000, Kathiravan *et al.*, 2012).

1.5.3.2 Protein synthesis inhibitors

Sordarins is a natural product first isolated from the mould *Sordaria araneosa* in 1971; it selectively inhibits fungal protein synthesis. It blocks the function of fungal translation Elongation Factor 2, which is a protein encoded by the Eukaryotic Elongation Factor 2 gene (eEF2). This protein is important and catalyses the movement of the ribosome along the mRNA, and for the translocation of peptidyl tRNA from the A to P site of the ribosome (Shastry *et al.*, 2001, Kathiravan *et al.*, 2012).

1.5.3.3 Microtubule synthesis inhibitors

Microtubules are dynamic polymers of α- and β-tubulin dimers. They form a highly organized cellular skeleton in all eukaryotic cells. The agents like griseofulvin and vinblastin interact with β-tubulin, which then inhibit fungal mitosis (Rathinasamy *et al.*, 2010, Kathiravan *et al.*, 2012).

1.6 Antibiotic resistance

1.6.1 In bacteria

Different kinds of resistance are developed by bacteria through the use of various mechanisms which keep evolving and confer resistance to currently used antibiotics. Some of these mechanisms include the chemical modification of the antibiotic, rendering the antibiotic ineffective by physically removing it from the cell,

modification of the target site and blocking or reducing uptake of the drug into the cell. The most common mechanism of antibiotic resistance is through the production of enzymes which are capable of inactivating the antibiotic. A good example is the production of penicillinase which cleaves the β-lactam ring of penicillins. Some bacteria carry out the modification of an existing cellular enzyme in such a way that it modifies the structure of the antibiotic by addition of an extra molecule, or it simply fails to interact with the target in an effective manner. Another mechanism of action which is used by several species of bacteria is the change or alteration of the target site. Some bacteria develop antibiotic resistance by making the target site unavailable to the antibiotic. This can be achieved by preventing penetration to targets like the cell wall and ribosomes. Antibiotic resistance can also be caused by simply shifting the cell metabolism to an alternative biochemical pathway. This mechanism of action is referred to as a metabolic by-pass and an example of this is resistance to trimethoprim (Guilfoile, 2006, Pelczar *et al.*, 2009, Aminov, 2010, Bauman, 2010).

Bacteria have the ability to control the entry of tiny molecules into the cell and this capacity is enhanced in Gram -ve bacteria due to their outer membranes. Gram +ve bacteria control the influx of antibiotics by physiological methods. Some antibiotics are active only when they are inside the body. Nitrofuran resistance is developed by mutations of genes which code for cellular reductase enzymes. *Enterococci* and *Staphylococcus aureus* develop resistance to the glycopeptide antibiotic vancomycin through the use of a strategy called antibiotic evasion (Jayaraman, 2009).

The antibiotic “resistome” is the sum of the possible mechanisms of resistance to bacterial drugs, via chemical modification or breakdown of antibiotics, target protection, efflux, or specific changes to the target (Figure 1.7). The source of the antibacterial resistance can be endogenous or exogenous. Endogenous is from ‘within’ the pathogen, by mutation. Exogenous resistance is where new resistance

mechanisms are acquired from other bacteria, (e.g. environmental organisms, antibiotic producers, commensals, nonhuman pathogens) by horizontal gene transmission. As an example of diverse resistance mechanisms, bacteria can have a class-specific efflux mechanism or target protection/modification to resist tetracycline as shown in Table 1.1 (Silver, 2011).

Table 1.1 Key Mechanisms of bacterial resistance against some drugs. *Source*: (Silver, 2011).

Origin	Mechanism	Examples of affected drug classes affected
Exogenous	Class-specific efflux	Tetracycline, macrolides
	Class-specific degradation/modification	β-Lactams, aminoglycosides, chloramphenicol, streptogramin A, metronidazole (for anaerobes), fosfomycin
	Target protection/modification	Tetracycline, macrolides, lincosamides, oxazolidinones, streptogramin B
	Replacement with reduced-affinity target	β-Lactams, vancomycin, trimethoprim, mupirocin, sulfonamides
	Sequestration of target	Fluoroquinolones, fusidic acid
Endogenous	Single mutations reducing target affinity	Rifamycin, streptomycin, trimethoprim (for Gram+ve organisms), fusidic acid
	Multistep mutations reducing affinity or remodeling of target	Fluoroquinolones, oxazolidinones, daptomycin, vancomycin, polymyxin, β-lactams (for transformable species)
	General efflux mechanism	Most classes for Pseudomonas; many classes for other species
	Reduced uptake (porin or permease loss)	Carbapenems, fosfomycin
	Loss of activation	Metronidazole (for *H. pylori*)
	Upregulation of target	Fosfomycin

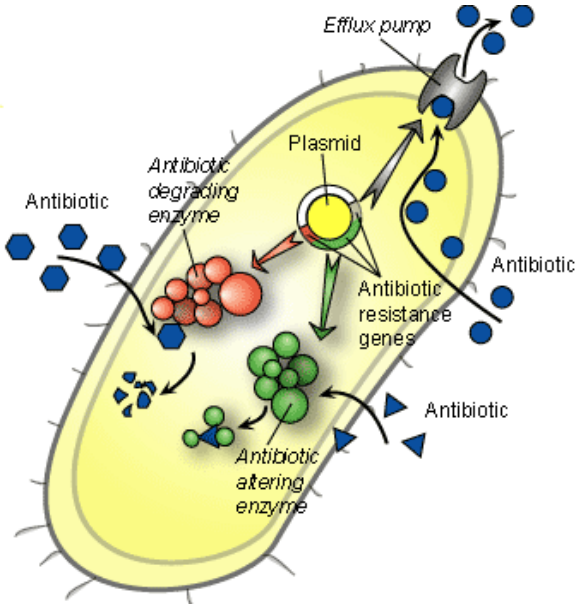


Figure 1.7 General mechanisms of antibiotic resistance in bacteria. *Source*: (Todar, 2008).

1.6.1.1 Increasing resistance in bacteria and the increasing financial cost

Rollo *et al* in 1952 noted the possibility of resistance emergence under laboratory conditions and concluded that: “Syphilis has now been treated with arsenicals for about 40 years without any indications of an increased incidence of arsenic-resistant infections, and this work gives grounds for hoping that the widespread use of penicillin will equally not result in an increasing incidence of infections resistant to penicillin”. But this did not apply to other pathogenic bacteria, where they found it not only became resistant to original penicillin, but even to the semi-synthetic penicillins such as cephalosporins, and newer carbapenems (Aminov, 2010). Earlier, when Fleming discovered penicillin, he found that some bacteria were already resistant and he warned that the misuse of penicillin could lead to increased resistance amongst bacteria (French, 2010).

Multidrug-resistant (MDR) pathogens constitute an emerging threat with increasing incidence and uncertain outcome. Examples of these pathogns are glycopeptide-

resistant MRSA and enterococci, and toxin-hyperproducing *Clostridium difficile* which is a species of Gram+ve bacteria that causes severe diarrhea and other intestinal disease. The characterisation of MDR is through their heterogeneity, increasing virulence, resistance to even reserve agents and spread within and between hospitals and the community (French, 2010). But after the introduction of methicillin and other penicillinase-stable penicillins in the 1960s, the global problem of the multidrug-resistant (MDR) 'hospital *Staphylococcus*' reduced, and outbreaks of gentamicin-resistant *Klebsiella* and other Gram -ve organisms seen in the 1970s, waned in the 1980s with the introduction of newer aminoglycosides, cephalosporins and quinolones (French, 2010).

The extensive resistance problems are due in part to negligent antibiotic use and poor infection control practice and it is an inevitable consequence of wide spread antibiotic use (French, 2010). There are some factors which increases the resistance rate; availability of viable resistant variants and the intensity of selection pressure imposed by antimicrobial treatment (Lipsitch, 2001).

An association has been found between antibiotic resistance and adverse outcomes in the order of a 1.3 to 2 times increase in mortality, morbidity, and costs for patient care with drug resistant infections, compared with susceptible infections (Franco, 2009).

Every year in the US, there are about 23 million kg of antibiotics prescribed in total, and the annual cost of treating antibiotic-resistant infections has been calculated at more than US $ 4 billion and 18% of the total antibacterial medicines prescribed in the US in 1992 were for respiratory illnesses (Franco, 2009).

The increasing prevalence of antibiotic resistance in bacteria is one of the greatest challenges in medicine (Figure 1.8). Despite extensive efforts, few totally new therapeutic agents have emerged over the past decade. There is thus great interest in developing derivatives of existing agents with enhanced activity, or that are less susceptible to bacterial resistance mechanisms. A study by Knight *et al.,* (2012) at

St George's Hospital, London, as cited in the report published by Department of Health in UK (2013), showed that when the hospital reduced the prescription of quinolones to paitents, the cases of meticillin-resistant *Staphylococcus aureus* (MRSA) in England declined (Figure 1.9) (Department of Health, 2013a).

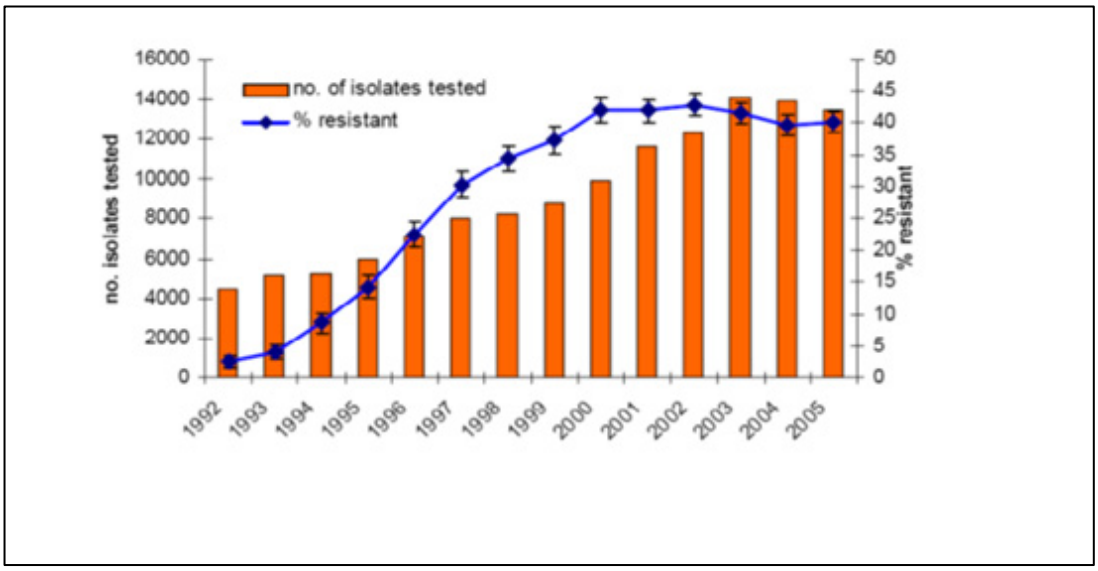


Figure 1.8 Rise in reports to Health Protection Agency of resistance to methicillin in *S. aureus* from bacteremia cases, England and Wales, 1992-2005 (voluntary laboratory reports). *Source:* (Health Protection Agency, 2006).

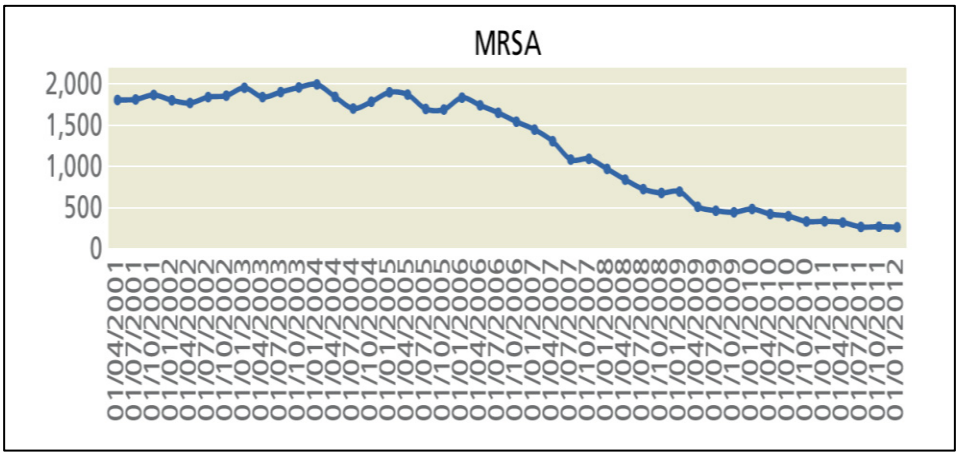


Figure 1.9 National decline in reported cases of meticillin-resistant Staphylococcus aureus (MRSA), England, 2001 to 2011. *Source*: (Department of Health, 2013a)

1.6.1.2 Challenges in development of new antibiotics

Excessive use of antibiotics like sulfonamides in the treatment of bacterial infections over several decades has led to the development of severe resistance in several bacterial species. When the species develop resistance to existing antibiotics, it becomes necessary to carry out research and development of new antibiotics as well as new testing procedures to identify strains that are resistant. The correlation between the increase in antibiotic resistance and the enormous cost to the patient and the health care system are growing problems and present major therapeutic challenges now and in the future.

One of these challenges is that many governments imposed new regulations for the pharmaceutical industry to invest in antibiotic research, such as increased regulatory conditions and strict price controls. These regulations have partly prevented developments in antibiotic research (Franco, 2009).

In the last three decades the development of such antibiotic resistance in bacteria has inflated the financial cost of development of new drugs which are effective against such infections. Linezolid and daptomycin are the only two new classes with antibiotics of novel mechanisms of action which have been introduced into the market. The rest of the new antibiotics are just chemical modifications of existing drugs as shown in Table 1.2 (Franco, 2009).

Table 1.2 Development of new antibiotics during the last three decades. *Source:* (Franco, 2009).

Antibiotic agent and brand name	Antibiotic class	Antimicrobial activity	Country and date of approval
Quinupristin and dalfopristin (Synercid®) I.V.	Streptogramins	Infections associated with vancomycin resistant *Enterococcus faecium* bacteremia	US, September 21, 1999
Dalbavancin, telavancin, oritavancin	Second-generation glycopeptides	Bactericidal activity against MRSA, VRSA, vancomycin-resistant *enterococcus* and drug-resistant *Streptococcus pneumoniae*	US, phase II, 2009
Daptomycin (Cubicin®) I.V.	Cyclic lipopeptide new class of antibiotic	Bactericidal activity against Gram +ve *S. aureus*, MRSA, *Staphylococcus epidermidis* including methicillin-resistant *S. epidermidis*, vancomycin-resistant *enterococcus*, penicillin-resistant *S. pneumoniae* and *Streptococcus pyogenes*	US, December 9, 2003
Tigecycline (Tygacil®) I.V.	Glycylcycline similar to the tetracyclines	Bacteriostatic activity against Gram+ve, Gram -ve and atypical anaerobic bacteria, and antibiotic-resistant, MRSA, vancomycin-resistant *enterococcus*, penicillin-resistant *S. pneumoniae*	US, June, 2005
Linezolid (Zyvox®) Oral and parenteral forms	Oxazolidinone	Bacteriostatic activity against enterococci and *staphylococci,* bactericidal activity against many drug-resistant streptococci, *S. pneumoniae* and infections caused by anaerobic bacteria	US, April 18, 2000
Telithromycin (Ketek®)	Erythromycin derivative	The FDA announced changes, including the removal of two of the three previously approved indications – acute bacterial sinusitis and acute bacterial exacerbations of chronic bronchitis. Ketek is now indicated for the treatment of community-acquired pneumoniae (of mild to moderate severity) due to *Streptococcus pneumoniae*, (including multi-drug resistant isolates), *Haemophilus infl uenzae*, *Moxarella catarrhalis*, *Chlamydophila pneumoniae*, or *Mycoplasma pneumoniae*	US, April 01, 2004 Last published revision: February 12, 2007

Antibiotic agent and brand name	Antibiotic class	Antimicrobial activity	Country and date of approval
Ertapenem (Invanz®) I.V. and intramuscular	Carbapenem	Activity against Gram +ve and Gram -ve bacteria, but shows only limited activity against *Enterococcus aspp*., *Pseudomonas aeruginosa* and other nonfermenting Gram +ve bacteria	US, November 21, 2001
Doripenem (Doribax®) I.V.	Carbapenem	Bactericidal activity, for the treatment of complicated urinary tract and intra-abdominal infections	US, October 12, 2007
Faropenem Oral	Carbapenem	It was developed for the treatment of community-acquired respiratory tract infections and later for uncomplicated skin and skin structure infections. It was shown to be active against penicillin-resistant *S. pneumoniae* and *β-lactamase*-producing *H. influenzae* and *M. catarrhalis*	Not yet FDA approved, 2008
Ceftobiprole (Zeftera®)	Cephalosporine	Bactericidal activity is mainly active against Gram +ve pathogens. For the treatment of complicated skin and soft tissue infections and nosocomial pneumonia caused by MRSAb*, enterococci* and *S. pneumoniae*	US, phase III, 2009
Iclaprim	Diaminopyrimidine New antibiotic	It has demonstrated *in vitro* activity against *S. aureus*, including MRSAb. Iclaprim was also active against one strain of VRSAc	US, phase III, 2009
Abbreviations: IV, intravenous presentation; MRSA, methicillin-resistant *S. aureus*; VRSA, vancomycin-resistant *S. aureus*.			

1.6.2 In fungi

Antifungal resistance has also been increasing and therefore there is an urgent need to develop new antifungal drugs. Moreover, some fungi could form biofilms which increases the resistance too. *Candida* species, which have the ability to form drug resistant biofilms, are a major factor in contributing to human disease. Different mechanisms of resistance are applied when the biofilm changes into a complex biofilm from an adherent phenotype. These mechanisms are extracellular matrix (ECM), efflux pump activity, persisters, cell density, overexpression of drug targets, stress responses, and the general physiology of the cell. The mechanisms of action of different classes of antifungal agents such as, azoles (AZL), polyenes (POL), and echinocandins (ECN) and their antifungal resistance, are summarised in the diagram below (Figure 1.10). First, it can be see that the layer of extracellular matrix (ECM) binds to the antifungal agents and shields the cells. ATP-binding cassette (ABC) and major facilitator superfamily (MFS) transporters in the membrane are efflux pumps for the antifungal agent. By changing the ergosterol biosynthesis pathway through mutation in genes such as; ERG, Cyp51, and FKS1 will change the drug target. Moreover, response to the antifungal agent stress can be altered by calcineurin, which is a Ca^{2+}-calmodulin-activated serine/ threonine-specific protein phosphatase, that plays many critical stress roles in the fungal cell (Ramage *et al.*, 2012, Sardi *et al.*, 2013).

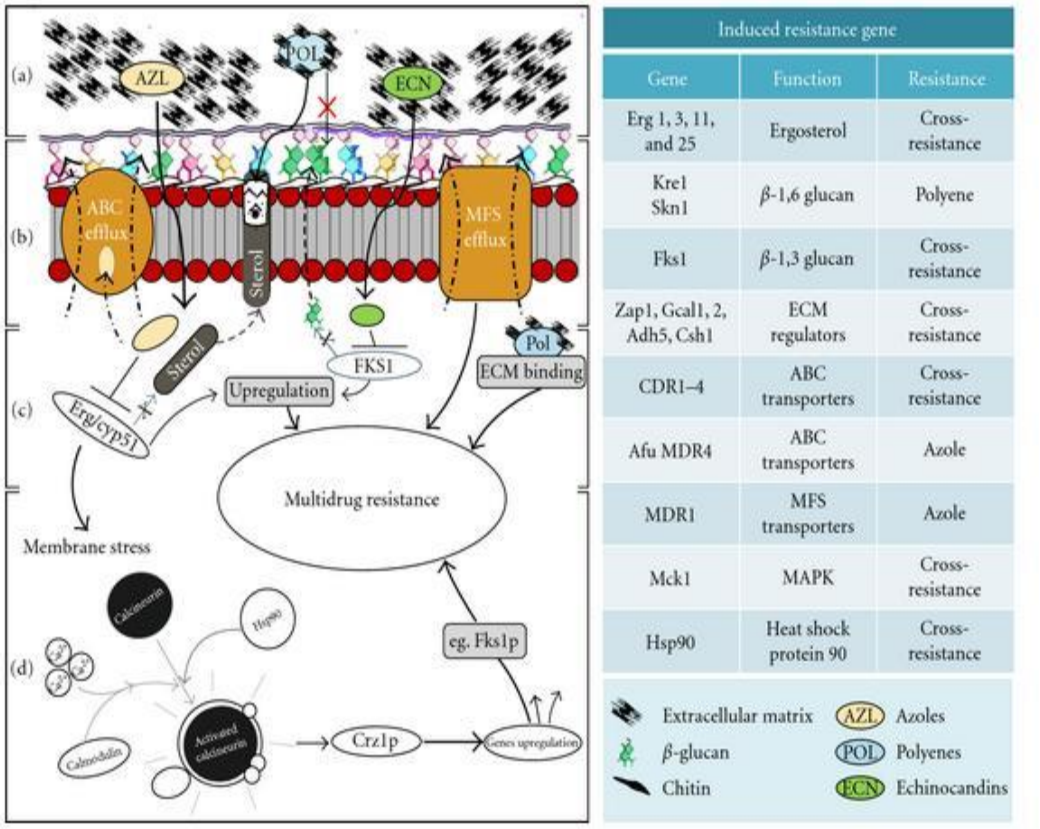


Figure 1.10 Molecular mechanisms of fungal biofilm resistance. *Source*: (Ramage *et al.*, 2012).

In the last 20 years other species such as *Aspergillus spp* have increased their resistance to the antifungal drugs, although the use of the antifungal drugs has also increased as well, which then partially results in the failure of the treatment. The extensive use of the antifungal drugs was clear in Denmark in using azoles, which is one of the major drug classes used against fungal infections. Their results doubled over the last decade (2002–2011) as summarised in Figure 1.11 (Hadrich *et al.*, 2012, Arendrup *et al.*, 2013).

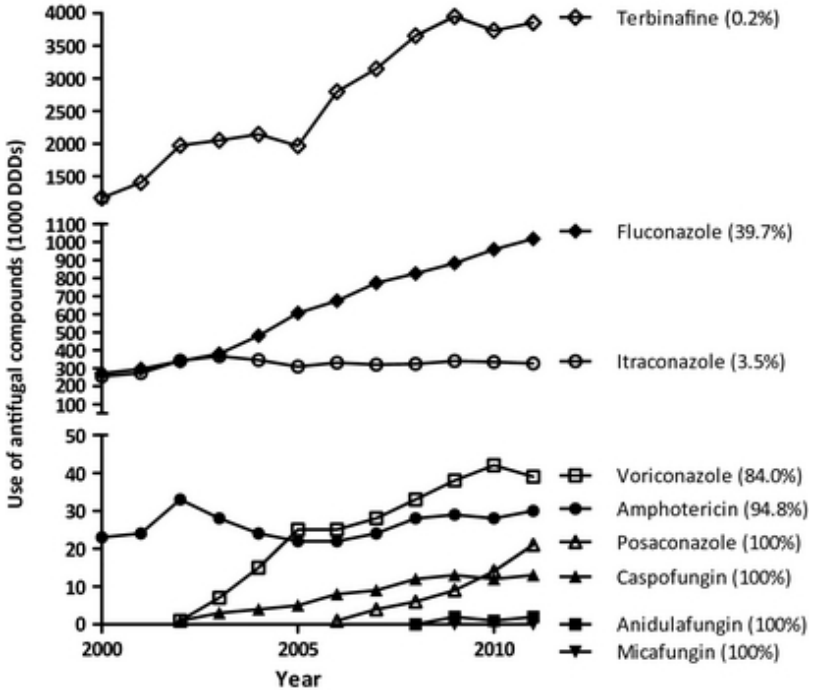


Figure 1.11 National antifungal consumption (1000 DDD) in the years 2000–11 in Denmark. The hospital use in percentage of the total use in the study period 2010–11 is indicated in parenthesis for each compound. *Source*: (Arendrup *et al.*, 2013).

1.7 Antimicrobial stewardship

Meanwhile, existing antimicrobials need to be preserved by controlling their usage. Therefore, an approach addressed by the Department of Health, antimicrobial resistance strategy in the UK 2013–2018 under the name of "Antimicrobial stewardship" has been instigated. This approach has three goals; improve the therapy for individual patients, prevent misuse, and control the increase of resistance at patient and community level. Over the past two decades there has been notable progress in UK hospitals in developing and implementing antimicrobial stewardship. The collected data of this approach is summarised in Table 1.3 (Department of Health, 2013a)

Table 1.3 Implementation of antimicrobial stewardship strategies in UK hospitals – progress over two decades. *Source: (Department of Health, 2013a)*

Criteria	**Percent of total hospitals using criteria**		
Year	**1994**	**2006**	**2012 (ongoing)**
Source	**BSAC**	**Wickens & Jacklin**	**ESGAP & ISC (unpublished)**
Sample	**n=427 UK hospitals**	**n=125 English hospitals**	**n=126 UK hospitals**
Guidelines for antibiotic therapy	62%	90%	100%
Guidelines for surgical prophylaxis	51%	87%	99%
Antibiotic formulary	79%	89%	99%
Restricted list	61%	69%	93%
Educational campaigns	52%	73%	100%
Automatic stop policy	26%	-	61% (stop/review)
Antibiotic committee	17%	56%	85%
Antibiotic audit	11%	78%	98%
IV-to-oral switch guidance	-	69%	92%
Microbiology ward rounds	64%	-	96%
Stewardship ward rounds	-	35%	86%
Antimicrobial consumption surveillance (WHO defined daily doses)	-	46%	69%
Dedicated antimicrobial prescription chart	1.5%	<1%	40%
Inflammatory marker testing (e.g. procalcitonin)	-	-	11%

1.8 The need to develop new drugs

According to what has been mentioned in Section **1.6**, there is a critical need to develop new drugs, but the investment available for such development is frequently lower than the required level. There are various reasons for this according to the annual Report of the Chief Medical Officer in UK, including the high cost for drugs that are effective against Gram -ve bacteria; type of drug which is used for short treatment courses, compared with drugs used for chronic conditions; reducing ineffective patent period and the investment for the current drug; and also there are challenges in the requirements for testing new drugs. The Chief Medical Officer for England; Professor Dame Sally Davies has described the dangers of the continuing increase of resistance to antibiotics, and listed it at the same level as “terrorism” on a list of threats to the community. Figure 1.12 illustrates that no new class of antibiotics have been developed since the late 80s (BBC, 2013).

Therefore, researchers only had focused on the chemical modification of existing drugs to inhibit multi drug resistance in bacteria. Also it is possible to use existing drugs to inhibit virulence rather than inhibit growth directly (Imperi *et al.*, 2013). As it will be describe in section 1.10, benzimidazole has been widely used in medicinal chemistry and researchers are actively seeking new uses and applications for it.

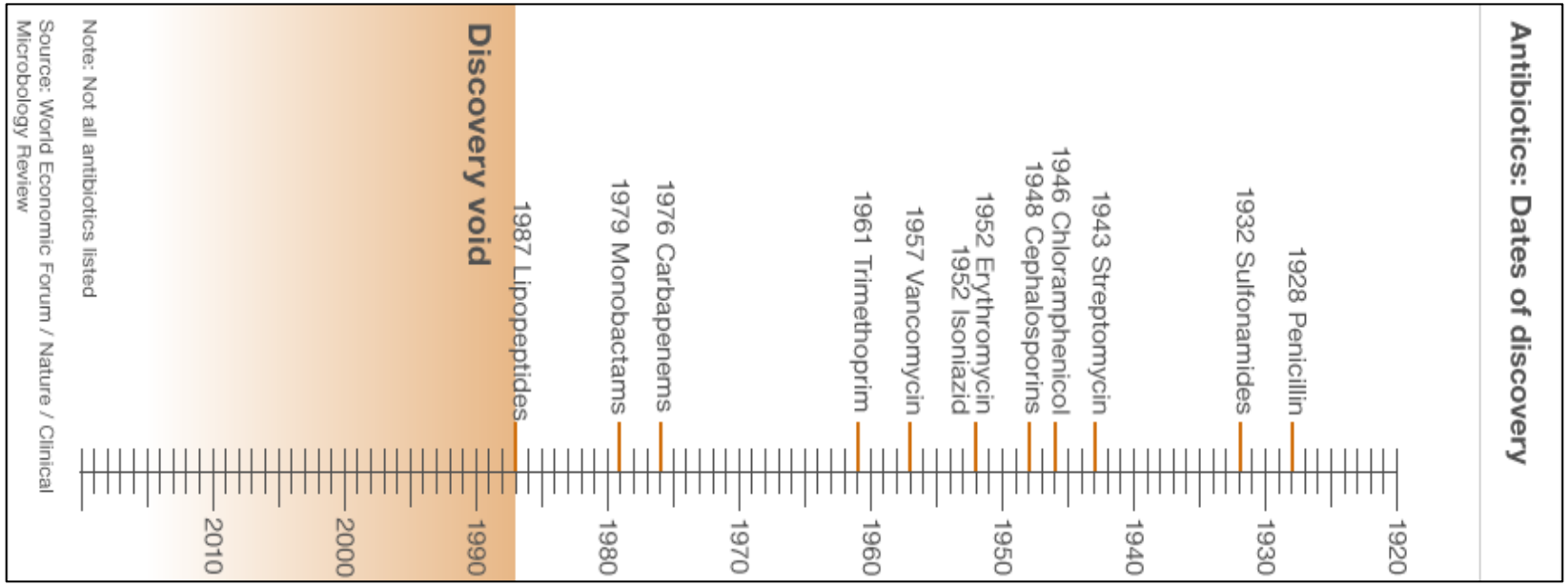
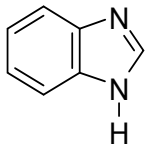


Figure 1.12 Antibiotics: dates of the discoveries. *Source*: (BBC, 2013).

1.9 Benzimidazoles in medicinal chemistry

1.9.1 The structure of benzimidazole

Benzimidazole (1) is a heterocyclic compound in which the benzene and imidazole rings are fused together. The chemical structure of benzimidazole is shown in Figure 1.13 (Wagner, 1943). This bicyclic compound has two nitrogen atoms from the imidazole nucleus and it has numerous medical and biological activities such as antibacterial, antifungal, antiviral, anticonvulsant, antidepressant, analgesic, anti-inflammatory and antidiabetic properties (Bansal and Silakari, 2012) (Figure 1.14).



(1)

Figure 1.13 The chemical structure of benzimidazole**.**

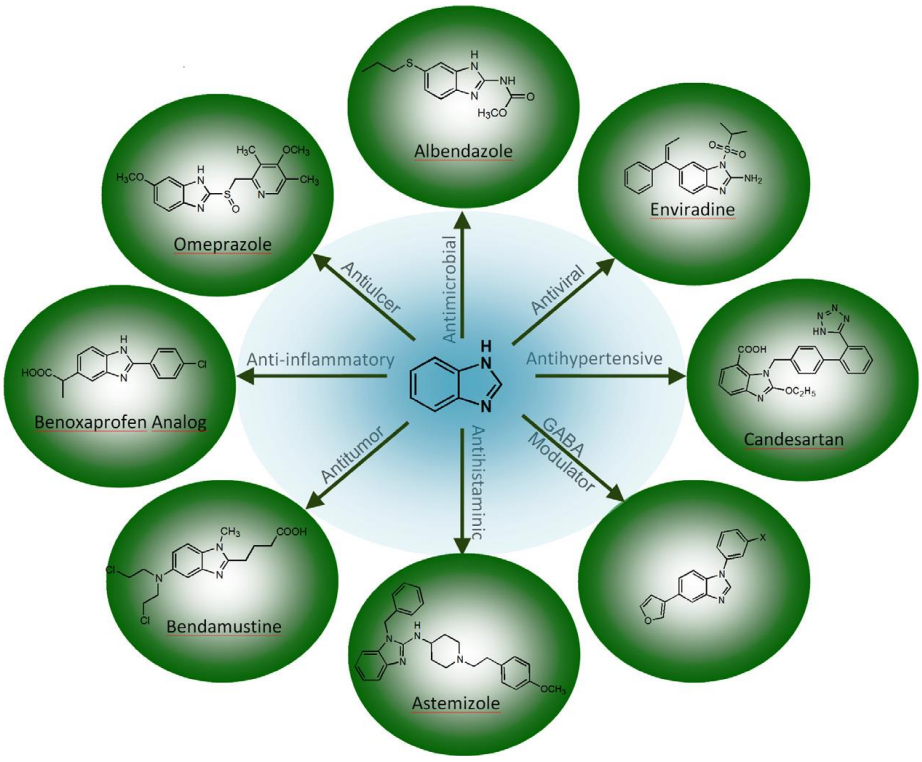


Figure 1.14 Benzimidazole, a multifunctional nucleus. *Source*:(Bansal and Silakari, 2012)

1.9.2 Properties of benzimidazole

Benzimidazole is a slightly beige coloured powder with a melting point of 172 ^{o}C and boiling point of 360 ^{o}C. It is sparingly soluble in water but readily soluble in ethanol. It is generally stable under normal temperature and pressure.

Since benzimidazole consists of a phenyl ring fused to imidazole, it is expected to form hydrogen bonding due to the nitrogen atoms of the imidazole ring and the H-bonding is illustrated in Figure 1.15.

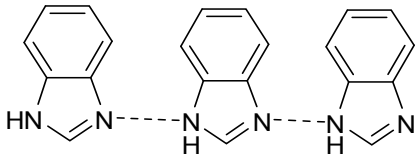


Figure 1.15 Hydrogen bonding network of benzimidazole

The nitrogen atoms of the imidazole ring possess a lone pair of electrons which indicates that benzimidazole and its derivatives have a tendency to donate electron pairs, but such tendency depends upon the substitution on the compound. Therefore, benzimidazoles act as bases and their properties can be compared with nitrogens of pyridine and pyrrole rings (Figure 1.16). Moreover, because of the tautomerism in benzimidazoles two non-equivalent structures can be written, but only one compound is known, as the situation of 5(or 6)-methylbenzimidazole.

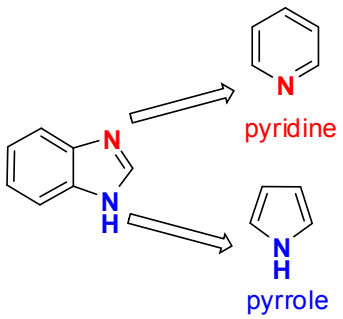
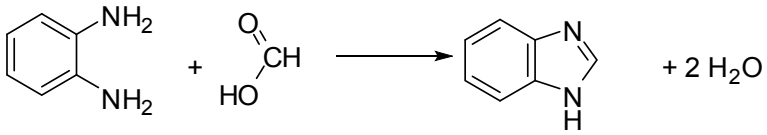


Figure 1.16 Pyridine and pyrrole type nitrogens of benzimidazole

1.9.3 Synthesis of benzimidazoles

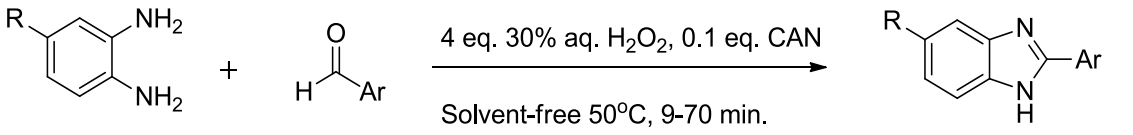
Benzimidazole (2) can be readily synthesised by heating *o*-phenylenendiamine with formic acid in absence of any catalyst as shown in Figure 1.17.



(2)

Figure 1.17 Synthesis of benzimidazole

Numerous derivatives of benzimidazole can be formed by altering the carboxylic acid especially for the formation of 2-substituted benzimidazoles. There are several methods available for the preparation of benzimidazole derivatives (3) (Khan, 2007). Only a few examples have been selected to illustrate the recent developments. Bahrami *et al.* (2008) proposed a method for synthesis of 2-substituted benzimidazole as shown in Figure 1.18. This is a very convenient method and involves quick reaction times with high yields and quick and easy isolation of the benzimidazole derivatives along with good chemoselectivity (Bahrami *et al.*, 2008).



R: H, CH_3, NO_2

Ar: Ph, CH_2-CH-Ph, Ph-NO_2, Ph-F

Yield: 95-98%

(3)

Figure 1.18 Synthesis of 2-substituted benzimidazole according to Bahrami *et al.,* (2008)

Another synthesis, which was proposed by Alinezhad *et al.,* (2012), involves ZnO nanoparticles as catalysts for the reaction of 1,2-phenylenediamine with formic acid at 70 °C and under solvent-free conditions (Figure 1.19) (Alinezhad *et al.*, 2012).

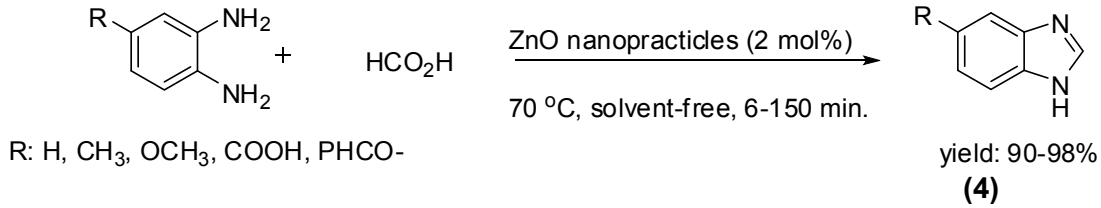


Figure 1.19 Synthesis of benzimidazole according to Alinezhad *et al.* (2012a)

Another excellent synthesis which has been proposed by Saha *et al*. (2009) involved a simple, efficient and ligand free reaction scheme for the synthesis of substituted benzimidazoles, 2-aminobenzimidazoles as well as benzothiazoles (5) (Saha *et al.*, 2009). The reaction is catalysed by CuO nanoparticles in DMSO and this heterogeneous catalyst can be recovered without losing its activity (Figure 1.20) (Saha *et al.*, 2009).

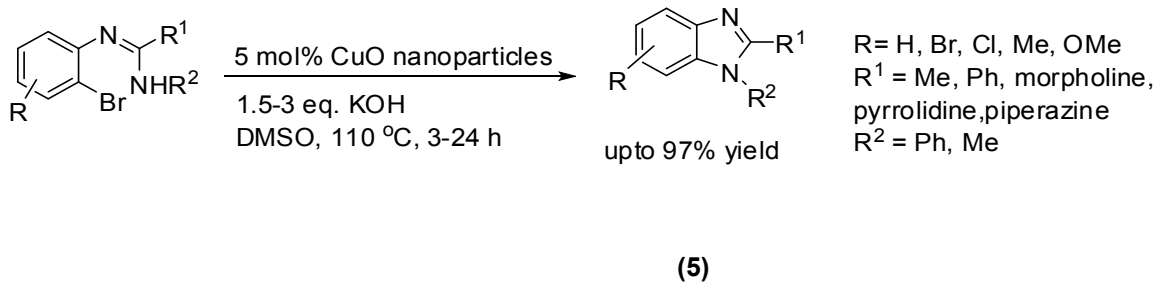


Figure 1.20 Synthesis of 2-substituted benzimidazole according to Saha *et al.* (2009)

In recent developments ultrasonic irradiation has been reported by Zhang *et al.,* (2012a), for the synthesis of 1,2-disubstitued benzimidazoles (6) and 2-substitued benzothiazoles (7), using rare-earth metal chlorides as a catalysts (Figure 1.21). This method has many advantages such as, economical catalysts, good yields, simple operation, solvent-free conditions, and ambient temperature (Zhang *et al.*, 2012a).

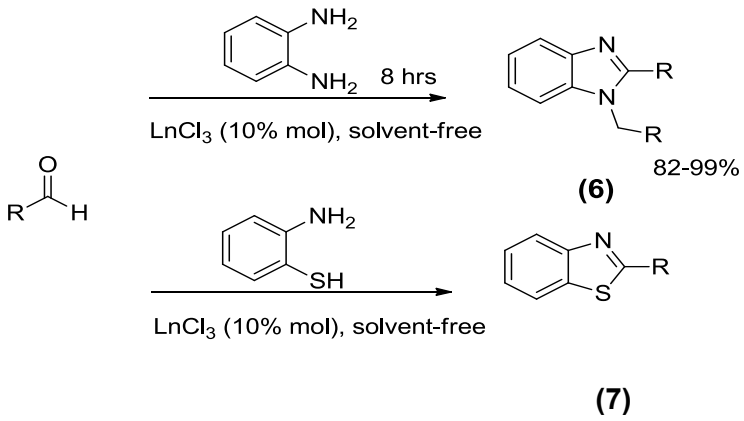


Figure 1.21 Synthesis of 1,2-substituted benzimidazole and benzothiazoles according to Zhang *et al.* (2012a)

1.10 Benzimidazoles in Medicinal Chemistry

As mentioned in section **1.10.1,** benzimidazole and its derivatives are very important in terms of their medicinal applications. Derivatives such as thiabendazole (8), cambendazole (9), parbendazole (10), mebendazole (11), albendazole (12), flubendazole (13) are very popular anti-helminth drugs used to cure people and animals of gastrointestinal worm infections (Cho and Lopes, (2011)) (Figure 1.22 and 1.23).

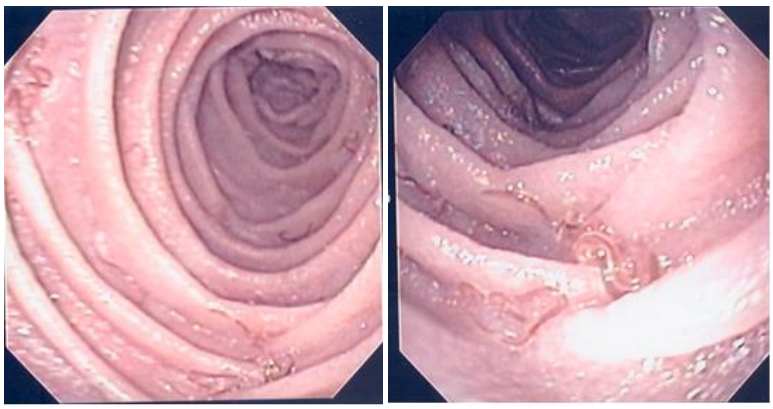


Figure 1.22 Endoscopic visualization of hookworms in the duodenum. *Source:* (Sudha and Christian, 2009)

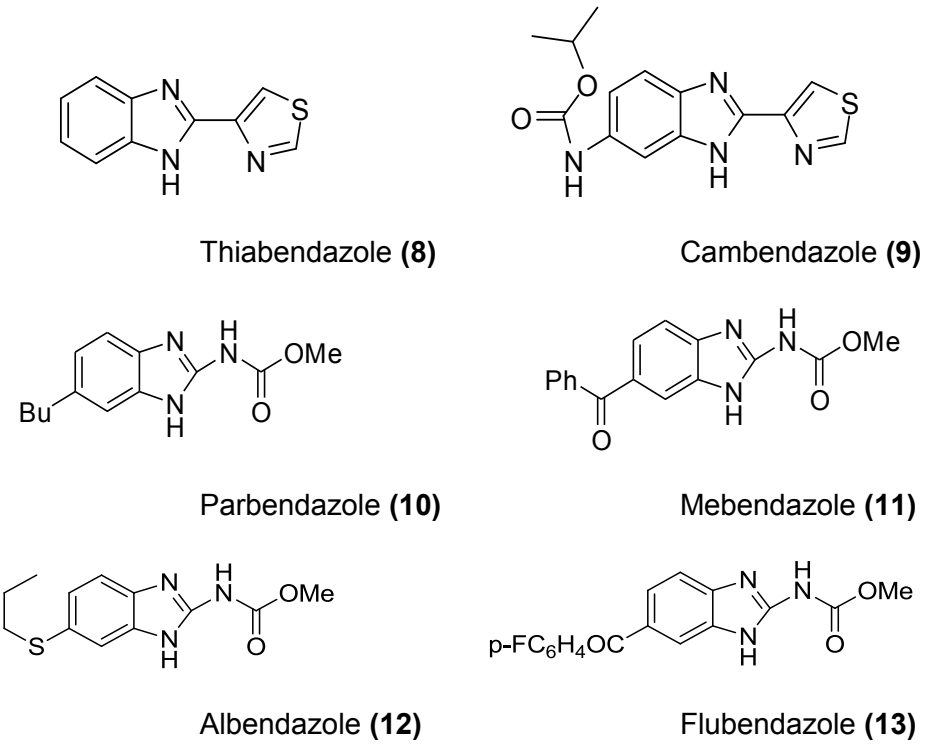


Figure 1.23 Examples of benzimidazole derivatives as anti-helminth drugs according to Cho and Lopes (2013)

Two mechanisms have been proposed for the action of benzimidazoles as anti-helminth drugs. Fumarate reductase is an important enzyme in helminths that helps in the oxidation of NADH to NAD. Benzimidazole derivatives are known to inhibit the activity of the enzyme and hence oxidative phosphorylation is uncoupled, which is an important event for ATP production (Prichard, 1970). The second mechanism involves binding of benzimidazoles with the protein tubulin resulting in reduced or no formation of microtubules. Due to reduced solubility of benzimidazoles in water, these are poorly adsorbed from the gut, which may act as a positive property of the drugs since primarily these drugs need to be concentrated in the intestinal tract for treating helminths. After consumption, these drugs undergo rapid metabolism in the liver and are excreted to the bile (Williams, 2008). Albendazole is quite unique as an anti-helminth due to the presence of a thio-ether group which helps in increasing sulfur oxidation. The metabolite formed by this drug is a highly potent anti-helminth.

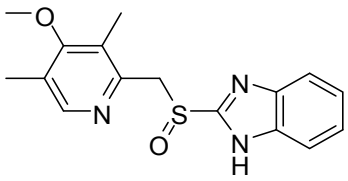
Mebendazole reduction gives a metabolite with a secondary alcohol substituent which increases the solubility of the drug into water and makes the drug quite active. Mintezol, tresaderm and arbotect are the trade names for thiabendazole and are commonly used in veterinary medicine for management and treatment of enterobiasis, strongloidiasis, ascariasis, unicinariasis and trichuriasis. Therefore, thiabendazole and its derivatives are less commonly used than albendazole or mebendazole. It is known to inhibit fumurate reductase with possible interaction with endogenous quinone, however it is not well established whether metal ions are involved in the inhibition mechanism or not. It also acts as a chelating agent and is useful to bind with metal ions and hence, medicinally useful for treating metal poisoning such as lead poisoning and mercury poisoning. Thiabendazole is known to be toxic and may lead to Strevens-Johnson syndrome and causes hepatotoxicity and crystalluria (Prichard, 1970).

A study (Kim *et al.*, 2008) has shown that 2-aminobenzimidazole acts as a potent glucogen receptor antagonist which causes resistance to glucagon. The glucogen receptor is a 62 kDa protein that is activated by a hormone of glucagon which is secreted by the pancreas, and causes the liver to convert stored glycogen into glucose. The results also showed excellent selectivity over other human glucagon-like peptide receptors and the human glucagon-like peptide 1 receptor which have a role in glucose-dependent insulin secretion.

Another study showed that 5,6-dichlorobenzilidazole-1-β-D-ribofuranoside (commonly known as DRB) and its 2-substituted derivatives have excellent activity against cytomegalovirus in humans (Devivar *et al.*, 1994). Some derivatives of benzimidazole such as 5,6-dinitrobenzimidazole are capable of replacing 5,6-dimethylbenzimidazole found in vitamin B_{12} in *Corynebacterium* species (Gram +ve bacterial genus) and other derivatives acts as an inhibitor for photosynthesis by decoupling oxidative phosphorylation in mitochondria (Burton *et al.,*1965 cited in Maske) (Maske *et al.*, 2012).

Since benzimidazole and its derivatives can act as excellent ligands due to an electron rich nitrogen atom present in the imidazole ring, the transition metal ions can form complexes with benzimidazoles and some have shown anti-cancer activities. Cu(II) complexes of 2-substituted benzimidazole have the ability to mimic copper, zinc superoxide dismutase (Cu, Zn-SOD) which is an antioxidant enzyme which helps cells to protect from the toxicity of superoxides by dismutation of oxygen and hydrogen peroxide through a series of biochemical reactions in biological systems. Due to the lower superoxide dismutase activity of tumour cells, as compared to the normal cells, Cu(II) complexes of 2-substituted benzimidazole can target tumour cells easily and have the ability to act as anticancer agents (Azam *et al.*, 2009).

Another very important and widely used benzimidazole derivative is omeprazole (14)(Figure 1.24) which acts as a proton pump inhibitor. It is widely used in treatment of dyspepsia, gastroesophageal reflux, laryngopharyngeal reflux and peptic ulcer disease. Due to its existence as a racemate (it exists in *R* as well as *S* configuration in a 1:1 ratio), it is converted to achiral products under acidic conditions in the stomach, which on reaction with cysteine residue of H+/K+ ATPase, inhibits the production of gastric acid. Omeprazole acts as a competitive inhibitor for certain enzymes that are associated with cytochrome P450 and hence is able to interact with other drugs that involve cytochrome P450 in metabolism. Examples include diazepam and warfarin. However, there are several side effects associated with its use such as headache, diarrhea, nausea, reduction in vitamin B_{12} and sleep deprivation. Its prolonged use may also cause inflammation of the kidney (tubulointerstitial nephritis) (Stedman and Barclay, 2000, Furuta, 2005, Ray *et al.*, 2010, Madanick, 2011).



(14)

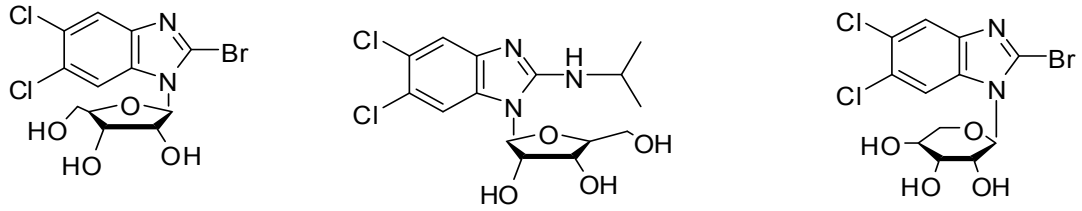
Figure 1.24 Omeprazole

1.10.1 Benzimidazole and derivatives as Antimicrobial and other medicinal agents

There have been some benzimidazole derivatives which have shown activity against Entamoeba indicating anti-protozoal efficacy of the drugs. 2-Methoxycarbonylamino derivatives of benzimidazole have shown better antiprotozoal activities against some protozoan parasites such as *Giardia lamblia* and *Entamoeba histolytica* by inhibiting the tubulin polymerization and hence making these better antiprotozoal agents than metronidazole and albendazole (Valdez *et al.*, 2002). Besides the above mentioned medicinal properties of benzimidazole and its derivatives, two groups of benzimidazole derivatives, namely 5,6-dinitro and 2-trifluromethyl derivatives, are known for use as antihelminth drugs (Stefanska *et al.*, 1999). Nitrogen and halogen substituted derivatives of benzimidazole and their analogues show potential chemotherapeutic activity against *Stenotrophomonas maltophilia* which is a Gram -ve opportunistic pathogen. Some analogues are highly effective against *Trichomonas vaginalis* which is an anaerobic, flagellated protozoan parasite, and are known to act in the same way as metronidazole behaves, via reduction of the nitro group through ferrodoxin.

Benzimidazole D-ribonucleosides such as 2, 5, 6-trichloro-(1-β-D-ribofuranosyl) benzimidazole (TCRB) as well as its bromo homologue (BDCRB) (15)have been found to be potent and selective inhibitors of human cytomegalovirus (HCMV). These drugs inhibit the processing and maturation of viral DNA and cause

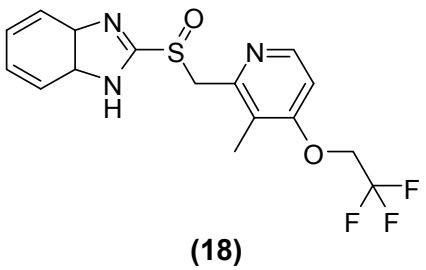
mutations in viral genes UL56 and UL89 and are better than the antiviral drugs used to date such as ganciclovir, cidoclovir and the pyrophosphate analogue foscarnet due to their improved oral bioavailability as well as fewer toxic side effects. These benzimidazole ribonucleoside derivatives, along with many additional analogues e.g. 1263W94 (16) and 275175X (17) (Figure 1.25) have been found to be more biologically stable and orally active (Williams *et al.*, 2003).



BDCRB **(15)** **1263W94** **(16)** **275175X** **(17)**

Figure 1.25 Analogues of benzimidazole ribonucleoside according to Williams *et al.* (2003**)**

Helicobacter pylori is a well-known Gram -ve bacterial human pathogen that is responsible for type B gastritis of the stomach and can lead to duodenal ulcers and even gastric cancer. This species is typically treated with bismuth salts in combination with antibiotics. However, lansoprazole (18) (Figure 1.26), a novel benzimidazole, has proven itself a better antagonist against *H. pylori* with a notable and highly selective activity which is much superior to bismuth salts or bismuth-antibiotic combinations. Lansoprazole acts as a proton pump inhibitor for stomach and hence useful for treatment of gastroduodenal diseases (Iwahi *et al.*, 1991).

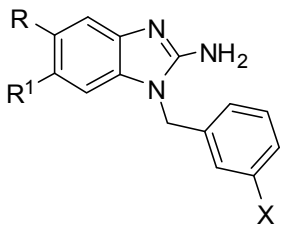


(18)

Figure 1.26 Lansoprazole

1.10.1.1 Benzimidazole derivatives possessing the amino group

In SAR, the amino group found at position 2 of the benzimidazole ring (19) (Figure 1.27) plays a significant role in the antibacterial activities of the benzimidazoles. However, the presence of some substituents are a disadvantage, when the methyl groups exist at positions 5 and 6, the antibacterial activity of the benzimidazoles are decreased, and even no activity was recorded for the methyl derivatives against *Pseudomonas aeruginosa*. However the activity against the Gram +ve strains, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923 and *Sarcina lutea* ATCC 9341, were about 1.5 to four fold less potent (MIC value 500 or 750 µg/ml) than the unsubstituted derivatives (Podunavac-Kuzmanovic and Cvetkovic, 2007).

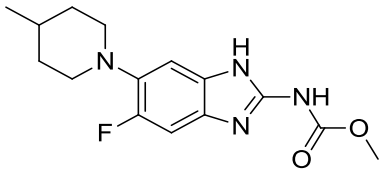


X = F, Cl
R= H, CH_3
R^1= H, CH_3

(19)

Figure 1.27 Structure of 2-amino benzimidazole derivatives synthesised by Podunavac-Kuzmanovic and Cvetkovic (2007)

When a series of derivatives related to 2-amino benzimidazole and 2-methyl benzimidazole were synthesised to evaluate their inhibitory activities against the yeast *Candida albicans,* antimicrobial activity was detected. The 2-amino derivatives (20) shown in Figure 1.28 increased the activity in contrast to the methyl group which decreased activity. The compound exhibited less antifungal activity than fluconazole and ketoconazole (comparator control drugs), with 15 mm inhibition zone diameter (Kus *et al.*, 2001).



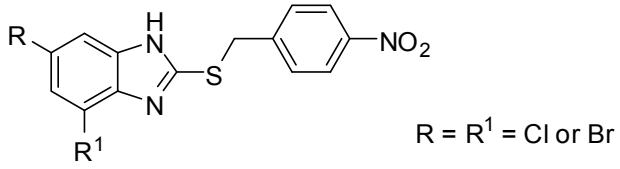
(20)

Figure 1.28 Structure of methyl 5-fluoro-6-(4-methylpiperidin- 1-yl)-1*H*-benzimidazole-2-carbamate according to Kus *et al.* (2001)

1.10.1.2 Bezimidazole derivatives possessing the sulfide group

Some of the derivatives of benzimidazole that possess sulfide groups show inhibitory activities against *Helicobacter spp*. with minimum bactericidal concentrations (MBCs) of 0.5 - 4 µg/mL (Kuhler *et al.*, 2002).

The derivatives of 4,6-dichloro(bromo)-2-(4-nitrobenzylthio)-1*H*-benzimidazole **(21)** as shown in Figure 1.29, displayed antibacterial activity against all selected Gram +ve bacteria; *Staphylococcus aureus* ATCC 6538P, *Staphylococcus. aureus* NCTC 4163, *Bacillus stearothermophilus* ATCC 7953, *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ML 98 with MICs within the range of 0.78–50 µg/ml, and also were more potent than nitrofurantion (control drug) by 4x - 32x except against *Enterococcus faecalis* ATCC 29212, which they were, respectively, 2 and 4 times less potent against (Andrzejewska *et al.*, 2004).

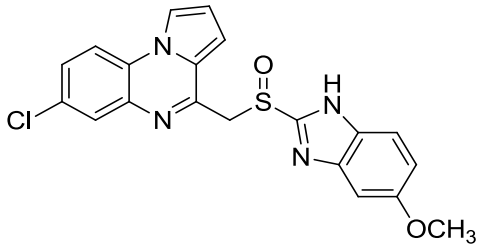


(21)

Figure 1.29 4,6-Dichloro(bromo)-2-(4-nitrobenzylthio)-1*H*-benzimidazole derivatives studied by Andrzejewska *et al.* (2004)

Several 2-[benzimidazol-2-yl-sulfanyl]acetyl amidino]thiazole derivatives, when tested against *Micrococcus luteus*, *Bacillus cereus*, *Staphylococcus aureus, Proteus vulgaris*, *Salmonella typhimurium*, *Escherichia coli*, *Candida albicans* and *Candida globrata*, showed significant levels of activity (MICs within the range of 3.9-250 µg/mL) (Kaplancikli *et al.*, 2004).

Some other analogues of omeprazole also showed some eukaryotic efflux pump inhibitor properties, and when combined with the norfloxacin, the MIC of the drug decreased by 4-16 times. It was also concluded that the compound (22) (Figure 1.30), was more effective than omeprazole, and particularly in restoring the bactericidal activity of norfloxacin over a prolonged period (Vidaillac *et al.*, 2007).

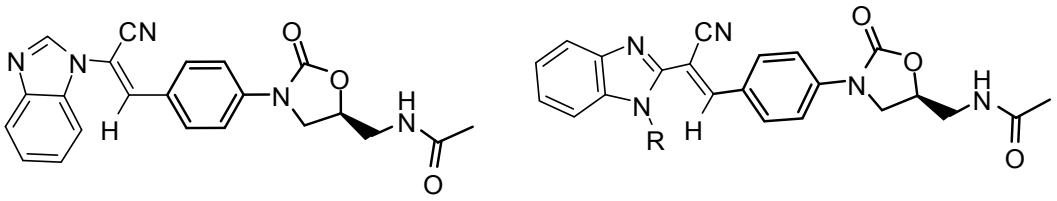


(22)

Figure1.30 7-Chloro-4-(((5-methoxy-*1H*-benzo[*d*]imidazol-2-yl)sulfinyl)methyl)pyrrolo[1,2-a]quinoxaline according to Vidaillac *et al*. (2007)

1.10.1.3 Benzimidazole possessing *N*-alkyl and *N*-acyl derivatives

The results of a study by Das *et al.* (2005) found that the *N*-linked benzimidazole (23,24) as shown in Figure 1.31, showed antibacterial activities (MIC values within the range of MICs 16- ≥32 µg/ml), comparable to *C*-linked benzimidazole, against selected strains; *Mycobacterium smegmatis* MTCC 006; *S. aureus* ATCC 29213 (MSSA); *S. aureus* ATCC 49951, *S. aureus* ATCC 33591 (MRSA); *Enterococcus. faecalis* ATCC 29212; *Enterococcus. faecalis* NCTC 12201 *Enterococcus. faecium* NCTC 12202 (VREfm) (Das *et al.*, 2005).

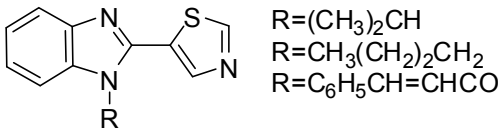


R= H, CH_3

(23) **(24)**

Figure 1.31 Structure of 1,2 disubstituted benzimidazole derivatives according to Das *et al.* (2005) (Das *et al.*, 2005)

In order to investigate the antimicrobial activities further, some of the *N*-alkyl and *N*-acyl derivatives (25) (Figure 1.32) were tested against some fungal strains and the research concluded that the presence of the isopropyl group at position-1 helped in enhancing sufficient amount of antifungal activity against *Aspergillus niger*, *Aspergillus flavus, Alternaria alternata* and *Fusarium oxysporum* (zone of inhibition within the range of 40-50 mm). A significant amount of antibacterial activity against *Bacillus mecarium*, *Bacillus japonecum* and *Pseudomonas fluorescence* was shown by the butyl (zone of inhibition within the range of 14-18 mm) and cinnamate derivatives (zone of inhibition within the range of 10-18 mm) (Pawar *et al.*, 2004).

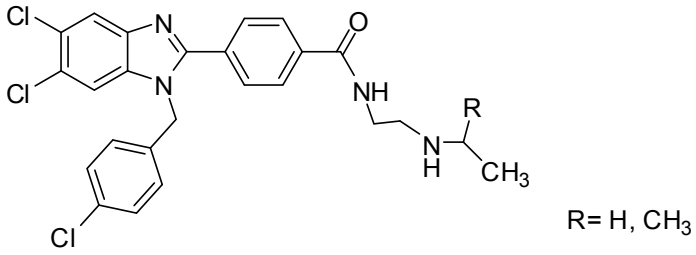


R=$(CH_3)_2CH$
R=$CH_3(CH_2)_2CH_2$
R=C_6H_5CH=CHCO

(25)

Figure 1.32 Structure of thiazolyl-benzimidazole derivatives according to Pawar *et al.* (2004)

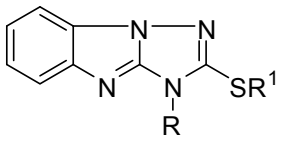
Two compounds of series 4-(5,6-dichloro-1*H*-benzimidazole-2-yl)-*N*-substituted benzimidazole derivatives (26) (Figure 1.33) possessed antibacterial activity with MIC values of just 3.12 µg/mL against *S. aureus*, methicillin-resistant *S. aureus* and methicillin-resistant *S. epidermidis* (Ozden *et al.*, 2004).



(26)

Figure 1.33 Structure of 4-(5,6-dichloro-1*H*-benzimidazole-2-yl)-*N*-substituted benzamidazole derivatives according to Ozden *et al*. (2004)

A series of 1,2,4-triazalo[2,3-a] benzimidazoles (27) displayed antibacterial as well as the antifungal activities. Their activity improved with an increase in the bulkiness of the introduced alkyl groups to position-1 and 2. The most active derivatives are shown in Figure 1.34 (1 zone of inhibition 6-18 mm) (Mohamed *et al.*, 2006).

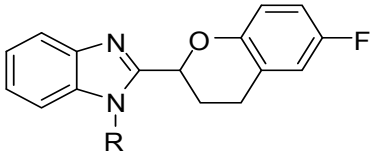


R	**R^1**
allyl	CH_3
C_2H_5	C_2H_5
allyl	C_2H_5
C_2H_5	allyl
allyl	allyl

(27)

Figure 1.34 Structure of 1,2,4-triazalo[2,3-a] benzimidazole derivatives synthesised by Mohamed *et al*. (2006)

Some 2-(6-flurochroman-2yl)-1-alkyl/ acyl/ aroyl 1*H*-benzimidazoles (28) as shown in Figure 1.35, have displayed promising antibacterial activity against *Salmonella typhimurium* (MICs within the range of MIC 200 to > 1000) (Kumar *et al.*, 2006).



R= CH_3, C_2H_5, C_3H_7, C_4H_9, -CO-O-C_2H_5, -CO-O-*tert*-Butyl, -CO-Phenyl, -SO_2-CF_3, -SO_2-Phenyl, -SO_2-C_6H_4-Me-*p*, -Benzyl, *p*-Fluorobenzyl, *p*-Bromobenzyl, *p*-Methylbenzyl, *p*-*tert*-Butylbenzyl

(28)

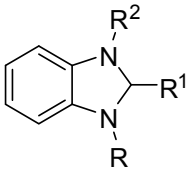
Figure 1.35 Structure of 2-(6-flurochroman-2yl)-1-alkyl/ acyl/ aroyl 1*H*-benzimidazoles studied by Kumar *et al.* (2006)

1.10.1.4 Benzimidazole complexes

According to a recent study by some US researchers, clay which contained iron showed antibacterial activity against *E. coli*. They believed that Fe^{2+} ions overwhelmed the bacteria's outer membranes and oxidised inside the cells to produce lethal hydroxyl (OH) radicals. This indicates that the complexes of iron metal were capable of inhibiting the bacteria (Cartwright, 2011). In addition, silver ion has been known as an antimicrobial agent from ancient times, and have been used for treatment infections of burns, open wounds, and chronic ulcers (Atiyeh *et al.*, 2007). Likewise benzimidazole itself has a broad spectrum of biological activity. Therefore, a complex of silver ions with benzimidazole could be useful as an antimicrobial agent. Silver in the metallic state is not active, but if present in an aqueous environment, it will then be as an ion which can then demonstrate activity against microbes (Cooper, 2004). Silver ions interact with a wide range of components of bacterial and fungal cells, resulting in inhibition of the microbial cells at very low concentrations. They do not possess a single mode of action; they can inactivate the vital enzymes by reacting with the thiol group, or prevent DNA replication by interaction with DNA. However, silver ions as metal with ligand contain sulfur will form a new and small molecule that causes changes on the cell structure (Matsumura *et al.*, 2003).

There are also reports on the synthesis and antimicrobial activity of some other transition metal complexes of benzimidazoles.(29) For example, some novel benzimidazole complexes of silver were synthesised and found to be effective against a series of bacteria and fungi. The compounds exhibited good activity against the selected bacteria for the chloro derivatives; *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922

and *Pseudomonas aeruginosa* ATCC 27853, (MIC ≤100 µg/ml), while the bromo derivatives were most active against the selected fungi; *Candida albicans* and *Candida tropicalis* (MIC12.5- 25 µg/ml) (Figure 1.36) (Özdemir *et al.*, 2010). For this reason, one of the aims of this project was to synthesise silver complexes of benzimidazole to explore the activity of these complexes against selected microorganisms.

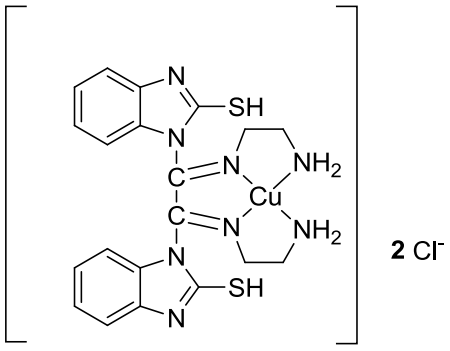


R	R^1	R^2
$-CH_2-CH_2-O-C_2H_5$	AgCl	Ph-CH-Ph
3,4,5-tri-methoxyphenyl	AgCl	Ph-CH-Ph
$-CH_2-CH_2$-1,3-dioxane	AgBr	2,4,6-tri-methyl-phenyl
$-CH_2-CH_2$-1,3-dioxane	AgBr	$-CH_2-CH_2-O-CH_3$

(29)

Figure 1.36 Structure of benzimidazol-2-ylidene carbene complexes of Ag(I) screened by Özdemir *et al.* (2010)

Many studies have also shown that the presence of metals connected with benzimidazole increase the activity of these compounds against a number of microorganisms. The copper complex (30) (Figure 1.37) was found to be active against *S. aureus* (with zone of inhibition of 19 mm), *E. coli* (with zone of inhibition of 17 mm) and *A. niger* (with zone of inhibition of 19 mm), and that can be due to the effect of the copper ion on the normal cell processes (Arjmand *et al.*, 2005).



2 Cl^-

(30)

Figure 1.37 Structure of Benzimidazole derived Cu(II) complex studied by Arjmand *et al.* (2005)

ZnL_2Cl_2 are complexes of zinc(II)chloride with 1-benzylbenzimidazole derivatives (L) (see Figure 1.27). Their biological activity showed that the Zn(II) complexes were more effective than the starting ligands against all the bacteria tested with the following results; *Bacillus cereus* ATCC 10876 (MIC 125- ≥500 µg/ml), *Staphylococcus aureus* ATCC 25923 (MIC 60- 250 µg/ml) and *Sarcina lutea* ATCC 9341 (MIC 60-250 µg/ml) and one gram negative isolate: *Pseudomonas aeruginosa* ATCC 27853 (MIC 125-250µg/ml) (Podunavac-Kuzmanovic and Cvetkovic, 2007).

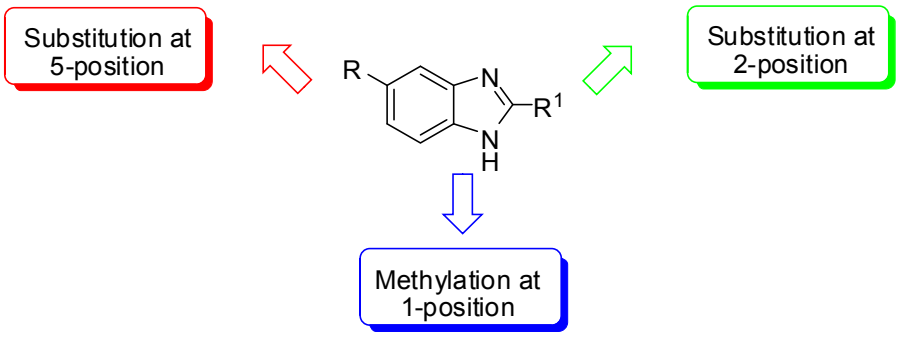
A series of Co(II) and Zn(II) complexes of benzimidazole (bz) were tested against various bacteria. The complex $[Zn(bz)_2Cl_2]$ 0.5 H_2O displayed antibacterial activity against *Micrococcus luteus* ATCC 9341 with zone of inhibition 10.4 mm (MIC value 1.6 µg/ml), whereas complex$[Co(bz)_2Br_2]$ showed no inhibition. Also, both complexes showed antibacterial activity against *E. coli* ATCC 6538P with zone of inhibition of 10 and 11.1 mm (MICs value 1.6 and 3.9µg/ml), respectively (Lopez-Sandoval *et al.*, 2008).

1.11 Programme of study

1.11.1 Design of an antimicrobial inhibitor

As mentioned in Section **1.4**, the mechanisms of action of antibiotics include the following; inhibition of cell wall synthesis, protein synthesis, synthesis of nucleic acids, the metabolic pathways and cell membrane functions (Hooper and Rubinstein, 2003, Lorian, 2005, Greenwood *et al.*, 2007, Pelczar *et al.*, 2009, Bauman, 2010, Karaiskos *et al.*, 2013, Rogers *et al.*, 2013). The mode of action of antifungal agents (Section **1.5**) include the following: fungal ergosterol synthesis inhibitors, squalene epoxidase inhibitors, ergosterol disruptors, glucan synthesis inhibitors, chitin synthesis inhibitors, nucleic acid synthesis inhibitors, protein synthesis inhibitors, and microtubules synthesis inhibitors (Kathiravan *et al.*, 2012). If further compounds were found to selectively block one of these targets, it could have potential as a chemotherapeutic drug.

It has been reported that the basic antimicrobial activity of the benzimidazoles is due to the presence of an amino group at position 2 of the benzimidazole ring (Podunavac-Kuzmanovic *et al.*, 1999). Disruption of the growth of bacteria through treatment with compounds with substitution of 2-alkylamine (Figure 1.38) could provide a new approach to antimicrobial inhibitors



R : H, CH_3, OCH_3, Cl, Br, F, CN, NO_2

R^1 : $\underline{C}H_2NH_2$, $(CH_3)\underline{C}HNH_2$, $\underline{C}H_2OH$,$\underline{C}OOH$, $\underline{C}H_2Cl$, $\underline{C}H_2SH$

Figure 1.38 1,2 and 5 benzimidazole derivatives available for this project

The biological activity of (1*H*-benzo[*d*]imidazol-2-yl)methanol FAS22, as mentioned in Table 2.4 (Section **2.4.3**), was previously studied. However, its antimicrobial activity was not investigated. Furthermore, the oxidation of 2-methanolbenzimidazole derivatives produces 2-carboxylic acid benzimidazole derivatives, which will decrease the pH, and may enhance the inhibition activity against different microorganisms, according to a study done by Talley *et al.* (2008), which found that the carboxylic analogue (1*H*-benzo[*d*]imidazole-2-carboxylic acid), inhibited the D-amino acid oxidase (Barden *et al.*, (2008)). Therefore this series of compounds are of interest for this study.

It has been found that the *N*-linked-benzimidazole showed antibacterial activities comparable to *C*-linked-benzimidazole (Das *et al.*, 2005). Thus, the alkylation of 5-substituted bezimidazole, and 2-methanolbenzimidazole derivatives could produce compounds which have antimicrobial activity (Figure 1.38).

It has also been found that the antibacterial activities of the chlorinated compounds are greater than the non-chlorinated compounds (Nandi *et al.*, 1984). According to this, the chlorine atom in 2-chloromethylbenzimidazole and its derivatives could enhance the antimicrobial activity of benzimidazole (Figure 1.38).

According to some literature which showed promising results for the silver ion, the silver complexes could be enhaced the biological activity of the benzimidazole as a ligand (Özdemir *et al.*, 2010).

Review of the literature therefore suggests that there is scope for the design of benzimidazole derivatives with antimicrobial activity, by including a number of different functional groups.

1.11.2 Aims and objectives

The focus of this project was to synthesise derivatives of benzimidazole with a degree of antimicrobial activity. A detailed study of the structure-activity relationship

of these derivatives will inform the design of more potent compounds. The activity will then be investigated against a range of pathogens to see if the compound has an enhanced spectrum of activity, relative to that of related structures. Efforts will then be made to enhance the activity by further chemical modification.

The objectives for the project included:

- Literature review of use of benzimidazole derivatives in medicinal chemistry and antimicrobial chemotherapy;
- Design, synthesis and characterisation (m.p., IR, ^{1}H and ^{13}C NMR, mass spectrometry, and elemental analysis), of a series of benzimidazole derivatives with varying functional groups at positions 1, 2 and 5 (Figure 1.39-42);
- Synthesis of some silver complexes as well.
- Developing the optimum method for the biological screening of the new compounds.
- Biological evaluation of the compounds against a bacterial reference collection that include 15 strains of *Staphylococcus* Spp. (inc 2 x *S. aureus,* 8 x MRSA, 2 x EMRSA, 2 x *S. epidermidis* and 1 x *S. haemolyticus*). 5 strains of *E. coli,* 3, 4 strains of *Ps. aeruginosa,* 1 strain of *Serratia marcescens,* and 1 strain of *Burkholderia cepacia.*
- Biological evaluation of the compounds against a fungal reference collection that include 4 unicellular fungi (4 x *Candida),* 6 filamentous fungi, (1x *Absidia*), (2 x *Aspergillus)*, (1 x *Mucor*), (1 x *Penicillium), (1 x Syncephalastrum)*.

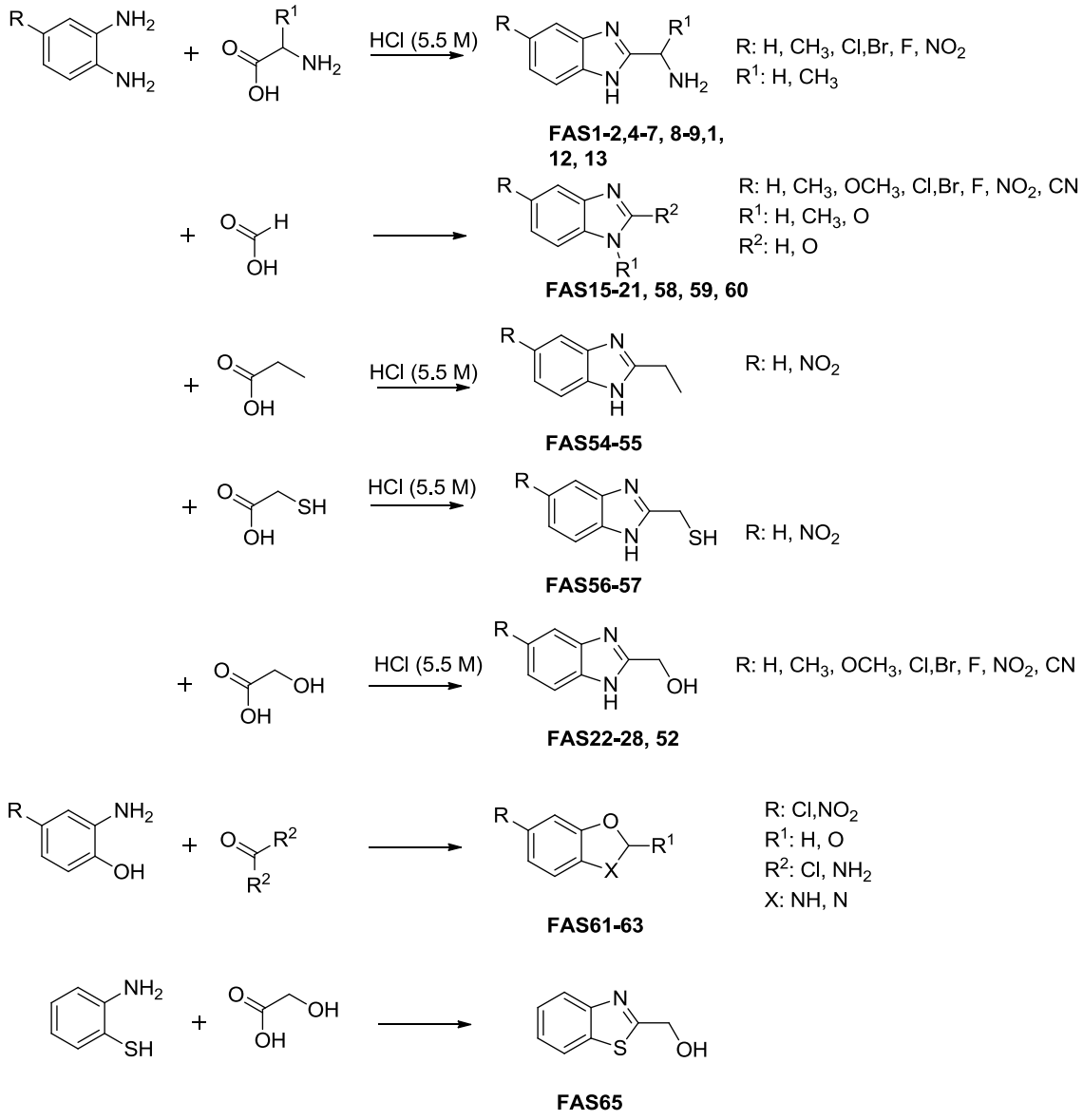


Figure 1.39 Group 1 target; benzimidazole derivatives

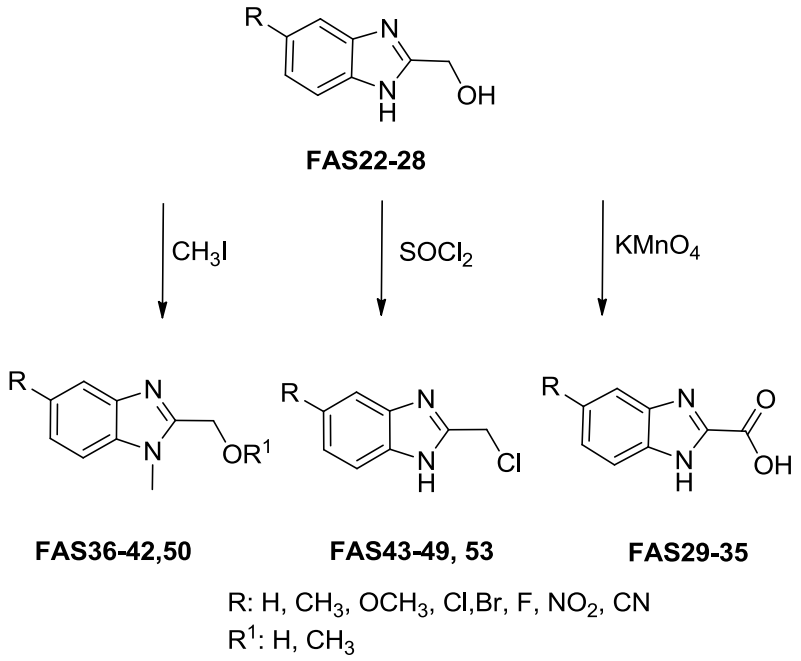


Figure 1.40 Group 2 target; 2-methanol benzimidazole derivatives

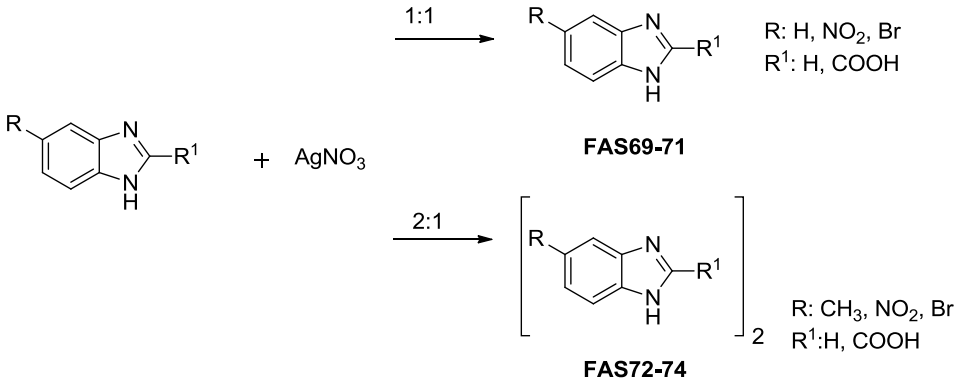


Figure 1.41 Group 3 target; silver benzimidazole complexes

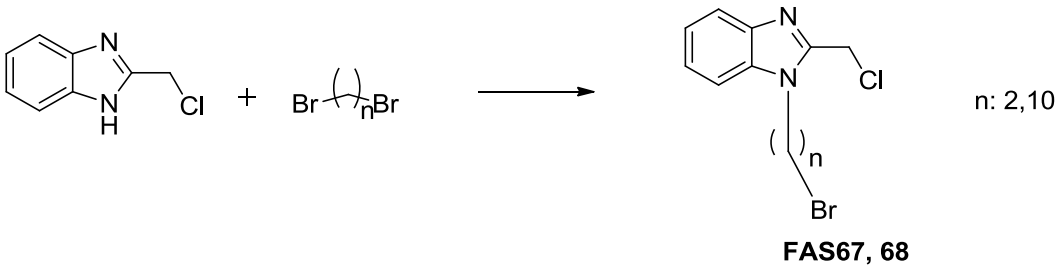


Figure 1.42 Group 4 target; *N*-bromoalkyl-benzimidazole derivatives

2 MATERIALS AND METHODS

The following section is an index of all compounds synthesised during this project, with full analysis data.

2.1 Index of compounds synthesised and purified

2.2 Materials for synthesis

2.2.1 Reagents

All reagents were purchased from Sigma Aldrich, Alfa Aesar, Goss Scientific or Merck unless otherwise stated. All solvents were purchased from Fisher and were of technical grade with the exception of dichloromethane and acetone, which were HPLC grade. All purchased reagents were used without further purification unless stated otherwise.

2.2.2 Purification of Solvents

All solvents were used without further purification except dichloromethane, which was distilled from anhydrous calcium chloride and stored over 4 Å molecular sieves (Sigma Aldrich, UK). Dimethylformamide was stored over 3 Å molecular sieves. Ethanol and chloroform were stored over 4 Å molecular sieves overnight before use. Triethylamine was heated under reflux and distilled from calcium chloride before storing over potassium hydroxide. Diethyl ether was stored over sodium wire.

2.2.3 Instrumentation

2.2.3.1 Nuclear magnetic spectroscopy (NMR)

^{1}H and ^{13}C NMR spectra were recorded using a Bruker DPX400 spectrometer. Coupling constants are given in Hertz (Hz). Deuterated solvents were obtained from Goss Scientific Instruments. Deuterated chloroform was stored over 4 Å molecular sieves. D_6-DMSO and D_2O were stored in silica gel desiccators. For ^{1}H NMR experiments, typically the sample (~20 mg) was dissolved in the appropriate deuterated solvent (0.7 ml). For ^{13}C NMR experiments, typically the sample (~40 mg) was dissolved in the appropriate deuterated solvent (0.7 ml).

2.2.3.2 Infrared spectra (IR)

IR of solid samples were recorded from KBr discs using 5 mg of dry sample and 35 mg of dry KBr. Spectra were recorded on a Nicolet 140 FTIR spectrophotometer in the range 4000- 400 cm^{-1}.

2.2.3.3 Mass spectrometry (MS)

Spectra were acquired by electron impact using an AEI MS902 mass spectrometer operated by Andrew Healey in the analytical centre of the University of Bradford.

2.2.3.4 Melting points (m.p.)

These were determined using an electronic melting point apparatus (Gallenkamp, Germany) and are uncorrected.

2.2.3.5 Elemental analysis

Elementalnalysis was carried out by the CHN microanalysis service using 2 mg of the sample, (MEDAC LTD, Analytical and chemical consultancy services, Chobham, Surrey, UK).

2.2.4 Chromatography

2.2.4.1 Thin layer chromatography (TLC)

TLC was performed on silica gel plates (60 F_{254}, Plastic sheets from MERCK) with UV indicator. Developed plates were visualised under UV light (366 nm).

2.2.4.2 Column chromatography

Column chromatography was performed using Silica gel, high-purity grade, pore size 60 Å, 70-230 mesh (Material Harvest, UK).

2.3 Procedures for synthesis

2.3.1 General procedure for the synthesis of 2-substituted benzimidazoles

The general method for the synthesis of benzimidazoles was based on the Phillips procedure (Phillips, 1928). The appropriate acid (1 equivalent) was added to a magnetically stirred solution of a 1,2-phenylenediamine (1 equivalent) and aqueous hydrochloric acid (typically 5.5 M). The mixture was heated under reflux until the 1,2-phenylenediamine was no longer detectable by NMR or until consumption of the starting materials had ceased. The characteristically bright blue reaction mixture was cooled to room temperature. The mixture was allowed to stand for several weeks until the desired 2-substituted benzimidazole precipitated out in its dihydrochloride salt form. The resulting solid was washed with acetone (3 x 20 ml) and recrystallised from water. Alternatively the reaction mixture was concentrated to one third under reduced pressure and cooled to produce the desired benzimidazole as a dihydrochloride salt. The free base was obtained by neutralisation of the reaction mixture with triethylamine or excess ammonia solution followed by extraction with ethyl acetate. The extract was evaporated to dryness and the solid residue was recrystallised from an appropriate solvent.

2.3.2 General Procedure for the synthesis of *N*-Methylbenzimidazoles

General method for the synthesis of *N*-methylbenzimidazoles was based on the Harisha procedure (Harisha *et al.*, 2009). A solution of 5-substituted-(*1H*-benzimidazole-2-yl)-methanol (0.01 mol) and anhydrous potassium carbonate (0.01 mol) in dry acetone (35 ml) was stirred for 30 min. Then, iodomethane (0.01 mol) was added to the mixture and the mixture was then stirred for 24 hours at room temperature. The reaction mixture was concentrated to 25% and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize excess

potassium carbonate. Then, the solid was washed with cold water (100 ml) and with aqueous ethanol. The desired product was then dried and recrystallized from an appropriate solvent.

2.3.3 General Procedure for the synthesis of *N*-bromoalkyl-2-(chloromethyl)-*1H*-benzo[*d*]imidazole

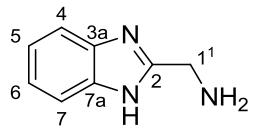
General method for the synthesis of these derivatives was based on Meyers procedure (Meyers *et al.*, 2005). 2-Chloromethylbenzimidazole derivatives (10 mmol) were combined with an excess of potassium hydroxide (13 mmol), and dissolved in ethanol (20 ml). An ethanol solution of dibromoalkane derivatives (1,4-1,6-1,8-1,10-1,12) (15 mmol) was added dropwise to the 2-chloromethylbenzimidazole solution, and the combined mixture was heated under reflux for 48 hrs. Water (50 ml) was added to the mixture, and then the product extracted with $CHCl_3$ (2 X 100 ml), then the organic phase was collected and dried over $MgSO_4$. The solvent was removed, and the remaining solid was purified by column chromatography (silica gel, 2:8 hexane/ ethyl acetate) yielding a light yellow solid.

2.3.4 General Procedure for the synthesis of silver complexes of benzimidazole

The general method for the synthesis of the silver complexes of benzimidazoles was based on the Podunavac-Kuzmanovic procedure (Podunavac-Kuzmanovic *et al.*, 2004). A solution of $AgNO_3$ (1 equivalent) in ethanol (5 ml) was added to a solution of 5-subistituted-*1H*-benzo[*d*]imidazole-2-carboxylic acid FAS29-30,33,35 (1 or 2 equivalent) in ethanol (5 ml) to form 1:1 and 1:2 M:L complexs. The reaction mixture was stirred at 50 ºC for 2 hrs. The resulting precipitate was filtered off, washed with diethyl ether, followed by warm ethanol. The formed complex was kept under dry conditions.

2.3.5 Synthesis of 2- aminomethylbenzimidazole derivatives

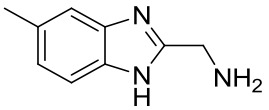
2-Aminomethylbenzimidazole dihydrochloride **(FAS1)**



. 2HCl

The compound FAS1 was prepared according to general procedure **2.3.1**, using 1,2-phenylenediamine (10.80 g, 0.10 mol) and glycine (12.65 g, 0.15 mol) dissolved in hydrochloric acid (70 ml, 5.5 M). The mixture was heated under reflux for 300 hours. The reaction mixture was then cooled to room temperature and left to evaporate slowly over several days. The resulting green crystals were washed with acetone. 2-Aminomethylbenzimidazole dihydrochloride was obtained as a grey powder (19.56 g; 89%), m.p. 270-272 ºC (lit., 268-270 ºC) (Cescon and Day, 1962). δ_H (400 MHz; DMSO-d_6) 7.85 (2H, dd, ^3J 6.00, ^4J 3.20, 4-H, 7-H), 7.53 (2H, dd, ^3J 6.40, ^4J 3.20, 5-H, 6-H), 4.59 (2H, s, CH_2), 9.287 (2H, br, s, NH, NH_2,); δ_C (400 MHz; DMSO-d_6) 150.16 (C-2), 132.20 (C-3a, C-7a), 125.47 (C-5, C-6), 114.48 (C-4, C-7), 38.78 (C-1'); υ_{max} /cm^{-1} (KBr) 3481 (N-H), 3053 (C-H, sp^2), 2850 (C-H, sp^3), 1485 and 1424 (C-H, bend).

(5-Methyl-*1H*-benzimidazole-2-yl)methanamine dihydrochloride **(FAS2)**

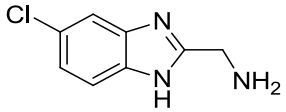


. 2HCl

(5-Methyl-*1H*-benzimidazole-2-yl)methanamine dihydrochloride (FAS2) was prepared according to general procedure **2.3.1**, using 4-methyl-1,2-phenylenediamine (12.22 g, 0.1 mol), and glycine (11.26 g, 0.1 mol) dissolved in hydrochloric acid (70 ml, 5.5 M). The mixture was heated under reflux for 170 hours. (5-Methyl-*1H*-benzimidazole-2-yl)methanamine dihydrochloride was isolated as a blue solid (12.05 g, 52%) after standing the reaction mixture for several weeks,

m.p. 208-210 °C(lit., 208-210 ºC)(Donkor, 2007). δ_H (DMSO-d_6) 9.34 (2H, br, s, N*H*$_2$), 8.37 (1H, br, s, N*H*), 7.38 (1H, d, ^3J 8.40, 7-H), 7.65 (1H, s, 4-H), 7.73 (1H, d, ^3J 8.40, 6-H), 4.58 (2H, s, C-1'), 2.48 (3H, s, C*H*$_3$); δ_C (DMSO-d_6) 168.1 (C-2), 146.2 (C-3a), 138.6 (C-7a), 133.3 (C-6),128.4 (C-5), 126.4 (C-4), 113.8 (C-7), 34.2 (C-1'), 20.9 (*C*H$_3$); υ_{max} /cm^{-1} (KBr) 3420 (N-H), 2994 (C-H, sp^2), 2863 (C-H, sp^3).

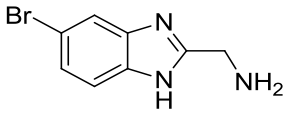
(5-Chloro-*1H*-benzimidazole-2-yl)methanamine dihydrochloride **(FAS4)**



. 2HCl

(5-Chloro-*1H*-benzimidazole-2-yl)methanamine (FAS4) was prepared according to general procedure **2.3.1**, using 4-chloro-1,2-phenylenediamine (3.50 g, 0.025 mol) and glycine (2.25 g, 0.03 mol) dissolved in hydrochloric acid (14 ml, 5.5 M). The mixture was heated under reflux for 216 hours. The reaction mixture was filtered to remove a black solid which had formed during the reaction. The filtrate was left to evaporate slowly whereupon (5-chloro-*1H*-benzimidazole-2-yl)methanamine dihydrochloride (2.51 g, 39%) was recovered as a brown solid, m.p. 252-254 °C (lit., 251-252 ºC)(Donkor, 2007). δ_H (DMSO-d_6) 8.99 (2H, br, s, N*H*$_2$), 6.52 (1H, br, s, N*H*), 7.49 (1H, d, ^4J 5.6, 4-H), 8.17 (1H, d, ^3J 9.2, 7-H), 7.83 (1H, dd, ^3J 8.80, ^4J 2.40, 6-H), 4.54 (2H, s, 1'-H); δ_C (DMSO-d_6) 156.6 (C-2), 140.15 (C-3a), 137.51 (C-7a), 130.2 (C-5), 133.62 (C-6), 130.50 (C-7), 128.18 (C-4), 35.32 (C-1'); υ_{max} /cm^{-1} (KBr) 3500 (N-H), 3040 (C-H, sp^2), 2836 (C-H, sp^3), 1113 C-Cl.

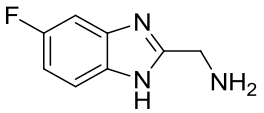
(5-Bromo-*1H*-benzimidazole-2-yl)methanamine **(FAS5)**



. 2HCl

(5-Bromo-*1H*-benzimidazole-2-yl)methanamine (FAS5) was prepared using general procedure **2.3.1**, using 4-bromo-1,2-phenylenediamine (3.74 g, 0.02 mol) and glycine (2.25 g, 0.03 mol) dissolved in hydrochloric acid (14 ml, 5.5 M). The reaction was heated under reflux for 216 hours and then was left to evaporate slowly until 5-bromo-*1H*-benzimidazole-2-yl)methanamine which was isolated as brownish orange precipitate (1 g, 22%), m.p. 258-260 °C (lit., 256-258 ºC) (Gillard.*et al.,*2004). δ_H (DMSO-d_6) 9.093 (2H, br, s, NH_2), 8.815 (1H, br, s, N*H*), 7.36 (1H, d, ^4J 4.8, 4-H), 8.11 (1H, d, ^3J 8.8, 7-H), 7.77 (1H, dd, ^3J 9.20, ^4J 1.60, 6-H), 4.38 (2H, s, 1'-H); δ_C (DMSO-d_6) 168.98 (C-2), 149.58 (C-3a, C-7a), 129.41 (C-6), 126.55 (C-4), 122.51 (C-7), 106.86 (C-5), 36.30 (C-1'); υ_{max} /cm^{-1} (KBr) 3233 (N-H), 3056 (C-H, sp^2), 2852 (C-H, sp^3), 1074 C-Br. MS (EI): m/z 226 ($M^{+\cdot}$, 100%) 228 (M+2, 100%).

(5-Fluoro-*1H*-benzimidazole-2-yl)methanamine dihydrochloride **(FAS6)**

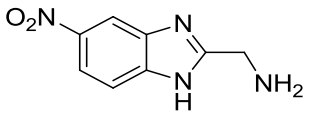


. 2HCl

(5-Fluoro-*1H*-benzimidazole-2-yl)methanamine (FAS6) was prepared using general procedure **2.3.1**, using 4-fluoro-1,2-phenylenediamine (1.50 g, 0.012 mol) and glycine (1.13 g, 0.015 mol) dissolved in hydrochloric acid (60 ml, 5.5 M). The reaction was heated under reflux for 216 hours. The resulting bright blue reaction mixture was left to crystallise at room temperature. After one week green crystals of (5-fluoro-*1H*-benzimidazole-2-yl)methanamine dihydrochloride (0.56 g, 16%) were

recovered, m.p. 262-264 °C (lit., 258-262 ºC)(Donkor, 2007).. δ_H (DMSO-d_6) 12.64 (2H, br, s, NH_2), 9.21 (1H, br, s, N*H*), 7.69 (1H, dd, ^3J 8.80, ^4J 2.40, 7-H), 7.82 (1H, dd, ^3J 8.80, ^4J 4.80, 4-H), 7.34 (1H, td, ^3J 9.40, ^4J, 2.40, 6-H), 4.51 (2H, s, CH_2). δ_C (DMSO-d_6) 164.30 (C-5), 149.10 (C-2), 134.4 (C-3a), 129.70 (C-7a), 115.3 (C-7), 113.2 (C-6), 106.3 (C-4), 34.5 (*C*-1'); υ_{max} /cm^{-1} (KBr) 3314 (N-H), 2928 (C-H, sp^2), 2858 (C-H, sp^3), 1139 (C-F).

5-Nitro-2-aminomethylbenzimidazole dihydrochloride **(FAS7)**

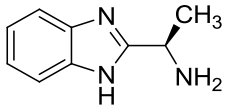


. 2HCl

5-Nitro-2-aminomethylbenzimidazole dihydrochloride (FAS7) was prepared according to general procedure **2.3.1**, using 4-nitro-1,2-phenylenediamine (7.65g; 0.05 mol) and glycine (9.13 g; 0.125 mol) dissolved in hydrochloric acid (35 ml, 5.5 M). The mixture was heated under reflux temperature for 300 hours. The bright orange reaction mixture was evaporated to dryness and (5-Nitro-*1H*-benzimidazole-2-yl)-methylamine) (FAS7) was isolated as a bright orange solid (2.83 g, 21%); m.p. 254- 256 ºC (lit., 248-251 ºC)(Donkor, 2007). δ_H (DMSO-d_6) 10.51 (1H, br, s, N*H*), 9.04 (2H, s, NH_2), 8.43 (2H, d, ^3J 10.00, 6-H & H-7), 8.10 (1H, s, 4-H), 4.41 (2H, s, CH_2); δ_C (DMSO-d_6) 152.81 (C-2), 143.9 (C-7a), 140.96 (C-5), 137.61 (C-3a), 118.23 (C-6), 115.14 (C-7), 111.64 (C-4), 36.16 (*C*H_2NH_2); υ_{max} /cm^{-1} (KBr); 3401 (N-H), 2966 (C-H, sp^2), 2851 (C-H, sp^3), 1509 and 1350 (N-O); MS (EI): m/z 192 ($M^{+\cdot}$, 100%) 163 (M-CH_2NH_2).

2.3.6 Synthesis of 2-ethanaminebenzimidazole derivatives

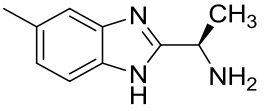
(*S*)-1-(*1H*-Benzimidazole-2-yl)ethanamine dihydrochloride **(FAS8)**



. 2HCl

(*S*)-1-(*1H*-Benzimidazole-2-yl)-ethylamine dihydrochloride (FAS8) was prepared according to general procedure **2.3.1** using 1,2-phenylenediamine (11.00 g, 0.102 mol) and *S*-alanine (16.00 g, 0.18 mol) in hydrochloric acid (70 ml; 5.5 M). The mixture was heated under reflux for 144 hours after which time a further portion of 1,2-phenylenediamine (5.00 g, 0.046 mol) was added and the mixture was heated under reflux for a further 120 hours. (*S*)-1-(*1H*-benzimidazole-2-yl)ethanamine was obtained as a blue solid (15.98 g, 67%), m.p. 138-140 °C (lit., 133-138 ºC) (Cescon and Day, 1962). δ_H (DMSO-d_6) 7.82 (2H, dd, ^3J 6.00, ^4J 3.20, 4-H, 7-H), 7.52 (2H, dd, ^3J 6.40, ^4J 3.20, 5-H, 6-H), 4.83 (1H, d, ^3J 6.80, C*H*), 1.83 (3H, d, ^3J 6.80, CH_3), 9.37 (2H, br, s, N*H*, NH_2,). δ_C (DMSO-d_6) 146.91 (C-2), 131.67 (C-3a, C-7a), 125.67 (C-5, C-6), 131.67 (C-4, C-7), 17.07 (C-1'), 40.44 (<u>*C*</u>H_3). υ_{max} /cm^{-1} (KBr) 3420(N-H), 3033 (C-H, sp^2), 2873 (C-H, sp^3), 1617 and 1476 (C=C).

(*S*)-1-(5-methyl-*1H*-benzimidazole-2-yl)ethanamine dihydrochloride **(FAS9)**

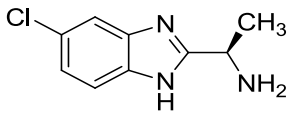


. 2HCl

(*S*)-1-(5-methyl-*1H*-benzimidazole-2-yl)ethanamine dihydrochloride (FAS9), was prepared according to general procedure **2.3.1** using 4-methyl-1,2-phenylenediamine (6.10 g, 50.00 mmol) and *L*-alanine (5.34 g, 60.00 mmol) in hydrochloric acid (60 ml; 5.5 M). (*S*)-1-(5-methyl-1*H*-benzimidazole-2-yl)ethanamine dihydrochloride (6.00 g, 68%) was obtained as a green solid, m.p. 212-214 °C (lit.,

212-213 ºC) (Donkor, 2007). δ_H (DMSO) 7.71 (1H, d, ^3J 8.00, 7-H), 7.62 (1H, s, 4-H), 7.36 (1H, d, ^3J 8.00, 6-H), 4.97 (1H, q, ^3J 8.00, C*H*, (1'-H), 2.48 (3H, s, 5a-H), 1.83 (3H, d, ^3J 8.00, 2'-H); 9.41 (2H, br, s, N*H*, NH_2,); δ_C (DMSO-d_6) 149.51 (C-2), 135.63 (C-3a), 132.02 (C-7a), 130.01 (C-5), 127.18 (C-6), 114.05 (C-4), 113.70 (C-7), 43.21 (C-1'), 21.11 (C-5a), 17.01 (C-2'); υ_{max} /cm^{-1} (KBr) 3411 (N-H), 2979 (C-H, sp^2), 2874 (C-H, sp^3), 1625 and 1487 (C=N).

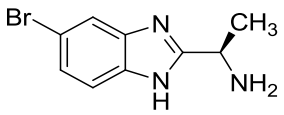
(S)-1-(5-Chloro-*1H*-benzimidazole-2-yl)ethanamine dihydrochloride **(FAS11)**



. 2HCl

(*S*)-1-(5-Chloro-*1H*-benzimidazole-2-yl)ethanamine (FAS11) was prepared according to general procedure **2.3.1** , using 4-chloro-1,2-phenylenediamine (0.17 g, 1.2 mmol), *S*-alanine (0.11 g, 1.23 mmol) and hydrochloric acid (20 ml, 5.5 M). The mixture was heated under reflux for 288 hours. The resulting dark brown solution was evaporated to dryness. (*S*)-1-(5-Chloro-*1H*-benzimidazole-2-yl)ethanamine dihydrochloride was obtained as a dark brown solid (0.13 g. 41%); m.p. 154-156 °C (lit., 152-154 ºC)(Donkor, 2007). δ_H (DMSO-d_6) 8.85 (1H, br, s, N*H*), 8.35 (2H, s, NH_2), 7.83-7.54 (2H, m, 4-H, 7-H), 7.25 (1H, m, 6-H), 4.55 (1H, br, s, 1'-H), 1.39 (3H, m, 2'-H). δ_C (DMSO-d_6) 153.03 (C-2), 135.76 (C-3a), 127.12 (C-7a), 123.61 (C-5), 120.74 (C-6), 117.75 (C-7), 115.24 (C-4), 47.8 (C-1'), 16.11.

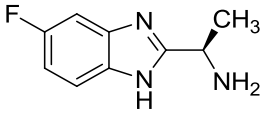
(*S*)-1-(5-Bromo-*1H*-benzimidazole-2-yl)ethanamine **(FAS12)**



(*S*)-1-(5-bromo-*1H*-benzimidazole-2-yl)ethanamine (FAS12) was prepared according to general procedure **2.3.1** using 4-bromo-1,2-phenylenediamine (0.22 g,

1.2 mmol), *S*-alanine (0.11 g, 1.23 mmol) and hydrochloric acid (20 ml, 5.5 M). The mixture was heated under reflux for 288 hours. The resulting dark brown solution was evaporated to dryness. (*S*)-1-(5-bromo-*1H*-benzimidazole-2-yl)ethanamine was obtained as a dark brown solid (0.07 g. 19%); m.p. 158-160 °C (lit., 160-162 ºC) (Gillard.*et al.,*2004) δ_H (DMSO-d_6) 8.13 (1H, t, ^3J 9.20, 7-H), 7.78 (1H, d, ^3J 9.20, 6-H), 7.33 (1H, d, ^4J 4.40, 4-H), 4.73 (1H, m, 1'-H), 1.39 (3H, d, ^3J 6.80, 2'). υ_{max} /cm^{-1} (KBr) 3401 (N-H), 3044 (C-H, sp^2), 2847 (C-H, sp^3), 1621 and 1471 (C=N). MS (EI): m/z 240 ($M^{+\cdot}$, 100%) 242 (M+2, 100%).

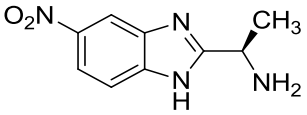
(*S*)-1-(5-Fuoro-*1H*-benzimidazole-2-yl)ethanamine **(FAS13)**



(*S*)-1-(5-Fluoro-*1H*-benzimidazole-2-yl)ethanamine (FAS13) was prepared according to procedure **2.3.1**, using 4-fluoro-1,2-phenylenediamine (0.15 g, 1.2 mmol), *S*-alanine (0.11 g, 1.23 mmol) and hydrochloric acid (20 ml, 5.5 M). The mixture was heated under reflux for 288 hours. The resulting green solution was evaporated to dryness. (*S*)-1-(5-Fluoro-1*H*-benzimidazole-2-yl)ethanamine (FAS13) was obtained as a dark brown solid (0.1 g, 33%). m.p. 260-262°C (lit., 262-264 ºC)(Donkor, 2007). δ_H (DMSO-d_6) 8.18 (1H, d, ^3J 8.80, 7-H), 7.62 (1H, s, 4-H), 7.54 (1H, d, ^3J 8.00, 6-H), 4.87 (1H, m, 1'-H), 1.39 (3H, d, ^3J 7.20, 2') (lit., 1.57 (3H, d) 4.39 (1H, q) 5.10 (3H, bs) 7.08.about.7.52 (3H, m)) (Shibata *et al.*, (1998)).

.

(*S*)-1-(5-Nitro-*1H*-benzimidazole-2yl)ethanamine **(FAS14)**

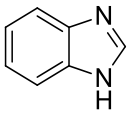


(*S*)-1-(5-Nitro-*1H*-benzimidazole-2yl)ethanamine (FAS14) was prepared according to general procedure **2.3.1** using 4-nitro-1,2-phenylenediamine (3.06 g, 20 mmol)

and *S*-alanine (2.00 g, 22.5 mmol) in hydrochloric acid (60 ml; 5.5 M). The mixture was heated under reflux for 336 hours. The reaction mixture was evaporated to dryness and dissolved in minimum ammonia solution. Water was added and the product was extracted with ethyl acetate. (*S*)-1-(5-Nitro-*1H*-benzimidazole-2yl)ethanamine (FAS14) containing unreacted 4-nitro-1,2-phenylenediamine (50% as determined by NMR) was recovered. δ_H (DMSO-d_6) 8.48 (1H, d, ^4J 2.00, 4-H), 8.10 (1H, dd, ^3J 8.00, ^4J 2.00 6-H), 7.74 (1H, d, ^3J 8.80, 7-H), 3.48 (1H, br, s, 1'-H), 1.77 (3H, s, 2'-H).

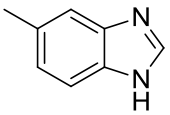
2.3.7 Synthesis of 5-substituted-benzimidazole derivatives

1H-Benzimidazole **(FAS15)**



1H-benzimidazole (FAS 15) was prepared according to general procedure **2.3.1**. 1,2-phenylenediamine (3.78 g, 35 mmol) was dissolved in formic acid (2.30 g, 50 mmol) and the mixture heated under reflux for 1 hour. *1H*-benzimidazole (FAS15) was obtained as a cream powder (3.08 g, 74%). m.p. 170-172 °C (lit.,170-172 °C) (Hojati *et al.*, 2011). δ_H (DMSO-d_6) 8.27 (1H, s, 2-H) ,7.63 (1H, dd, ^3J 6.90, ^4J 3.20, 7-H & H-4), 7.20 (1H, dd, ^3J 6.90, ^4J 3.20, 5-H & H-6). δ_C (DMSO-d_6) 141.93 (C-2), 138.09 (C-3a & C-7a), 121.71 (C-4 & C7), 115.30 (C-5 & C-6); υ_{max} /cm^{-1} (KBr) 3468 (N-H), 3062 (C-H, sp^2).

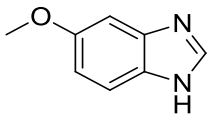
5-Methylbenzimidazole **(FAS16)**



5-Methylbenzimidazole (FAS 16) was prepared according to procedure **2.3.1.** 4-mehyl-1,2-phenylenediamine (4.20 g, 35 mmol) was dissolved in formic acid (2.30

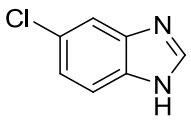
g, 50 mmol) and the mixture heated under reflux for 1 hour. 5-Methylbenzimidazole (FAS16) was obtained as a cream powder (4.06 g, 87%). m.p. 112-114 °C, (lit., 112-114 °C) (Mohammadpoor-Baltork *et al.*, 2007). δ_H (DMSO-d_6) 8.18 (1H, s, 2-H) , 7.50 (1H, d, ^3J 8.00, 7-H), 7.40 (1H, s, 4-H), 7.01 (1H, d, ^3J 8.00, 6-H), 2.41 (3H, s, CH_3); δ_C (DMSO-d_6) 141.55 (C-2), 130.93 (C-3a & C-7a), 123.15 (C-5), 116.06 (C-6), 113.44 (C-4 & C-7), 21.06 (C-5a); υ_{max} /cm^{-1} (KBr) 3429 (N-H), 3073 (C-H, sp^2), 2805 (C-H, sp^3).

5-Methoxy-*1H*-benzo[*d*]imidazole **(FAS17)**



5-Methoxybenzimidazole (FAS 17) was prepared according to procedure **2.3.1.** 4-Mehoxy-1,2-phenylenediamine (1.20 g, 8.75 mmol) was dissolved in formic acid (0.57 g, 12.5 mmol) and the mixture heated under reflux for 1 hour. 5-Methyoxybenzimidazole (FAS17) was obtained as a cream powder (1.10 g, 85 %). m.p. 116-118 °C,(lit., 117-120 °C) (Tanaka *et al.*, 1981). δ_H (DMSO-d_6) 8.09 (1H, s, 2-H) , 7.53 (1H, d, ^3J 8.00, 7-H), 7.07 (1H, s, 4-H), 6.91 (1H, d, ^3J 8.00, 6-H), 3.79 (3H, s, CH_3); δ_C (DMSO-d_6) 141.55 (C-2), 130.93 (C-3a & C-7a), 156.1 (C-5), 113.06 (C-6), 97.6 (C-4) 116.44 (C-7), 55.30 (C-5a).

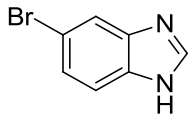
5-Chloro-*1H*-benzo[*d*]imidazole **(FAS18)**



5-Chlorobenzimidazole (FAS 18) was prepared according to procedure **2.3.1.** 4-chloro-1,2-phenylenediamine (2 g, 14 mmol) was dissolved in formic acid (10 ml) and the mixture heated under reflux for 3 hours. 5-Chlorobenzimidazole (FAS18) was obtained as a brown crystals (2.15 g, 100%). m.p. 123-125 °C (lit., 124-126 °C)

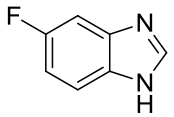
(Liu *et al.*, 2011). δ_H (DMSO-d_6) 8.32 (1H, s, 2-H), 7.60 (1H, d, ^3J 8.40, 7-H), 7.66 (1H, d, ^4J 2.00, 4-H), 7.20 (1H, dd, ^3J 8.80, ^4J 2.00, 6-H). δ_C (DMSO-d_6) 143.40 (C-2), 139.16 (C-3a) 136.48 (C-7a), 126.19 (C-5), 122.02 (C-6), 115.14 (C-7) 116.34 (C-4); υ_{max}/cm^{-1} (KBr) 3429 (N-H), 3103 (C-H, sp^2), 1057 (C-Cl).

5-Bromo-*1H*-benzo[*d*]imidazole **(FAS19)**



5-Bromobenzimidazole (FAS 19) was prepared according to procedure **2.3.1.** 4-chloro-1,2-phenylenediamine (2.61 g, 14 mmol) was dissolved in formic acid (10 ml) and the mixture heated under reflux for 2 hours. 5-Bromobenzimidazole (FAS19) was obtained as brown crystals (2.69 g, 98%). m.p. 130-133 °C (lit., 130-131°C) (Evans and et al., 1978). δ_H (DMSO-d_6) 8.27 (1H, s, 2-H), 7.56 (1H, d, ^3J 8.40, 7-H), 7.81 (1H, d, ^4J 2.00, 4-H), 7.32 (1H, dd, ^3J 8.40, ^4J 2.00, 6-H). δ_C (DMSO-d_6) 143.29 (C-2), 139.92 (C-3a) 136.89 (C-7a), 124.54 (C-6), 118.17 (C-5), 113.95 (C-7) 116.81 (C-4); υ_{max}/cm^{-1} (KBr) 3437 (N-H), 3087 (C-H, sp^2), 1046 C-Br.

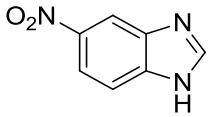
5-Fluoro-1*H*-benzo[*d*]imidazole **(FAS20)**



5-Flourobenzimidazole (FAS 20) was prepared according to procedure **2.3.1.** 4-Fluoro-1,2-phenylenediamine (0.5g, 4 mmol) was dissolved in formic acid (10 ml) and the mixture heated under reflux for 3 hours. 5-Flourobenzimidazole (FAS20) was obtained as beige powder (0.49 g, 90 %). m.p. 130-132 °C (lit., 132 °C) (Fisher and Joullie, 1958, Rao and Kondal Reddy, 1979). δ_H (DMSO-d_6) 9.05 (*1*H, s, 2-H) , 7.56 (1H, d, ^3J 8.80, ^4J 2.40, 7-H), 7.25 (1H, d, ^4J 2.00, 4-H), 7.75 (1H, dd, ^3J 8.40,

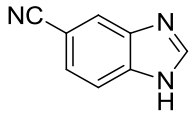
^{4}J 4.80, 6-H); δ_C (DMSO-d_6) 142.26 (C-2), 134.22 (C-3a) 130.52 (C-7a), 158.12 (C-5), 115.94 (C-7), 112.51 (C-67), 101.00 (C-4).

5-Nitro-*1H*-benzo[*d*]imidazole **(FAS21)**



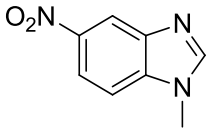
5-Nitrobenzimidazole (FAS 21) was prepared according to procedure **2.3.1.** 4-Nitro-1,2-phenylenediamine (3.06 g, 20 mmol) was dissolved in formic acid (5 ml) and the mixture heated under reflux for 8 hours. 5-Nitrobenzimidazole (FAS21) was obtained as a cream powder (3.28 g, 100%). m.p. 203-205 °C (lit., 204-205 °C) (Liu *et al.*, 2011). δ_H (DMSO-d_6) 8.54 (1H, s, 2-H) , 7.74 (1H, d, ^{3}J 9.2, 7-H), 8.49 (1H, d, ^{4}J 2.00, 4-H), 8.07 (1H, dd, ^{3}J 8.80, ^{4}J 2.00, 6-H). δ_C (DMSO-d_6) 142.47 (C-2), 138.63 (C-3a) 141.75 (C-7a), 146.82 (C-5), 114.63 (C-7), 117.38 (C-6) 112.70 (C-4); υ_{max} /cm^{-1} (KBr) 3104 (C-H, sp^2), 1514 and 1345 (N-O).

1H-Benzo[*d*]imidazole-5-cyano **(FAS51)**



5-Cyano-*1H*-benzimidazole (FAS 51) was prepared according to procedure **2.3.1.** of 4-Cyano-1,2-phenylenediamine (2.66 g, 20 mmol) was dissolved in formic acid (5 ml) and the mixture heated under reflux for 24 hours. 5-Cyanobenzimidazole (FAS51) was obtained as a cream powder (1.26 g, 44%). m.p. 230-232 °C (lit., 230-233 °C)(Elshihawy, 2008). δ_H (DMSO-d_6) 8.48 (1H, s, 2-H), 7.75 (1H, d, ^{3}J 8.00, 7-H), 8.16 (1H, s, 4-H), 7.58 (1H, d, ^{3}J 8.00, 6-H), 12.97 (1H, br, s, N*H*); δ_C (DMSO-d_6) 145.21 (C-2), 142.02 (C-3a &C-7a), 103.78 (C-5), 119.75 (C-7), 125.26 (C-6) 123.40 (C-4) 123.21 (CN).

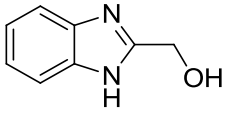
1-Methyl-5-nitro-*1H*-benzo[*d*]imidazole **(FAS58)**



N-Methyl-5-nitrobenzimidazole (FAS 58) was prepared according to general procedure **2.3.2.** 5-Nitro-*1H*-benzo[*d*]imidazole (FAS 21) (1.63 g, 0.01 mol) was added to sodium hydroxide (0.01 mol, 0.4 g) in dry acetone (10ml) and stirred for 30 min. Then, Iodomethane (0.01 mmol, 1.41 g) was added to the mixture and stirring was continued for 24 hours. The reaction mixture was concentrated to a quarter and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize the excess potassium carbonate. The solid was washed with cold water (10 ml) and with aqueous ethanol (50%). The desire product (FAS58) was then dried and purified by column chromatography (2:8 cyclohexane/ethyl acetate) to give the desired product to give a beige precipitate (0.02 g, 25 %). m.p. 212-214 °C (lit., 211-215 ºC)(Piersanti *et al.*, 2007). δ_H (DMSO) 7.78 (1H, d, ^3J 8.00, 7-H), 8.58 (1H, d, ^4J 4.00, 4-H), 8.16 (1H, d, ^3J 8.00, 6-H), 8.51 (1H, q, ^3J 8.00, ^4J4.00, 2-H), 3.93 (3H, d, ^3J 16.00 CH_3). δ_C (DMSO-d_6) 142.68(C-2), 142.61 (C-3a) 142.39 (C-7a), 148.73 (C-5), 119.52 (C-6), 117.79 (C-4), 110.85 (C-7), 31.18 ($\underline{C}H_3$).

2.3.8 Synthesis of 2-hydroxymethylbenzimidazole derivatives

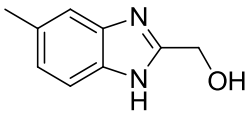
(*1H*-benzimidazol-2-yl)-methanol **(FAS22)**



2-Hydroxymethylbenzimidazole (FAS22) was prepared according to general procedure **2.3.1.** 1,2-Phenylenediamine (10.80 g, 0.10 mol) and glycolic acid 50% (30 ml) were dissolved in hydrochloric acid (70 ml; 5.5 M) and the mixture heated under reflux for 3 hours. The reaction mixture was neutralised with ammonia

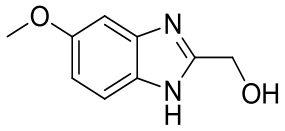
solution and the cream precipitate was recrystallised from water. 2-Hydroxymethylbenzimidazole (14.15 g; 96%) was obtained as pure brown crystals. m.p. 172-174 °C (lit. 170-172 °C) (Lahlou *et al.*, 2003). δ_H (DMSO-d_6) 7.50 (2H, br,s, 4-H, 7-H), 7.14 (2H, dd, ^3J 6.00, ^4J 3.20, 5-H, 6-H), 5.73 (1H, t, ^3J 5.56, O*H*,), 4.71 (2H, d, ^3J 5.12, CH_2), 12.40 (1H, br, s, NH). δ_C (400 MHz; DMSO-d_6) 155.02 (C-2), 121.27 (C-3a, C-7a), 118.14 (C-5, C-5), 111.12 (C-4, C-7), 57.51 (C-1'); υ_{max} /cm^{-1} (KBr) 3259 (O-H), 3060 (C-H, sp^2), 2856 (C-H, sp^3), 1440 and 1345 (C-H, bend).

((5-Methyl-1*H*-benzimidazole-2-yl)-methanol) **(FAS23)**



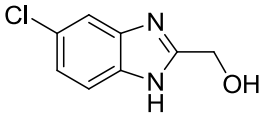
(5-Methyl-1*H*-benzimidazole-2-yl)-methanol (FAS23) was prepared according to procedure **2.3.1.** 4-Methyl-1,2-phenylenediamine (12.22 g; 0.1 mol) and glycolic acid (11.40 g, 0.15 mmol) in hydrochloric acid (50 ml, 5.5 M) were heated under reflux with for 3 hours. The reaction mixture was cooled to room temperature and ammonia solution was added and the mixture cooled in ice until a bright brown precipitate formed. The resulting solid was recrystallised from aqueous ethanol to give (5-methyl-1*H*-benzimidazole-2-yl)-methanol as a pale creamy powdery solid (16.2g; 100%). m.p. 194-196 ºC (lit. 203 ºC) (Dellweg *et al.*, 1956). δ_H (DMSO-d_6) 7.38 (1H, d, ^3J 8.40, 7-H), 7.29 (1H, s, 4-H), 6.97 (1H, d, ^3J 8.40, 6-H), 4.69 (2H, s, CH_2), 2.39 (3H, s, CH_3), NH not observed; δ_C (DMSO-d_6) 154.73 (C-2), 137.96 (C-3a), 136.6 (C-7a), 130.4 (C-6),122.9 (C-5), 114.64 (C-4), 114.13 (C-7), 57.52 (C-1'), 21.20 (CH_3); υ_{max} /cm^{-1} (KBr) 3481 (O-H), 3314 (NH) 3050 (C-H, sp^2), 2850 (C-H, sp^3).

((5-Methyl-*1H*-benzimidazole-2-yl)-methanol **(FAS24)**



(5-Methoxy-1*H*-benzimidazole-2-yl)-methanol (FAS24) was prepared according to procedure **2.3.1.** 4-Methoxy-1, 2-phenylenediamine (0.7 g, 5 mmol) was heated under reflux temperature with glycolic acid (0.4 g, 5.25 mmol) in hydrochloric acid (15 ml, 5.5 M) for 6 hours. The reaction mixture was cooled to room temperature and ammonia solution was added and the mixture cooled in ice until a bright brown precipitate formed. The resulting solid was recrystallised from aqueous ethanol to give 5-methoxy-*1H*-benzimidazole-2-yl)-methanol as a bright brown solid (0.89 g; 100%). m.p. 182-184 ºC. δ_H (DMSO-d_6) 7.37 (1H, d, ^3J 8.72, 7-H), 6.99 (1H, d, ^4J 2.36, 4-H), 6.76 (1H, dd, ^3J 8.64, ^4J 2.48, 6-H), 4.75 (2H, s, CH_2), 3.75 (3H, s, OCH_3), 5.65 (1H, br, s, OH), 12.20 (1H, br, s, NH). δ_C (DMSO-d_6) 115.74 (C-2), 155.20 (C-3a,C-7a), 97.27 (C-5), 110.56 (C-4, C-6), 154.50 (C-7), 57.68 (C-1'), 55.35 (O<u>C</u>H_3); MS (EI): m/z 177 (M-1, 100%), 162 (M-OH, 10%). υ_{max} /cm^{-1} (KBr) 3392 (O-H), 3110 (C-H, sp^2), 3038 (C-H, sp^3), 1459 and 1378 (C-H, bend); MS (EI): m/z 178 ($M^{+\cdot}$, 100%). Found; C, 60.47 %; H, 5.30 %; N, 15.92 %, requires; C, 60.66 %; H, 5.66 %; N, 15.72 %.

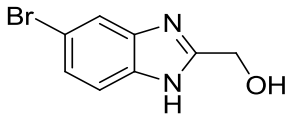
5-Chloro-*1H*-benzimidazol-2-yl)-methanol **(FAS25)**



5-Chloro-*1H*-benzimidazole-2-yl) methanol (FAS25) was prepared according to procedure **2.3.1.** using 4-chloro-1,2-phenylenediamine (2.12 g, 14.84 mmol), glycolic acid (1.30 g, 17 mmol) and hydrochloric acid (40 ml, 5.5 M). The solution was heated under reflux for 8 hours. 5-Chloro-*1H*-benzimidazole-2-yl)methanol was recovered as brownish red coloured crystals (2.58 g, 95%). m.p. 200- 202 °C (lit.,

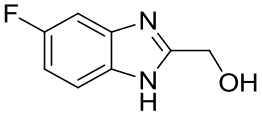
202 °C) (Lahlou *et al.*, 2003). δ_H (DMSO-d_6) 7.54 (1H, d, ^4J 1.88, 4-H), 7.49 (1H, d, ^3J 8.52, 7-H), 7.16 (1H, dd, ^3J, 8.00, ^4J 2.00, 6-H), 5.80 (1H, br, s, OH), 4.70 (2H, s, CH_2), 12.50 (1H, br, s, NH); δ_C (DMSO-d_6) 114.75 (C-2), 121.52 (C-3a, C-7a), 156.67 (C-5), 125.64 (C-7), 156.67 (C-4, C6), 57.60 (C-1'); υ_{max} /cm^{-1} (KBr) 3441 (O-H), 3136 (C-H, sp^2), 3041 (C-H, sp^3), 1441 and 1362 (C-H, bend), 1044 (C-Cl).

(5-Bromo-*1H*-benzimidazole-2-yl)methanol **(FAS26)**



(5-Bromo-*1H*-benzimidazole-2-yl) methanol (FAS26) was synthesised according to general procedure **2.3.1** using 4-bromo1,2-phenylenediamine (0.65 g, 3.5 mmol) and glycolic acid (0.305 g, 4 mmol) dissolved in hydrochloric acid (10 ml, 5.5 M). The mixture was heated under reflux for 8 hours. The product was obtained as an orange precipitate (0.67 g, 85%) m.p. 208- 210 °C (lit., 208 °C)(Khan *et al.*, 1972).δ_H (DMSO-d_6) 7.67 (1H, d, ^4J 1.80, 4-H), 7.45 (1H, d, ^3J 8.48, 7-H), 7.27 (1H, dd, ^3J, 8.52, ^4J 1.96, 6-H), 5.75 (1H, br, s, OH), 4.70 (2H, s, CH_2), 12.50 (1H, br, s, NH). δ_C (DMSO-d_6) 121.40 (C-2), 117.58 (C-3a), 116.19 (C-7a), 156.20 (C-5), 113.46 (C-7), 124.13 (C-4, C6), 57.58 (C-1'); υ_{max} /cm^{-1} (KBr) 3376 (O-H), 3130 (C-H, sp^2), 3089 (C-H, sp^3), 1445 and 1320(C-H, bend), 1081 (C-Br); MS (EI): m/z 227 ($M^{+\cdot}$, 100%), 229 (M+2, 100%).

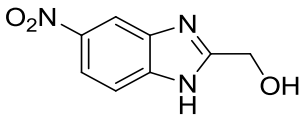
(5-Fluoro-*1H*-benzimidazol-2-yl)-methanol **(FAS27)**



(5-Fluoro-*1H*-benzimidazol-2-yl)methanol (FAS27) was synthesised according to general procedure **2.3.1** using 4-fluoro-1,2-phenylenediamine (1.26 g, 10 mmol) and glycolic acid (0.80 g, 10.5 mmol) dissolved in hydrochloric acid (30 ml, 5.5 M). The

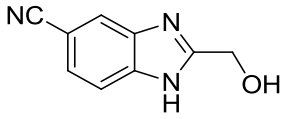
mixture was heated under reflux for 8 hours. The brown crude product was decolourised using activated charcoal and recrystallised from ethanol to give 5-fluoro-*1H*-benzimidazol-2-yl)methanol as brown crystals (1.15 g, 70%) . m.p. 182-184 °C (lit., 182 °C) (Wagner and et al., 1972). δ_H (DMSO-d_6) 7.49 (1H, dd, ^3J 8.72, ^4J 4.96, 7-H), 7.28 (1H, dd, ^{3}J 9.56, ^4J 2.48 4-H), 6.99 (1H, m, ^3J 9.32, 6-H), 4.70 (2H, s, 1'-H), N*H,* O*H* not observed; δ_C (DMSO-d_6) 159.38 (C-2), 156.79 (C-5), 138.9 (C-3a), 134.9 (C-7a), 115.25 (C-7), 109.30 (C-6), 100.87 (C-4), 57.59 (C-1'); υ_{max} /cm^{-1} (KBr) 3256 (C-H, sp^2), 3079 (C-H, sp^3), 1455 and 1355 (C-H, bend), 1076 (C-F).

(5-Nitro-1(3)*H*-benzimidazol-2yl)-methanol **(FAS28)**



5-Nitro-2-hydroxymethylbenzimidazole (FAS28) was prepared according to procedure **2.3.1** 4-nitro-1,2-phenylenediamine (8.00 g; 52 mmol) and glycolic acid 50% in water (30 ml) were dissolved in hydrochloric acid (70 ml; 5.5 M) and the mixture heated under reflux for 48 hours. The reaction mixture was neutralised with ammonia solution and the brown pale yellow precipitate was recrystallised from water to give 5-nitro-2-hydroxymethylbenzimidazole as a yellow powder (5.65 g, 56%). m.p. 198-200 ºC (lit., 198-200 °C)(Donkor, 2007). δ_H (DMSO-d_6) 8.39 (1H, d, 4-H, ^4J 2.22), 8.08 (1H, dd, ^3J 8.86, ^4J 2.2, 6-H), 7.66 (1H, d, ^3J 8.88, 7-H), N*H* not observed, 4.76 (2H, s, CH_2), 3.74 (1H, s, OH); δ_C (DMSO-d_6) 160.3 (C-2), 144.1 (C-7a), 138.9 (C-5), 134.7 (C-3a), 119.8 (C-6), 115.2 (C-7), 111.5 (C-4), 56.9 (C-1'); υ_{max} /cm^{-1} (KBr) 3586 (O-H), 3488 (N-H), 3121 (C-H, sp^2), 2850 (C-H, sp^3), 1507 and 1353 (N-O).

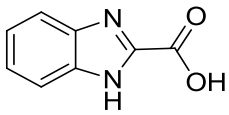
2-(Hydroxymethyl)-*1H*-benzo[*d*]imidazole-5-cyano **(FAS52)**



(5-Cyano-*1H*-benzimidazole-2-yl)-methanol (FAS52) was prepared according to procedure **2.3.1.** 4-Cyano-1, 2-phenylenediamine (0.66 g, 5 mmol) was heated under reflux temperature with glycolic acid (0.4 g, 5.25 mmol) in hydrochloric acid (15 ml, 5.5 M) for 1 hour. The reaction mixture was cooled to room temperature and ammonia solution was added and the mixture cooled in ice until a bright orange precipitate formed. The resulting solid was recrystallised from aqueous ethanol to 5-cyano-*1H*-benzimidazole-2-yl)-methanol as a bright orange solid (0.49g; 57%). m.p. 170-172 ºC; (lit. 172-173 ºC) (Rangarajan *et al.*, 2000). δ_H (DMSO-d_6) 7.64 (1H, d, ^3J 8.00, 7-H), 8.00 (1H, s, 4-H), 7.52 (1H, d, ^3J 8.00, 6-H), 4.73 (2H, s, CH_2), N*H* & O*H* not observed; δ_C (DMSO-d_6) 142.27 (C-2), 136.44 (C-3a,C-7a), 102.98 (C-5), 120.16(C-4), 128.88 (C-6), 108.55 (C-7), 57.60 (C-1'), 118.16 (CN).

2.3.9 Synthesis of 2-carboxylic acid benzimidazole derivatives

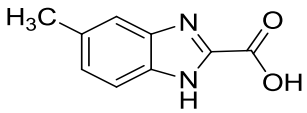
2-Carboxylic acid benzimidazole **(FAS29)**



2-Hydroxymethylbenzimidazole (FAS22) (2.96 g, 20 mmol) was dissolved in water warm (150 ml). Potassium permanganate (4.80 g, 30 mmol) in water (150 ml) was added slowly and the solution stirred until the purple potassium permanganate colour had been discharged. The potassium oxide was filtered off to leave a slightly pale brown solution. The filtrate was concentrated by two thirds and the solution acidified with concentrated hydrochloric acid. The resulting white precipitate was recrystallised from aqueous ethanol to give 2-carboxylic acid benzimidazole

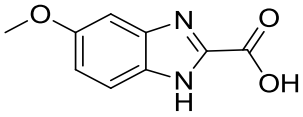
(FAS29) as fine white crystals (1.76 g, 44%). m.p. 170-172 °C (lit. 168-169 °C) (Prostakov *et al.*, 1990). δ_H (DMSO-d_6) 10.61 (1H, s, NH), 8.30 (1H, s, OH), 7.67 (1H, m, ^3J 6.16, ^4J 3.20, 4-H), 7.63 (1H, m, ^3J 6.02, ^4J 3.20, 7-H), 7.38 (1H, m, ^3J 6.12, ^4J 3.20, 6-H), 7.22 (1H, m, ^3J 6.02, ^4J 3.08, 5-H); δ_C (DMSO-d_6) 159.14 (C=O), 144.59 (C-2), 141.86 (C-3a), 137.79 (C-7a), 136.49 (C-5), 124.22 (C-6), 121.81 (C-4), 115.59 (C-7). υ_{max} /cm^{-1} (KBr) 3539 (O-H), 3032 (C-H, sp^2), 1647 (C=O), 1342 (C-O).

5-Methyl-*1H*-benzimidazole-2-carboxylic acid **(FAS30)**



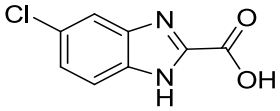
((5-Methyl-1 H-benzimidazole-2-yl)-methanol) (FAS23) (0.81 g, 5 mmol) was dissolved in acetone (35 ml). Potassium permanganate (0.95 g, 6 mmol) in water (160 ml) was added slowly and the solution stirred until the purple potassium permanganate colour had been discharged. The potassium oxide was filtered off to leave a clear solution. The solution was evaporated to dryness and the resulting solid dissolved in the minimum amount of water. The solution was acidified with concentrated hydrochloric acid and the resulting white precipitate recrystallised from aqueous ethanol to give 5-methyl-*1H*-benzimidazole-2-carboxylic acid (FAS30) as a white solid (0.88 g, 83%). m.p.140-142 °C, (lit., 140 °C) (Kempe *et al.*, 1989). δ_H (DMSO-d_6) 8.20 (2H, br, s, O*H,* NH), 7.55 (1H, d, ^3J 8.36, 7-H), 7.49 (1H, ^3J 8.20, 6-H), 7.43 (1H, s, 4-H), 2.42 (3H, s, CH_3); δ_C (DMSO-d_6) 158.68 (C=O), 143.01 (C-2), 136.44 (C-3a), 134.37 (C-7a), 131.10 (C-5), 124.53 (C-6), 115.5 (C-4), 114.6 (C-7), 21.21 (*C*H_3); υ_{max} /cm^{-1} (KBr) 3488 (O-H), 3024 (C-H, sp^2), 2861 (C-H, sp^3), 1624 (C=O), 1300 (C-O).

5-Methoxy-*1H*-benzimidazole-2-carboxylic acid **(FAS31)**



((5-Methoxy-1 H-benzimidazole-2-yl)-methanol) (FAS24) (0.89 g, 5 mmol) was dissolved in acetone (35 ml). Potassium permanganate (0.95 g, 6 mmol) in water (160 ml) was added slowly and the solution stirred until the purple potassium permanganate colour had been discharged. The potassium oxide was filtered off to leave a clear solution. The solution was evaporated to dryness and the resulting solid dissolved in minimum water. The solution was acidified with concentrated hydrochloric acid and the resulting cream solid recrystallised from aqueous ethanol to give 5-methyl-*1H*-benzimidazole-2-carboxylic acid (FAS31) as a white solid (0.96 g, 84%). m.p.150-152°C (lit., 152-154 ºC) (Thakurdesai *et al.*, 2007). δ_H (DMSO-d_6) 7.50 (3H, br, s, H-4, H-6 & H-7s), 3.65 (3H, s, OCH_3); δ_C (DMSO-d_6) 157.33 (C=O), 143.46 (C-2), 139.89 (C-3a), 132.67 (C-7a), 124.16 (C-5), 117.75 (C-6), 115.18 (C-4), 96.32 (C-7), 55.63 ($O\underline{C}H_3$); υ_{max} /cm^{-1} (KBr) 3130 (O-H), 3110 (C-H, sp^2) 1641 (C=O), 1300 (C-O) 1401 (C=N).

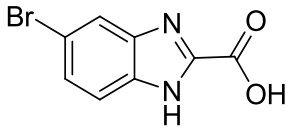
5-Chloro-*1H*-benzimidazole-2-carboxylic acid **(FAS32)**



Synthesis of 5-chloro-*1H*-benzimidazole-2-carboxylic acid (FAS32) was prepared, using 5-chloro-*1H*-benzimidazole-2-yl)methanol (FAS25) (0.365 g, 2.0 mmol) was dissolved in acetone (20 ml) and potassium permanganate was dissolved in water (20 ml). The crude product was recrystallised from water. 5-Chloro-*1H*-benzimidazole-2-carboxylic acid (FAS32) (0.11 g, 24 %) was recovered as a creamy white powder. m.p. 158-160 °C (lit., 159-160ºC) (Thakurdesai *et al.*, 2007). δ_H (DMSO-d_6) 8.75 (1H, br, s, O*H*), 7.73 (1H, d, ^4J 1.80, 4-H), 7.70 (1H, d, ^3J 8.00, 7-

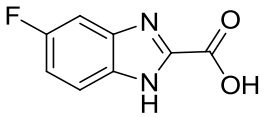
H), 7.38 (1H, dd, ^{3}J 8.00, ^{4}J 1.80, 6-H). δ_C (DMSO-d_6) 160.01 (C=O), 143.53 (C-2), 141.36 (C-3a), 139.80 (C-7a), 130.01 (C-5), 124.52 (C-6), 117.56 (C-7), 115.59 (C-4). υ_{max} /cm^{-1} (KBr) 3499 (O-H), 3096 (C-H, sp^2), 1741 (C=O), 1331 (C-O), 1063 (C-Cl).

5-Bromo-*1H*-benzimidazole-2-carboxylic acid **(FAS33)**



Synthesis of 5-bromo-*1H*-benzimidazole-2-carboxylic acid hydrochloride (FAS33) was prepared using 5-bromo-*1H*-benzimidazole-2-yl)-methanol (FAS26) (0.23 g, 1.0 mmol) dissolved in acetone (10 ml) and potassium permanganate (0379, 2.4 mmol) dissolve in water (10 ml). The crude product was recrystallised from water. 5-Bromo-*1H*-benzimidazole-2-carboxylic acid (FAS33) (0.10 g, 37 %) was recovered as a creamy white powder. m.p. 162-164 °C. δ_H (DMSO-d_6) 10.80 (1H, s, N*H*), 8.44 (1H, s, O*H*), 7.84 (1H, d, ^{4}J 3.20, 4-H), 7.62 (1H, d, ^{3}J 8.80, 7-H), 7.47 (1H, dd, ^{3}J 6.80, ^{4}J 5.40, 6-H). δ_C (DMSO-d_6) 160.1 (C=O), 144.30 (C-2), 139.80 (C-3a), 137.80 (C-7a), 127.30 (C-5), 124.80 (C-6), 119.70 (C-7), 116.30 (C-4); υ_{max} /cm^{-1} (KBr) 3062 (C-H, sp^2), 1744 (C=O), 1046 (C-Br); (lit., ESI+ 241) (ISHII *et al.*, (2010)).

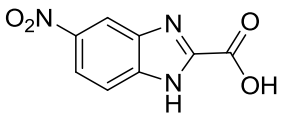
5-Fluoro-*1H*-benzimidazole-2-carboxylic acid **(FAS34)**



5-Fluoro-*1H*-benzimidazole-2-carboxylic acid (FAS34) was synthesised using 5-fluoro-*1H*-benzimidazole-2-yl)methanol (FAS 27) (0.10 g, 0.60 mmol) dissolved in acetone (20 ml) and potassium permanganate (0.10 g, 0.63 mmol) dissolved in water (10 ml). 5-Fluoro-*1H*-benzimidazole-2-carboxylic acid (FAS34) (0.05 g, 38%) was recovered as bright white crystals. m.p. (166-168 °C). δ_H (DMSO-d_6) 12.14 (1H,

br, s, O*H*), 7.80 (1H, ^{3}J 8.00, ^{4}J 3.20, 7-H), 7.53 (1H, d, ^{3}J 8.00, 4-H), 7.32 (1H, t, ^{3}J 8.00, 6-H); δ_C (DMSO-d_6) 165.43 (C=O), 159.68 (C-5), 142.78 (C-2), 141.80 (C-3a), 132.30 (C-7a), 112.02 (C-7), 113.90 (C-6), 104.20 (C-4); υ_{max} /cm^{-1} (KBr) 3433 (O-H), 3108 (C-H, sp^2), 1748 (C=O), 1335 (C-O), 1199 (C-F) ; (lit., ESI+ 181) (ISHII *et al.*, (2010)).

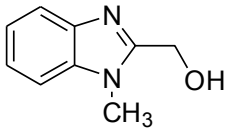
5-Nitro-*1H*-benzimidazole-2-carboxylic acid **(FAS35)**



2-Hydroxymethyl-5-nitrobenzimidazole (FAS 28) (0.96 g, 5 mmol) was dissolved in acetone (35 ml). Potassium permanganate (0.95 g, 6 mmol) in water (160 ml) was added slowly and the solution stirred until the potassium permanganate colour had been discharged. The solution was filtered to leave a pale yellow solution which then was acidified with concentrated hydrochloric acid and the volume reduced until a precipitate formed. The resulting yellow precipitate was recrystallised from aqueous ethanol to give 5-Nitro-*1H*-benzimidazole-2-carboxylic acid (FAS35) as a yellow powder (0.67 g, 55%), m.p. (198-200 °C) (lit., 198-200 ºC)(Donkor, 2007). δ_H (DMSO-d_6) 8.52 (1H, s, br , 4-H), 8.10 (1H, s, br, 6-H), 7.77 (1H, s, br, 7-H), 2.50 (1H, s, OH), 13.10 (1H, s, NH). δ_C (DMSO-d_6) 159.80 (C=O), 143.64 (C-7a), 142.37 (C-5), 142.64 (C-2), 136.90 (C-3a), 116.39 (C-6), 113.24 (C-7), 111.34 (C-4). υ_{max} /cm^{-1} (KBr) 3550 (O-H), 3117 (C-H, sp^2), 1629 (C=O), 1343 (C-O), 1509 and 1393 (N-O).

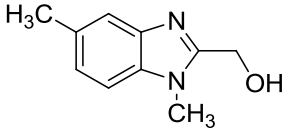
2.3.10 Synthesis of *N*-methyl-2-methanolbenzimidazole derivatives

(*N*-Methyl-*1H*-benzimidazole-2-yl)-methanol **(FAS36)**



N-Methyl-2-methanolbenzimidazole (FAS36) was prepared according to general procedure **2.3.2.** A solution of (*1H*-benzimidazole-2-yl)-methanol (FAS22) (1.48 g, 0.01 mol), and sodium hydroxide (0.40 g, 0.01 mol) was stirred in dry acetone (30 ml) for 30 min. Then, iodomethane (0.01 mol, 1.41g) was added to the mixture and continued stirring for 24 hours. The reaction mixture was concentrated to a quarter and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize the excess sodium hydroxide and washed the solid with cold water (100 ml) and with aqueous ethanol. The desired product (FAS36) was then dried and recrystallized from ethanol to give white crystals (0.7 g, 43%). m.p. (128-130 °C) (lit., 125-130ºC) (Bednyagina and Postovskii, 1960). δ_H (DMSO-d_6) 7.60 (1H, d, ^3J 8.00, 7-H), 7.53 (1H, d, ^3J 8.00, 4-H), 7.25 (1H, dt, ^3J 7.80, ^4J 1.20, 5-H), 7.19 (1H, dt, ^3J 8.00, ^4J 1.20, 6-H), 5.63 (1H, t, ^3J 5.60, O*H*,), 4.72 (2H, d, ^3J 5.20, CH_2), 3.83 (3H, s, CH_3); δ_C (DMSO-d_6) 155.02 (C-4, C-7), 121.27 (C-5, C-6), 118.14 (C-3a, C-7a), 111.12 (C-2), 57.51 (C-1'); υ_{max}/cm^{-1} (KBr) 3441 (O-H), 3139 (C-H, sp^2), 2837 (C-H, sp^3), 1482and 1334 (C-H, bend); MS (EI): m/z 162 ($M^{+\cdot}$, 100%).

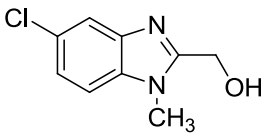
(*N*-Methyl-5-methyl-*1H*-benzimidazole-2-yl)-methanol **(FAS37)**



N-Methyl-2-methanol-5-methylbenzimidazole (FAS37) was prepared according to general procedure **2.3.2.** A solution of 5-methyl-(*1H*-benzimidazole-2-yl)-methanol (FAS23) (1.62 g, 0.01 mol), and sodium hydroxide (0.40 g, 0.01 mol) were stirred in

dry acetone (30 ml) was stirred for 30 min. Then, iodomethane (1.41g, 0.01 mol) was added to the mixture and the mixture was stirred for 24 hours. The reaction mixture was concentrated to a quarter and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize the excess potassium carbonate. Then, wash the solid with cold water (100 ml) and then aqueous ethanol. The product was purified by column chromatography (9:1 chloroform/ethanol) to give the desired product (FAS37) as a brown powder (0.82 g, 46%). m.p. (122-124 °C). δ_H (DMSO-d_6) 7.46 (1H, d, ^3J 8.00, 7-H), 7.31 (1H, s, 4-H), 7.39 (1H, dd, ^3J 9.80, ^4J 2.80, 6-H), 5.59 (1H, s, O*H*,), 4.67 (2H, s, CH_2), 3.79 (3H, s, N-CH_3), 2.40 (3H, s, CH_3); δ_C (DMSO-d_6) 153.8 (C-2), 142.06 (C-3a), 136.24 (C-7a), 131.34 (C-6),123.44 (C-5), 118.63 (C-4), 109.64 (C-7), 55.40 (C-1'), 21.39 (*C*H_3), 29.76 (*C*H_3); υ_{max}/cm^{-1} (KBr) 3154 (C-H, sp^2), 2853 (C-H, sp^3). MS (EI): m/z 176 ($M^{+\cdot}$, 100%).

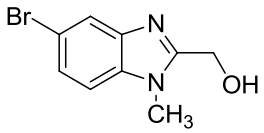
(*N*-Methyl-5-chloro-*1H*-benzimidazole-2-yl)-methanol **(FAS38)**



N-Methyl-2-methanol-5-chlorobenzimidazole (FAS38) was prepared according to general procedure **2.3.2.** A solution of 5-chloro-(*1H*-benzimidazole-2-yl)-methanol (FAS25) (1.50 g, 8.20 mmol), and sodium hydroxide (0.33 g, 8.2 mmol) were stirred in dry acetone (30 ml) for 30 min. Then, iodomethane (1.41g, 8.20 mmol) was added and the mixture was stirred for 24 hours. The reaction mixture was concentrated to a quarter and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize the excess potassium carbonate. The solid was washed with cold water (100 ml) and aqueous ethanol. The product was purified by column chromatography (9:1 chloroform/ethanol) to give the desired product (FAS38) as bright orange crystals (0.14 g, 9 %). m.p. (136-138 °C). δ_H (DMSO-d_6) 7.64 (1H, s, 4-H), 7.58 (1H, d, ^3J 8.00, ^4J 2.80 7-H), 7.19 (1H, d, ^3J, 8.00,

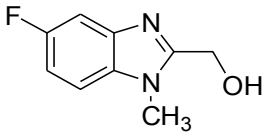
6-H), 5.64 (1H, br, s, OH), 4.71 (2H, s, CH_2), 3.82 (3H, s, N-CH_3); δ_C (DMSO-d_6) 155.57 (C-2), 142.51 (C-3a), 136.78 (C-7a), 126.66 (C-5), 122.19(C6), 120.09 (C-7), 111.40 (C-4) 56.31 (C-1') 30.03 (N-CH_3); υ_{max} /cm^{-1} (KBr) 3154 (C-H, sp^2), 2853 (C-H, sp^3), 1439 and 1334 (C-H, bend), 1134 (C-Cl). MS (EI): m/z 197 ($M^{+\cdot}$, 100%),199 (M+2, 40%). Found; C, 54.65 %; H, 4.64 %; N, 14.12 %, requires; C, 54.97 %; H, 4.61 %; N, 14.25 %.

(*N*-Methyl-5-bromo*1H*-benzimidazole-2-yl)-methanol **(FAS40)**



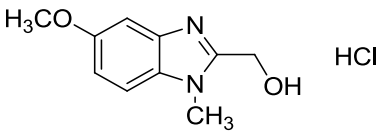
N-Methyl-2-methanol 5-bromobenzimidazole (FAS 40) was prepared according to general procedure **2.3.2.** A solution of 5-bromo-(*1H*-benzimidazole-2-yl)-methanol (FAS26) (0.60 g, 2.60 mmol), and sodium hydroxide (0.10 g, 2.60 mmol) were stirred in dry acetone (10 ml) for 30 min. Then, iodomethane (0.37 g, 2.60 mmol) was added and the mixture was stirred for 24 hours. The reaction mixture was concentrated to a quarter and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize the excess potassium carbonate. The solid was washed with cold water (100 ml) and with aqueous ethanol. The product was purified by column chromatography (9:1 chloroform/ethanol) to give the desired product (FAS40) as a bright yellow crystals (0.10 g, 16 %). m.p. (158-160 °C). δ_H (DMSO-d_6) 7.88 (1H, s, 4-H), 7.58 (1H, d, ^3J 8.00,7-H), 7.43 (1H, dd, ^3J 8.00, ^4J 1.60 6-H), 5.68 (1H, br, s, OH), 4.75 (2H, s, CH_2), 3.86 (3H, s, N-CH_3); δ_C (DMSO-d_6) 155.42 (C-2), 143.10 (C-3a), 137.27 (C-7a), 124.74 (C-5), 121.66 (C6), 120.55 (C-7), 111.90 (C-4) 56.27 (C-1') 30.03 (N-CH_3); υ_{max} /cm^{-1} (KBr) 3122 (C-H, sp^2), 2851 (C-H, sp^3), 1479 and 1348 (C-H, bend), 1105 (C-Br); MS (EI): m/z 241 ($M^{+\cdot}$, 100%), 243 (M+2, 100%).

(*N*-Methyl-5-fluoro *1H*-benzimidazole-2-yl)-methanol **(FAS41)**



N-Methyl-2-methanol-5-flourobenzimidazole (FAS41) was prepared according to general procedure **2.3.2.** A solution of (5-fluoro-*1H*-benzimidazole-2-yl) methanol (FAS27) (0.42g, 2.5 mmol), and sodium hydroxide (0.1 g, 2.5 mmol) were stirred in dry acetone (8 ml) for 30 min. Then, iodomethane (0.35 g, 2.5 mmol) was added and the mixture was stirred for 24 hours. The reaction mixture was concentrated to a quarter and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize the excess potassium carbonate. The solid was washed with cold water (25 ml) and aqueous ethanol. The desired product (FAS41) was dried and recrystallized from ethanol to give white pale cream crystals (0.24 g, 53%). m.p. 132-134 °C. δ_H (DMSO-d_6) 7.60 (1H, d, ^3J 8.00, 7-H), 7.53 (1H, d, ^3J 8.00, 4-H), 7.25 (1H, dt, ^3J 7.80, ^4J 1.20, 5-H), 7.19 (1H, dt, ^3J 8.00, ^4J 1.20, 6-H), 5.63 (1H, t, ^3J 5.60, O*H*,), 4.73 (2H, d, ^3J 4.80, CH_2), 3.83 (3H, s, CH_3); δ_C (400 MHz; DMSO-d_6) 155.02 (C-4, C-7), 121.27 (C-5, C-6), 118.14 (C-3a, C-7a), 111.12 (C-2), 57.51 (C-1'); υ_{max} /cm^{-1} (KBr) 3170 (C-H, sp^2), 2865 (C-H, sp^3), 1434 and 1343 (C-H, bend), 1137 (C-F). MS (EI): m/z 181 (M +1, 100%), 182 (M+ 2, 15%), 163 (M-OH, 15%).

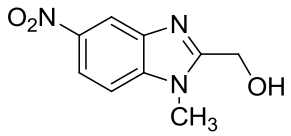
(*N*-Methyl-5-methoxy-*1H*-benzimidazole-2-yl)-methanol **(FAS42)**



N-Methyl-2-methanol-5-methoxybenzimidazole (FAS 42) was prepared according to general procedure **2.3.2.** A solution of (5-methoxy-1 *H*-benzimidazole-2-yl)-methanol (FAS24) (0.06g, 0.43 mmol), and sodium hydroxide (0.017 g, 0.43 mmol) were stirred in dry acetone (1 ml) for 30 min. Then, iodomethane (0.06 g, 0.43

mmol) was added and the mixture was stirred for 24 hours. The reaction mixture was concentrated to a quarter and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize the excess potassium carbonate. The solid was washed with cold water (10 ml) and aqueous ethanol. The product was purified by column chromatography (9:1 chloroform/ethanol) to give the desired product (FAS42) as a dark green precipitate (0.02 g, 25 %). m.p. 182-184 °C (lit., 183 ºC) (Khristich *et al.*, 1982). δ_H (DMSO) 7.93 (1H, d, ^3J 9.20, 7-H), 7.58 (1H, d, ^4J 2.40, 4-H), 7.30 (1H, dd, ^3J 8.00, ^4J 2.40, 6-H), 5.01 (2H, d, ^3J 10.40, CH_2), 4.05 (3H, s, OCH_3), 3.90 (3H, s, CH_3); δ_C (DMSO-d_6) 126.27 (C-2), 158.30 (C-3a,C-7a), 96.52 (C-5), 113.86 (C-4, C-6), 152.32 (C-7), 56.08 (C-1'), 51.43 (O<u>C</u>H_3), 31.77 (<u>C</u>H_3); υ_{max} /cm^{-1} (KBr) 3411 (OH) 3000 (C-H, sp^2), 2834 (C-H, sp^3), 1425 and 1356 (C-H, bend), 1105 (C-Br); MS (EI): m/z 193 (M + 1, 100%), 177 (M-CH_3). Found; C, 48.09 %; H, 4.45 %; N, 10.61 %, requires; C, 48.69 %; H, 6.13 %; N, 11.36 %.

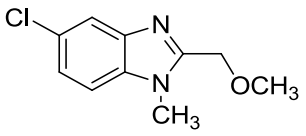
(*N*-Methyl-5-nitro-*1H*-benzimidazole-2-yl)-methanol **(FAS50)**



N-Methyl-2-methanol-5-nitrobenzimidazole (FAS50) was prepared according to general procedure **2.3.2.** A solution of 5-nitro-(*1H*-benzimidazole-2-yl)-methanol (FAS28) (0.86 g, 4.50 mmol), and sodium hydroxide (0.18 g, 4.50 mmol) were stirred in dry acetone (20 ml) for 30 min. Then, iodomethane (0.64 g, 4.50 mmol) was added and the mixture was stirred for 24 hours. The reaction mixture was concentrated to a quarter and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize the excess potassium carbonate. The solid was washed with cold water (100 ml) and aqueous ethanol. The product was purified by column chromatography (9:1 chloroform/ethanol) to give the desired product (FAS50) as an yellow crystals (0.18 g, 19 %). m.p. (160-162 °C) (lit., 167-

168 ºC) (Khristich *et al.*, 1982). δ_H (DMSO-d_6) 7.47 (1H, d, ^4J 1.60 4-H), 7.77 (1H, d, ^3J 8.00,7-H), 7.16 (1H, dd, ^3J 8.00, ^4J 1.60 6-H), 5.76 (1H, br, s, OH), 4.78 (2H, s, CH_2), 3.91 (3H, s, N-CH_3); δ_C (DMSO-d_6) 153.31 (C-2), 142.52 (C-3a), 140.89 (C-7a), 140.49 (C-5), 117.80 (C6), 115.06 (C-7), 110.58 (C-4) 56.36 (C-1') 30.45 (N-CH_3). υ_{max} /cm^{-1} (KBr) 3104 (C-H, sp^2), 2853 (C-H, sp^3), 1436 and 1347 (C-H, bend), 1531 and 1339 (N-O).

N-methyl -5-chloro-2-methoxymethyl-*1H*-benzimidazole **(FAS39)**

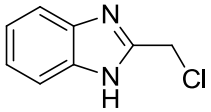


. 3HCl

N-Methyl-5-chloro-2-methoxymethybenzimidazole (FAS39) was prepared according to general procedure **2.3.2.** A solution of 5-chloro-*1H*-benzimidazole-2-yl)-methanol (FAS25) (0.91 g, 0.005 mol), and sodium hydroxide (0.2 g, 0.005 mol) were stirred in dry acetone (15 ml) for 30 min. Then, iodomethane (0.71 g, 0.005 mol) was added and the mixture was stirred for 24 hours. The reaction mixture was concentrated to a quarter and then poured into ice-cold water. The solid was filtered and washed with 50% HCl to neutralize the excess potassium carbonate. The solid was washed with cold water (50 ml) and aqueous ethanol. The desired product (FAS39) was dried and recrystallized from ethanol to give a cream crystals (0.46 g, 46 %). m.p. (138-140 °C). δ_H (DMSO-d_6) 8.01 (1H, d, ^3J 8.80, 7-H), 7.89 (1H, d, ^4J 2.00, 4-H), 7.64 (1H, dd, ^3J 8.80, ^4J 2.00, 6-H), 5.06 (2H, s, CH_2), 3.53 (3H, s, CH_3) , 4.03 (3H, s, OCH_3); δ_C (DMSO-d_6) 114.38 (C-2), 125.85 (C-3a, C-7a), 130.08 (C-5), 131.42 (C-7), 144.28 (C-6), 152.19 (C-4), 64.42 (C-1'), 59.34 (O*C*H_3), 32.36 (*C*H_3); υ_{max} /cm^{-1} (KBr) 3451 (OH) 3027 (C-H, sp^2), 2851 (C-H, sp^3), 1425 and 1338 (C-H, bend), 1123 (C-Cl); MS (EI): m/z 211 ($M^{+\cdot}$, 100%), 213 (M+2, 40%); Found; C, 37.61 %; H, 4.09 %; N, 7.62 %, requires; C, 37.53 %; H, 4.41 %; N, 8.75 %.

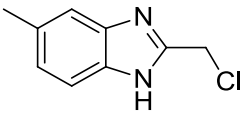
2.3.11 Synthesis of 2- Chloromethylbenzimidazole derivatives

2-Chloromethylbenzimidazole **(FAS43)**



Thionyl chloride (2.5 ml) was added dropwise to finely ground 2-hydroxymethylbenzimidazole (FAS22) (2.5 g, 0.0167 mol). The orange solution was heated under reflux for 3 hours. The reaction was allowed to cool to room temperature before any excess thionyl chloride was destroyed by the addition of methanol in small portions. The methanol was removed in vacuo and the resulting orange/red solid dissolved in dichloromethane (25 ml) and water (25 ml). The aqueous phase was separated and evaporated to dryness. The creamy white solid was recrystallised from water to give yellow crystals (FAS43) (2.00 g; 72%). m.p. 142-144 ºC (lit, 140-141 ºC) (Prostakov *et al.*, 1990). δ_H (DMSO) 7.81 (2H, dd, ^3J 6.00, ^4J 3.20 4-H, 7-H), 7.54 (2H, dd, ^3J 6.00, ^4J 3.20, 5-H, 6-H), 5.28 (1H, s, CH_2); δ_C (DMSO-d_6) 148.29 (C-2), 130.74 (C-3a, C-7a), 125.84 (C-5, C-6), 114.03(C-4, C-7), 33.79 (C-1'); υ_{max}/cm^{-1} (KBr) 3088 (C-H, sp^2), 2851 (C-H, sp^3), 1253 ($\underline{C}H_2$-Cl).

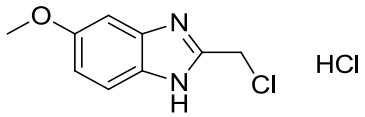
2-(Chloromethyl)-5-methyl-*1H*-benzimidazole **(FAS44)**



5-Methyl-1 H-benzimidazole-2-yl)-methanol) (FAS23) (5 g, 25 mmol) was dissolved in thionyl chloride (30 ml). 2-(Chloromethyl)-5-methyl-*1H*-benzimidazole (FA 44) (2.75g, 61%) was recovered as yellow powder. m.p. 128-130 °C; (lit., 127-132 °C)(Wilson and Hunt, 1983). δ_H (DMSO-d_6) 7.66 (1H, d, ^3J 7.60, 7-H), 7.57 (1H, s, 4-H), 7.34 (1H, d, ^3J 7.60, 6-H), 5.25 (2H, s, CH_2), 2.44 (3H, s, CH_3); δ_C (DMSO-d_6) 153.95 (C-2), 147.64 (C-3a), 135.83 (C-7a), 130.95 (C-6),128.76 (C-5), 127.33 (C-

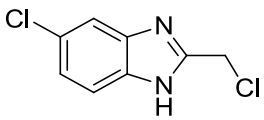
4), 113.47 (C-7), 33.76 (C-1'), 21.11 ($\underline{C}H_3$); υ_{max} /cm^{-1} (KBr) 3420 (NH) 3089 (C-H, *sp*^{2}), 2846 (C-H, *sp*^{3}), 1275 ($\underline{C}H_2$-Cl).

2-(Chloromethyl)-5-methoxy-*1H*-benzimidazole **(FAS45)**



Thionyl chloride (27.5 mmol, 2 ml) was added slowly to a solution of 5-methoxy-*1H*-benzimidazole-2-yl)-methanol) (FAS24) (0.58 g, 3.31 mmol) in dichloromethane (10 ml) at 10 ºC, the mixture was stirred until no presence of the starting material. The solvent was then evaporated, and the residue was triturated with DCM, and suction filtered, then was washed with dichloromethane and ether. 5-Methoxy-2-(chloromethyl)-5-methyl-*1H*-benzimidazole (FAS45)(0.58 g, 89%) was recovered as a green powder. m.p. 200-202 °C. δ_H (DMSO-d_6) 7.72 (1H, d, ^3J 9.20, 7-H), 7.25 (1H, d, ^4J 2.00, 4-H), 7.16 (1H, d, ^3J 9.00, ^4J 2.40 6-H), 5.22 (2H, s, CH_2), 3.85 (3H, s, OCH_3). δ_C (DMSO-d_6) 157.44 (C-2), 146.77 (C-3a), 131.50 (C-7a), 124.76 (C-6),115.64 (C-5), 114.54 (C-4), 95.47 (C-7), 35.15 (C-1'), 33.26 (O$\underline{C}H_3$). υ_{max} /cm^{-1} (KBr) 3401 (NH) 3093 (C-H, *sp*^{2}), 2850 (C-H, *sp*^{3}), 1272 ($\underline{C}H_2$-Cl). MS (EI): m/z 197 ($M^{+\cdot}$, 100%), 199 (M+2, 40%). Found; C, 45.78%; H, 4.08 %; N, 11.83 %, requires; C, 46.37 %; H, 4.32 %; N, 12.02 %.

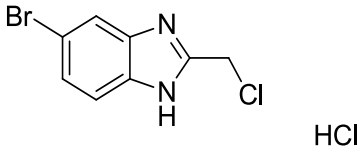
2-(Chloromethyl)-5-chloro-*1H*-benzimidazole **(FAS46)**



Thionyl chloride (27.5 mmol, 5 ml) was added slowly to a solution of 5-chloro-1 *H*-benzimidazole-2-yl)-methanol) (FAS25) (1.51 g, 8.27 mmol) in dichloromethane (25 ml) at 10 ºC, the mixture was stirred until no presence of the starting material. The solvent was evaporated and then the residue was triturated with dichloromethane,

suction filtered, washed with dichloromethane and ether. 2-(Chloromethyl)-5-chloro-*1H*-benzimidazole (FAS46) (1.32 g, 80%) was recovered as a light brown powder. m.p. 210-212 °C (lit., 213-214°C) (King *et al.*, 1949). δ_H (DMSO-d_6) 7.78 (1H, d, ^3J 8.40, 7-H), 7.85 (1H, d, ^4J 1.60, 4-H), 7.49 (1H, d, ^3J 8.80, ^4J 2.00 6-H), 5.16 (2H, s, CH_2); δ_C (DMSO-d_6) 156.12 (C-2), 150.21 (C-3a), 134.09 (C-7a), 129.72 (C-6),125.91 (C-5), 116.11 (C-4), 114.42 (C-7), 35.11 (C-1'); υ_{max} /cm^{-1} (KBr) 3420 (NH) 3075 (C-H, sp^2), 2790 (C-H, sp^3), 1199 ($\underline{C}H_2$-Cl). MS (EI): m/z 201 ($M^{+\cdot}$, 100%), 203 (M+2, 70%).

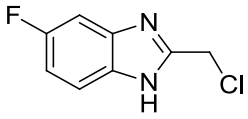
2-(Chloromethyl)-5-bromo-*1H*-benzimidazole **(FAS47)**



HCl

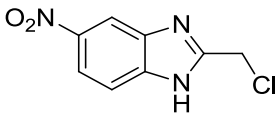
Thionyl chloride (68.75 mmol, 5 ml) was added slowly to a solution of 5-bromo-1*H*-benzimidazole-2-yl)-methanol) (FAS26) (1.51 g, 8.27 mmol) in dichloromethane (25 ml) at 10 ºC, the mixture was stirred until no presence of the starting material. The solvent was then evaporated and the residue was triturated with dichloromethane, suction filtered, washed with dichloromethane and ether. 2-(Chloromethyl)-5-bromo-*1H*-benzimidazole (FAS47) (2.03 g , 92 %) was recovered as a brown pale orange powder. m.p. 248-250 °C; (lit., 249-250 °C)(Dandegaonker and Shastri, 1965). δ_H (DMSO-d_6) 7.73 (1H, d, ^3J 8.80, 7-H), 7.99 (1H, d, ^4J 0.80, 4-H), 7.61 (1H, d, ^3J 8.80, ^4J 1.20 6-H), 5.17 (2H, s, CH_2). δ_C (DMSO-d_6) 150.16 (C-2), 134.37 (C-3a), 132.15 (C-7a), 129.47 (C-6),125.54 (C-5), 117.30 (C-4), 116.09 (C-7), 35.00 (C-1'); υ_{max} /cm^{-1} (KBr) 3436 (NH) 3070 (C-H, sp^2), 2867 (C-H, sp^3), 1208 ($\underline{C}H_2$-Cl); MS (EI): m/z 245 ($M^{+\cdot}$, 80%), 247 (M+2, 100%), 249(M+4, 20%); Found; C, 34.95%; H, 2.61 %; N, 9.84 %, requires; C, 34.08 %; H, 2.50 %; N, 9.94 %.

2-(Chloromethyl)-5-flouro-*1H*-benzimidazole **(FAS48)**



Thionyl chloride (27.5 mmol, 2 ml) was added slowly to a solution of 5-flouro-1*H*-benzimidazole-2-yl)-methanol) (FAS27) (0.55 g, 3.31 mmol) in dichloromethane (10 ml) at 10 ºC, the mixture was stirred until no presence of the starting material. The solvent was then evaporated, and the residue was triturated with dichloromethane, suction filtered, washed with dichloromethane and ether. 2-(Chloromethyl)-5-flouro-*1H*-benzimidazole (FAS48) (0.49 g, 80%) was recovered as a brown powder. m.p. 212-214. δ_H (DMSO-d_6) 7.82 (1H, d, ^3J 8.80, 7-H), 7.67 (1H, d, ^4J 2.00, 4-H), 7.38 (1H, d, ^3J 8.80, ^4J 2.00 6-H), 5.18 (2H, s, CH_2) (lit., 4.87 (2H, s, CH_2), 7.05 (1H, td, ^3J 9.00, ^4J 3.00) 7.27 (1H, dd, ^3J 9.00, ^4J 3.00), 7.51-7.55 (1H, m)) (Cowart *et al.*, 2004); δ_C (DMSO-d_6) 158.63 (C-2), 149.99 (C-3a), 132.97 (C-7a), 129.33.14 (C-6),116.14 (C-5), 114.17 (C-4), 101.11 (C-7), 35.00 (C-1'); υ_{max} /cm^{-1} (KBr) 3434 (NH) 3083 (C-H, sp^2), 2797 (C-H, sp^3), 1219 ($\underline{C}H_2$-Cl); MS (EI): m/z 185 ($M^{+\cdot}$, 100%), 187 (M+2, 30%).

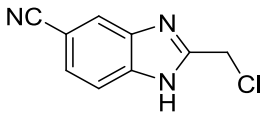
2-(Chloromethyl)-5-nitro-*1H*-benzimidazole **(FAS49)**



Thionyl chloride (275 mmol, 20 ml) was added slowly to a solution of 5-nitro-1 *H*-benzimidazole-2-yl)-methanol) (FAS28) (6.38 g, 33.1 mmol) in dichloromethane (100 ml) at 10 ºC, the mixture was stirred until no presence of the starting material. The solvent was then evaporated, and the residue was triturated with dichloromethane, suction filtered, washed with dichloromethane and ether. 2-(Chloromethyl)-5-nitro-*1H*-benzimidazole (FAS49) (6.14 g, 88%) was recovered as an orange crystals. m.p. 170-172 °C (lit., 174 °C)(Labas *et al.*, 2011). δ_H (DMSO-d_6)

7.74 (1H, d, ^{3}J 8.80, 7-H), 7.48 (1H, d, ^{4}J 2.00, 4-H), 8.11 (1H, d, ^{3}J 7.52, ^{4}J 2.20 6-H), 5.01 (2H, s, CH_2). δ_C (DMSO-d_6) 142.63 (C-2 &C-3a &C-7a), 118.10 (C-6 & C-5 & C-4 & C-7), 37.82 (C-1'). υ_{max} /cm^{-1} (KBr) 3503 (NH) 3090 (C-H, sp^2), 2854 (C-H, sp^3), 1313 (CH_2-Cl). MS (EI): m/z 212 ($M^{+\cdot}$, 100%), 214 (M+2, 30%).

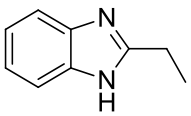
2-(Chloromethyl)-5-cyano-*1H*-benzimidazole **(FAS53)**



Thionyl chloride (27.5 mmol, 2 ml) was added slowly to a solution of 5-cyano-1 *H*-benzimidazole-2-yl)-methanol) (FAS52) (0.57 g, 3.31 mmol) in dichloromethane (10 ml) at 10 ºC, the mixture was stirred until no presence of the starting material. The solvent was then evaporated then the residue was triturated with dichloromethane, suction filtered, washed with dichloromethane and ether. 2-(Chloromethyl)-5-cyano-*1H*-benzimidazole (FAS53) (0.36 g, 57 %) was recovered as a beige powder, m.p. 230-232 °C (lit., 230-232 °C)(Elshihawy, 2008). δ_H (DMSO-d_6) 7.52 (1H, d, ^{3}J 8.40, 7-H), 7.99 (1H, s, 4-H), 7.64 (1H, d, ^{3}J 8.00, 6-H), 4.66 (2H, s, CH_2); δ_C (DMSO-d_6) 142.63 (C-2), 140.99 (C-3a), 130.97 (C-7a), 120.33.14 (C-6),115.14 (C≡N) , 113.78 (C-4), 112.11 (C-7), 110.20 (C-5), 57.00 (C-1').

2.3.12 Synthesis of 2-ethylbenzimidazole derivatives

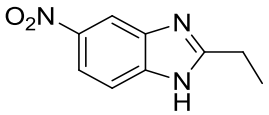
2-Ethyl-*1H*-benzo[*d*]imidazole **(FAS54)**



1,2-Phenylenediamine (2.16 g, 20 mmol) was dissolved in hydrochloric acid (20 ml, 5.5 M). Propionic acid (1.48 g, 20 mmol) was then added and the mixture heated under reflux for 24 hours. The reaction mixture was cooled in ice before the

ammonia solution was added. 2-Ethyl-*1H*-benzimidazole (FAS54) was recovered as creamy powder (1.75 g, 60 %). m.p. 164-166 °C (lit., 164-165 °C) (Aridoss and Laali, 2011). δ_H (DMSO-d_6) 7.47 (2H dd, ^3J 8.00, ^4J 2.40, 4-H, 7-H), 7.11 (2H, dd, ^3J 8.00, ^4J 2.20, 5-H, 6-H), 1.33 (3H, t, ^3J 6.00, CH_3,), 2.84 (2H, q, ^3J 6.00, CH_2), 12.24 (1H, br, s, NH); δ_C (400 MHz; DMSO-d_6) (156.13) (C-2), 120.99 (C-5, C-6), 138.68 (C-3a, C-7a), 114.16 (C-4, C-7), 21.95 (C-1') 12.18 ($\underline{C}H_3$).

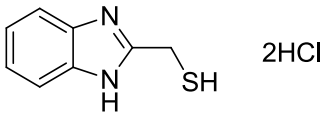
2-Ethyl-5-nitro-*1H*-benzo[*d*]imidazole **(FAS55)**



4-Nitro-1,2-phenylenediamine (3.06 g, 20 mmol) was dissolved in hydrochloric acid (20 ml, 5.5 M). Propionic acid (1.48 g, 20 mmol) was then added and the mixture was heated under reflux for 24 hours. The reaction mixture was cooled in ice before the ammonia solution was added. The resulting dark brown oil was dissolved in ethanol and treated with decolourising using charcoal. 2-Ethyl-5-nitro-*1H*-benzimidazole (FAS55) was recovered as yellow crystals (1.50 g, 40%). m.p. 175-177 °C (lit., 178-179 °C) (Willitzer *et al.*, 1978). δ_H (DMSO-d_6) 8.33 (1H, s, 4-H), 8.01 (1H, d, ^3J 8.80, ^4J 2.20 6-H), 7.59 (1H, d, ^3J 8.80, 7-H), 2.89 (2H, q, ^3J 6.00, CH_2), 1.33 (3H, t, ^3J 5.70, CH_3), 12.85 (1H, br, s, NH); δ_C (DMSO-d_6) 161.06 (C-2), 142.01 (C-7a), 139.26 (C-5), 117.06 (C-3a), 114.21 (C-6), 110.98 (C-7), 107.39 (C-4), 22.05 (C-1'), 11.67 ($\underline{C}H_3$).

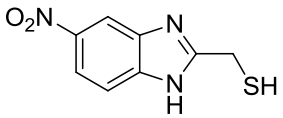
2.3.13 Synthesis of 2-methanethiolbenzimidazole derivatives

(*1H*-Benzo[*d*]imidazol-2-yl)methanethiol **(FAS56)**



(1*H*-Benzo[d]imidazol-2-yl)methanethiol (FAS56) was prepared according to general procedure **2.3.1.** 1,2-Phenylenediamine (10.80 g, 0.10 mol) and 2-mercaptoacetic acid (50%) were dissolved in hydrochloric acid (70 ml, 5.5 M) and the mixture was heated under reflux for 60 hours. The reaction mixture was neutralised with ammonia solution to give a precipitate. The crude product was recrystallised from water. (*1H*-Benzo[*d*]imidazol-2-yl)methanethiol (FAS56) was obtained as olive coloured crystals(13.46 g; 82 %). m.p. 170-172 °C (lit. 173 °C) (Gowda *et al.*, 2011). δ_H (DMSO-d_6) 7.54 (2H, s, 4-H, 7-H), 7.16 (2H, s, 5-H, 6-H), 4.21 (2H, s, CH_2), 12.72 (1H, br, s, NH), S*H* not observed. δ_C (DMSO-d_6) 114.50 (C-4, C-7), 118.51 (C-5, C-6), 121.76 (C-3a, C-7a), 150.59 (C-2), 35.72 (C-1');). MS (EI): m/z 165 (M+1, 100%). Found; C, 41.88 %; H, 3.56 %; N, 11.23 %; requires; C, 40.52 %; H, 4.25 %; N, 11.81 %.

(5-Nitro-*1H*-benzo[*d*]imidazol-2-yl)methanethiol **(FAS57)**

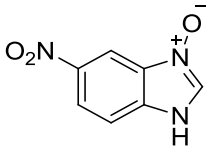


5-Nitro-2-methanethiolbenzimidazole (FAS57) was prepared according to procedure **2.3.1.**, 4-Nitro-1,2-phenylenediamine (7.65 g, 50 mmol) and 2-mercaptoacetic acid (50%) were dissolved in hydrochloric acid (70 ml, 5.5 M) and the mixture was heated under reflux for 70 hours. The reaction mixture was neutralised with ammonia solution and give 5-nitro-2- methanethiolbenzimidazole (FAS57) as bright orange crystals (5.00 g, 48%). m.p. 195-197 ºC (lit., 195 ºC) (Gowda *et al.*, 2011). δ_H (DMSO-d_6) 8.41 (1H, d, 4-H, ^4J 2.22), 8.09 (1H, d, ^3J 8.40, ^4J 2.2, 6-H), 7.69 (1H,

d, ^{3}J 8.40, 7-H), 4.27 (2H, s, CH_2), 3.60 (1H, s, SH), 13.14 (1H, br, s, NH). δ_C (DMSO-d_6) 155.31 (C-2), 142.47 (C-7a), 139.99 (C-5), 135.50 (C-3a), 117.69 (C-6), 114.34 (C-7), 111.93 (C-4), 35.39 (C-1').

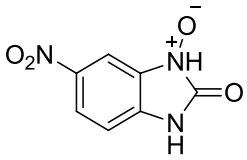
2.3.14 Synthesis of 1-oxidebenzimidazole derivatives

5-Nitro-1*H*-benzo[*d*]imidazole 3-oxide **(FAS59)**



5-Nitro-1*H*-benzo[*d*]imidazole 3-oxide (FAS59) was prepared by using 5-nitro -*1H*-benzo[*d*]imidazole (FAS 21) (1.63 g, 10 mmol), which was reacted with hydrogen peroxide (30% v/v). 5-Nitro-3-oxido-*1H*-benzo[*d*]imidazole (FAS59) was obtained as a cream powder (0.95 g, 49%). m.p. 200-202 °C(lit., 201-203 °C)(Elshihawy, 2008) . δ_H (DMSO-d_6) 8.55 (1H, s, 2-H) , 7.77 (1H, d, ^{3}J 8.00, 7-H), 8.51 (1H, s, 4-H), 8.12 (1H, d, ^{3}J 8.00, 6-H), 13.90 (1H, s, NH). δ_C (DMSO-d_6) 146.68 (C-2), 142.60 (C-7a) 138.51(C-3a), 133.12 (C-5), 117.55 (C-4), 115.28 (C-6) 112.30 (C-7).

5-Nitro-2-oxo-1,2-dihydro-1*H*-benzo[*d*]imidazol-3-oxide **(FAS60)**

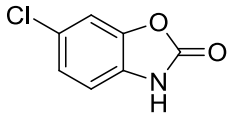


5-Nitro-2-oxo-1,2-dihydro-1*H*-benzo[*d*]imidazol-3-oxide (FAS60) was prepared by using 5-nitro-*1H*-benzo[*d*]imidazol-2(3H)-one (1.79 g, 10 mmol), which was reacted with hydrogen peroxide (30% v/v). 5-Nitro-2-oxo-1,2-dihydro-1*H*-benzo[*d*]imidazol-3-oxide (FAS60) was obtained as a yellow powder (0.98 g, 50 %). m.p. 298-300 °C (lit., 296-298 °C)(Elshihawy, 2008). δ_H (DMSO-d_6) 7.09 (1H, d, ^{3}J 8.00, 7-H), 7.69 (1H, d, ^{3}J 2.00, 4-H), 7.92 (1H, dd, ^{3}J 8.00, ^{4}J 2.00 6-H), 11.19 (1H, s, NH). δ_C

(DMSO-d_6) 155.42 (C-2), 141.60 (C-7a) 135.66 (C-3a), 129.6 (C-5), 117.71 (C-4), 108.01 (C-6) 103.58 (C-7).

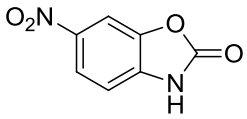
2.3.15 Synthesis of benzoxazole derivatives

6-Chlorobenzo[*d*]oxazol-2(3*H*)-one **(FAS61)**



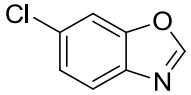
6-Chlorobenzo[*d*]oxazol-2(3*H*)-one (FAS61) was prepared by mixing 5-chloro-2-hydroxyaniline (1.43 g, 10 mmol) with phosgene (4.95 g, 50 mmol) and the mixture reacted at 180 °C under reflux for 2 hours. 6-Chlorobenzo[*d*]oxazol-2(3*H*)-one was obtained as peach coloured crystals (1.27 g, 75 %). m.p. 154-156 °C (lit., 155-157 °C)(Elshihawy, 2008). δ_H (DMSO-d_6) 7.24 (1H, d, ^3J 8.00, 7-H), 7.11 (1H, s, 4-H), 6.82 (1H, dd, ^3J 8.00, ^4J 2.00 6-H), 8.15 (1H, s, NH); δ_C (DMSO-d_6) 154.26 (C-2), 143.41 (C-7a) 136.94 (C-3a), 129.10 (C-5), 116.70 (C-4), 113.05 (C-6) 106.93 (C-7); MS (EI): m/z 169.5 (M^+, 100%).

6-Nitrobenzo[*d*]oxazol-2(3*H*)-one **(FAS62)**



6-Nitrobenzo[*d*]oxazol-2(3*H*)-one (FAS 62) was prepared by mixing 5-nitro-2-hydroxyaniline (1.54 g, 10 mmol) with urea (3.00 g, 50 mmol), and the mixture reacted at 180 °C under reflux for 2 hours. 6-Nitrobenzo[*d*]oxazol-2(3*H*)-one (FAS62) was obtained as a cream powder (1.29 g, 72%). m.p. 240-242 °C (lit., 238-241 °C) (Abdelaal *et al.*, 1992). δ_H (DMSO-d_6) 7.25 (1H, d, ^3J 8.00, 7-H), 8.14 (1H, d, ^4J 2.00, 4-H), 8.09 (1H, dd, ^3J 8.00, ^4J 2.00 6-H), 12.38 (1H, s, NH); δ_C (DMSO-d_6) 154.16 (C-2), 142.73 (C-7a) 142.01 (C-3a), 136.76 (C-5), 120.75 (C-4), 109.33 (C-6) 105.39 (C-7).

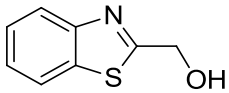
6-Chlorobenzo[*d*]oxazole **(FAS63)**



6-Chlorobenzo[*d*]oxazole (FAS 63) was prepared according to procedure **2.3.1.** 5-Chloro-2-hydroxyaniline (2.00 g, 14 mmol) was dissolved in formic acid (10 ml) and the mixture was heated under reflux for 3 hours. 6-Chlorobenzo[*d*]oxazole (FAS63) was obtained as a brown powder (1.93 g, 90%). m.p. 58-60 °C (lit., 61-62 °C)(Wertz *et al.*, 2011)δ_H (DMSO-d_6) 6.85 (1H, d, ^3J 8.00, 7-H), 8.31 (1H, s, 4-H), 6.95 (1H, dd, ^3J 8.00, ^4J 2.00 6-H), 9.72 (1H, s, 2-H); δ_C (DMSO-d_6) 160.29 (C-2), 145.38 (C-7a) 127.20 (C-3a), 123.28 (C-5), 122.15 (C-4), 119.73 (C-6) 115.89 (C-7).

2.3.16 Synthesis of benzothiozole derivative

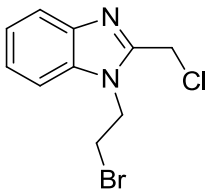
Benzo[*d*]thiazol-2-ylmethanol **(FAS65)**



2-Aminothiophenol (12.5 g, 0.10 mol) was added to glycolic acid (99%) (11.40 g, 0.15 mol) in the absence of a solvent. Then it was heated under reflux for 5 hours. After the reaction mixture was cooled to room temperature, ethanol (30 ml) was added with water (100 ml). The product was recrystallised from aqueous ethanol. 2-Hydroxymethylbenothiazole (FAS65) was isolated as bright green needles (15.0 g, 91%). m.p.100-102 °C (lit., 100-102 °C) (Asselin *et al.*, 2010). δ_H (DMSO) 8.11 (1H, d, ^3J 8.00, ^4J 2.00, 4-H), 7.93 (1H, d, ^3J 8.00, 7-H), 7.44 (2H, m, ^3J 8.00, ^4J 2.00, 5-H, 6-H), 6.26 (1H, t, ^3J 6.00, O*H*), 4.88 (2H, d, ^3J 6.00, CH_2); δ_C (400 MHz; DMSO-d_6) 153.05 (C-3a) 122.27 (C-4) 122.19 (C-7), 125.94 (C-5), 124.63 (C-6), 134.17 (C-7a), 175.55 (C-2), 61.25 (C-1'); MS (EI): m/z 165 ($M^{+\cdot}$, 100%); Found; C, 58.24 %; H, 4.60 %; N, 8.47 %; requires; C, 58.16 %; H, 4.27 %; N, 8.48 %.

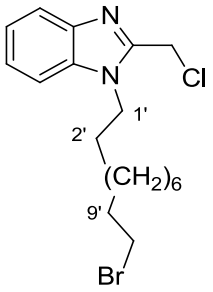
2.3.17 Synthesis of *N*-bromoalkyl-2-chloromethylbenzimidazole derivatives

1-(2-Bromoethyl)-2-(chloromethyl)-*1H*-benzo[*d*]imidazole **(FAS67)**



2-Chloromethylbenzimidazole (FAS43) (1.66 g, 10mmol) was combined with an excess of potassium hydroxide (0.73 g, 13 mmol), and dissolved in ethanol (20 ml). An ethanol solution of 1,2-dibromoethane (2.79 g, 15 mmol) was added dropwise to the 2-chloromethyl-1*H*-benzimidazole (FAS43) solution, and the combined mixture was refluxed for 48 hrs. Water (50 ml) was added to the mixture, and then was extract the product with $CHCl_3$ (2 X 100 ml), then collected the organic phase and dried it over $MgSO_4$. The solvent was removed, and the remaining solid was purified by column chromatography (2:8 hexane/ ethyl acetate) to obtained a bright yellow solid (FAS67) (0.28 g, 10%). m.p. 108-110 ºC δ_H (DMSO) 7.52 (2H, dd, ^3J 5.80, ^4J 3.22 4-H, 7-H), 7.17 (2H, dd, ^3J 5.90, ^4J 3.10, 5-H, 6-H), 4.66 (1H, s, CH_2Cl), 4.04 (1H, t, ^3J 7.16, CH_2Br), 3.55 (1H, t, ^3J 7.00, CH_2N).

1-(10-bromodecyl)-2-(chloromethyl)-*1H*-benzo[*d*]imidazole **(FAS68)**



2-Chloromethylbenzimidazole **(**FAS 43) (0.83 g, 5 mmol) was combined with an excess of potassium carbonate (2.76 g, 20 mmol), and was dissolved in ethanol (20 ml). An ethanol solution of 1,10-dibromodecane (1.80 g, 6 mmol) was added dropwise to the the 2-chloromethyl-1*H*-benzimidazole (FAS43) solution, and the combined mixture was heated under reflux for 48 hours. Water (50 ml) was added to the mixture, and then was extracted the product with $CHCl_3$ (2 X 100 ml), then collected the organic phase and dried it over $MgSO_4$. The solvent was removed, and the remaining solid was purified by column chromatography (9:1 chloroform/ ethanol) to obtained a bright yellow solid chloroform/ ethanol) to obtained a bright yellow solid (FAS68) (0.05 g, 3%). m.p. 154-156 ºC. δ_H (DMSO) 7.65 (2H, dd, ^3J 8.40, ^4J 4.40 4-H, 7-H), 7.21 (2H, dd, ^3J 8.00, ^4J 4.20, 5-H, 6-H), 4.72 (1H, s, CH_2Cl), 3.50 (1H, t, ^3J 6.80, CH_2Br), 4.23 (1H, t, ^3J 7.60, CH_2N), 1.28 (12H, m, 3'-H,4'-H,5'-H,6'-H,7'-H and 8'-H), 1.78 (4H, p, 2'-H & 9'-H).). MS (EI): m/z 307 (M-Br, 100%).

2.3.18 Synthesis of silver complexs of 2-carboxylic acid-benzimidazole derivatives

Silver complex of *1H*-benzo[*d*]imidazole-2-carboxylic acid **(FAS69)**

$$\left[AgC_8H_7N_2O_3\right]$$

A solution of $AgNO_3$ (0.5 g, 3 mmol) in ethanol (5 ml), was added to a solution of *1H*-benzo[*d*]imidazole-2-carboxylic acid (FAS29) (0.49 g, 3mmol) in ethanol (5 ml) to form 1:1 M:L complex. The reaction mixture stirred at 50 ºC, for 2 hrs. The resulting precipitate was filtered off, washed with diethyl ether, followed by warm ethanol. The formed complex (FAS69) was kept under dry conditions. The complex was obtained as white solid (0.52 g, 63 %), m.p. >300ºC. υ_{max} /cm^{-1} (KBr) 3224 (O-H), 1631 (C=O), 1339 (C-O). δ_H (DMSO-d_6) 7.70 (2H, s, 4-H, 7-H), 7.35 (2H, s, 6-H, 5-H).Elemental analysis based on the structure above, found; C, 33.93 %; H, 2.00 %; N, 10.98 %; requires; C, 34.40 %; H, 2.32 %; N, 10.03 %.

Silver complex of 5-bromo-*1H*-benzo[*d*]imidazole **(FAS70)**

$$\left[AgC_7H_5N_2BrCl\right]$$

A solution of $AgNO_3$ (0.5 g, 3 mmol) in ethanol (5 ml), was added to a solution of 5-bromo-*1H*-benzo[*d*]imidazole (FAS19) (0.72 g, 3mmol) in ethanol (5 ml) to form 1:1 M:L complex. The reaction mixture was stirred at 50 ºC, for 2 hrs. The resulting precipitate was filtered off, washed with diethyl ether, followed by warm ethanol. The formed complex was kept under dry condition, to obtained the product as yellow solid (FAS70) (0.20 g, 20%). m.p. 258-260 ºC. υ_{max} /cm^{-1} (KBr) 3464 (N-H), 1046 C-Br. δ_H (DMSO-d_6) 8.66 (1H, s, 2-H), 8.01 (1H, s, 4-H), 7.50 (1H, dd, ^{3}J 8.00, ^4J 2.20, 6-H), 7.47 (1H, d, ^3J 8.00, 7-H). Elemental analysis based on the structure above, found; C, 26.75 %; H, 1.53 %; N, 10.19 %; requires; C, 27.75 %; H, 1.65 %; N, 9.19 %.

Silver complex of *1H*-benzo[*d*]imidazole **(FAS71)**

$$\left[AgC_7H_5N_3Cl\right]$$

A solution of $AgNO_3$ (0.5 g, 3 mmol) in ethanol (5 ml), was added to a solution of 5-nitro-*1H*-benzo[*d*]imidazole (FAS21) (0.62 g, 3mmol) in ethanol (5 ml) to form 1:1 M:L complex. The reaction mixture was stirred at 50 ºC, for 2 hours. The resulting precipitate was filtered off, washed with diethyl ether, followed by warm ethanol. The formed complex (FAS71) was kept under dry condition. The complex formed as yellow solid (FAS 71) (0.3 g, 33%). m.p. >300 ºC. υ_{max} /cm^{-1} (KBr) 3432 (NH), 3103 (C-H, sp^2), 1520 and 1341 (N-O). δ_H (DMSO-d_6) 8.71 (1H, s, 2-H), 8.65 (1H, s, 4-H), 8.19 (1H, dd, ^{3}J 8.00, ^4J 2.20, 6-H), 7.84 (1H, d, ^3J 8.00, 7-H). Elemental analysis based on the structure above, found; C, 27.22 %; H, 1.56 %; N, 13.86 %; requires; C, 27.43 %; H, 1.64 %; N, 13.71 %.

Silver complex of bis(5-methyl- *1H*-benzo[*d*]imidazole-2-carboxylic acid) **(FAS72)**

$$\left[AgC_{18}H_{16}N_4O_4Cl\right]$$

A solution of $AgNO_3$ (0.18 g, 1.07 mmol) in ethanol (5 ml), was added to a solution of 5-methyl-*1H*-benzo[*d*]imidazole-2-carboxylic acid (FAS30) (0.38 g, 2.15 mmol) in ethanol (5 ml) to formed 1:2 M:L complex. The reaction mixture was stirred at 50 ºC, for 2 hours. The resulting precipitate was filtered off, washed with diethyl ether, followed by warm ethanol. The formed complex (FAS72) was kept under dry condition. υ_{max} /cm^{-1} (KBr) 3406 (N-H), 3052 (C-H, sp^2), 1624 (C=O), 1336 (C-O). The complex formed as pink solid (FAS 72) (0.33 g, 31%). m.p. 288-290ºC. δ_H (DMSO-d_6) 8.20 (2H, br, s, O*H,* NH), 7.52 (1H, s, br, 7-H), 7.45 (1H, s, br, 6-H), 7.19 (*1H*, s, br, 4-H), 2.45 (3H, s, CH_3). Elemental analysis based on the structure above, found; C, 43.61 %; H, 3.17 %; N, 11.43 %; requires; C, 43.62 %; H, 3.25 %; N, 11.30 %.

Silver complex of bis(5-bromo-*1H*-benzo[*d*]imidazole) **(FAS73)**

$$\left[AgC_{14}H_8N_5O_3Br\right]$$

A solution of $AgNO_3$ (0.13 g, 0.8 mmol) in ethanol (5 ml), was added to a solution of 5-bromo-*1H*-benzo[*d*]imidazole (FAS19) (0.38 g, 1.6 mmol) in ethanol (5 ml) to formed 1:2 M:L complex. The reaction mixture was stirred at 50 ºC, for 2 hours. The resulting precipitate was filtered off, washed with diethyl ether, followed by warm ethanol. The formed complex (FAS73) was kept under dry condition. υ_{max} /cm^{-1} (KBr) 3468 (N-H), 3052 (C-H, sp^2), 1045 C-Br, 1585 and 1345 (N-O). The complex formed as yellow solid (FAS73)(0.29 g, 33%). m.p.138-140 ºC. δ_H (DMSO-d_6) 8.64 (1H, s, 2-H), 8.00 (1H, s, 4-H), 7.70 (1H, d, ^3J 8.00, 7-H), 7.48 (1H, dd, ^3J 8.00, ^4J 2.40, 6-H). Elemental analysis based on the structure above, found; C, 29.58 %; H, 1.81 %; N, 11.76 %; requires; C, 29.92 %; H, 1.43 %; N, 12.46 %.

Silver complex of bis(5-nitro- *1H*-benzo[d]imidazole) **(FAS74)**

$$\left[AgC_{14}H_{10}N_7O_3\right]$$

A solution of $AgNO_3$ (0.16 g, 0.97 mmol) in ethanol (5 ml), was added to a solution of 5-nitro-*1H*-benzo[*d*]imidazole (FAS21) (0.40 g, 1.95 mmol) in ethanol (5 ml) to formed 1:2 M:L complex. The reaction mixture was stirred at 50 ºC, for 2 hours. The resulting precipitate was filtered off, washed with diethyl ether, followed by warm ethanol. The formed complex (FAS74) was kept under dry condition. υ_{max} /cm^{-1} (KBr) 3468 (NH), 3105 (C-H, sp^2), 1514 and 1350 (N-O). The complex formed as yellow solid (FAS 74) (0.37 g, 39 %). m.p. >300 ºC. δ_H (DMSO-d_6) 8.80 (1H, s, 2-H), 8.72 (1H, s, br, 4-H), 8.21 (1H, dd, ^3J 8.00, ^4J 2.40, 6-H), 7.89 (1H, d, ^3J 8.20, 7-H). Elemental analysis based on the structure above, found; C, 33.55 %; H, 2.05 %; N, 18.91 %; requires; C, 33.89 %; H, 2.03 %; N, 19.76 %.

2.4 Materials and methods for microbiological evaluation

2.4.1 Screening for Antibacterial activity

2.4.1.1 Materials

Tryptone Soya Agar (CM0131, Oxid Ltd., Basingstoke, UK)

Iso-sensitest agar (CM0471, Oxid Ltd., Basingstoke, UK)

Dimethylsulphoxide (DMSO), Sigma-Aldrich D5879

Whatman grade AA discs, 6 mm diameter (Fisher Scientific).

2.4.1.2 Bacterial strains used in this project

All reference and clinical strains were obtained from Dr Anna Snelling, University of Bradford. Details are given in Table 2.1. Overall, 15 strains of *Staphylococcus* Spp. (inc 2 x *S. aureus,* 8 x MRSA, 2 x EMRSA, 2 x *S. epidermidis* and 1 x *S. haemolyticus*), 5 strains of *E. coli,* 4 strains of *Ps. aeruginosa,* 1 strain of *Serratia marcescens,* and 1 strain of *Burkholderia cepacia* were used (Table 2.1).

2.4.1.3 Culture media and incubation conditions

Culture media was prepared and treated according to manufacturer's guidelines and sterlised by autoclaving at 121°C for 15 minutes at 15 lbs/sq inch pressure. The strains were routinely grown on Tryptone Soya Agar or Iso-Sensitest agar, incubated at 37 ˚C in air for 16-18 hrs. Stock culture plate were stored at 4 ˚C.

2.4.1.4 Ciprofloxacin

The control drug used for the antibacteria screening was ciprofloxacin. The drug was purchased as discs: 5µg from Oxid Ltd (Basingstoke, UK) (CIP 5µg) (CT0425B) (Figure 2.1).

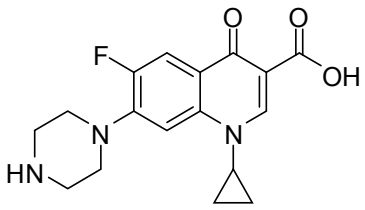


Figure 2.1 Structure of ciprofloxacin

2.4.1.5 Disc diffusion tests

Disc diffusion assays for antimicrobial susceptibility testing were carried out in-line with the standard method first described by Bauer (1966), to assess if the compounds had any antibacterial activities. Bacterial suspensions were prepared from fresh, overnight, agar cultures in sterile distilled water, and adjusted to a 0.5 McFarland turbidity standard (Remel). These were used to seed Iso-sensitest agar plates evenly using a sterile swab (Bauer R. W. *et al.*, 1966).

For test compounds, each was measured to 10 mg/ ml in DMSO, and different amounts of each (typically 10, 100 and 200 µg) were loaded from the working stock solution onto 6 mm diameter sterile Whatmann discs (Fisher Scientific, UK) and allowed to dry. The loaded discs were placed on the surface of the agar lawns with the help of a needle and forceps. Each test plate comprised of 5 discs, three impregnated discs, one control (Ciprofloxacin 5 µg), and one negative control (impregnated with 20 µl of 100 % DMSO). The plates were incubated at 37˚C for 24 hours. At the end of incubation, the plates were examined for zone of inhibition. Diameters of inhibition zones were measured (in mm) and recorded. If any activity was observed, the test was repeated on a separate day, using a fresh culture.

Table 2.1. Details of strains of bacteria used in this project

Bacteria species / strain	Gram +ve or – ve	Notes
***Staphylococcus aureus* (Oxford) NCTC 6571**	+	Reference control strain for antibiotic sensitivity testing
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	
Methicillin-resistant *Staphylococcus aureus* (HG-1)	+	
EMRSA-15 NCTC 13142	+	EMRSA type EMRSA-15
EMRSA-16 NCTC 13143	+	EMRSA type EMRSA-16
MRSA BIG 0043	+	
MRSA BIG 0044	+	
MRSA BIG 0045	+	
MRSA BIG 0047	+	
MRSA BIG 0050	+	Clinical isolate, ciprofloxacin resistant
MRSA BIG 0052	+	Clinical isolate, ciprofloxacin resistant
MRSA BIG 0053	+	
Staphylococcus epidermidis NCTC 11047	+	Type strain of genus
Staphylococcus epidermidis NCTC 2749	+	Commensal skin isolate
Staphylococcus haemolyticus NCTC 11042	+	
***Escherichia coli* NCTC 10418**	-	
Escherichia coli BIG 0046	-	
Coliform BIG 0048	-	
Coliform BIG 0049	-	
Coliform BIG 0051	-	
***Pseudomonas aeruginosa* NCTC 6749 (=BIG 0028)**	-	=ATCC 19582 Reference strain for testing disinfectants and sterilants
Pseudomonas aeruginosa (Environmental) BIG 0039	-	
Pseudomonas aeruginosa NCTC 10662	-	Reference control strain for antibiotic sensitivity testing
Pseudomonas aeruginosa BIG 0063	-	Clinical isolate
Serratia marcescens BIG 0011 = NCTC 1377	-	
Burkholderia cepacia BIG 0009 = NCTC 10744	-	

2.4.1.6 Agar dilution method for determining Minimum Inhibitory Concentration (MICs)

The MIC of the synthesised compounds was determined by the agar dilution technique using Iso-sensitest agar. Agar dilution was on the range of: 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 µg/ml. Iso-sensitest agar was prepared in 19 ml aliquots, using sufficient base powder for 20 ml. Once it had cooled to approximately 50˚C, appropriate amount of the test compound were added in a volume of 1 ml.

The 19 ml aliquots in universal bottles were double steamed instead of autoclaving; to ensure that there was no loss of the volume of water in them. The lids were tightend, and the bottles were then steamed for 2 hours; left to cool and set, then on the following day steamed again for another 2 hours to kill any vegetative cells that had germinated from spores. Eventually, the agar was ready to be used for MIC tests. A few extra universals were prepared to make the control plates (Iso-sensitest aga only). One of them was inoculated at the start and one at the end of a run; to ensure there is no splash over or cross contamination and that the strains grow.

Strains were challenged against a series of doubling dilutions of the test compounds (from 512 through to 0.5 µg/ml) details are given in Table 2.2. A bacterial suspension was prepared in bijoux bottles in sterile water with the turbidity equivalent to a 0.5 McFarland standard. Then, 0.5 ml of each suspension was transferred to a seprate, sterile well of 32-well multipoint inoculator base.

Microorganisms were seeded on to the freshly prepared MIC agar plates using a multi-point inoculator (Denley). The device has a 37 pin head.

After briefly allowing the innocula to dry, the plates were incubated overnight at 37 ˚C and the MIC was defined as the lowest concentration of compound that inhibited bacterial growth completely, as indicated by the absence of any visible growth in the inoculation spot on the agar surface.

Table 2.2. Details of how the doubling dilution plates were prepared for the MIC assays

Tube	Stock tubes										
	1	2	3	4	5	6	7	8	9	10	11
DMSO	4ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml
Amount per ml	10.24 mg/ml	5.12 mg/ml	2.65 mg/ml	1.28 mg/ml	0.64 mg/ml	0.32 mg/ml	0.16 mg/ml	0.08 mg/ml	0.04 mg/ml	0.02 mg/ml	0.01 mg/ml
1ml to plate 2ml to tube 2		1ml to plate 2ml to tube 3	1ml to plate 2ml to tube 4	1ml to plate 2ml to tube 5	1ml to plate 2ml to tube 6	1ml to plate 2ml to tube 7	1ml to plate 2ml to tube 8	1ml to plate 2ml to tube 9	1ml to plate 2ml to tube 10	1ml to plate 2ml to tube 11	1ml to plate
Concentration in 20 ml plate	512 µg/ml	265 µg/ml	128 µg/ml	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml	2 µg/ml	1 µg/ml	0.5 µg/ml

2.4.2 Screening for antifungal activity

2.4.2.1 Materials

Peptone Primatone®

RL, Sigma-Aldrich P4963

Agar powder, Sigma-Aldrich 05040

Malt extract, Sigma-Aldrich 70167

D-(+)-Glucose monohydrate, Sigma-Aldrich 49159

Dimethylsulphoxide (DMSO) *ReagentPlus*®, ≥99.5% , Sigma-Aldrich D5879

Amphotericin B solubilised, Sigma-Aldrich A9528

2.4.2.2 Fungal strains used in this project

The strains were obtained from the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt and this part of the practical work was done there as well. Reference isolates for the antifungal screening; included representatives of **unicellular fungi**; namely, *Candida albicans* RCMB 05035, *Candida krusei* RCMB 05051, *Candida parapsilosis* RCMB 05065, *Candida tropicalis* RCMB 05049, and **filamentous fungi**; namely, *Absidia corymbifera* RCMB 09635, *Aspergillus clavatus* RCMB 2593, *Aspergillus fumigatus* RCMB 02564, *Mucor circinelloides* RCMB 07328, *Mucor circinelloides* RCMB 07328*, Absidia corymbifera* RCMB 09635, *Penicillium marneffei* RCMB 01267, *Syncephalastrum racemosum* RCMB 05922 (Table 2.3).

Table 2.3. Details of fungal strains used in this project

Fungal species / strain	Type
Aspergillus fumigatus RCMB 02564	filamentous
Aspergillus clavatus RCMB 02593	filamentous
Mucor circinelloides RCMB 07328	filamentous
Absidia corymbifera RCMB 09635	filamentous
Penicillium marneffei RCMB 01267	filamentous
Syncephalastrum racemosum RCMB 05922	filamentous
Candida albicans RCMB 05035	unicellular
Candida tropicalis RCMB 05049	unicellular
Candida krusei RCMB 05051	unicellular
Candida parapsilosis RCMB 05065	unicellular

RCMB: Regional Center for Mycology and Biotechnology, Cairo, Egypt.

2.4.2.3 Culture media and incubation conditions

For culturing, maintenance of axenic strains and antifungal screening, Malt Extract Agar (Malt extracts, 20 g; Peptone, 1 g; Glucose, 20 g; Agar, 25 g per litre) was used. The medium was prepared by dissolving the solid ingredients in one litre of cold distilled water, and then heating to 60-70 °C with stirring. It was sterilized by autoclaving at 121°C for 15 minutes at 15 lbs/sq inch pressure and when cool, poured into sterile glass Petri-dishes. For maintenance of stock cultures, it was poured into slants, autoclaved, and then tilted whilst the agar set to provide a slope for growth of stock cultures. A cork borer was used to remove a piece of mycelia growth from an older plate, and transplant it onto the fresh agar. A liquid Malt Extract (broth) medium was prepared by using the same ingredients without the agar.

2.4.2.4 Amphotericin B

Amphotericin B was the control drug compartor used in the antifungal screening, see Figure 2.2. A stock solution was prepared in DMSO at 2.5 mg/ml and filters sterilized.

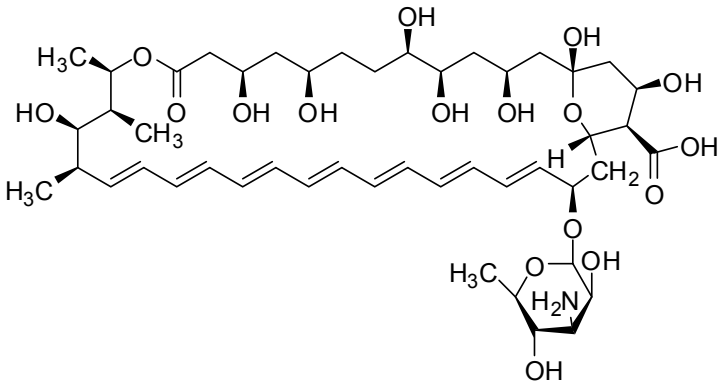


Figure 2.2 . Structure of amphotericin B

2.4.2.5 Screening for antifungal activity by the well diffusion method

Antimicrobial activity was determined by the well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) methodology (National Committee for Clinical Laboratory Standards, 1993). Petri plates containing 20 ml of Malt extract Agar were seeded with 2-3 day old cultures of fungal inoculums (suspensions of spores in sterile water). Wells (6 mm in diameter) were cut by cork borer into the agar, and 50 µl of diluted compound in DMSO was added at a concentration of 5 mg/ml (250 µg per well) and incubated at 37°C (unicellular strains) or 28°C (filamentous strains) for 3-7 days depending on growth rate of each strain. The antifungal activity was determined based on measurement of the diameter of the inhibition zone formed around each well.

2.4.2.6 Determination of Minimum Inhibitory Concentration Test (MIC) of compounds against fungi

The chemical compounds were diluted in DMSO, and then serial doubling dilutions 1:1, with a sterile diluent (typically appropriate broth) was inoculated in each well of a microtiter tray (96 well). After the compounds was diluted, a volume of the

standardized inoculum equal to the volume of the diluted antimicrobial agent was added to each well, bringing the microbial concentration to approximately 5 X 10^5 cells per milliliter. The trays were incubated at an appropriate temperature (28°C or 37°C) for the test species for a pre-set period. After incubation, the series of dilution wells was observed for microbial growth, usually indicated by turbidity and/or a pellet of fungi in the bottom of the well. The last well in the dilution series that didn't demonstrate growth corresponds with the minimum inhibitory concentration (MIC) of the test compound.

2.4.3 The series of compounds screened for Antimicrobial activity

The compounds were synthesised, purified and used for antimicrobial screening assays; after first being divided into 13 main series as shown below according to their structures (Table 2.4).

Table 2.4. Details of the series of compounds screened for antimicrobial activity

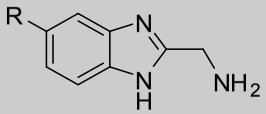
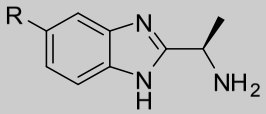
Series 1: Synthesis of 2-aminomethylbenzimidazole derivatives	R	Compound code	Notes	
			Synthesis (Reference)	Biological
	H	FAS1	89% (Galal *et al.*, 2009)	inhibition of tumor cell growth, and protease activity, virus induced cell killing(CEM-SS cells; genetically modified/infected with: HIV-1), RT of HIV-1 (Galal *et al.*, 2009)
	CH_3	FAS2	60% (Wilson and Hunt, 1983)	-
	Cl	FAS4	(Bernard R. and Elizabeth M., 2001)	-
	Br	FAS5	-	-
	F	FAS6	(Donkor, 2007)	-
	NO_2	FAS7	(Donkor, 2007)	-
Series 2: Synthesis of 2-ethanamine benzimidazole derivatives	**R**	**Compound code**	**Synthesis (Reference)**	**Biological**
	H	FAS8	(Trova *et al.*, (1995))	-
	CH_3	FAS9	(Donkor, 2007)	-
	Cl	FAS11	(Donkor, 2007)	-
	Br	FAS12	-	-
	F	FAS13	(Donkor, 2007, Shibata *et al.*, (1998))	-

Table 2.4 (Continued)

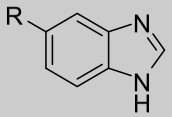
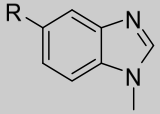
Series 3: Synthesis of 5-substituted benzimidazole derivatives	R	Compound code	Synthesis (Reference)	
	H	FAS15	(Maquestiau *et al.*, 1978)	-
	CH_3	FAS16	94% (Alinezhad *et al.*, 2012)	Inhibition of xanthine oxidase, (Hsieh *et al.*, 2007)
	OCH_3	FAS17	98% (Alinezhad *et al.*, 2012)	Binding to pantothenate synthetase (Hung *et al.*, 2009) Inhibition of Mca-KPLGL-Dpa-AR-NH2 cleavage (Nordstroem *et al.*, 2008)
	Cl	FAS18	89% (Valdez *et al.*, 2002)	No activity against *Escherichia coli* ATCC-25922, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* ATCC-27853, *Staphylococcus aureus* ATCC-25923, *Aspergillus flavus* NCIM No. 524, *Aspergillus fumigatus* NCIM No. 902, *Penicillium marneffei*, *Trichophyton mentagrophytes* (Karuvalam *et al.*, 2012), protein binding affinity (Drinkwater *et al.*, 2010), anthelmintic against *Trichinella spiralis larvae*, antiparasitic against *Entamoeba histolytica*, and *Giardia lamblia* (Valdez *et al.*, 2002), Antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*: inhibition, zone size 6-8 mm (Varma *et al.*, 1980)
	Br	FAS19	(Phillips, 1931)	-
	F	FAS20	(Smith and Steinle, 1953)	inhibition of aminopyrine N-demethylase activity(Murray *et al.*, 1982)
	NO_2	FAS21	89% (Rowlands and Taylor, (1979))	No activity against *Escherichia coli* ATCC-25922, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* ATCC-27853, *Staphylococcus aureus* ATCC-25923, *Aspergillus flavus* NCIM No. 524, *Aspergillus fumigatus* NCIM No. 902, *Penicillium marneffei*, *Trichophyton mentagrophytes* (Karuvalam *et al.*, 2012), inhibition of xanthine oxidase (Hsieh *et al.*, 2007). milk xanthine oxidase (EC 1.2.3.2) (Rudnicka *et al.*, 2004). No activity against *Staphylococcus aureusactive* against *Escherichia coli* ATCC 25922 (18mm), *Escherichia coli* NCTC 8196 (22mm), *Bordetella bronchiseptica* ATCC 4617 (16-19), no activity against *Proteus vulgaris* NCTC 4635 , *Candida albicans* ATCC 10231 , *Micrococcus flavus* NCIB 8166 , *Enterococcus faecium* ATCC 6057, *Pseudomonas aeruginosa* NCTC 6749 , and *Saccharomyces cerevisiae* RW1-4D (Stefanska *et al.*, 1999),
	CN	FAS51	82% (Quattropani *et al.*, (2009))	-
	NO_2	FAS58	(Thomson *et al.*, (2006))	-

Table 2.4 (Continued)

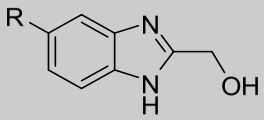
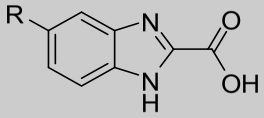
Series 4: Synthesis of 2-methanol-benzimidazole derivatives	R	Compound code	Synthesis (Reference)	Biological
	H	FAS22	40% (Christopher *et al.*, (2002))	Inhibition of cell viability (breast cancer MCF-7 cells of human, colon cancer HT29 cells of human, ovarian cancer SK-OV-3 cells of human; pre-existing medical conditions: cisplatin resistant, prostate cancer DU145 cells of human) (O'Connor *et al.*, 2012). Inhibition of human L-xylulose reductase (EC 1.1.1.10) (Carbone *et al.*, 2005). truncated glyceraldehyde 3-phosphate dehydrogenase-S (tGAPDHS) (O'Brien and Eddy, (2005)). Antiamoebic activity against Entamoeba histolytica HM-1/1MSS (Bharti *et al.*, 2002). Insecticidal (Culex pipiens larvae) (Lahlou *et al.*, 2003)
	CH_3	FAS23	(Siegart and Day, 1957)	-
	OCH_3	FAS24	(Roderick *et al.*, 1972)	-
	Cl	FAS25	63%(Lahlou *et al.*, 2003)	Insecticidal (Culex pipiens larva) at value 31.60 mg/l (Lahlou *et al.*, 2003)
	Br	FAS26	(Khan *et al.*, 1972)	-
	F	FAS27	(Wagner and et al., 1972)	-
	NO_2	FAS28	(Siegart and Day, 1957)	-
	CN	FAS52	66 % (Rangarajan *et al.*, 2000)	-
Series 5: Synthesis of 2-carboxylic acid benzimidazole derivatives	**R**	**Compound code**	**Synthesis (Reference)**	**Biological**
	H	FAS29	64.9% (Xia *et al.*, 2013)	Inhibition of D-amino acid oxidase (DAO) (Barden *et al.*, (2008))
	CH_3	FAS30	48% (Buchstaller *et al.*, (2005))	Inhibition of PPIase activity (Potter *et al.*, 2010)
	OCH_3	FAS31	(PRICE and LANE, (2006))	-
	Cl	FAS32	(Allen *et al.*, (2011))	-
	Br	FAS33	(ISHII *et al.*, (2010))	-
	F	FAS34	(Allen *et al.*, (2011))	-
	NO_2	FAS35	49% (PEAT *et al.*, (2008))	-

Table 2.4 (Continued)

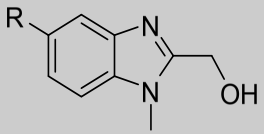
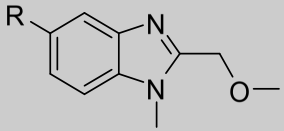
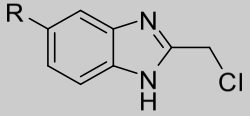
Series 6: Synthesis of *N*-methyl-2-methanol-benzimidazole derivatives	R	Compound code	Synthesis (Reference)	Biological
	H	FAS36	86% (Popov, 1996)	-
	CH_3	FAS37	(Woo *et al.*, 2012)	-
	OCH_3	FAS42	(Woo *et al.*, 2012)	-
	Cl	FAS38	(Woo *et al.*, 2012)	-
	Br	FAS40	-	-
	F	FAS41	-	-
	NO_2	FAS50	(Woo *et al.*, 2012)	-
	Cl	FAS39	-	-
Series 7: Synthesis of 2- chloromethylbenzimidazole derivatives	**R**	**Compound code**	**Synthesis (Reference)**	**Biological**
	H	FAS43	(Hughes and Lions, 1938)	Inhibition growth of *Bacillus proteus* (> 512 µg/ml), *Bacillus subtilis* (> 512 µg/ml) , *Candida albicans* (512 µg/ml), *Candida mycoderma* (> 512 µg/ml), *Eberthella typhosa* (512 µg/ml), *Escherichia coli* DH52(256 µg/ml), *Micrococcus luteus* ATCC 4698(512 µg/ml), *Pseudomonas aeruginosa* (>512 µg/ml), *Shigella dysenteriae* (512 µg/ml), *Staphylococcus aureus* ATCC 25923 (>512 µg/ml), *Staphylococcus aureus* N315; pre-existing medical conditions: methicillin resistant (>512 µg/ml) (Zhang *et al.*, 2012b), no activity against *Escherichia coli* ATTC-25922, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* ATCC-27853, *Staphylococcus aureus* ATTC-25923. Active against *Aspergillus flavus* NCIM No. 524(12.5µg/ml), *Aspergillus flavus* NCIM No. 524 (6-12 mm), *Aspergillus fumigatus* NCIM No. 902 (12.5 µg/ml), *Aspergillus fumigatus* NCIM No. 902(6-12 mm), *Penicillium marneffei*(6-12 mm) (Karuvalam *et al.*, 2012). Anthelmenthic activity against *Pheretima posthuma*.(Vaidehi and

Table 2.4 (Continued)

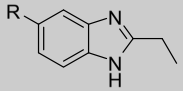
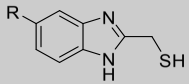
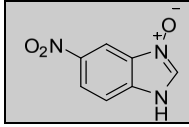
				Deepika, 2012)
	CH_3	FAS 44	(Wilson and Hunt, 1983)	-
	OCH_3	FAS45	(IBRAHIM *et al.*, (2008))	-
	Cl	FAS46	(IBRAHIM *et al.*, (2008))	-
	Br	FAS47	54% (Wilson and Hunt, 1983)	-
	F	FAS48	86% (Matsumura *et al.*, (2008))	-
	NO_2	FAS49	100% (Dirk *et al.*, (2009))	-
	CN	FAS53	55% (Komoriya *et al.*, 2004)	-
Series 8: Synthesis of 2- ethylbenzimidazole derivatives	**R**	**Compound code**	**Synthesis (Reference)**	**Biological**
	H	FAS54	97% (Kaul *et al.*, 2007)	*in vivo* influence on the system of mouse liver microsomal monooxygenases (Prikhod'ko and Astashkin, 1992), spasmolytic activity (isolated rat ileum) (Komissarov *et al.*, 1982), inhibition of aryl hydrocarbon hydroxylase (AHH) and aminopyrine N-demethylase (APDM) activities in hepatic microsomes from phenobarbitone-treated rats (Little and Ryan, 1982), spasmolytic activity on isolated sections of rat intestines: pA2 = 3.68 (Dadali *et al.*, 1981)
	NO_2	FAS55	(Willitzer *et al.*, 1978)	-
Series 9: Synthesis of 2- methanethiolbenzimidazole derivatives	**R**	**Compound code**	**Synthesis (Reference)**	**Biological**
	H	FAS56	87% (Gowda *et al.*, 2011)	-
	NO_2	FAS57	75% (Gowda *et al.*, 2011)	-
Series 10: Synthesis of 1-oxide-benzimidazole derivatives	**R**	**Compound code**	**Synthesis (Reference)**	**Biological**
	NO_2	FAS59	(Elshihawy, 2008)	-

Table 2.4 (Continued)

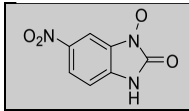
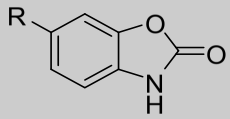
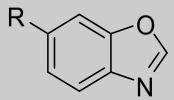
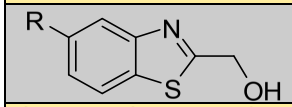
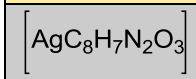
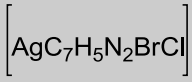
	NO_2	FAS60	(Elshihawy, 2008)	-
Series 11: Synthesis of benzoxazole derivatives	**R**	**Compound code**	**Synthesis (Reference)**	**Biological**
	H	FAS61	(Olesen and Watjen, (1993))	Active against *Escherichia coli* ATCC 25922 (128 µg/ml), *Escherichia coli* ATCC 352189128 µg/ml), *Klebsiella pneumoniae* RSKK 574(64 µg/ml), *Pseudomonas aeruginosa* ATCC 27853(64 µg/ml), isolates of *Escherichia coli*(64 µg/ml), isolates of *Klebsiella pneumonia*(128 µg/ml), isolates of *Pseudomonas aeruginosa*; pre-existing medical conditions: resistant to ceftriaxone(32 µg/ml), inhibition of biofilm formation of *Pseudomonas aeruginosa* PA01 (60%), reduction of enzyme production of *Pseudomonas aeruginosa* PA01(20.4 %) (Miandji *et al.*, 2012)
	NO_2	FAS62	(Rawson *et al.*, (2007))	antimicrobial activities against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, and antifungal activity against *Candida albicans* (Bravo *et al.*, 1997)
	Cl	FAS63	78% (Wertz *et al.*, 2011)	-
Series 12: Synthesis of benzothiozole derivative	**R**	**Compound code**	**Synthesis (Reference)**	**Biological**
	H	FAS65	(Lingam *et al.*, (2011))	-
Series 13: Synthesis of silver complex of benzimidazole derivatives	**R**	**Compound code**	**Synthesis (Reference)**	**Biological**
	H	FAS69	-	-
	Br	FAS70	-	-

Table 2.4 (Continued)

$\left[AgC_7H_5N_3Cl\right]$	NO_2	FAS71	-	-
$\left[AgC_{18}H_{16}N_4O_4Cl\right]$	CH_3	FAS72	-	-
$\left[AgC_{14}H_8N_5O_3Br\right]$	Br	FAS73	-	-
$\left[AgC_{14}H_{10}N_7O_3\right]$	NO_2	FAS74	-	-

3 Synthesis

3.1 Introduction

The Introduction (Section **1.10**) highlighted the need for new chemotherapeutic drugs to treat microbial infections. It is possible to design a wide range of potential microbial inhibitors by replacing the hydrogen at various positions of the benzimidazole ring with different functional groups. However the most accessible derivatives are those with substituents at the 1-, 2- and 5-positions. Retrosynthetic analysis of a 2,5-disubstituted benzimidazole identified 2 fragments which explains why these particular substituted benzimidazoles are easy to prepare (Figure 3.1). The substitution at the 1-position occurs readily after the ring has formed.

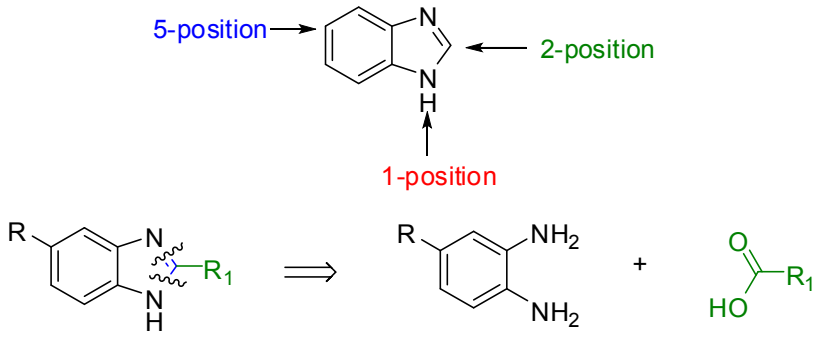


Figure 3.1 Retrosynthetic analysis of benzimidazole

Benzimidazole is synthesised from the reaction of a 1,2-phenylenediamine and a carboxylic acid. It is through this route that the structural diversity arises on both sides of the fused bicyclic ring, i.e., the imidazole and benzene rings (Figure 3.1). The use of different substituted 1,2-phenylenediamines creates variation on the benzene ring whereas substitution on the imidazole side chain can be achieved through the use of various carboxylic acids. It is also possible to create structural diversity in the desired potential inhibitors, through the modification on the side chain of benzimidazole. The reaction between various carboxylic acids with a variety of 1,2-phenylenediamine derivatives would ultimately lead to numerous

benzimidazoles as potential microbial inhibitors. In this project, the design of these inhibitors focused on 5-substituted benzimidazoles (Section **3.3.1**.) followed by synthesis of 2,5-disubstiututed analogues based on 1*H*-benzimidazole-2-yl)methanol derivatives (Section **3.3.2**), (1*H*-benzimidazole-2-yl)alkylamines derivatives (Section **3.4**), (1*H*-benzimidazole-2-yl)-ethyl derivatives (Section **3.5**), (1*H*-benzimidazole-2-yl)-methanthiol derivatives (Section **3.6**), (1*H*-benzothiozole-2-yl)-methanol (Section **3.7**), *N*-alkylbromo-(1*H*-benzimidazole-2-yl)-chloromethyl (Section **3.8**), 1(3)-oxide-benzimidazole derivatives (Section **3.9**), benzoxazole derivatives (Section **3.10**) and silver complexes (Section **3.11**).

3.2 Characterisation of products

3.2.1 ^{1}H NMR spectroscopy

The aromatic region of the ^{1}H NMR spectra of benzimidazoles contained characteristic splitting and coupling constants. In all the benzimidazoles unsubstituted in the fused benzene ring, peaks corresponding to the 4-H, 7-H and a signal for 5-H, 6-H were observed. In benzimidazoles unsubstituted in the 2-position, a singlet is also observed for 2-H. These peaks all appeared in the aromatic region of the spectrum. The 2-H peak will be absent in benzimidazoles with groups in the 2-position and will exhibit signals corresponding to the side chain. Simple splitting for a 5-substituted benzimidazole would be peaks arising from 4-H and two doublets arising from 6-H and 7-H. However, 4-H appears as a doublet, 6-H as a doublet of doublets and 7-H as a doublet. This occurs due to the coupling of 4-H and 6-H protons. Coupling of the aromatic benzimidazole proton occurs with both adjacent protons and protons *meta* to it (Figure 3.2). The ^{3}J values occur from *ortho* coupling while ^{4}J values occur from *meta* coupling.

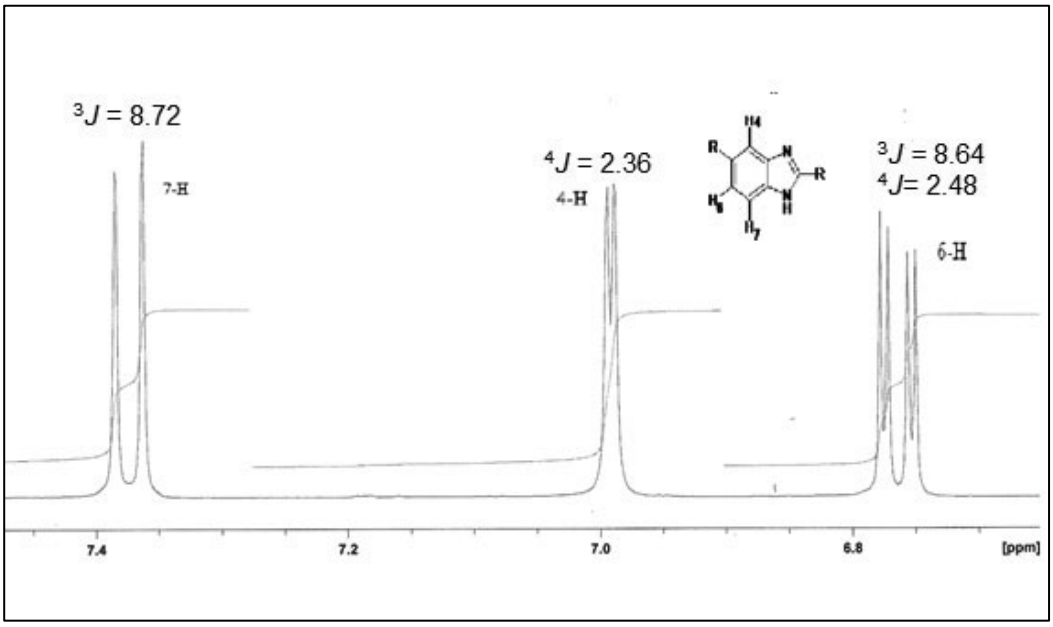


Figure 3.2 An expansion of the aromatic region of the ^{1}H spectrum of FAS24

The addition of a substituent in the 5-position, affects the chemical shifts of the aromatic ring protons. For example, an electron-withdrawing nitro group in the 5-position reduces the electron density around the aromatic protons. As a result these protons are deshielded from the external magnetic field and appear downfield.

Likewise, the addition of a substituent in the 2-position, affects the chemical shifts of these protons. For example, ethyl, methanthiol and methanol groups in the 2-position increase the electron density around the imidazole ring. As a result these protons are shielded from the external magnetic field and appear upfield, unlike the aminoalkyl groups which decrease the electron density around the imidazole ring. As a result these protons are deshielded from the external magnetic field and appear downfield. After the chlorination or oxidation of the 2-methanolbenzimidazole, the chloromethyl and carboxylic acid groups decrease the electron density around the imidazole ring. As a result these protons are deshielded from the external magnetic field and appear downfield.

The addition of a substituent in the 1-position affects the chemical shifts of these protons. For example, an electron withdrawing oxygen group in the 1-position

reduces the electron density around the imidazole ring. As a result these protons are deshielded and appear downfield. Moreover, the addition of methyl group in the 1-position increases the electron density around the imidazole ring and appears upfield.

The NH signal in the imidazole ring was sometimes absent in ^{1}H NMR spectra. The OH peak of alcohols and acids were broad due to rapid intermolecular proton exchange and their presence was confirmed by deuterium exchange experiments.

The aromatic region of the ^{1}H NMR spectra of benzothiazole FAS65, which is unsubstituted in the fused benzene ring, showed two doublets corresponding to the 4-H, 7-H and two doublet of doublets for 5-H and 6-H protons. These peaks all appeared in the aromatic region of the spectrum. FAS65 which has the methanthiol group in the 2-position, exhibits signals for SH and CH_2 in the spectrum.

Simple splitting for a 5-substituted benzoxazole FAS63 would be expected to be a a singlet arising from 4-H and another doublet arising from 7-H and 6-H as doublet of doublets. However, for compound FAS62, 4-H appears as a doublet, 6-H as a doublet of doublets and 7-H as a doublet. This occurs due to the coupling of 4-H and 6-H protons. Coupling of an aromatic benzoxzole proton occurs with both adjacent protons and protons *meta* to it (Figure 3.3).

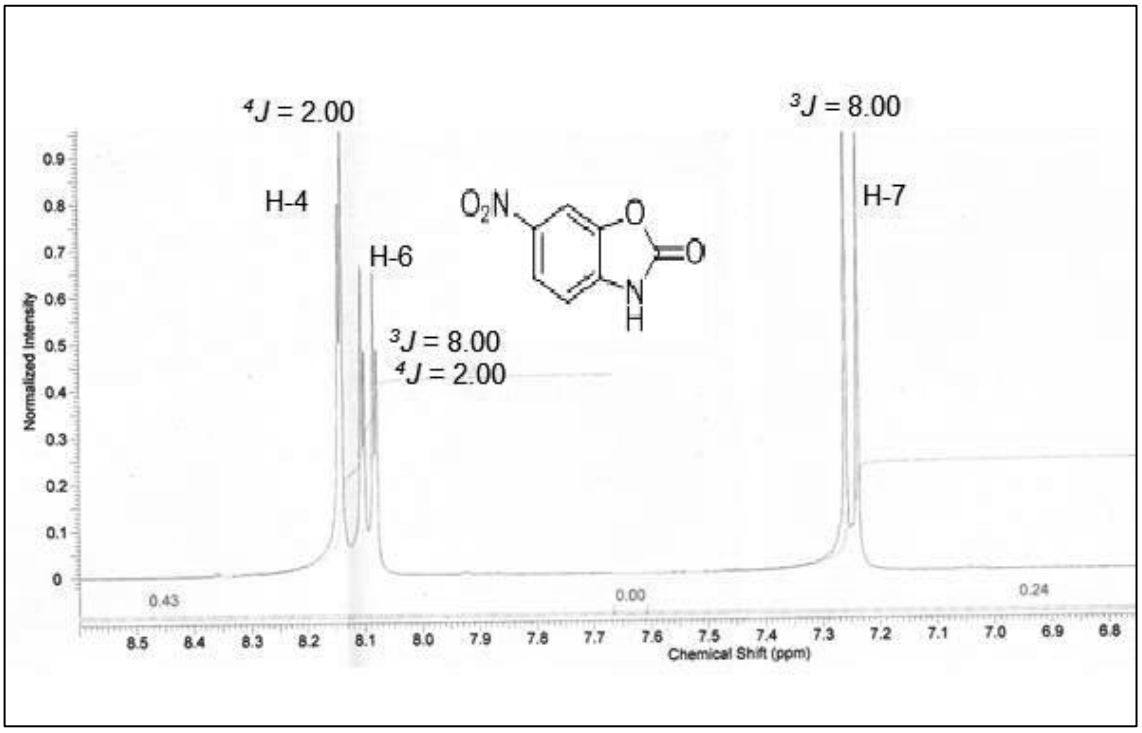


Figure 3.3 An expansion of the aromatic region of the ^{1}H spectrum of FAS62

The addition of a substituent in the 5-position affects the chemical shifts of the aromatic ring protons. For example, an electron-withdrawing nitro group in the 5-position reduces the electron density around the aromatic protons. As a result these protons are deshielded from the external magnetic field and appear downfield.

Compounds FAS61 and FAS62 contains the carbonyl group in the 2-position which also affects the chemical shifts and decreases the electron density around the oxazole ring by the resonance with the electron pair of the nitrogen atom. Therefore, the peaks appear to be shielded. Moreover, the 2-H peak will be absent in these compounds. On the other hand the N*H* appears as a single peak. In the ^{13}C NMR spectrum, the the carbonyl group appears at 154 ppm.

3.2.2 Mass spectra of benzimidazoles

Mass fragmentation patterns of the benzimidazoles synthesised showed similar characteristics and confirmed the structure. The major fragments present in the mass spectra of all the synthesised compounds were similar to the mass spectrum of 5-nitro-2-chloromethylbenzimidazole FAS49, which is shown in Figure 3.10. The chlorine isotope is seen as expected and supports the structure. The ratio of the M and M + 2 is 3: 1. The molecular ion was stable and fragmentation proceeded with loss of chlorine atom and then loss proton. The nitro group was lost then HC_2N as demonstrated in the scheme (Figure 3.4).

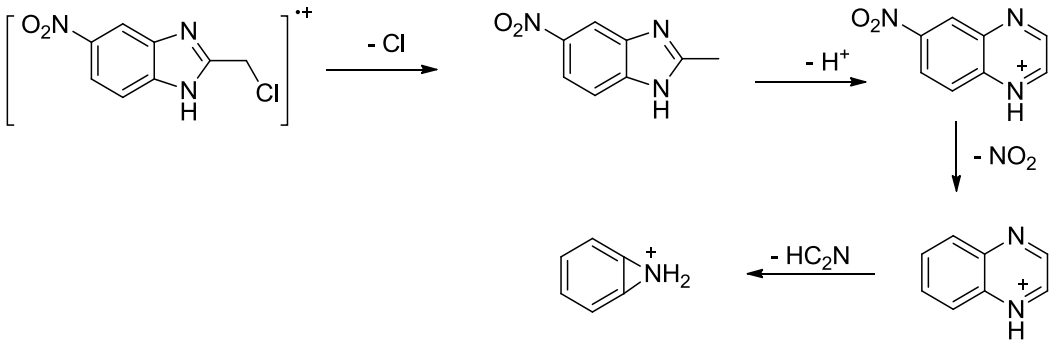


Figure 3.4 Fragmentations pattern of compound FAS49

3.2.3 IR spectra

The infrared spectra of the compounds exhibit the bands at 3250-3590 cm^{-1} and 2900-3040 cm^{-1}, assigned to ν(NH, OH) and ν(C-H, sp^2) of the benzimidazole ring, respectively. The band appearing at about 2830-2790 cm^{-1} is assigned to ν(C-H, sp^3) vibrations. The compounds containing carbonyl groups in their structures, exhibited a vibration in the range of 1624-1748 cm^{-1}, while the peak at 1300-1343 cm^{-1} is also attributed to C-O stretching vibrations.

The silver complexes were fromed with benzimidazole derivatives as a ligand. The bands are shifted towards lower and higher frequencies respectively, depending on

the ligands. The IR spectra of the complexes are similar to those of the corresponding ligands. An upward shift of ν(C=N) in the spectra of complexes FAS69, 70, 72, 73 and FAS 74 as compared to their values for the free ligand, suggests coordination through the imidazole nitrogen of the benzimidazole derivatives of FAS19, 21, 29, and 30.The other bands in the spectrum of each complex are similar to those for the corresponding ligand, except for slight shifts in their positions and changes in their intensities due to coordination

3.3 Synthesis of benzimidazoles

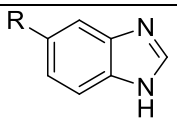
The well-known method for the synthesis of the benzimidazole ring is the Phillips condensation, reported by Phillips in 1928 (Phillips, 1928). The formation of benzimidazoles by this procedure is achieved by the heating of a carboxylic acid with a 1,2-phenylenediamine in the presence of an acid catalyst. This method was selected to synthesise the target compounds, as it is reported to produce benzimidazoles in moderate yields.

3.3.1 5-Substituted benzimidazoles

Some of the target compounds required for this study were benzimidazoles which only varied at the 5-position. These compounds were synthesised from 4-substituted-1,2-phenylenediamine and formic acid to give benzimidazole derivatives FAS15-21, 51. The reaction time for compounds FAS16-21 was between 60-480 min (Table 3.1). On the other hand, the synthesis of benzimidazole itself FAS15, from the reaction of 1,2-phenylenediamine with formic acid, is achieved within 30 min (Phillips, 1928). However, the reaction for 1*H*-benzo[*d*]imidazole-5-cyano FAS51, was much longer than the synthesis of other derivatives. The reaction commences in the absence of an acid catalyst. The proposed mechanism of formation of the benzimidazole ring is described in (Figure

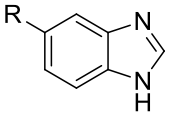
3.5). The yield here is obviously dependent on the nature of the substituent on 4-position of the 1,2-phenylenediamine.

Table 3.1 Reaction times, yields and melting points for 5-substituted benzimidazoles



Compound	R	Reaction time (min)	Yield (%)	mp/ °C	Lit. mp/ °C
FAS15	H	30	74	170-172	170 (Phillips, 1928)
FAS16	CH_3	60	87	112-114	112-114 (Mohammadpoor-Baltork *et al.*, 2007)
FAS17	OCH_3	60	85	116-118	117-120 (Tanaka *et al.*, 1981)
FAS18	Cl	180	100	123-125	124-126 (Liu *et al.*, 2011)
FAS19	Br	180	97.5	130-133	130-131 (Evans and et al., 1978)
FAS20	F	180	90.0	130-132	132 (Fisher and Joullie, 1958)
FAS21	NO_2	480	100	203-205	204-205 (Liu *et al.*, 2011)
FAS51	CN	1440	44	230-232	230-233 (Elshihawy, 2008)

Table 3.2 Assignment of the ^{1}H NMR spectra of 5-substituted benzimidazoles



Compound	δ/ppm					
	R	H^4	H^5	H^6	H^7	H^2
FAS15	H	7.63	7.20	7.20	7.63	8.27
FAS16	CH_3	7.40	-	7.01	7.50	8.18
FA 17	OCH_3	7.07	-	6.91	7.53	8.09
FAS18	Cl	7.66	-	7.20	7.60	8.32
FAS19	Br	7.81	-	7.32	7.56	8.27
FA 20	F	7.25	-	7.75	7.56	9.05
FAS21	NO_2	8.49	-	8.07	7.74	8.54
FAS51	CN	8.16	-	7.58	7.75	8.48

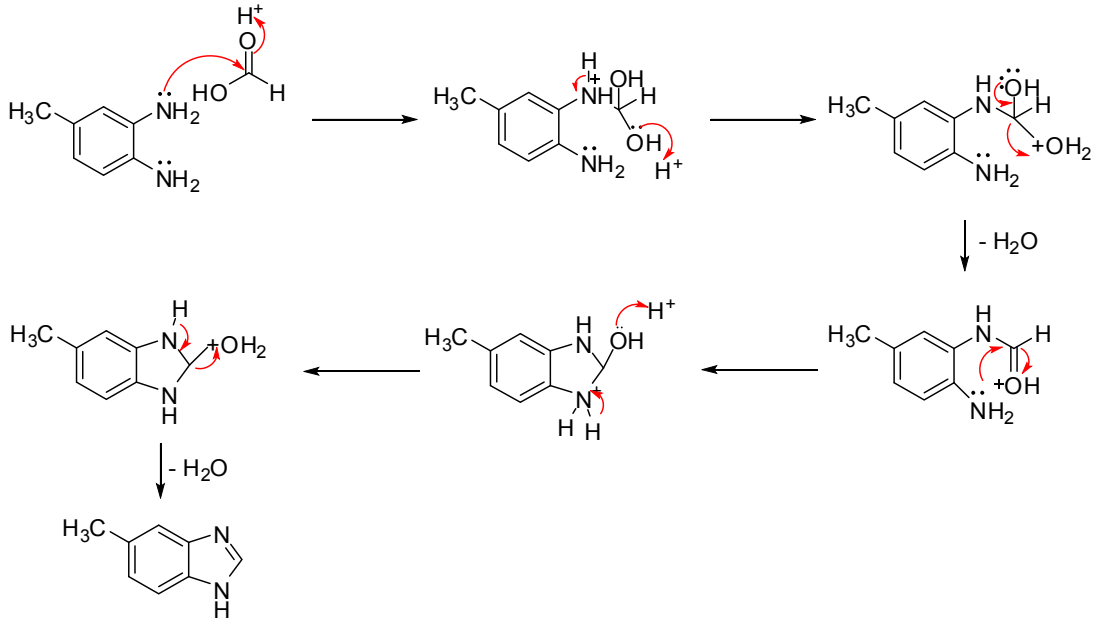
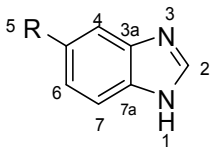


Figure 3.5 Proposed mechanism for the formation of 5-methylbenzimidazole

Table 3.3 Assignment of the ^{13}C NMR spectra of 5-substituted benzimidazoles



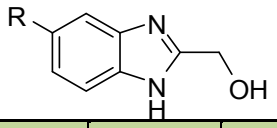
Compound	δ/ppm							
	R	C^4	C^5	C^6	C^7	C^2	C^{3a}	C^{7a}
FAS15	H	121.7	115.3	115.3	121.7	141.9	138.1	138.1
FAS16	CH_3	113.4	123.2	116.1	113.4	141.6	130.9	130.9
FAS17	OCH_3	97.6	156.1	113.1	116.4	141.6	130.9	130.9
FAS18	Cl	116.3	126.2	122.0	115.1	143.4	139.2	136.5
FAS19	Br	116.8	118.2	125.5	113.9	143.3	139.9	136.9
FAS20	F	101.0	158.1	112.5	115.9	142.3	134.2	130.5
FAS21	NO_2	112.7	146.8	117.4	114.6	142.5	138.6	141.8
FAS51	CN	123.4	103.8	125.3	119.8	145.2	142.0	142.0

The structures of these compounds were verified by ^1H NMR spectroscopy. The presence of a singlet peak at around 8 ppm for the hydrogen at the 2-position was indicative of successful benzimidazole ring formation (Table 3.2). The melting points also matched the literature data. The chemical shifts in the ^{13}C NMR spectra were as expected, and the presence of the peak around 141 ppm for the carbon at position 2 confirmed the structure (Table 3.3).

3.3.2 (1*H*-Benzimidazole-2-yl)-methanol derivatives

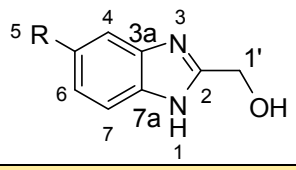
(1*H*-Benzimidazole-2-yl)-methanol derivatives (**FAS 22-28, 52**) were synthesised by the ring closure of 1,2-phenylenediamines with glycolic acid (2-hydroxyacetic acid) (Figure 3.7). Complete reaction was typically achieved within 10 hours even for the most electron deprived 1,2-phenylenediamines. The (1*H*-benzimidazole-2-yl)-methanol derivatives were precipitated from the reaction mixture as brown solids on treatment with ammonia solution. They were purified by initially treating them with decolourising charcoal and followed by recrystallisation. 5-Nitro FAS28, 5-chloro FAS25, 5-bromo FAS26**,** 5-fluoro FAS27, 5-methyl FAS23**,** 5-methoxy FAS24**,** 5-cyano FAS52**,** and (1*H*-benzimidazole-2-yl)methanol FAS22**,** were all successfully synthesised in good yields (Table 3.4). The structures of these compounds were confirmed by ^{1}H NMR (Table 3.5) and ^{13}C NMR spectroscopy (Table 3.6). These compounds all gave characteristic singlets around 4.7 ppm corresponding to the CH_2 group and a broad signal corresponding to the hydroxyl group in the ^{1}H NMR spectra. The CH_2 peak of the starting material appears at a lower chemical shift (4.13 ppm) (Justino *et al.*, 2000).

Table 3.4 Reaction time, yields and melting points of (1*H*-benzimidazole-2-yl)methanol and derivatives



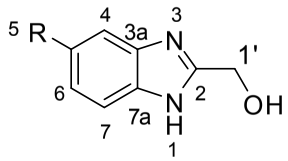
Compound	R	Reaction time (min)	Yield (%)	m.p/ °C	Lit. m.p/ °C
FAS22	H	180	96	172-174	170-172 (Lahlou *et al.*, 2003)
FAS23	CH_3	180	100	194-196	202-203 (Donkor, 2007)
FAS24	OCH_3	480	100	182-184	-
FAS25	Cl	480	95	200- 202	202 (Lahlou *et al.*, 2003)
FAS26	Br	480	85	208- 210	208 (Khan *et al.*, 1972)
FAS27	F	480	70	182-184	182 (Wagner and et al., 1972)
FAS28	NO_2	2880	56	198-200	198-200 (Donkor, 2007)
FAS52	CN	60	57	170-172	172-173 (Rangarajan *et al.*, 2000)

Table 3.5 Assignment of the ^{1}H NMR spectra of the *1H*-Benzimidazole-2-yl)methanol derivatives



Compound	δ/ppm				
	R	H4	H5	H6	H7
FAS22	H	7.50	7.14	7.14	7.50
FAS23	CH3	7.29	-	6.97	7.38
FAS24	OCH3	6.99	-	6.76	7.37
FAS25	Cl	7.54	-	7.16	7.49
FAS26	Br	7.67	-	7.27	7.45
FAS27	F	7.28	-	6.99	7.49
FAS28	NO2	8.39	-	8.08	7.66
FAS52	CN	8.00	-	7.52	7.64

Table 3.6 Assignment of the ^{13}C NMR spectra of the *1H*-Benzimidazole-2-yl)methanolderivatives



Compound	δ/ppm								
	R	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}	C-1'
FAS22	H	111.1	118.1	155.0	121.3	121.3	155.0	118.1	57.5
FAS23	CH_3	154.7	137.9	114.6	122.9	130.4	114.1	136.6	57.5
FAS24	OCH_3	115.7	155.2	110.5	97.27	110.5	154.5	155.2	57.6
FAS25	Cl	114.7	121.5	156.6	156.6	156.6	125.6	121.5	57.6
FAS26	Br	121.4	117.5	124.1	156.2	124.1	113.4	116.1	57.5
FAS27	F	159.3	138.9	100.8	156.7	109.3	115.2	134.9	57.5
FAS28	NO_2	160.3	134.7	111.5	138.9	119.8	115.2	144.1	56.0
FAS52	CN	142.3	136.4	120.2	103.0	128.9	105.6	136.4	57.6

3.3.3 Reactions of (*1H*-benzimidazole-2-yl)methanol

3.3.3.1 Conversion to chloromethyl group

The hydroxyl group of (1*H*-benzimidazole-2-yl)methanol, was converted into a chloride through a reaction with thionyl chloride (Figure 3.7) and the proposed mechanism is shown in (Figure 3.6). The 2-chloromethylbenzimidazoles were required for the biological studies to provide a direct comparison with other functional groups in the 2-position of benzimidazole. 2-(Chloromethyl)-1*H*-benzimidazole derivatives FAS43-49, 53 were formed from the reaction of the (1*H*-benzimidazole-2-yl)methanol FAS22-28, 52 with thionyl chloride according to the method developed by Setoi which involved heating the mixture under reflux (Setoi *et al.*, (2001)) or according to the Dirk method which involved stirring the reaction mixture at ambient temperature (Dirk *et al.*, (2009)). The difference between the two methods is clear in terms of the purity of the synthesised compounds (Table. 3.7).

2-Chloromethylbenzimidazoles are able to undergo reactions with amines and other nucleophiles to produce a new series of compounds. Two methods have been used in the synthesis of these derivatives. In the first method, according to Lohman *et al.* (1999), a solution of the 5-subistituted-2-hydroxymethyl-benzimidazole derivatives in thionyl chloride was heated under reflux until the starting material no longer exists (Lohmann *et al.*, (1999), Dirk *et al.*, (2009)). The second method, developed by Dirk *et al.*, is similar except dichloromethane was required as a solvent and the reaction was conducted at ambient temperature (Lohmann *et al.*, (1999), Dirk *et al.*, (2009)). The procedure was superior in that the were products purer.

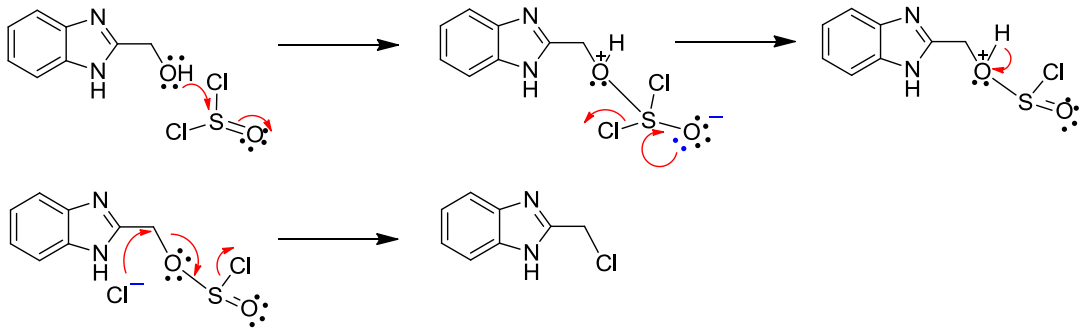
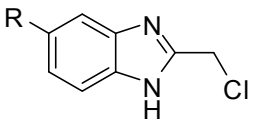


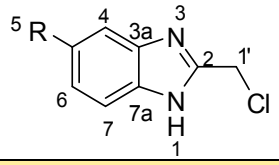
Figure 3.6 Proposed mechanism for the chlorination of hydroxyl group

Table 3.7 Comparison of reaction times, yields and melting points for 5-substituted-2-*H*-benzimidazoles.



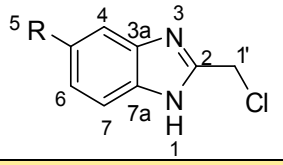
Compound	R	Reaction time (min)	Yield (%)	Method	mp/ °C	Lit. mp/ °C
FAS43	H	180	72	Hiroyuki, 2001	142-144	140-141
FAS44	CH_3	180	61	Hiroyuki, 2001	128-130	127-132
FAS45	OCH3	180-	89	Dirk, 2009	200-202	-
FAS46	Cl	300-	79	Dirk, 2009	210-212	213-214 (King *et al.*, 1949)
FAS47	Br	300-	91	Dirk, 2009	248-250	249-250 (Dandegaonker and Shastri, 1965)
FAS48	F	300-	80	Dirk, 2009	212-214	-
FAS49	NO_2	480-	88	Dirk, 2009	170-172	174 (Labas *et al.*, 2011)
FAS53	CN	560-	57	Dirk, 2009	230-232	230-232 (Elshihawy, 2008)

Table 3.8 Assignment of the ^{1}H NMR spectra of the 2-chloromethylbenzimidazole derivatives



Compound	δ/ppm				
	R	**H4**	**H5**	**H6**	**H7**
FAS43	H	7.81	7.54	7.54	7.81
FAS44	CH3	7.57	-	7.34	7.66
FAS45	OCH_3	7.25	-	7.16	7.72
FAS46	Cl	7.85	-	7.49	7.78
FAS47	Br	7.99	-	7.61	7.73
FAS48	F	7.67	-	7.38	7.82
FAS49	NO_2	7.48	-	8.11	7.74
FAS53	CN	7.99	-	7.64	7.52

Table 3.9 Assignment of the ^{13}C NMR spectra of the 2-chloromethylbenzimidazole derivatives



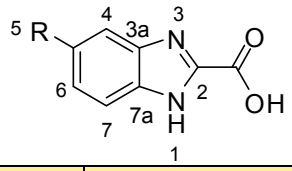
Compound	δ/ppm								
	R	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}	C-1’
FAS43	H	148.2	130.7	114.0	125.8	125.8	114.0	130.7	33.79
FAS44	CH_3	153.9	147.6	127.3	128.7	130.9	113.4	135.8	33.76
FAS45	OCH_3	157.4	146.8	114.5	115.6	124.8	95.47	131.5	35.15
FAS46	Cl	156.1	150.2	116.1	125.9	129.7	114.4	134.1	35.1
FAS47	Br	150.2	134.4	117.3	125.5	129.5	116.1	132.2	35.0
FAS48	F	158.6	150.0	114.2	116.2	129.3	101.1	133.0	35.0
FAS49	NO_2	142.6	142.6	118.1	118.1	118.1	118.1	142.6	37.8
FAS53	CN	142.6	141.0	113.8	110.2	120.2	112.1	131.0	57.0

The structures of these compounds were confirmed by ^{1}H NMR (Table 3.8) and ^{13}C NMR spectroscopy (Table 3.9). These compounds all gave characteristic singlets around 5.15 ppm corresponding to the CH_2 group and the broad signal corresponding to the hydroxyl group of the starting compounds had disappeared. The CH_2 peak of the starting material appears at a lower chemical shift (4.7 ppm) (Karickal *et al.*, 2010).

3.3.3.2 Oxidation of the hydroxyl group

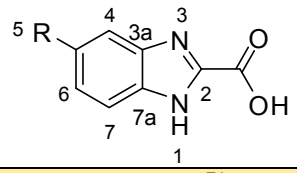
Benzimidazole-2-carboxylic acids are another series of compounds synthesised through the transformation of (1*H*-benzimidazole-2-yl)-methanol. (1*H*-Benzimidazole-2-yl)-methanol derivatives can be readily converted into their corresponding acids through oxidation (Figure 3.7) using potassium permanganate. NMR spectroscopy confirmed the structure. The proton spectra of the benzimidazole-2-carboxylic acids lacked the peak at approximately 4.73 ppm corresponding to the CH_2 group, which confirmed the conversion of the primary alcohol to the carboxylic acid. The position of the aromatic ring proton signals are shifted downfield as the electron-withdrawing nature of the 5-substituent is increased (Table 3.10). Also a broad peak corresponding to the carboxylic acid proton was observed. The carbon spectra gave rise to the carbonyl carbon signal at approximately 160 ppm which is also shifted downfield as the electron-withdrawing nature of the 5-substituent is increased (Table 3.11). All the products were obtained in high yields. The benzimidazole-2-carboxylic acids were not reacted further.

Table 3.10 Assignment of the ^{1}H NMR spectra of the benzimidazole-2-carboxylic acids



Compound		δ/ppm			
	R	H4	H5	H6	H7
FAS29	H	7.67	7.22	7.38	7.63
FAS30	CH_3	7.43	-	7.49	7.55
FAS31	OCH_3	7.50	-	7.50	7.50
FAS32	Cl	7.73	-	7.38	7.70
FAS33	Br	7.84	-	7.47	7.62
FAS34	F	7.53	-	7.32	7.80
FAS35	NO_2	8.52		8.10	7.77

Table 3.11 Assignment of the ^{13}C NMR spectra of the benzimidazole-2-carboxylic acids



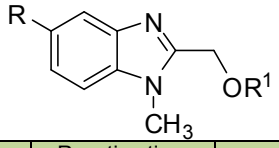
Compound	δ/ppm								
	R	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}	C=O
FAS29	H	144.5	141.8	121.8	136.4	124.2	115.5	137.7	159.1
FAS30	CH_3	143.0	136.4	115.5	131.1	124.5	114.6	134.3	158.6
FAS31	OCH_3	143.5	139.9	115.2	124.2	117.8	96.3	132.7	157.3
FAS32	Cl	143.5	141.3	115.5	130.0	124.5	117.5	139.8	160.0
FAS33	Br	144.3	139.8	116.3	127.3	124.8	119.7	137.8	160.1
FAS34	F	142.7	141.8	104.2	159.6	113.9	112.0	132.3	165.4
FAS35	NO_2	142.6	136.9	111.3	142.3	116.3	113.2	143.6	159.8

3.3.3.3 *N*-Alkylation and *O*-alkylation of (1*H*-benzimidazole-2-yl)methanol

Many studies (Das *et al.*, 2005) showed that the *N*-linked benzimidazole displayed antibacterial activities comparable to *C*-linked benzimidazole. Some of the target compounds required for this study were *N*-alkyl-(1*H*-benzimidazole-2-yl)-methanol FAS36-38, 40-42, 50 and the *N*- and *O*-methyl derivative FAS39**.** The *N*-alkylation and *O*-alkylation of (1*H*-benzimidazole-2-yl)methanol, occurs by the addition of iodomethane in presence of acetone and sodium hydroxide, to give the corresponding *N*-methyl and *O*-methyl-5-substituted (1*H*-benzimidazole-2-yl)methanol (Figure 3.7). Complete reaction was typically achieved within 24 hours. The *N*-methyl and *O*-methyl-(1*H*-benzimidazole-2-yl)-methanol derivatives were purified by recrystallisation. 5-Methyl FAS37**,** 5-chloro FAS38, 5-bromo FAS40, 5-fluoro FAS41, 5-methoxy FAS42, 5-nitro FAS50, *N*-methyl(1*H*-benzimidazole-2-yl)methanol FAS 36, and *N*-methyl(1*H*-benzimidazole-2-yl)methanol FAS39 were all successfully synthesised in good yields (Table 3.12). The structure of these compounds was confirmed by ^1H NMR spectroscopy (Table 3.13). These compounds all gave characteristic singlets around 3.80 ppm, which corresponds to the *N*-CH_3 group. For compounds FAS39 and FAS42 another singlet around 4.03 ppm, which corresponds to the OCH_3 group was also observed in the ^1H NMR

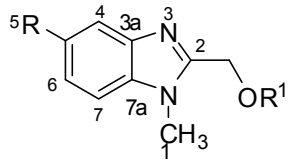
spectrum. The carbon spectra displayed the *N*CH_3 signal at approximately 31 ppm and around 55 ppm for the *O*CH_3 signal (Table 3.14).

Table 3.12 Reaction time, yields and melting points of *N*-methyl (1*H*-benzimidazole-2-yl)-methanol and derivatives



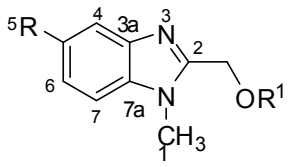
Compound	R	R^1	Reaction time (hrs)	Yield (%)	m.p./ °C	Lit. mp/ °C
FAS36	H	H	24	43.4	128-130	125-130 (Bednyagina and Postovskii, 1960)
FAS37	CH_3	H	24	46.5	122-124	-
FAS38	Cl	H	24	8.8	136-138	-
FAS39	Cl	CH_3	24	46	138-140	-
FAS40	Br	H	24	15.8	158-160	-
FAS41	F	H	24	53	132-134	-
FAS42	OCH_3	H	24	25	182-184	183 (Khristich *et al.*, 1982)
FAS50	NO_2	H	24	19.4	160-162	167-168 (Khristich *et al.*, 1982)

Table 3.13 Assignment of the ^{1}H NMR spectra of the *N*-methyl-(*1H*-benzimidazole-2-yl)methanol derivatives



Compound			δ/ppm			
	R	R^1	H4	H5	H6	H7
FAS36	H	H	7.53	7.25	7.19	7.60
FAS37	CH_3	H	7.31	-	7.39	7.46
FAS38	Cl	H	7.64	-	7.19	7.58
FAS39	Cl	CH_3	7.89	-	7.64	8.01
FAS40	Br	H	7.88	-	7.43	7.58
FAS41	F	H	7.28	-	6.99	7.49
FAS42	OCH_3	H	6.99	-	6.76	7.37
FAS50	NO_2	H	7.47	-	7.16	7.77

Table 3.14 Assignment of the ^{13}C NMR spectra of the *N*-methyl-(1*H*-benzimidazole-2-yl)methanol



Compound	δ/ppm									
	R	R^1	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}	C-1'
FAS36	H	H	111.1	118.1	155.0	121.3	121.3	155.0	118.1	57.5
FAS37	CH_3	H	153.8	142.1	118.6	123.4	131.3	109.6	136.2	55.4
FAS38	Cl	H	155.6	142.5	111.4	126.6	122.2	120.1	136.8	56.3
FAS39	Cl	CH_3	114.4	125.9	152.2	130.1	144.3	131.4	125.9	64.4
FAS40	Br	H	155.4	143.1	111.9	124.7	121.6	120.5	137.3	56.3
FAS41	F	H	114.8	121.5	156.7	156.7	156.7	125.6	121.5	57.6
FAS42	OCH_3	H	159.4	138.9	100.9	156.8	109.3	115.3	134.9	57.6
FAS50	NO_2	H	153.3	142.5	110.6	140.5	117.8	115.1	140.9	56.4

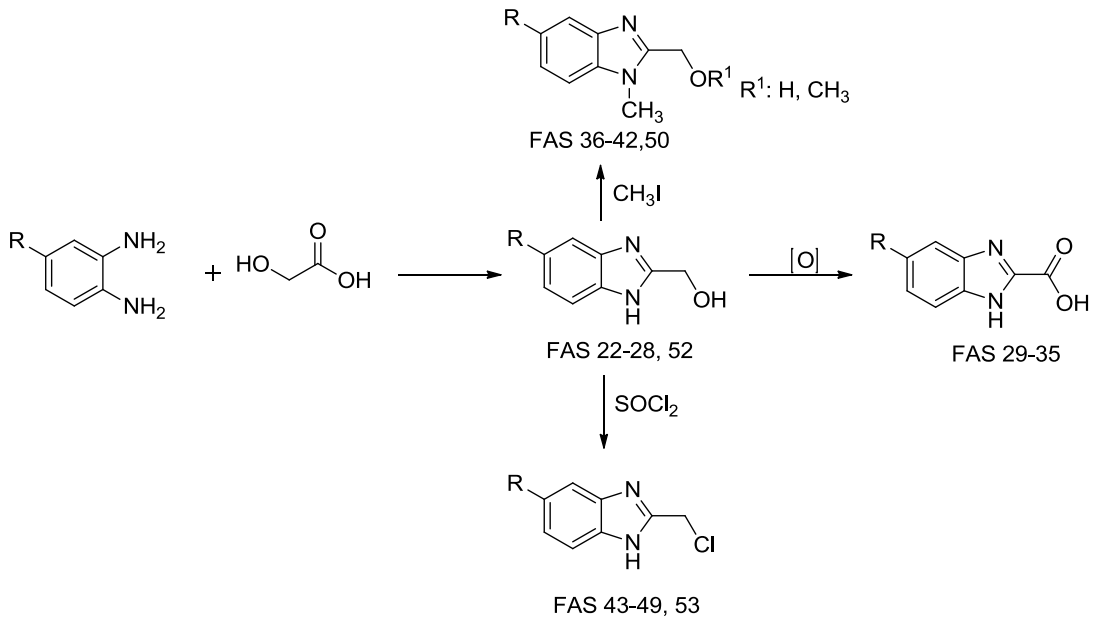


Figure 3.7 Summary of the reactions of (1*H*-benzimidazole-2-yl)-methanol

3.4 1*H*-(benzimidazole-2-yl)alkylamines

3.4.1 Formation of (1*H*-benzimidazole-2-yl)alkylamines

(1*H*-benzimidazole-2-yl)alkylamine FAS1 can be produced from the condensation of 1,2-phenylenediamine and the appropriate amino acid as described for the

formation of simple benzimidazoles. However, amino acids are very reluctant to undergo ring closure with 1,2-phenylenediamines to produce the corresponding benzimidazoles, more so when the substituent in the 5 position of the 1,2-phenylenediamine is an electron withdrawing, e.g. nitro group. This explains the low yield of the 5-nitro derivative FAS7.

3.4.2 Glycine and alanine derived benzimidazoles

The Phillips procedure was used to produce (1*H*-benzimidazole-2-yl)alkylamines and the derivatives prepared is shown in Figure 3.8. The mechanism of the ring closure between a 1,2-phenylenediamine and an amino acid is simiar to the mechanisim illustrated in Figure 3.5. The hydrochloric acid used in the reaction acts both as a catalyst to complete the reaction, and to convert the amines into their hydrochloride salts.

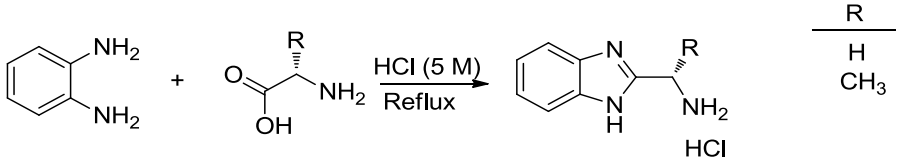
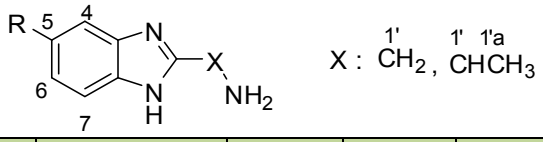


Figure 3.8 Synthesis of (1*H*-benzimidazole-2-yl)alkylamine

The effect of a substituent in the 5-position of 1,2-phenylenediamine affects the rate and ease of the ring closure of the reaction. An electron donating methyl group in the 5-position increases the rate of reaction whereas an electron-withdrawing nitro group makes the ring closure difficult. The reaction of 4-nitro-1,2-phenylenediamine with glycine is an extremely slow reaction. In the synthesis of (*S*)-1-(1*H*-benzimidazole-2-yl)ethanamine dihydrochloride FAS8 from 1,2-phenylenediamine and alanine, completion was achieved after 264 hours reaction

time with high yield (Table 3.15). The progress of the reaction was monitored by TLC and the reaction was continued until all the starting material was consumed.

Table 3.15 Reaction times, yields and melting points of (1*H*-benzimidazole-2-yl)alkylamines



	R	Amino acid	Reaction time (h)	Yield (%)	mp/ °C	Lit. mp/ °C
FAS1	H	Gly	300	89	270-272	268-270 (Cescon and Day, 1962)
FAS2	CH_3	Gly	170	52	208-210	208-210 (Donkor, 2007)
FAS4	Cl	Gly	216	39	252-254	251-252 (Donkor, 2007)
FAS5	Br	Gly	216	22	258-260	-
FAS6	F	Gly	216	16	262-264	258-262 (Donkor, 2007)
FAS7	NO_2	Gly	300	21	254- 256	248-251 (Donkor, 2007)
FAS8	H	*L*-Ala	264	67	138-140	133-138 (Cescon and Day, 1962)
FAS9	CH_3	*L*-Ala	264	68.5	212-214	212-213 (Donkor, 2007)
FAS11	Cl	*L*-Ala	288	41	154-156	152-154 (Donkor, 2007)
FAS12	Br	*L*-Ala	288	19	158-160.	-
FAS13	F	*L*-Ala	288	33	296-298	-

When the crude product was isolated as a solid, it was washed with acetone to remove the unreacted 1,2-phenylenediamine. However, in some cases the crude product was obtained as a bright blue thick sticky tar. In this case, the crude material was treated with triethylamine to convert the product to the free base which changed the bright blue hydrochloride reaction mixture into a bright red solution. This was followed by extraction with ethyl acetate. The organic phase was washed with water, and then filtered.

3.4.3 Spectral characterisation of (1*H*-benzimidazole-2-yl)alkylamines

The (1*H*-benzimidazole-2-yl)alkylamines were fully characterized by spectroscopy (Table 3.16). The cyclization of a 1,2-phenylenediamine with a carboxylic acid resulted in a change in the aromatic region of the NMR spectra. The aromatic benzene type protons of 1,2-phenylenediamine appear between 6-7 ppm whereas those of the imidazole ring appear downfield between 7-9 ppm. This difference in

chemical shift was also useful in determining how much starting material remained in the reaction mixture and gave a rough idea of the rate of reaction. The expansion of the NMR spectra (Figure 3.9) shows the incomplete reaction of 4-nitro-1,2-phenylenediamine and alanine. The comparison of the benzimidazole peaks (downfield) with those of the starting material show the reaction had gone to about 50% completion. The ^{13}C NMR spectra obtained are also consistent with the structure of the desired target compounds (Table 3.17). The absence of a carbonyl signal proved that the amino acid reacted completely. Another very important signal in the carbon spectra was that arising from C-2, and confirmed successful ring closure.

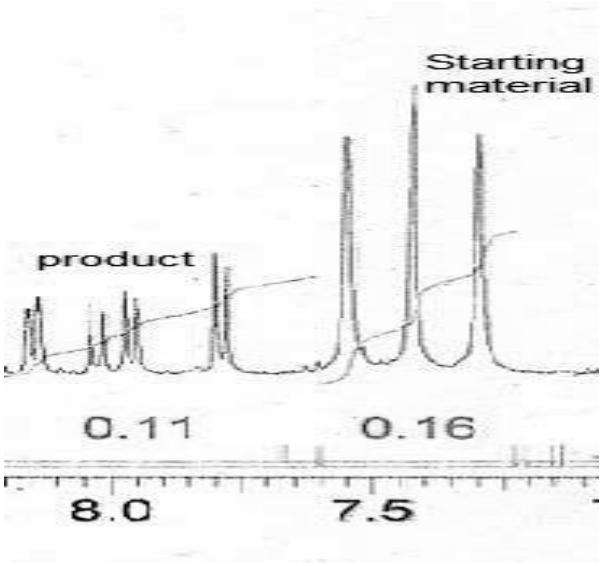
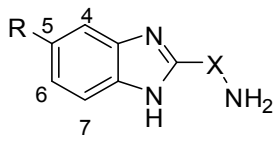


Figure 3.9 Expansion of aromatic region showing the incomplete ring closure of a *(S)*-1-(5-nitro-*1H*-benzimidazole-2-yl)ethanamine (FAS14)

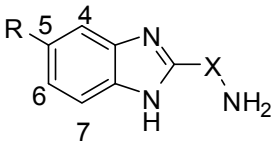
Table 3.16 Assignment of the ^{1}H NMR spectra of (1*H*-benzimidazole-2-yl)alkylamines



X : $\overset{1'}{CH_2}$, $\overset{1'}{CH}\overset{1'a}{CH_3}$

compound	δ/ppm							
	R	H^4	H^5	H^6	H^7	$H^{1'}$	$H^{1'a}$	$H^{5'a}$
FAS1	H	7.85	7.53	7.53	7.85	4.59	-	-
FAS2	CH_3	7.65	-	7.73	7.38	4.58	-	2.48
FAS4	Cl	7.49	-	7.83	8.17	4.54	-	-
FAS5	Br	7.36	-	7.77	8.11	4.38	-	-
FAS6	F	7.82	-	7.34	7.69	4.51	-	-
FAS7	NO_2	8.10	-	8.43	8.43	4.41	-	-
FAS8	H	7.82	7.52	7.52	7.82	4.83	1.83	-
FAS9	CH_3	7.62	-	7.36	7.71	4.97	1.83	2.48
FAS11	Cl	7.83-7.54	-	7.25	7.83-7.54	4.55	1.39	-
FAS12	Br	7.33	-	7.78	8.13	4.73	1.39	-
FAS13	F	7.62	-	7.54	8.18	4.87	1.39	-
FAS14	NO_2	8.48	-	8.10	7.74	3.48	1.77	-

Table 3.17. Assignment of the ^{13}C NMR spectra of selected (1*H*-benzimidazole-2-yl)alkylamines



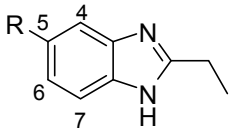
X : $\overset{1'}{CH_2}$, $\overset{1'}{CH}\overset{1'a}{CH_3}$

compound	δ/ppm										
	R	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}	$C^{1'}$	$C^{1'a}$	C^{5a}
FAS1	H	150.16	132.20	114.48	125.47	125.47	114.48	132.20	38.78	-	-
FAS2	CH_3	168.1	146.2	126.4	128.4	133.3	113.8	138.6	34.2	-	20.9
FAS4	Cl	156.6	140.15	128.18	130.2	133.62	130.50	137.51	35.3	-	-
FAS5	Br	168.98	149.58	126.55	106.86	129.41	122.51	149.58	36.3	-	-
FAS6	F	149.10	134.4	106.3	164.30	113.2	115.3	129.70	34.5	-	-
FAS7	NO_2	152.81	137.61	111.64	140.96	118.23	115.14	143.9	36.2		
FAS8	H	146.91	131.67	131.67	125.67	125.67	131.67	131.67	40.4	17.1	-
FAS9	CH_3	149.5	135.6	114.1	130.0	127.2	113.7	132.0	43.2	17.0	21.1
FAS11	Cl	153.03	135.76	115.24	123.61	120.74	117.75	127.12	47.8	16.1	-
FAS13	F	149.9	136.6	114.6	130.1	128.1	114.3	132.2	43.7	17.5	-
FAS14	NO_2	161.7	140.7	115.2	126.1	122.6	116.3	138.9	46.3	15.2	-

3.5 (1*H*-benzimidazole-2-yl)-ethyl derivatives

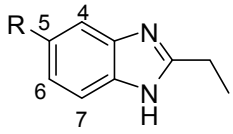
(1*H*-benzimidazole-2-yl)-ethyl derivatives (FAS54, 55) were synthesised by the ring closure of 1,2-phenylenediamines with propionic acid. Complete reaction was typically achieved within 24 hours even for the most electron deprived 1,2-phenylenediamines. The (1*H*-benzimidazole-2-yl)-ethyl derivatives were precipitated from the reaction mixture after treating the reaction mixture with ammonia solution. They were purified by the initial treatment with decolourising charcoal and then recrystallised. 5-Nitro FAS55, and (1*H*-benzimidazole-2-yl)-ethyl FAS54 were all successfully synthesised in good yields (Table 3.18). The structures of these compounds were confirmed by ^{1}H NMR (Table 3.19), and ^{13}C NMR spectrocsopy (Table 3.20).These compounds all gave a characteristic singlet around 2.8 ppm corresponding to the CH_2 group and another signal corresponding to the methyl group at around 1.3 ppm in the ^{1}H NMR spectra.

Table 3.18 Reaction time, yields and melting points of 2- ethylbenzimidazole derivatives



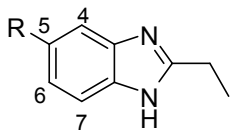
Compound	R	Reaction time (hrs)	Yield (%)	m.p/ °C	Lit. mp/ °C
FAS54	H	24	60	164-166	164-165(Aridoss and Laali, 2011)
FAS55	NO_2	24	40	175-177	178-179 (Willitzer *et al*., 1978)

Table 3.19 Assignment of the ^{1}H NMR spectra of the 2- ethylbenzimidazole derivatives



Compound	δ/ppm				
	R	H4	H5	H6	H7
FAS54	H	7.47	7.11	7.11	7.47
FAS55	NO_2	8.33	-	8.01	7.59

Table 3.20 Assignment of the ^{13}C NMR spectra of the 2- ethylbenzimidazole derivatives

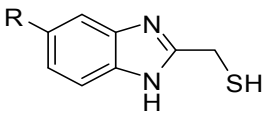


Compound	δ/ppm								
	R	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}	C-1'
FAS54	H	156.1	138.7	114.2	121.0	121.0	114.2	138.7	21.95
FAS55	NO_2	161.1	117.1	107.4	139.3	114.2	111.0	142.0	22.1

3.6 (1*H*-benzimidazole-2-yl)-methanthiol derivatives

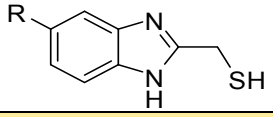
(1*H*-benzimidazole-2-yl)-methanthiol derivatives (FAS56, 57) were synthesised by the ring closure of 1,2-phenylenediamines with 2-mercaptoacetic acid. Complete reaction was typically achieved within 60-70 hours and isolated upon basic workup. (1*H*-benzimidazole-2-yl)-methanthiol FAS56 and 5-nitro FAS57 were successfully synthesised in good yields (Table 3.21) and characterized (Table 3.22 and Table 3.23). The characteristic singlet around 4.2 ppm corresponded to the CH_2 group and the thiol H appeared around 3.6 in the ^1H NMR spectra. Comparing the methanthiol derivatives to the 2-ethyl, amino methyl, chloromethyl and methanol derivatives, it can be see that the peak corresponding to CH_2 at position 1 is dependent on the nature of the attached substituents, R^1. The SH, NH_2, and OH have dual effects; one through the electron donating and the second through the inductive effect of the sulfur, nitrogen and oxygen. The inductive effect could be decreased when the electronegativity is decreased as well. Therefore, the substituents have the following inductive effect; Cl > OH > NH_2 > SH. The methyl group also has the inductive effect. Accordingly the peak is deshielded and appears downfield in the spectra due to the effect of R^1 (CH_3, SH, NH_2, OH, Cl) as shown in Table 3.24. Whereas in the ^{13}C NMR spectra for these compounds, it can be see that the most deshielded CH_2 is when it is attached to OH, while the most shielded one is when it is attached to CH_3, but when CH_2 is attached to SH, NH_2 or Cl, the chemical shifts are similar to each other (Table 3.25).

Table 3.21 Reaction time, yields and melting points of 2- methanethiolbenzimidazole derivatives



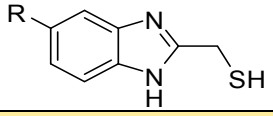
Compound	R	Reaction time (hrs)	Yield (%)	m.p/ °C	Lit. mp/ °C
FAS56	H	60	82	170-172	173 (Gowda *et al.*, 2011)
FAS57	NO_2	70	47.8	195-197	195 (Gowda *et al.*, 2011)

Table 3.22 Assignment of the ^{1}H NMR spectra of the 2- methanethiolbenzimidazole derivatives



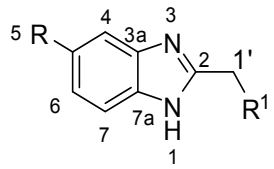
Compound		δ/ppm			
	R	H4	H5	H6	H7
FAS56	H	7.54	7.16	7.16	7.54
FAS57	NO_2	8.41	-	8.09	7.69

Table 3.23 Assignment of the ^{13}C NMR spectra of the2- methanethiolbenzimidazole derivatives



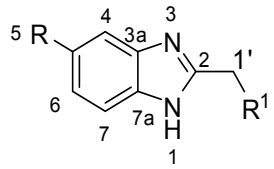
Compound	δ/ppm								
	R	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}	C-1'
FAS56	H	150.6	121.7	114.5	118.5	118.5	114.5	121.7	35.7
FAS57	NO_2	155.3	135.5	112.0	140.0	117.7	114.3	142.5	35.4

Table 3.24 Comparison of ^{1}H NMR spectra of 2- methanethiolbenzimidazole derivatives with their analogues



Compound	δ/ppm		
	R	R^1	H1'
FAS56	H	SH	4.21
FAS57	NO_2	SH	4.27
FAS54	H	CH_3	2.84
FAS55	NO_2	CH_3	2.89
FAS1	H	NH_2	4.59
FAS7	NO_2	NH_2	4.41
FAS43	H	Cl	5.28
FAS49	NO_2	Cl	5.01
FAS22	H	OH	4.71
FAS28	NO_2	OH	4.76

Table 3.25 Comparison of ^{13}C NMR spectra of 2- methanethiolbenzimidazole derivatives with their analogues

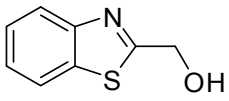


Compound	δ/ppm		
	R	R^1	C1'
FAS56	H	SH	35.72
FAS57	NO_2	SH	35.39
FAS54	H	CH_3	21.95
FAS55	NO_2	CH_3	22.05
FAS1	H	NH_2	38.78
FAS7	NO_2	NH_2	36.16
FAS43	H	Cl	33.79
FAS49	NO_2	Cl	37.82
FAS22	H	OH	57.51
FAS28	NO_2	OH	56.90

3.7 1*H*-benzothiazole-2-yl)-methanol derivatives

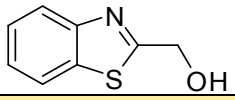
(1*H*-Benzothiazole-2-yl)-methanol (FAS65) was synthesised by the ring closure of 2-aminothiophenol with glycolic acid (2-hydroxyacetic acid) in excellent yield (Table 3.26). The NMR spectra (Table 3.27 and Table 3.28) confirmed the structure. This compound gave a characteristic doublet at 4.88 ppm, corresponding to the CH_2 group, and a triplet signal at 6.26 ppm, corresponding to the hydroxyl group, in the ^{1}H NMR spectrum.

Table 3.26 Reaction time, yields and melting points of benzothiazole derivative



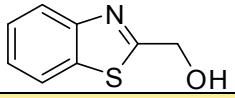
Compound	Yield (%)	m.p/ °C	Lit. mp/ °C
FAS65	91	100-102	100-102 (Asselin *et al.*, 2010)

Table 3.27 Assignment of the ^{1}H NMR spectra of the benzothiazole derivative



Compound	δ/ppm			
	H4	H5	H6	H7
FAS65	8.11	7.44	7.44	7.93

Table 3.28 Assignment of the ^{13}C NMR spectra of the benzothiazole derivative

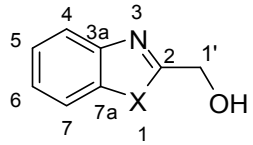


Compound	δ/ppm						
	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}
FAS65	175.5	153.1	122.3	125.9	124.6	122.2	134.2

Due to the electronic effects of the sulfur atom with a large β-effect, which results in the appearance of all the proton peaks in the ^{1}H NMR spectrum to move downfield when compared to the bezimidazole derivative FAS22 (Table 3.29). This is also

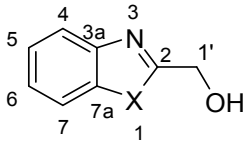
observed in the the ^{13}C NMR spectra, which showed that the signals for FAS65 to be more deshielded than the signals for FAS22 (Table 3.30) (Abraham and Reid, 2002).

Table 3.29 Comparison of ^{1}H NMR spectra of benzothaizole derivatives with benzimidazole derivatives



Compound	δ/ppm							
	X	H1'	OH	H2	H4	H5	H6	H7
FAS22	NH	8.32	5.73	4.71	7.50	7.14	7.14	7.50
FAS65	S	9.72	6.26	4.88	8.11	7.41	7.49	7.93

Table 3.30 Comparison of ^{13}C NMR spectra of benzothaizole derivatives with benzimidazole derivatives



Compound	δ/ppm								
	X	C^{2}	C^{3a}	C^{4}	C^{5}	C^{6}	C^{7}	C^{7a}	C-1'
FAS22	NH	155.0	121.3	111.1	118.1	118.1	111.1	121.3	57.5
FAS65	S	175.5	153.1	122.3	125.9	124.6	122.2	134.2	61.25

3.8 *N*-Bromoalkylation of (1*H*-benzimidazole-2-yl)-chloromethyl

Some of the target compounds required for this study were *N*-alkylbromo-(1*H*-benzimidazole-2-yl)-chloromethyl FAS67-68 derivatives**.** The *N*-bromoalkylation of (1*H*-benzimidazole-2-yl)chloromethyl FAS43 was achieved by addition of the dibromoalkane in the presence of ethanol and potassium hydroxide and stirring for 48 hours at room temperature. The *N*-bromoalkyl-(1*H*-benzimidazole-2-yl)-chloromethyl derivatives were purified by column chromatography. The structure of these compounds was confirmed by ^{1}H NMR spectra (Table 3.32).

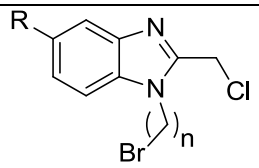
Some new *N*-bromoalkyl substituted bromides of (*E*)-4-azachalcone revealed good antimicrobial activity against some Gram +ve and Gram -ve strains. Moreover, the antimicrobial activity of these compounds relied on the length of the *N*-bromoalkyl chain. When the number of the carbon atoms in the bromoalkyl chain was increased, the antimicrobial activity also increased (Nowakowska *et al.*, 2001). And as the substituent at the *N* position of the heterocyclic rings was shown to be significant from the biological point of view, it was important to investigate this in this project. There are not many studies on the synthesis of *N*-bromoalkyl-2-chloromethylbenzimidazole derivatives in the literature. Several methods were investigated. One of the methods was based on Meyers procedure, and it was successful for the reaction of (1,2- and 1,10) dibromoalkanes with 2-chloromethylbenzimidazole (Meyers *et al.*, 2005). 2-Chloromethylbenzimidazole was combined with an excess of potassium hydroxide and dissolved in ethanol. Then an ethanol solution of dibromoalkane derivatives was added dropwise to the 2-chloromethylbenzimidazole solution, and the combined mixture was heated under reflux for 48 hours. After workup, the crude product was purified by column chromatography. The disadvantage about this method was the number of side products formed which required multiple rounds of column chromatography to be performed in order to isolate the pure compounds. This resulted in poor yields.

The Romashkina method (2011) is another method for the synthesis of these derivatives. This method involved using sodium hydride as a base in DMF. The difficulty of this method was that the use of sodium hydride, a strong base, could react with any acidic proton in the starting material. In addition, DMF has a high boiling point and it was difficult to get rid of this solvent. The high temperature required to remove the DMF may have contributed to the impurities which were very difficult to separate from the desired compound. Therefore, this method had been avoided and other methods were explored. In addition, calcium hydride was also

tried as the base but this resulted in the isolation of starting material only (Romashkina *et al.*, 2011).

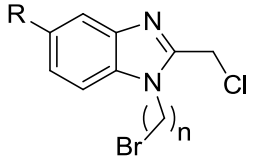
Another method explored the use of ionic liquids. The synthesis of *N*-bromodecyl-2-chloromethylbenzimidazole FAS68 was attempted using a modification of the method by Wang *et al.* (Wang *et al.*, 2012). KOH and 2-chloromethylbenzimidazole FAS43 were added to tri-*n*-butylphosphonium octyl bromide FAS 75 (the ionic liquid) and the mixture was stirred for 5 min. Then, 1,10-dibromodecane was added and the mixture was stirred for another 5 hours and no reaction was observed even when the reaction time was increased.

Table 3.31 Comparison of yields and melting points of *N*-bromoalkyl-2-chloromethylbenzimidazole derivatives



Compound	n	Reaction time (hrs)	Yield (%)	m.p/ °C	Lit. mp/ °C
FAS67	2	48	10	108-110	-
FAS68	10	48	3	154-156	-

Table 3.32 Assignment of the ^{1}H NMR spectra of the *N*-bromoalkyl-2-chloromethylbenzimidazole derivatives



Compound		δ/ppm			
	n	H4	H5	H6	H7
FAS67	2	7.52	7.17	7.17	7.52
FAS68	10	7.65	7.21	7.21	7.65

3.9 5-Nitro-1(3)-oxidebenzimidazoles

Some of the target compounds required for this study were benzimidazoles with oxidation at position 3 to assess the importance of position 1 in the biological studies. These compounds were synthesised from 5-nitro-(1*H*-benzimidazole), and 5-nitro-(1*H*-benzimidazole)-2(3*H*)-one, and hydrogen peroxide to give the desired benzimidazole derivatives FAS59-60. Comparing compound FAS59 to its *N*-methyl analogue FAS58, both compounds revealed the same pattern in the ^{1}H NMR spectra except for the signal for *N*-CH_3 group in FAS58 (Table 3.36). The ^{13}C NMR chemical shifts showed the same pattern too apart for the methyl group signal (Table 3.37).

Table 3.33 Comparison of yields and melting points of 1-oxidebenzimidazole derivatives

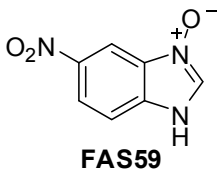
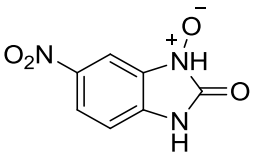
	 FAS59		 FAS60	
Compound	X	Yield (%)	m.p/ °C	Lit. mp/ °C
FAS59	H	48.7	200-202	201-203 (Elshihawy, 2008)
FAS60	O	50	298-300	296-298 (Elshihawy, 2008)

Table 3.34 Assignment of the ^{1}H NMR spectra of the 1-oxidebenzimidazole derivatives

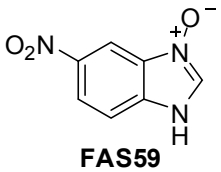
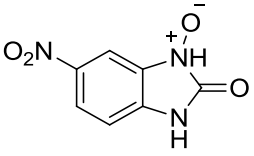
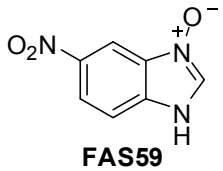
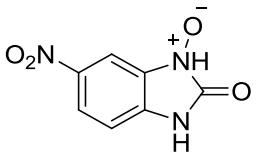
	 FAS59		 FAS60		
Compound		δ/ppm			
	X	H4	H5	H6	H7
FAS59	H	8.51	-	8.12	7.77
FAS60	O	7.69	-	7.92	7.09

Table 3.35 Assignment of the ^{13}C NMR spectra of the 1-oxidebenzimidazole derivatives



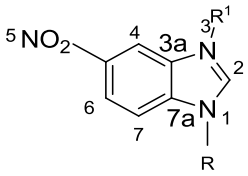
FAS59



FAS60

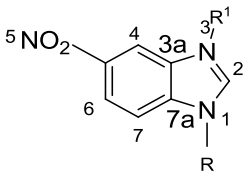
Compound	δ/ppm							
	X	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}
FAS59	H	146.7	138.5	117.5	133.1	115.3	112.3	142.6
FA 60	O	155.4	135.6	117.7	129.6	108.0	103.6	141.6

Table 3.36 Comparison of ^1H NMR spectra of 1-oxidebenzimidazole derivative with its *N*-methyl analogue



Compound	δ/ppm						
	R	R^1	H1	H2	H4	H6	H7
FAS58	CH_3	-	3.93	8.51	8.58	8.16	7.78
FAS59	H	O	-	8.55	8.51	8.12	7.77

Table 3.37 Comparison of ^{13}C NMR spectra of 1-oxidebenzimidazole derivative with its *N*-methyl analogue



Compound	δ/ppm								
	R	R^1	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}
FAS58	31.18	-	146.7	138.5	117.5	133.1	115.3	112.3	142.6
FAS59	-	O	142.68	142.61	117.79	148.73	119.52	110.85	142.39

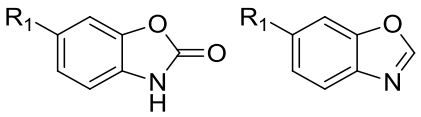
3.10 Benzoxazole derivatives

Benzoxazole derivatives were some of the target compounds required for this study in order to compare their antimicrobial activity to benzimidazole. These compounds

were synthesised from 4-substituted-2-hydroxyaniline and phosgene or urea to give benzoxazole derivatives FAS61-62. On the other hand, the reaction of 5-substituted-2-hydroxyaniline with formic acid gave benzoxazole derivative FAS63. The reaction time for compounds FAS61-63 was between120-180 min. The reaction commences in the absence of an acid catalyst. The proposed mechanism for the formation of the benzoxazole ring is similar to the mechanism described in Figure 3.5.

The ^{1}H NMR spectrum confirmed the structure and the peak of CH at position 2 appeared at downfield spectra for the benzoxazole derivative FAS63 (Table 3.41). In addition, the ^{13}C NMR spectrum also support this by displaying the chemical shift for OCH (position 2) which is more deshielded than the NCH (position 2) peak in the benzimidazole derivative FAS 18 (Table 3.42).

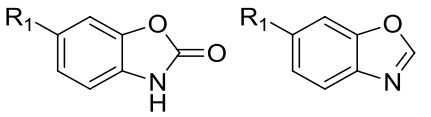
Table 3.38 Comparison of yields and melting points of benzoxazole derivatives



FAS61,62 **FAS63**

Compound	R^1	Time (min)	Yield (%)	m.p/ °C	Lit. mp/ °C
FAS61	Cl	120	75	154-156	155-157(Elshihawy, 2008)
FAS62	NO_2	120	71.6	240-242	238-241 (Abdelaal *et al.*, 1992)
FAS63	Cl	180	90	58-60	61-62 (Wertz *et al.*, 2011)

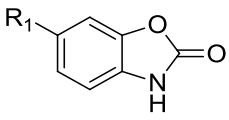
Table 3.39 Assignment of the ^{1}H NMR spectra of the benzoxazole derivatives



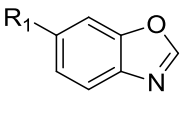
FAS61,62 **FAS63**

Compound	δ/ppm				
	R^1	H4	H5	H6	H7
FAS61	- Cl	7.11	-	6.82	7.24
FAS62	- NO_2	8.14	-	8.09	7.25
FAS63	H Cl	8.31	-	6.95	6.85

Table 3.40 Assignment of the ^{13}C NMR spectra of the benzoxazole derivatives



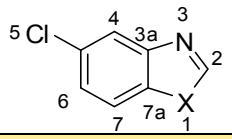
FAS61,62



FAS63

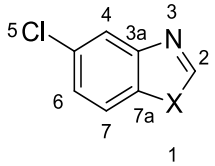
Compound	δ/ppm							
	R^1	C^2	C^{3a}	C^4	C^5	C^6	C^7	C^{7a}
FAS61	Cl	154.3	136.9	116.7	129.1	113.0	106.9	143.4
FAS62	NO_2	154.2	142.0	120.8	136.7	109.3	105.4	142.7
FAS63	Cl	160.3	127.2	122.2	123.3	119.7	115.9	145.4

Table 3.41 Comparison of ^1H NMR spectra of benzoxazole derivative with its analogue



Compound	δ/ppm					
	X	H2	H4	H5	H6	H7
FAS18	NH	8.32	7.66	-	7.20	7.60
FAS63	O	9.72	8.31	-	6.95	6.85

Table 3.42 Comparison of ^{13}C NMR spectra of benzoxazole derivative with its analogue



Compound	δ/ppm							
	X	C^4	C^5	C^6	C^7	C^2	C^{3a}	C^{7a}
FAS18	NH	116.3	126.2	122.0	115.1	143.4	139.2	136.5
FAS63	O	122.2	123.3	119.7	115.9	160.3	127.2	145.4

3.11 Synthesis of silver complex of benzimidazole derivatives

As mentioned in (Section **1.10.1.4**) some silver complexes of benzimidazoles were effective against a series of bacteria and fungi (Özdemir *et al.*, 2010). For this reason, one of the aims of this project was to synthesise silver complexes of benzimidazole to explore the activity of these complexes against the selected microorganisms.

The complexes were synthesised according to Podunavac-Kuzmanovic (Podunavac-Kuzmanovic *et al.*, 2004), by stirring a mixture at 50 ºC of an ethanolic solution of $AgNO_3$ with an ethanolic solution of 5-substituted-1*H*-benzo[*d*]imidazole and 5-substituted-1*H*-benzo[*d*]imidazole-2-carboxylic acid FAS 19,21,29-30 (1 or 2 equivalent) to form 1:1 and 1:2 M:L complexes (Figure 3.10).

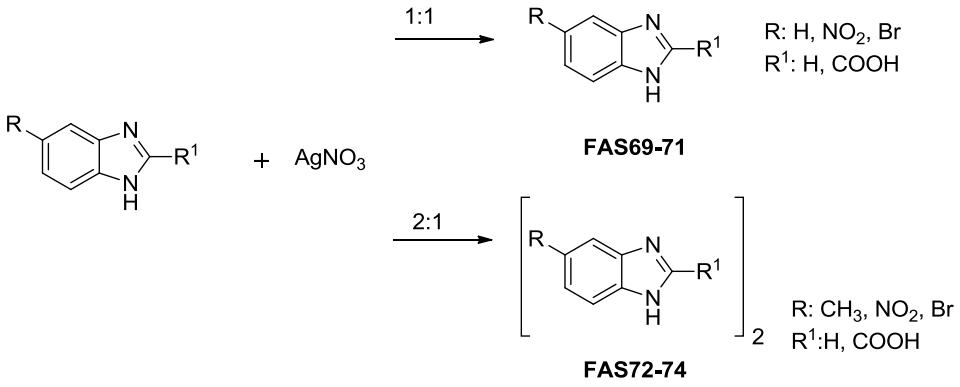
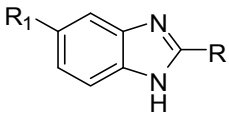


Figure 3.10 Synthesis of silver complexes

The silver complexes were fromed from chelating ring of benzimidazolewith the silver ion. The IR spectrum of complex FAS69 showed strong bands at 1631 and 1520 cm^{-1} that can be assigned to the groups ν(C=O) and ν (C=N), respectively. The spectrum of ligand FAS29 showed the bands of these groups at 1647 and 1516 cm^{-1}. The bands are shifted towards lower and higher frequencies, respectively. On the other hand, the silver salt of the complexes FAS70-74 formed a bond with the nitrogen atom of the benzimidazole ring. The IR spectra of complexes FAS70,71,73, and 74 showed band for the ν (C=N) which are shifted towards lower or higher frequencies depending on the structure of the formed complexes (Table 3.44). The elemental analysis data were sometime in good agreement with the calculated values and confirmed the structure.

Table 3.43 Assignment of the ^{1}H NMR spectra of the silver complexes



= Ligand

Compound	δ/ppm					
	R	R^1	H4	H5	H6	H7
FAS69	H	H	7.70	7.35	7.35	7.70
FAS70	Br	Br	8.01	-	7.50	7.71
FAS71	NO_2	NO_2	8.65	-	8.19	7.84
FAS72	CH_3	CH_3	7.19	-	7.45	7.52
FAS73	Br	Br	8.01	-	7.48	7.70
FAS74	NO_2	NO_2	8.72	-	8.21	7.89

^{1}H NMR spectroscopy also confirmed the structure of the desired complexes and was similar to the spectrum of the ligand but either deshielded or shielded. The proton spectra of the complexes FAS70, 71, 73 and 74 showed the peak at approximately 8.70 ppm corresponding to the proton of CH at position 2 (Table 3.43).

Table 3.44 The IR [cm^{-1}] data for the ligand benzimidazole FAS29, 30, 19, 21 and its complexes FAS69-74.

IR(cm^{-1}) / Compound code	FAS29	FAS30	FAS19	FAS21	FAS69	FAS70	FAS71	FAS72	FAS73	FAS74
C=O	1647	1624	-	-	1631	-	-	1624	-	-
C=N	1516	1516	1582	1527	1520	1584	1520	1522	1585	1591

4 Biological evaluation

4.1 Introduction

The synthesised compounds were evaluated *in vitro* for their antimicrobial activity against a set of reference isolates of bacteria and fungi as listed in sections **2.4.1 and 2.4.2 (Table 2.1 and Table 2.3).** The methods used were namely; the disc (or well) diffusion test as described in sections **2.4.1.5 and 2.4.2.5**, and the Agar dilution method as described in sections **2.4.1.6 and 2.4.2.6.** The biological results for these compounds are reported in this chapter as; the antibacterial study (section **4.2**) which combined the results for disc diffusion tests, and Agar dilution MIC tests (sections **4.2.1 and 4.2.2**). This is followed by the antifungal study (section **4.3**) which includes the results for well diffusion tests, and MIC determination (section **4.3.1 and 4.3.2**).

4.2 Antibacterial study

4.2.1 Screening for antibacterial activity by disc diffusion tests

The strains used in this project included Reference and clinical isolates, and it has to be noted that some strains were resistant to Ciprofloxacin, the comparater drug that is widely used in medicine. Ciprofloxacin is one of the synthetic chemotherapeutic antibiotics from the fluoroquinolone drug class, which have a broad range of therapeutic indications against Gram +ve and -ve bacteria. Thus, ciprofloxacin resistant strains were included to see if any of the synthesised compounds had activity against them, as such strains are increasingly problematic to inhibit

Activity was determined through measuring diameters of the inhibition zones surrounding discs saturated with 10 µg, 100 µg, and 200 µg of test compounds

(Figure 4.1). The formation of the zones represents the dynamic interaction between the diffusing compound and bacteria trying to grow on the surface of the agar. A lack of activity is confirmed when bacterial growth occurs upto the edge of the disc. The results of disc diffusion tests are given in Tables 4.1-8. It should be noted that the filter paper discs themselves have a diameter of 6 mm, but for clarity,growth up to the edge of the disc (i.e. no antibacterial activity) is indicated by a diameter of “0 mm” in the tables. Control discs saturated with just the DMSO solvent showed no inhibition of growth. Moreover, 12 mm diameter (i.e. no growth within 3 mm of the disc) was selected to be the cutoff point for an ‘appreciable’ level of activity, so if the zone of inhibition was wider than this, it was considered as a promising result, and is highlighted on the tables.

Due to a lack of observed activity, results for screening at 10 µg are not shown in most of the tables, except for compounds FAS12 and FAS56.

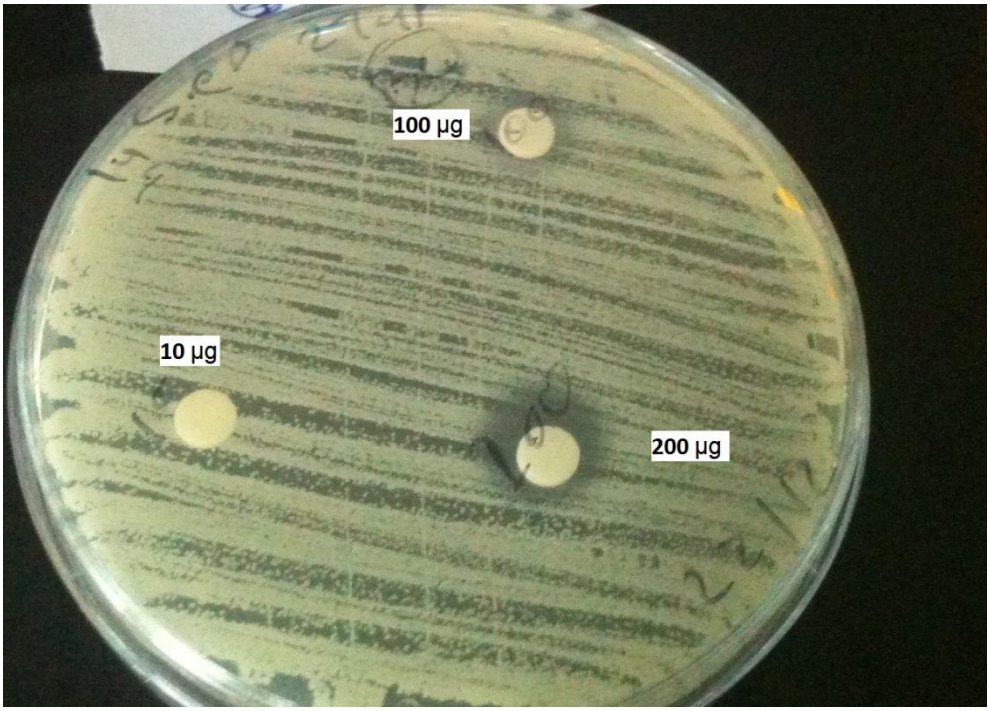


Figure 4.1 Example of disc diffusion test:

Activity of 2- carboxylic acid benzimidazole (FAS30) against *S. epidermidis* NCTC 2749

4.2.1.1 Results of disc diffusion tests of 2- aminoalkylbenzimidazole derivatives (series 1 and 2)

The results for the 11 tested compounds from the series 2- amino-methane benzimidazole derivatives showed positive results for seven compounds from series 1(FAS1, FAS2, FAS6, FAS7), and from series 2 (FAS8, FAS11, and FAS12), and the results for the most active of these are shown in Table 4.1. However, there was no activity with compounds FAS4, FAS5, FAS9, and FAS13. Weak antibacterial activity was exhibited by compound FAS1 against (one Gram +ve) *S. epidermidis* NCTC 2749 and against (one Gram -ve) *Burkholderia cepacia* NCTC 10744 only. There was a very borderline result for compound FAS2 against *S. epidermidis* NCTC 2749, and for compounds FAS6 and FAS7 against just *B. cepacia* NCTC 10744 (at 200 µg only). Only 2 compounds (FAS11 and FAS12) as shown in Table 4.1, were active against multiple strains, giving zone diameters of 12 – 14 mm. Antibacterial activity was exhibited by these compounds against some Gram +ve and Gram -ve strains, including some ciprofloxacin resistant strains. Compound FAS12 exhibited activity against some strains even at 10 µg with zone diameters of 12 – 13 mm.

Table 4.1 Disc diffusion test results for the most active compounds of 2-amino-alkylbenzimidazole derivatives

Bacteria species / strain	Gram	Cip.	FAS1		FAS11		FAS12		
			100 µg	200 µg	100 µg	200 µg	10 µg	100 µg	200 µg
Staphylococcus aureus (Oxford) NCTC 6571	+	27	0	0	0	10	10	11	11
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	33	0	0	7	12	10	12	12
Methicillin-resistant *Staphylococcus aureus* (HG-1)	+	0	0	0	0	10	9	12	11
EMRSA-15 NCTC 13142	+	28	0	0	0	11	11	12	10
EMRSA-16 NCTC 13143	+	0	0	0	0	10	11	12	13
MRSA BIG 0043	+	0	0	0	0	11	11	12	10
MRSA BIG 0044	+	0	0	0	0	9	11	12	12
MRSA BIG 0045	+	0	0	0	0	11	12	13	11
MRSA BIG 0047	+	0	0	0	8	12	11	12	13
MRSA BIG 0050	+	0	0	0	0	10	11	12	12
MRSA BIG 0052	+	0	0	0	0	10	12	12	13
MRSA BIG 0053	+	0	0	0	0	10	13	13	11
Staphylococcus epidermidis NCTC 11047	+	32	0	0	9	10	0	0	11
Staphylococcus epidermidis NCTC 2749	+	35	12	17	0	9	11	14	13
Staphylococcus haemolyticus NCTC 11042	+	34	0	0	8	12	10	12	10
Escherichia coli NCTC 10418	-	33	0	0	0	0	0	0	0
Escherichia coli BIG 0046	-	0	0	0	0	0	0	0	0
Coliform BIG 0048	-	0	0	0	0	0	0	0	0
Coliform BIG 0049	-	0	0	0	0	0	0	0	0
Coliform BIG 0051	-	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	33	0	0	0	0	0	0	0
Pseudomonas aeruginosa (Environmental) BIG 0039	-	34	0	0	0	0	0	0	0
Pseudomonas aeruginosa NCTC 10662	-	32	0	0	0	0	0	0	0
Pseudomonas aeruginosa BIG 0063	-	30	0	7	0	0	0	0	0
Serratia marcescens BIG 0011 = NCTC 1377	-	32	0	0	0	0	0	0	0
Burkholderia cepacia BIG 0009 = NCTC 10744	-	27	0	12	0	11	11	12	10

Cip. =Ciprofloxacin
The results are the average of two independent readings.
Zone diameters ≥ 12 mm are highlighted.

4.2.1.2 Results of Disc diffusion tests of 2-methanolbenzimidazole derivatives (series 4)

The results for the eight compounds from the series of 2-methanolbenzimidazole derivatives showed antibacterial activity with only three compounds; namely FAS24, FAS25 and FAS28 as shown in Table 4.2. Very weak antibacterial activity was exhibited against two Gram -ve strains; *Serratia marcescens* NCTC 1377 and *Burkholderia cepacia* NCTC 10744 with a range of just 9 – 10 mm.

4.2.1.3 Results of Disc diffusion tests of 2-carboxylic acid-benzimidazole derivatives (series 5)

The results for the seven compounds from the series of 2-carboxylic acid-benzimidazole derivatives showed a positive result for only FAS30 as shown in Table 4.3. Antibacterial activity was exhibited by this compound against the Gram +ve *S.epidermidis* NCTC 2749, and against one Gram –ve *B. cepacia* NCTC 10744, but only when 200 µg of compound was on the disc.

4.2.1.4 Results of Disc diffusion tests of *N*-methyl-2-(methanol, methoxy)benzimidazole derivatives (series 6)

The results for the eight scompounds from the series of *N*-methyl-2-(methanol, methoxy)benzimidazole derivatives showed some activity with only two compounds (FAS39 and FAS42) as shown in Table 4.4. Antibacterial activity was exhibited by these compounds against some Gram +ve and Gram –ve strains, especially against the ciprofloxacin resistant strains. FAS42 recorded good activity with a range of zone diameter of 12 – 17 mm.

Table 4.2 Disc diffusion test results of the most active 2-methanolbenzimidazole derivatives

Bacteria species / strain	Gram	Cip.	FAS24		FAS25		FAS28	
			100 µg	200 µg	100 µg	200 µg	100 µg	200 µg
Staphylococcus aureus (Oxford) NCTC 6571	+	27	0	0	0	0	0	0
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	33	0	0	0	0	0	0
Methicillin-resistant *Staphylococcus aureus* (HG-1)	+	0	0	0	0	0	0	0
EMRSA-15 NCTC 13142	+	28	0	0	0	0	0	0
EMRSA-16 NCTC 13143	+	0	0	0	0	0	0	0
MRSA BIG 0043	+	0	0	0	0	0	0	0
MRSA BIG 0044	+	0	0	0	0	0	0	0
MRSA BIG 0045	+	0	0	0	0	0	0	0
MRSA BIG 0047	+	0	0	0	0	0	0	0
MRSA BIG 0050	+	0	0	0	0	0	0	0
MRSA BIG 0052	+	0	0	0	0	0	0	0
MRSA BIG 0053	+	0	0	0	0	0	0	0
Staphylococcus epidermidis NCTC 11047	+	32	0	0	0	0	0	0
Staphylococcus epidermidis NCTC 2749	+	35	0	0	0	10	0	0
Staphylococcus haemolyticus NCTC 11042	+	34	0	0	0	0	0	0
Escherichia coli NCTC 10418	-	33	0	0	0	0	0	0
Escherichia coli BIG 0046	-	0	0	0	0	0	0	0
Coliform BIG 0048	-	0	0	0	0	0	0	0
Coliform BIG 0049	-	0	0	0	0	0	0	0
Coliform BIG 0051	-	0	0	0	0	0	0	0
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	33	0	0	0	0	0	0
Pseudomonas aeruginosa (Environmental) BIG 0039	-	34	0	0	0	0	0	0
Pseudomonas aeruginosa NCTC 10662	-	32	0	0	0	0	0	0
Pseudomonas aeruginosa BIG 0063	-	30	0	0	0	10	0	9
Serratia marcescens BIG 0011 = NCTC 1377	-	32	0	0	0	0	0	0
Burkholderia cepacia BIG 0009 = NCTC 10744	-	27	0	9	0	0	0	0

Cip. = Ciprofloxacin)
The results are the average of two independent readings
Zone diameters ≥ 12 mm are highlighted.

Table 4.3 Disc diffusion test result of the most active 2-carboxylic acid-benzimidazole derivative

Bacteria species / strain	Gram	Cip.	FAS30	
			100 µg	200 µg
Staphylococcus aureus (Oxford) NCTC 6571	+	27	0	0
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	33	0	0
Methicillin-resistant *Staphylococcus aureus* (HG-1)	+	0	0	0
EMRSA-15 NCTC 13142	+	28	0	0
EMRSA-16 NCTC 13143	+	0	0	0
MRSA BIG 0043	+	0	0	0
MRSA BIG 0044	+	0	0	0
MRSA BIG 0045	+	0	0	0
MRSA BIG 0047	+	0	0	0
MRSA BIG 0050	+	0	0	0
MRSA BIG 0052	+	0	0	0
MRSA BIG 0053	+	0	0	0
Staphylococcus epidermidis NCTC 11047	+	32	0	0
Staphylococcus epidermidis NCTC 2749	+	35	10	13
Staphylococcus haemolyticus NCTC 11042	+	34	0	0
Escherichia coli NCTC 10418	-	33	0	0
Escherichia coli BIG 0046	-	0	0	0
Coliform BIG 0048	-	0	0	0
Coliform BIG 0049	-	0	0	0
Coliform BIG 0051	-	0	0	0
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	33	0	0
Pseudomonas aeruginosa (Environmental) BIG 0039	-	34	0	0
Pseudomonas aeruginosa NCTC 10662	-	32	0	0
Pseudomonas aeruginosa BIG 0063	-	30	0	0
Serratia marcescens BIG 0011 = NCTC 1377	-	32	0	0
Burkholderia cepacia BIG 0009 = NCTC 10744	-	27	0	19

Cip. = Ciprofloxacin)
The results are the average of two independent readings
Zone diameters ≥ 12 mm are highlighted.

Table 4.4 Disc diffusion test results of the most active *N*-methyl-2-methanolbenzimidazole derivatives

Bacteria species / strain	Gram	Cip.	FAS39		FAS42	
			100 µg	200 µg	100 µg	200 µg
Staphylococcus aureus (Oxford) NCTC 6571	+	27	0	9	12	14
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	33	0	10	14	17
Methicillin-resistant *Staphylococcus aureus* (HG-1)	+	0	0	8	11	13
EMRSA-15 NCTC 13142	+	28	0	11	13	15
EMRSA-16 NCTC 13143	+	0	0	10	12	13
MRSA BIG 0043	+	0	0	0	10	12
MRSA BIG 0044	+	0	0	8	10	12
MRSA BIG 0045	+	0	0	8	12	13
MRSA BIG 0047	+	0	0	0	12	13
MRSA BIG 0050	+	0	0	8	10	13
MRSA BIG 0052	+	0	0	9	10	12
MRSA BIG 0053	+	0	0	0	10	13
Staphylococcus epidermidis NCTC 11047	+	32	0	0	10	12
Staphylococcus epidermidis NCTC 2749	+	35	0	9	10	13
Staphylococcus haemolyticus NCTC 11042	+	34	0	9	13	15
Escherichia coli NCTC 10418	-	33	0	0	12	14
Escherichia coli BIG 0046	-	0	0	0	8	9
Coliform BIG 0048	-	0	0	0	9	11
Coliform BIG 0049	-	0	0	0	8	8
Coliform BIG 0051	-	0	0	0	9	10
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	33	0	0	0	10
Pseudomonas aeruginosa (Environmental) BIG 0039	-	34	0	0	0	8
Pseudomonas aeruginosa NCTC 10662	-	3.2	0	0	0	8
Pseudomonas aeruginosa BIG 0063	-	30	0	0	0	9
Serratia marcescens BIG 0011 = NCTC 1377	-	32	0	0	11	13
Burkholderia cepacia BIG 0009 = NCTC 10744	-	27	0	8	10	12

Cip. = Ciprofloxacin)
The results are the average of two independent readings
Zone diameters ≥ 12 mm are highlighted.

4.2.1.5 Results of Disc diffusion tests of 2-chloromethylbenzimidazole derivatives (series 7)

The results for the five most active compounds from this series tested, is shown in Table 4.5. Antibacterial activity was exhibited against some Gram +ve and Gram -ve strains. Interestingly, this was observed against the ciprofloxacin resistant strains. These compounds recorded good activity with zones of 12 – 17 mm. FAS47 was the most active compound of this series with zone diameters of 13 – 17 mm (at 200 µg) against some strains.

4.2.1.6 Compounds which did not display any antibacterial activity (series 3 and 13)

None of the compounds tested from the series of 5-sbstituted-benzimidazole, 2-ethanebenzimidazole derivatives, and the silver complexes showed any activity against the selected strains.

Table 4.5 Disc diffusion test results of the most active Chloromethybenzimidazole derivatives

Bacteria species / strain	Gram	Cip.	FAS43		FAS44		FAS46		FAS47		FAS48	
			100 µg	200 µg	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg
Staphylococcus aureus (Oxford) NCTC 6571	+	27	9	13	0	9	8	9	0	9	0	9
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	33	0	7	0	7	0	0	0	0	0	8
Methicillin-resistant *Staphylococcus aureus* (HG-1)	+	0	0	0	0	0	0	0	11	13	0	9
EMRSA-15 NCTC 13142	+	28	0	0	0	0	0	9	1	14	0	10
EMRSA-16 NCTC 13143	+	0	0	0	0	0	9	10	12	15	0	9
MRSA BIG 0043	+	0	0	9	0	10	0	9	9	11	0	8
MRSA BIG 0044	+	0	0	10	0	10	7	9	12	15	0	0
MRSA BIG 0045	+	0	0	0	10	12	0	9	9	11	0	8
MRSA BIG 0047	+	0	0	0	7	11	0	9	9	11	0	9
MRSA BIG 0050	+	0	0	0	8	9	0	9	11	15	0	7
MRSA BIG 0052	+	0	0	0	0	9	0	9	11	14	0	8
MRSA BIG 0053	+	0	0	0	0	10	0	9	11	14	0	10
Staphylococcus epidermidis NCTC 11047	+	32	0	10	10	12	0	0	0	0	0	0
Staphylococcus epidermidis NCTC 2749	+	35	0	9	0	10	11	14	8	9	0	7
Staphylococcus haemolyticus NCTC 11042	+	34	0	11	7	11	0	0	0	0	0	0
Escherichia coli NCTC 10418	-	33	9	13	0	9	7	9	0	9	8	10
Escherichia coli BIG 0046	-	0	8	9	8	9	0	8	0	0	7	9
Coliform BIG 0048	-	0	12	15	7	9	7	8	0	8	9	11
Coliform BIG 0049	-	0	9	11	0	0	0	7	0	8	7	10
Coliform BIG 0051	-	0	10	12	0	9	0	7	0	0	8	10
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	33	0	10	0	0	0	0	0	0	8	9
Pseudomonas aeruginosa (Environmental) BIG 0039	-	34	0	0	0	0	0	0	0	0	7	9
Pseudomonas aeruginosa NCTC 10662	-	32	7	10	0	0	0	0	0	0	0	8
Pseudomonas aeruginosa BIG 0063	-	30	0	10	0	0	0	0	0	0	0	8
Serratia marcescens BIG 0011 = NCTC 1377	-	32	11	15	0	8	7	9	0	8	8	10
Burkholderia cepacia BIG 0009 = NCTC 10744	-	27	0	0	9	12	12	15	15	17	0	8

Cip. = Ciprofloxacin)
The results are the average of two independent readings
Zone diameters ≥ 12 mm are highlighted

4.2.1.7 Results of Disc diffusion tests of 2-methylthiolbenzimidazole derivatives (series 9)

The results of the two compounds tested from the series of 2-methanolbenzimidazole derivatives showed that both had some activity (Table 4.6). Antibacterial activity was exhibited by FAS56 against most of the Gram +ve strains, with zone diameters of 13-16 mm (100 µg discs) to 12-18 mm (200 µg discs). The results for Gram -ve strains were only against the *B. cepacia* isolate (12-15 mm diameter). Compound FAS57 had good activity only against one strain of the *S. epidermidis* with zone of inhibition of 20 mm (100 µg discs), and 25 mm (200 µg discs) respectively.

4.2.1.8 Results of Disc diffusion tests of benzoxazole derivatives (series 11)

The three compounds tested from this series gave very few positive results as shown in Table 4.7. Appreciable antibacterial activity was exhibited against by just two strains, with zone diameter of 16– 20 mm when using the 200 µg discs.

4.2.1.9 Results of Disc diffusion tests of benzothiazole derivative (series 12)

The results of the one compound tested FAS65 showed activity against all but three of the Gram +ve strains as shown in Table 4.8. Also, there was a weak inhibition of the Gram -ve *B. cepacia* NCTC 10744.

Table 4.6 Disc diffusion test results of 2-methylthiolbenzimidazole derivatives

Bacteria species / strain	Gram	Cip.	FAS56			FAS57	
			10 µg	100 µg	200 µg	100 µg	200 µg
Staphylococcus aureus (Oxford) NCTC 6571	+	27	0	10	11	0	0
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	33	13	15	16	0	0
Methicillin-resistant *Staphylococcus aureus* (HG-1)	+	0	0	11	12	0	0
EMRSA-15 NCTC 13142	+	28	0	10	12	0	0
EMRSA-16 NCTC 13143	+	0	0	15	15	0	0
MRSA BIG 0043	+	0	0	10	11	0	0
MRSA BIG 0044	+	0	0	11	12	0	0
MRSA BIG 0045	+	0	0	13	14	7	8
MRSA BIG 0047	+	0	0	11	12	0	0
MRSA BIG 0050	+	0	0	10	11	0	0
MRSA BIG 0052	+	0	0	10	11	0	0
MRSA BIG 0053	+	0	0	11	13	0	0
Staphylococcus epidermidis NCTC 11047	+	32	0	0	0	20	25
Staphylococcus epidermidis NCTC 2749	+	35	0	11	13	8	8
Staphylococcus haemolyticus NCTC 11042	+	34	10	16	18	8	9
Escherichia coli NCTC 10418	-	33	0	0	0	0	0
Escherichia coli BIG 0046	-	0	0	0	0	0	0
Coliform BIG 0048	-	0	0	0	0	0	0
Coliform BIG 0049	-	0	0	0	0	0	0
Coliform BIG 0051	-	0	0	0	0	0	0
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	33	0	0	0	0	0
Pseudomonas aeruginosa (Environmental) BIG 0039	-	34	0	0	0	0	0
Pseudomonas aeruginosa NCTC 10662	-	32	0	0	0	0	0
Pseudomonas aeruginosa BIG 0063	-	30	0	0	0	0	0
Serratia marcescens BIG 0011 = NCTC 1377	-	32	0	0	0	0	0
Burkholderia cepacia BIG 0009 = NCTC 10744	-	27	10	15	15	9	10

Cip. = Ciprofloxacin)
The results are the average of two independent readings.
Zone diameters ≥ 12 mm are highlighted.

Table 4.7 Disc diffusion test results of benzoxazole derivatives

Bacteria species / strain	Gram	Cip.	FAS61			FAS62		FAS63	
			10 µg	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg
Staphylococcus aureus (Oxford) NCTC 6571	+	27	13	15	16	0	0	0	0
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	33	0	9	10	0	0	0	0
Methicillin-resistant *Staphylococcus aureus* (HG-1)	+	0	0	0	0	0	0	0	0
EMRSA-15 NCTC 13142	+	28	0	0	0	9	10	0	0
EMRSA-16 NCTC 13143	+	0	0	0	0	10	11	0	0
MRSA BIG 0043	+	0	0	0	0	0	0	0	0
MRSA BIG 0044	+	0	0	0	0	0	0	0	0
MRSA BIG 0045	+	0	0	0	0	0	0	0	0
MRSA BIG 0047	+	0	0	10	11	0	0	0	0
MRSA BIG 0050	+	0	0	0	0	0	9	0	0
MRSA BIG 0052	+	0	0	0	0	9	10	0	0
MRSA BIG 0053	+	0	0	0	0	0	11	0	0
Staphylococcus epidermidis NCTC 11047	+	32	0	0	0	0	0	0	0
Staphylococcus epidermidis NCTC 2749	+	35	0	0	0	0	0	0	0
Staphylococcus haemolyticus NCTC 11042	+	34	0	0	0	0	0	0	0
Escherichia coli NCTC 10418	-	33	0	0	0	0	0	0	0
Escherichia coli BIG 0046	-	0	0	0	0	0	0	9	9
Coliform BIG 0048	-	0	0	0	0	0	0	0	8
Coliform BIG 0049	-	0	0	0	0	0	0	9	10
Coliform BIG 0051	-	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	33	0	0	0	0	0	0	0
Pseudomonas aeruginosa (Environmental) BIG 0039	-	34	0	0	0	0	0	0	0
Pseudomonas aeruginosa NCTC 10662	-	32	0	0	0	0	0	0	0
Pseudomonas aeruginosa BIG 0063	-	30	0	0	0	0	0	0	0
Serratia marcescens BIG 0011 = NCTC 1377	-	32	0	0	0	0	0	19	20
Burkholderia cepacia BIG 0009 = NCTC 10744	-	27	0	0	0	0	0	0	0

Cip. = Ciprofloxacin)
The results are the average of two independent readings.
Zone diameters ≥ 12 mm are highlighted.

Table 4.8 Disc diffusion tests of a benzothaizole derivative

Bacteria species / strain	Gram	Ciprofloxacin	FAS65	
			100 µg	200 µg
Staphylococcus aureus (Oxford) NCTC 6571	+	27	0	0
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	33	12	13
Methicillin-resistant *Staphylococcus aureus* (HG-1)	+	0	11	12
EMRSA-15 NCTC 13142	+	28	11	12
EMRSA-16 NCTC 13143	+	0	11	13
MRSA BIG 0043	+	0	12	13
MRSA BIG 0044	+	0	12	13
MRSA BIG 0045	+	0	11	13
MRSA BIG 0047	+	0	11	13
MRSA BIG 0050	+	0	10	13
MRSA BIG 0052	+	0	10	11
MRSA BIG 0053	+	0	10	13
Staphylococcus epidermidis NCTC 11047	+	32	12	14
Staphylococcus epidermidis NCTC 2749	+	35	0	0
Staphylococcus haemolyticus NCTC 11042	+	34	14	15
Escherichia coli NCTC 10418	-	33	0	0
Escherichia coli BIG 0046	-	0	0	0
Coliform BIG 0048	-	0	0	0
Coliform BIG 0049	-	0	0	0
Coliform BIG 0051	-	0	0	0
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	33	0	0
Pseudomonas aeruginosa (Environmental) BIG 0039	-	34	0	0
Pseudomonas aeruginosa NCTC 10662	-	32	0	0
Pseudomonas aeruginosa BIG 0063	-	30	0	0
Serratia marcescens BIG 0011 = NCTC 1377	-	32	0	0
Burkholderia cepacia BIG 0009 = NCTC 10744	-	27	10	10

Cip. = Ciprofloxacin)
The results are the average of two independent readings.
Zone diameters ≥ 12 mm are highlighted.

4.2.1.10 Conclusion

A cut off point of ≥ 12 mm zone diameter was used to define 'activity'. The Disc diffusion tests of the 67 compounds revealed that 14 had some degree of antibacterial activity; 12 compounds were active against some of two *S. aureus* species, and the eight against MRSA species. Five compounds were active against the two strains of EMRSA. Nine compounds were active against the strains of *S. epidermidis*. Five compounds were active against the single strain of *S. haemolyticus*. Regarding the Gram -ve bacteria, none of the compounds tested were active against any of the five strains of *E. coli*, or the four strains of *P. aeruginosa*. Three compounds were active against the single strain of *Serratia marcescens*. Eight compounds had some activity against the single strain of *B. cepacia* used in the screening panel.

The most active compounds overall were selected on the basis of a broad spectrum of activity, and / or wide zones of inhibition, or novel chemical structure. These compounds are summarised in Table 4.9, and were investigated further in minimum inhibitory concentration (MIC) assays to quantify their activity against the reference isolates.

4.2.2 Results of agar dilution MIC tests

The MIC of the synthesised compounds was determined by the agar dilution technique as outlined in section **2.4.1.6**. The agar dilution range used was: 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 µg/ml. Bacteria were seeded onto agar containing different conecntrations of the compounds using a multipoint inoculator (using the 37 pin head-each pin delivers 1 µl of the test organism onto the agar). The plates were then incubated overnight at 37˚C and the MIC was defined as the lowest concentration of the compounds which inhibited bacterial growth completely,

as indicated by the absence of any visible colony formation on the agar surface (Figure 4.2). Moreover, 256 µg/ml was selected to be the cutoff point for an appreciable level of activity, so if the MIC was lower than this, it should be considered as a promising result and is highlighted in the tables.

Table 4.9 The most active compounds which were taken forward to determination of MICs

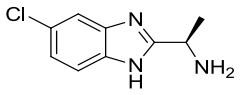
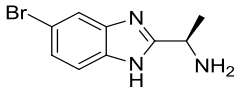
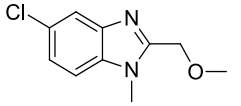
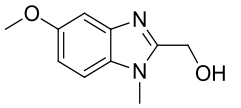
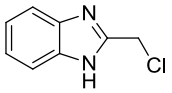
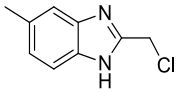
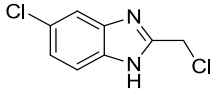
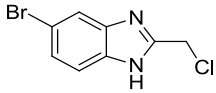
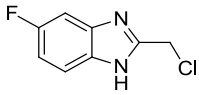
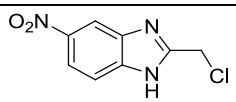
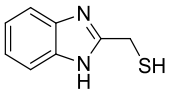
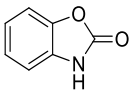
Compound	Chemical structure	Note
FAS11		➢ Active against multiple strains with zone diameter of 12 – 14 mm. ➢ Antibacterial activity was exhibited against some Gram +ve and Gram -ve strains, and included some ciprofloxacin resistant strains
FAS12		➢ Active against multiple strains with zone diameter of 12 – 14 mm. ➢ Antibacterial activity was exhibited against some Gram +ve and Gram -ve strains, and included some ciprofloxacin resistant strains ➢ Exhibited activity against some strains even at 10 µg with zone diameter of 12 – 13 mm
FAS39		➢ Slightly active against multiple strains
FAS42		➢ Antibacterial activity was exhibited against some Gram +ve and Gram -ve strains, and included some ciprofloxacin resistant strains ➢ Recorded good activity with a range of zone diameter of 12 – 17 mm
FAS43		➢ Antibacterial activity was exhibited against some Gram -ve strains with zone diameter of 12-15 mm ➢ Selectively active against one strain of the *S.aureus* (Oxford) with zone of diameter of 13 mm at 200 µg
FAS44		➢ Some activity against some Gram +ve and Gram –ve strains
FAS46		➢ Selectively active against one strani of *S. epidermidis* with zone diameter of 14 at 200 µg ➢ Selectively active against the *B. cepacia isolate* with zone diameter of 12, and 15 mm at 100 µg and 200 µg respectively
FAS47		➢ The most active member of this series against some strains with zone diameters of 13 – 17 mm at 200 µg.
FAS48		➢ Slightly active against multiple strains
FAS49		➢ To study the variation in the antibacterial activity of the compounds of this series
FAS56		➢ Antibacterial activity against most of the Gram +ve strains, with zone diameter of 13-16 mm (100 µg discs) to 12-18 mm (200 µg discs). ➢ The results against Gram -ve strains were only against the *B. cepacia* isolate with a range of 12-15 mm diameter.
FAS61		➢ Recorded activity against *S. aureus* (Oxford) with zone of diameter of 13, 15, and 16 mm at 10, 100, and 200 µg respectively

Table 4.9 (continued)

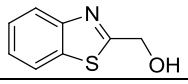
Compound	Chemical structure	Note
FAS65		➢ Recorded some activity against some Gram +ve strains with zone of diameter of 12-14mm at 100 µg, and 12-15 mm at 200 µg respectively

Figure 4.2 MIC screening by agar dilution of FAS47 at 64µg/ml VS the growth control

4.2.2.1 MIC results of the 2-amino-alkylbenzimidazole derivatives (series 1 and 2)

The results of the two tested compounds from the series 2-amino-ethanebenzimidazole derivatives FAS11 and FAS12, showed promising results especially against Methicillin-resistant *Staphylococcus aureus* (HG-1), and MRSA BIG 0050; with an MIC of 32 µg/ml, which is equivalent to the MIC of the ciprofloxacin (control drug) against this strains. The antibacterial activity of these compounds against the rest of the selected strains was in the range of 32 to > 512 µg/ml. The results are summarised in Table 4.10.

4.2.2.2 MIC results of the *N*-methyl-2-methanolbenzimidazole derivatives (series 6)

The results of the most active compounds from this series (FAS39 and FAS42), where the methoxy is present either at the position 2 or 5, is not promising. The

results are shown in Table 4.11. The antibacterial activity of these compounds against all selected strains was on the range of 128 to > 512 µg/ml.

4.2.2.3 MIC results of the 2-cholromethylbenzimidazole derivatives (series 7)

Six compounds from this series were the most active in the initial screen and therefore were selected to determine their MICs. The results for FAS43, FAS44, FAS46, FAS47, FAS48, and FAS49, are presented in Table 4.12. The antibacterial activity of these compounds against all selected strains was on the range of to 32 > 512µg/ml. Only FAS46 and FAS47 were capable of inhibiting some strains at a concentration below 256 µg/ml.

4.2.2.4 MIC results of the 2-methylthiolbenzimidazole derivatives (series 9)

Only one compound FAS56 was active against most of the Gram +ve strains, in the range 32 - 64 µg/ml. The results against all Gram -ve strains showed MICs ≥ 512 µg/ml, except when it was against the *B. cepacia*; where the MIC was 64 µg/ml (Table 4.13).

4.2.2.5 MIC results of the benzoxazole derivatives (series 11)

Only one compound FAS61 was active against most of the Gram +ve strains, with MIC≥ 64 µg/ml. The results against Gram -ve strains were ≥ 256 µg/ml (Table 4.14).

4.2.2.6 MIC results of the benzothaizole derivative (series 12)

Compound FAS65 showed no activity against the refernce selected for this study with MICs ≥ 512, the results are presented in Table 4.15.

Table 4.10 MIC tests of the most active 2-alkylaminobenzimidazole derivatives

Bacteria species / strain	Gram	ciprofloxacin	FAS11	FAS12
Staphylococcus aureus (Oxford) NCTC 6571	+	≤0.5	32	32
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	>32	256	256
Methicillin-resistant Staphylococcus aureus (HG-1)	+	32	32	32
EMRSA-15 NCTC 13142	+	≤0.5	256	256
EMRSA-16 NCTC 13143	+	32	256	256
MRSA BIG 0043	+	>32	256	256
MRSA BIG 0044	+	>32	>512	>512
MRSA BIG 0045	+	8	256	256
MRSA BIG 0047	+	8	256	256
MRSA BIG 0050	+	32	32	32
MRSA BIG 0052	+	≤0.5	256	256
MRSA BIG 0053	+	>32	256	256
Staphylococcus epidermidis NCTC 11047	+	≤0.5	256	256
Staphylococcus epidermidis NCTC 2749	+	≤0.5	256	256
Staphylococcus haemolyticus NCTC 11042	+	8	256	265
Escherichia coli NCTC 10418	-	≤0.5	>512	>512
Escherichia coli BIG 0046	-	32	>512	>512
Coliform BIG 0048	-	8	>512	>512
Coliform BIG 0049	-	8	>512	>512
Coliform BIG 0051	-	32	>512	>512
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	≤0.5	>512	>512
Pseudomonas aeruginosa (Environmental) BIG 0039	-	≤0.5	>512	>512
Pseudomonas aeruginosa NCTC 10662	-	≤0.5	>512	>512
Pseudomonas aeruginosa BIG 0063	-	≤0.5	>512	>512
Serratia marcescens BIG 0011 = NCTC 1377	-	≤0.5	>512	>512
Burkholderia cepacia BIG 0009 = NCTC 10744	-	≤0.5	32	32

MICs < 256 µg/ml are highlighted.

Table 4.11 MIC tests of the most active *N*-methyl- 2-methanolbenzimidazole derivatives

Bacteria species / strain	Gram	Cip.	FAS39	FAS42
Staphylococcus aureus (Oxford) NCTC 6571	+	≤0.5	512	256
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	>32	512	256
Methicillin-resistant Staphylococcus aureus (HG-1)	+	32	128	256
EMRSA-15 NCTC 13142	+	≤0.5	128	256
EMRSA-16 NCTC 13143	+	32	512	256
MRSA BIG 0043	+	>32	512	256
MRSA BIG 0044	+	>32	>512	>512
MRSA BIG 0045	+	8	512	256
MRSA BIG 0047	+	8	512	256
MRSA BIG 0050	+	32	128	256
MRSA BIG 0052	+	≤0.5	512	256
MRSA BIG 0053	+	>32	512	256
Staphylococcus epidermidis NCTC 11047	+	≤0.5	>512	512
Staphylococcus epidermidis NCTC 2749	+	≤0.5	>512	512
Staphylococcus haemolyticus NCTC 11042	+	8	>512	512
Escherichia coli NCTC 10418	-	≤0.5	>512	>512
Escherichia coli BIG 0046	-	32	>512	>512
Coliform BIG 0048	-	8	>512	>512
Coliform BIG 0049	-	8	>512	>512
Coliform BIG 0051	-	32	>512	>512
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	≤0.5	>512	>512
Pseudomonas aeruginosa (Environmental) BIG 0039	-	≤0.5	>512	>512
Pseudomonas aeruginosa NCTC 10662	-	≤0.5	>512	>512
Pseudomonas aeruginosa BIG 0063	-	≤0.5	>512	>512
Serratia marcescens BIG 0011 = NCTC 1377	-	≤0.5	>512	>512
Burkholderia cepacia BIG 0009 = NCTC 10744	-	≤0.5	512	256

Cip. = (Ciprofloxacin)
MICs < 256 µg/ml are highlighted

Table 4.12 MIC tests of the 2-chloromethylbenzimidazole derivatives

Bacteria species / strain	Gram	Cip.	FAS43	FAS44	FAS46	FAS47	FAS48	FAS49
Staphylococcus aureus (Oxford) NCTC 6571	+	≤0.5	512	256	128	64	512	>512
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	>32	512	256	128	64	512	>512
Methicillin-resistant Staphylococcus aureus (HG-1)	+	32	512	256	265	64	512	>512
EMRSA-15 NCTC 13142	+	≤0.5	512	256	128	64	512	>512
EMRSA-16 NCTC 13143	+	32	512	512	256	64	512	>512
MRSA BIG 0043	+	>32	512	256	256	64	512	>512
MRSA BIG 0044	+	>32	512	>512	256	64	512	>512
MRSA BIG 0045	+	8	>512	512	256	>512	512	>512
MRSA BIG 0047	+	8	512	512	256	64	512	>512
MRSA BIG 0050	+	32	512	512	256	64	512	>512
MRSA BIG 0052	+	≤0.5	512	256	256	64	512	>512
MRSA BIG 0053	+	>32	512	512	256	64	512	>512
Staphylococcus epidermidis NCTC 11047	+	≤0.5	512	>512	256	32	>512	>512
Staphylococcus epidermidis NCTC 2749	+	≤0.5	512	>512	256	64	512	>512
Staphylococcus haemolyticus NCTC 11042	+	8	512	>512	256	32	512	>512
Escherichia coli NCTC 10418	-	≤0.5	>512	>512	>512	>512	>512	>512
Escherichia coli BIG 0046	-	32	>512	>512	>512	>512	>512	>512
Coliform BIG 0048	-	8	512	>512	>512	>512	>512	>512
Coliform BIG 0049	-	8	>512	>512	>512	>512	>512	>512
Coliform BIG 0051	-	32	>512	>512	>512	>512	>512	>512
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	≤0.5	512	>512	512	265	512	>512
Pseudomonas aeruginosa (Environmental) BIG 0039	-	≤0.5	512	>512	512	512	512	>512
Pseudomonas aeruginosa NCTC 10662	-	≤0.5	512	>512	512	512	512	>512
Pseudomonas aeruginosa BIG 0063	-	≤0.5	512	>512	512	512	512	>512
Serratia marcescens BIG 0011 = NCTC 1377	-	≤0.5	>512	>512	>512	>512	>512	>512
Burkholderia cepacia BIG 0009 = NCTC 10744	-	≤0.5	512	512	256	64	512	>512

Cip. = (Ciprofloxacin)
MICs < 256 µg/ml are highlighted

Table 4.13 MIC testsof the most active compound of the2-methylthiolbenzimidazole derivatives

Bacteria species / strain	Gram	Cip.	FAS56
Staphylococcus aureus (Oxford) NCTC 6571	+	≤0.5	64
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	>32	64
Methicillin-resistant Staphylococcus aureus (HG-1)	+	32	64
EMRSA-15 NCTC 13142	+	≤0.5	64
EMRSA-16 NCTC 13143	+	32	64
MRSA BIG 0043	+	>32	64
MRSA BIG 0044	+	>32	64
MRSA BIG 0045	+	8	64
MRSA BIG 0047	+	8	64
MRSA BIG 0050	+	32	64
MRSA BIG 0052	+	≤0.5	64
MRSA BIG 0053	+	>32	64
Staphylococcus epidermidis NCTC 11047	+	≤0.5	32
Staphylococcus epidermidis NCTC 2749	+	≤0.5	64
Staphylococcus haemolyticus NCTC 11042	+	8	32
Escherichia coli NCTC 10418	-	≤0.5	512
Escherichia coli BIG 0046	-	32	>512
Coliform BIG 0048	-	8	512
Coliform BIG 0049	-	8	>512
Coliform BIG 0051	-	32	>512
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	≤0.5	>512
Pseudomonas aeruginosa (Environmental) BIG 0039	-	≤0.5	>512
Pseudomonas aeruginosa NCTC 10662	-	≤0.5	>512
Pseudomonas aeruginosa BIG 0063	-	≤0.5	>512
Serratia marcescens BIG 0011 = NCTC 1377	-	≤0.5	>512
Burkholderia cepacia BIG 0009 = NCTC 10744	-	≤0.5	64

MICs < 256 µg/ml are highlighted

Table 4.14 MIC tests of the most active compound of the benzoxazole derivative

Bacteria species / strain	Gram	Cip.	FAS61
Staphylococcus aureus (Oxford) NCTC 6571	+	≤0.5	64
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	>32	128
Methicillin-resistant Staphylococcus aureus (HG-1)	+	32	128
EMRSA-15 NCTC 13142	+	≤0.5	128
EMRSA-16 NCTC 13143	+	32	128
MRSA BIG 0043	+	>32	256
MRSA BIG 0044	+	>32	128
MRSA BIG 0045	+	8	256
MRSA BIG 0047	+	8	128
MRSA BIG 0050	+	32	256
MRSA BIG 0052	+	≤0.5	128
MRSA BIG 0053	+	>32	256
Staphylococcus epidermidis NCTC 11047	+	≤0.5	256
Staphylococcus epidermidis NCTC 2749	+	≤0.5	256
Staphylococcus haemolyticus NCTC 11042	+	8	512
Escherichia coli NCTC 10418	-	≤0.5	256
Escherichia coli BIG 0046	-	32	512
Coliform BIG 0048	-	8	256
Coliform BIG 0049	-	8	512
Coliform BIG 0051	-	32	512
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	≤0.5	256
Pseudomonas aeruginosa (Environmental) BIG 0039	-	≤0.5	>512
Pseudomonas aeruginosa NCTC 10662	-	≤0.5	>512
Pseudomonas aeruginosa BIG 0063	-	≤0.5	>512
Serratia marcescens BIG 0011 = NCTC 1377	-	≤0.5	512
Burkholderia cepacia BIG 0009 = NCTC 10744	-	≤0.5	256

MICs < 256 µg/ml are highlighted

Table 4.15 MIC tests of the benzothaizole derivative

Bacteria species / strain	Gram	Cip.	FAS65
Staphylococcus aureus (Oxford) NCTC 6571	+	≤0.5	>512
Staphylococcus aureus NCTC 10399 (=BIG 0010)	+	>32	512
Methicillin-resistant Staphylococcus aureus (HG-1)	+	32	>512
EMRSA-15 NCTC 13142	+	≤0.5	>512
EMRSA-16 NCTC 13143	+	32	512
MRSA BIG 0043	+	>32	>512
MRSA BIG 0044	+	>32	>512
MRSA BIG 0045	+	8	>512
MRSA BIG 0047	+	8	>512
MRSA BIG 0050	+	32	>512
MRSA BIG 0052	+	≤0.5	>512
MRSA BIG 0053	+	>32	>512
Staphylococcus epidermidis NCTC 11047	+	≤0.5	512
Staphylococcus epidermidis NCTC 2749	+	≤0.5	512
Staphylococcus haemolyticus NCTC 11042	+	8	512
Escherichia coli NCTC 10418	-	≤0.5	>512
Escherichia coli BIG 0046	-	32	>512
Coliform BIG 0048	-	8	>512
Coliform BIG 0049	-	8	>512
Coliform BIG 0051	-	32	>512
Pseudomonas aeruginosa NCTC 6749 (=BIG 0028)	-	≤0.5	>512
Pseudomonas aeruginosa (Environmental) BIG 0039	-	≤0.5	>512
Pseudomonas aeruginosa NCTC 10662	-	≤0.5	>512
Pseudomonas aeruginosa BIG 0063	-	≤0.5	>512
Serratia marcescens BIG 0011 = NCTC 1377	-	≤0.5	>512
Burkholderia cepacia BIG 0009 = NCTC 10744	-	≤0.5	>512

MICs < 256 µg/ml are highlighted

4.2.2.7 Conclusion

The agar dilution tests of the most active compounds (13 compounds out of 67) revealed that two (FAS11and FAS12) were active against multiple strains with zone diameter of 12 – 14 mm. Also, activity was exhibited against some Gram +ve and Gram -ve strains, and, for compounds FAS11, FAS12, and FAS42, this included some ciprofloxacin resistant strains. Moreover, FAS11 exhibited activity against some strains even at 10 µg with zone diameter of 12 – 13 mm. FAS47 was the most active compound of the series of 2-chloromethylbenzimidazole derivatives against some strains with zone diameter of 13 – 17 mm at 200 µg. FAS61 recorded good activity against *Staphylococcus aureus* (Oxford) with zone diameter of 13, 15, and 16 mm at 10, 100, and 200 µg respectively.The rest of the compounds were unlike this and therefore were not better than ciprofloxacin (control drug).

To summarise, 5 out of the 13 compounds exhibited antibacterial activity against two MRSA strains with MIC values which correspond to the control drug. This is significant and provides lead compounds for further development.

4.3 Antifungal study

4.3.1 Screening for antifungal activity by well diffusion tests

The strains used were unicellular fungi; *Candida albicans* RCMB 05035, *Candida krusei* RCMB 05051, *Candida parapsilosis* RCMB 05065, *Candida tropicalis* RCMB 05049, and the filamentous fungi; *Absidia corymbifera* RCMB 09635, *Aspergillus clavatus* RCMB 2593, *Aspergillus fumigatus* RCMB 02564, *Mucor circinelloides* RCMB 07328, *Penicillium marneffei* RCMB 01267, and *Syncephalastrum racemosum* RCMB 05922, and all were clinical isolates. Amphotericin B was used as a comparator drug. Amphotericin B is a polyene macrolide antifungal agent derived from the actinomycete *Streptomyces nodosus*. Out of 200 known polyene

agents, amphotericin B is the only one with toxicities that are sufficiently limited to permit intravenous administration (Gallis *et al.*, 1990). Moreover, it has a broad spectrum of action and is useful in treating cases of candidosis, cryptococcosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, coccidioidomycosis, aspergillosis, extracutaneous sporotrichosis and mucormycosis, and some cases of hyalohyphomycosis and phaeohyphomycosis (Ellis, 2002). However, it has been studied as an empirical antifungal agent in patients with persistent fever and neutropenia and the results showed it has a limited spectrum of activity. It has to be noted that there is a need for new classes of antifungal agents to use in the therapy of patients with persistent fever and neutropenia (Walsh *et al.*, 2004).

Activity was determined through measuring diameters of the inhibition zones surrounding the wells in the agar loaded with 250 µg of the compound (see section **2.4.2.6**). The formation of the zones represents the dynamic interaction between the diffusing compound and fungus trying to grow on the surface of the agar. A lack of activity is confirmed when fungal growth can be seen right up to the edge of the well. The results of the zone of inhibition tests are given in Tables 4.12-23. It should be noted that the wells themselves had a diameter of 6 mm. Growth up to the edge of the well (i.e. no activity) is indicated by a diameter of “0 mm” in the tables. Control wells containing just the DMSO solvent showed no inhibition of growth. Furthermore, 15 mm was selected to be the cutoff point, so if the zone of inhibition was wider than this, then it was considered a promising result, which is highlighted on the tables.

4.3.1.1 Results of well diffusion tests of the 2-amino alkylbenzimidazole derivatives (series 1 and 2)

The results from the 11 compounds tested from this series o showed activity for eight of them (FAS1, FAS2, FAS4, FAS5, FAS6, FAS7, FAS8, and FAS9), and the

results are shown in Table 4.16. However, there was no activity displayed by compounds FAS11, FAS12, and FAS13. Slight activities were exhibited by compounds FAS1 and FAS2 against all the filamentous fungi selected for the study except *Syncephalastrum racemosum* RCMB 05922, although good activity, which is equivalent to the control drug, was recorded against this species by compound FAS7. Moreover, compound FAS7 was the most active compound of this series, especially against *Mucor circinelloides* RCMB 07328 and *Absidia corymbifera* RCMB 09635, which showed activity more than amphotericin B with zone of inhibition 20.85 ± 0.05 mm . No activity was recorded for compounds FAS1 and FAS2 against the unicellular fungi.

4.3.1.2 Results of well diffusion tests of the 5-substituted-benzimidazole derivatives (series 3)

The eight compounds tested showed activity for all compounds, especially for compounds FAS18, FAS19, and FAS20 which were more active against most of the fungal strains selected. This was more than the control drug (zone of inhibition was 19.2 to 28.7 mm) (Table 4.17).

Table 4.16 Antifungal activity of the 2-aminoalkylbenzimidazole derivatives

Fungal	Diameter of zone of inhibition (mm)								
	FAS1	**FAS2**	**FAS4**	**FAS5**	**FAS6**	**FAS7**	**FAS8**	**FAS9**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	9.3	6.2	19.3	12.3	19.8	22.6	18.6	15.4	23.9
Aspergillus clavatus RCMB 02593	10.4	9.8	19.4	12.6	18.7	21.4	17.3	14.6	22.4
Mucor circinelloides RCMB 07328	10.9	10.4	17.6	12.6	17.3	20.8	16.5	13.1	17.9
Absidia corymbifera RCMB 09635	11.2	9.2	14.2	13.1	18.7	20.9	13.6	15.2	19.8
Penicillium marneffei RCMB 01267	12.1	10.1	16.3	12.4	12.9	19.3	15.2	13.1	20.6
Syncephalastrum racemosum RCMB 05922	0	0	18.2	0	18.1	19.4	17.4	0	19.7
Candida albicans RCMB 05035	0	0	20.6	13.1	20.2	21.9	19.2	15.4	21.9
Candida tropicalis RCMB 05049	0	0	20.2	13.7	21.7	22.3	19.4	16	25.4
Candida krusei RCMB 05051	0	0	14	0	11.7	17.3	13.6	0	19.4
Candida parapsilosis RCMB 05065	0	0	19.3	14.5	22.1	23.9	18.9	13.5	18.4

Table 4.17 Antifungal activity for the 5-substituted-benzimidazole derivatives

Fungal	Diameter of zone of inhibition (mm)								
	FAS15	**FAS16**	**FAS17**	**FAS18**	**FAS19**	**FAS20**	**FAS21**	**FAS51**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	9.3	21.6	21.4	24.2	25.8	23.2	18.6	20.3	23.9
Aspergillus clavatus RCMB 02593	11.4	20.3	20.5	23.1	23.4	22.6	17.3	21.6	22.4
Mucor circinelloides RCMB 07328	8.2	20.4	20.7	21.2	22.6	20.7	16.5	24.6	17.9
Absidia corymbifera RCMB 09635	10.1	20.8	20.7	22.1	24.9	23.2	14.9	0	19.8
Penicillium marneffei RCMB 01267	12.4	19.1	19	22.3	24.8	23.4	16.8	20.3	20.6
Syncephalastrum racemosum RCMB 05922	0	18.8	18.6	20.2	20.4	19.8	12.9	20.6	19.7
Candida albicans RCMB 05035	0	21.3	21	24.3	25.8	23.3	20.6	19.2	21.9
Candida tropicalis RCMB 05049	0	22.1	22.2	24.9	26	25	20.5	19.9	25.4
Candida krusei RCMB 05051	0	16.8	16.4	18.9	19.2	17.6	14.3	20.2	19.4
Candida parapsilosis RCMB 05065	0	21.4	21.3	24.8	28.7	22.8	19.3	17.4	18.4

The results are the average of three independent readings
Zone diameters ≥ 15 mm are highlighted

4.3.1.3 Results of well diffusion tests of the 2-methanolbenzimidazole derivatives (series 4)

All seven compounds tested from this series showed some activity (Table 4.18). However, there was no activity of any of the compounds against *Syncephalastrum racemosum* RCMB 05922 except compound FAS26 which was more active than the control drug with +0.3 mm on the zone of inhibition. Compound FAS 26 was slight active compound of this series against two species of *Candida*; *Candida krusei* RCMB 05051 and *Candida parapsilosis* RCMB 05065 with the zone of inhibition on the range of 19.4 to ≤20.6 mm.

4.3.1.4 Results of well diffusion tests of the 2-carboxylic acid-benzimidazole derivatives (series 5)

The results of the seven compounds tested from the series showed activity for only five compounds. Compound FAS33 was the only compound which that showed activity more than amphotericin B by +1.2 mm on the zone of inhibition (Table 4.19). No activity was seen with compounds FAS 32 and FAS 35. Furthermore, all the other compounds did not inhibit two strains; *Syncephalastrum racemosum* RCMB 05922, and *Candida krusei* RCMB 05051.

4.3.1.5 Results of well diffusion tests of the *N*-methyl-2-methanolbenzimidazole derivatives (series 6)

In this series, only two compounds FAS42, and FAS50 were active against all the strains except against *Absidia corymbifera* RCMB 09635, and were more active than the control drug against the following species; *Mucor circinelloides* RCMB 07328, *Penicillium marneffei* RCMB 01267, *Syncephalastrum racemosum* RCMB 05922, and *Candida krusei* RCMB 05051 (zone of inhibition 20.6 - 24.5 mm). No

activity was observed for FAS38 (Table 4.20). Compound FAS50 was the most active compound against *Aspergillus clavatus* RCMB 02593, and more active than amphotericin B with zone of inhibition + 0.4 mm. FAS41 was the most active compound against *Absidia corymbifera* RCMB 09635, which showed greater activity compared to amphotericin B with zone of inhibition +0.3 mm.

4.3.1.6 Results of well diffusion tests of the 2-chloromethylbenzimidazole derivatives (series 7)

All the compounds from this series showed some antifungal activity, especially compounds FAS44, FAS45, and FAS49 which were the most active compounds against *Mucor circinelloides* RCMB 07328, and they were more active than amphotericin B (zone of inhibition 18.1-19.9 mm). Moreover, FAS49 was the most active compound against *Absidia corymbifera* RCMB 09635. FAS45 was not active against all the unicellular fungi selected for the study (Table 4.21).

4.3.1.7 Results of well diffusion tests of the 2-ethylbenzimidazole derivatives (series 8)

The results for the two compounds tested of the series of 2-methanebenzimidazole derivatives showed good activity for compound FAS54 and compound FAS55 showed slight activity. Both had some activity but not against *Absidia corymbifera* RCMB 09635. FAS55 was active against some species namely; *Aspergillus clavatus* RCMB 02593, *Mucor circinelloides* RCMB 07328, *Syncephalastrum racemosum* RCMB 05922, *Candida albicans* RCMB 05035, *Candida krusei* RCMB 05051, and *Candida parapsilosis* RCMB 05065. The activities were greater than amphotericin B (zone of inhibition in the range 19.9 - 23.9 mm). Moreover, compound FAS55 had no activity against the unicellular fungi (Table 4.22).

4.3.1.8 Results of well diffusion tests of the 2-methanthiolbenzimidazole derivatives (series 9)

Both compounds tested from this series showed some activity, but not against *Absidia corymbifera* RCMB 09635. Moreover, compound FAS57 had no activity against all the unicellular fungi except for weak inhibition of *Candida tropicalis* RCMB 05049, with zone diameter of just 14.5 mm (Table 4.23). Furthermore, FAS57 was more active than amphotericin B active against *Mucor circinelloides* RCMB 07328 and *Syncephalastrum racemosum* RCMB 05922 (zone of inhibition 19.8 - 20.9 mm).

4.3.1.9 Results of well diffusion testsof the silver complexes (series 13)

The results for the six complexes tested showed that all had some antifungal activity. None were active against *Absidia corymbifera* RCMB 09635, except for compound FAS72, which revealed some limited activity with zone diameter of 16.3 mm. Moreover, compounds FAS69, FAS70, and FAS71 had higher activity compared to the control drug against *Syncephalastrum racemosum* RCMB 05922 (zone of inhibition 20.8 - 22.6 mm) (Table 4.24).

4.3.1.10 Results of well diffusion test of the *N*-oxidebenzimidazole derivatives (series 10)

Both *N*-oxidebenzimidazoles had activity. Compound FAS60 was more active than amphotericin B against some species namely; *Mucor circinelloides* RCMB 07328, *Penicillium marneffei* RCMB 01267, *Syncephalastrum racemosum* RCMB 05922, *Candida albicans* RCMB 05035, *Candida krusei* RCMB 05051, and *Candida parapsilosis* RCMB 05065 (zone of inhibition 20.9 - 25.8 mm). However, both compounds lacked activity against *Absidia corymbifera* RCMB 09635 (Table 4.25).

4.3.1.11 Results of well diffusion tests of the benzoxazole derivatives (series 11)

Only one compound, FAS63 recorded good activity against all the strains except against *Absidia corymbifera* RCMB 09635. Moreover, it was more active than amphotericin B against *Mucor circinelloides* RCMB 07328, *Syncephalastrum racemosum* RCMB 05922, *Candida krusei* RCMB 05051, and *Candida parapsilosis* RCMB 05065, in terms of zone diameter (zone of inhibition 19.9 - 23.7 mm) (Table 4.26).

4.3.1.12 Results of well diffusion tests of the benzothiazole derivative (series 12)

The results for the single benzothiazole derivative tested showed FAS65 had some antifungal activity. Weak activity was recorded against all the strains except against *Absidia corymbifera* RCMB 09635, and *Candida krusei* RCMB 05051 (Table 4.27). It was more active against *Candida tropicalis* RCMB 05049 with zone diameter 17.9 mm, although this was less than the activity of amphotericin B (zone diameter of 25.4 mm).

Table 4.18 Antifungal activity for 2-methanolbenzimidazole-derivatives

Fungal	Diameter of zone of inhibition (mm)							
	FAS22	**FAS23**	**FAS24**	**FAS25**	**FAS26**	**FAS27**	**FAS28**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	10.3	13.7	13.6	14.2	17.8	15.7	15.6	23.9
Aspergillus clavatus RCMB 02593	12.1	14.5	12.6	14.8	18.3	13.8	12.7	22.4
Mucor circinelloides RCMB 07328	0	0	0	14.3	14.9	16.9	16.3	17.9
Absidia corymbifera RCMB 09635	11.3	13.6	10.4	14	19.4	17.2	15.8	19.8
Penicillium marneffei RCMB 01267	12.9	12.9	12.6	12.9	17.3	18.3	17.2	20.6
Syncephalastrum racemosum RCMB 05922	0	0	0	0	20	0	0	19.7
Candida albicans RCMB 05035	0	13.9	0	14.5	20.3	17.9	18.9	21.9
Candida tropicalis RCMB 05049	0	14.4	0	14.9	21.3	18.5	19.4	25.4
Candida krusei RCMB 05051	0	0	0	0	19.4	0	0	19.4
Candida parapsilosis RCMB 05065	0	15	0	15.6	20.6	19.1	16.4	18.4

Table 4.19 Antifungal activity for 2-carboxylic acid-benzimidazole-derivatives

Fungal	Diameter of zone of inhibition (mm)					
	FAS29	**FAS30**	**FAS31**	**FAS33**	**FAS34**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	14.2	15	15.6	16.7	16.8	23.9
Aspergillus clavatus RCMB 02593	12.7	12.6	13.9	14.8	15.6	22.4
Mucor circinelloides RCMB 07328	11.6	12.1	12.2	13.6	14.4	17.9
Absidia corymbifera RCMB 09635	13.6	14.3	16.4	18.3	16.2	19.8
Penicillium marneffei RCMB 01267	15.2	14.6	15	17.4	14	20.6
Syncephalastrum racemosum RCMB 05922	0	0	0	0	0	19.7
Candida albicans RCMB 05035	16.7	16.7	17.3	19.9	16.2	21.9
Candida tropicalis RCMB 05049	17	17.5	17.8	18.8	16.9	25.4
Candida krusei RCMB 05051	0	0	0	0	0	19.4
Candida parapsilosis RCMB 05065	14.2	17.7	18.4	19.6	12.5	18.4

The results are the average of three independent readings
Zone diameters ≥ 15 mm are highlighted

Table 4.20 Antifungal activity of *N*-methyl-2-methanolbenzimidazole derivatives

Fungal	Diameter of zone of inhibition (mm)								
	FAS36	**FAS37**	**FAS38**	**FAS39**	**FAS40**	**FAS41**	**FAS42**	**FAS50**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	11.3	14.2	0	13.6	12.7	18.4	20.3	20.9	23.9
Aspergillus clavatus RCMB 02593	12.6	12.7	0	11.4	13.1	17.6	21.6	22.8	22.4
Mucor circinelloides RCMB 07328	14.8	11.6	0	14.2	14	19.8	23.4	24.5	17.9
Absidia corymbifera RCMB 09635	16.7	13.6	0	15.1	0	20.1	0	0	19.8
Penicillium marneffei RCMB 01267	15.8	15.2	0	17.1	11.7	18.6	20.6	21.7	20.6
Syncephalastrum racemosum RCMB 05922	0	0	0	0	12	0	23.4	24.2	19.7
Candida albicans RCMB 05035	13.7	16.7	0	15.9	0	19.2	20.6	21.5	21.9
Candida tropicalis RCMB 05049	13.9	17	0	16.8	0	20.3	22.4	23.6	25.4
Candida krusei RCMB 05051	0	0	0	0	0	0	21.2	22.4	19.4
Candida parapsilosis RCMB 05065	14.6	14.2	0	17.4	0	16.3	17.9	18.3	18.4

Table 4.21 Antifungal activity of 2-chloromethylbenzimidazole derivatives

Fungal	Diameter of zone of inhibition (mm)							
	FAS43	**FAS44**	**FAS45**	**FAS46**	**FAS47**	**FAS48**	**FAS49**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	12.6	16.8	17.6	13.6	13.6	14.2	20.2	23.9
Aspergillus clavatus RCMB 02593	13.4	14.7	19.4	11.4	11	14.9	19.6	22.4
Mucor circinelloides RCMB 07328	14.8	18.3	19.9	14.1	13.4	13.6	18.1	17.9
Absidia corymbifera RCMB 09635	17.2	18.5	0	0	0	16.8	20	19.8
Penicillium marneffei RCMB 01267	17.9	17.4	17.7	12	12.4	18.1	19.8	20.6
Syncephalastrum racemosum RCMB 05922	0	0	19.3	17.1	16.3	0	19.3	19.7
Candida albicans RCMB 05035	14.1	18.9	0	11.4	11.2	16.8	21.6	21.9
Candida tropicalis RCMB 05049	14.6	20.3	0	12.7	13	17.9	22.3	25.4
Candida krusei RCMB 05051	0	0	0	13.6	13.9	0	18.4	19.4
Candida parapsilosis RCMB 05065	16.2	21.2	0	0	0	18.2	22.1	18.4

The results are the average of three independent readings
Zone diameters ≥ 15 mm are highlighted

Table 4.22 Antifungal activity of 2-methanebenzimidazole derivatives

Fungal	Diameter of zone of inhibition (mm)		
	FAS54	**FAS55**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	22.6	12.3	23.9
Aspergillus clavatus RCMB 02593	23.2	9.3	22.4
Mucor circinelloides RCMB 07328	23.9	10.5	17.9
Absidia corymbifera RCMB 09635	0	0	19.8
Penicillium marneffei RCMB 01267	21.4	11.6	20.6
Syncephalastrum racemosum RCMB 05922	23.6	12.4	19.7
Candida albicans RCMB 05035	23.2	0	21.9
Candida tropicalis RCMB 05049	21.2	0	25.4
Candida krusei RCMB 05051	20.4	0	19.4
Candida parapsilosis RCMB 05065	19.9	0	18.4

Table 4.23 Antifungal activity of 2-methanthiolbenzimidazole derivatives

Tested microorganisms	Diameter of zone of inhibition (mm)		
	FAS56	**FAS57**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	18.2	16.3	23.9
Aspergillus clavatus RCMB 02593	19.3	15.2	22.4
Mucor circinelloides RCMB 07328	19.8	17.3	17.9
Absidia corymbifera RCMB 09635	0	0	19.8
Penicillium marneffei RCMB 01267	16.3	15.2	20.6
Syncephalastrum racemosum RCMB 05922	20.9	17.4	19.7
Candida albicans RCMB 05035	16.8	0	21.9
Candida tropicalis RCMB 05049	15.4	11.2	25.4
Candida krusei RCMB 05051	17.2	0	19.4
Candida parapsilosis RCMB 05065	14.5	0	18.4

The results are the average of three independent readings
Zone diameters ≥ 15 mm are highlighted

Table 4.24 Antifungal activity of silver complexes

Fungal	Diameter of zone of inhibition (mm)						
	FAS69	**FAS70**	**FAS71**	**FAS72**	**FAS73**	**FAS74**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	15.7	18.9	16.8	20.4	14.9	17.3	23.9
Aspergillus clavatus RCMB 02593	17.4	20.2	18.6	20.9	16.4	19.4	22.4
Mucor circinelloides RCMB 07328	13.9	16.8	16.8	18.9	14.7	15.3	17.9
Absidia corymbifera RCMB 09635	0	0	0	16.3	0	0	19.8
Penicillium marneffei RCMB 01267	16.8	19.2	18.2	16.4	16.2	14.2	20.6
Syncephalastrum racemosum RCMB 05922	15.9	20.8	16.2	22.6	15.3	19.4	19.7
Candida albicans RCMB 05035	13.4	18.3	19.3	20.2	13.7	16.3	21.9
Candida tropicalis RCMB 05049	12.7	19.9	18.3	22.1	15	21	25.4
Candida krusei RCMB 05051	14.3	18	17.3	0	0	0	19.4
Candida parapsilosis RCMB 05065	10.5	15.7	13.6	18.3	10	13	18.4

Table 4.25 Antifungal activity of *N*-oxidbenzimidazole derivatives

Fungal	Diameter of zone of inhibition (mm)		
	FAS59	**FAS60**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	15.7	22.3	23.9
Aspergillus clavatus RCMB 02593	17.2	20.3	22.4
Mucor circinelloides RCMB 07328	19.8	25.8	17.9
Absidia corymbifera RCMB 09635	0	0	19.8
Penicillium marneffei RCMB 01267	16.2	23.4	20.6
Syncephalastrum racemosum RCMB 05922	19.8	25.1	19.7
Candida albicans RCMB 05035	13.6	22.6	21.9
Candida tropicalis RCMB 05049	16.8	21.9	25.4
Candida krusei RCMB 05051	16.5	22.8	19.4
Candida parapsilosis RCMB 05065	0	20.9	18.4

The results are theaverage of three independent readings
Zone diameters ≥ 15 mm are highlighted

Table 4.26 Antifungal activity of benzoxazole derivative

Fungal	Diameter of zone of inhibition (mm)	
	FAS63	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	20.6	23.9
Aspergillus clavatus RCMB 02593	21.1	22.4
Mucor circinelloides RCMB 07328	21.9	17.9
Absidia corymbifera RCMB 09635	0	19.8
Penicillium marneffei RCMB 01267	20.6	20.6
Syncephalastrum racemosum RCMB 05922	23.7	19.7
Candida albicans RCMB 05035	20.9	21.9
Candida tropicalis RCMB 05049	21.4	25.4
Candida krusei RCMB 05051	20.1	19.4
Candida parapsilosis RCMB 05065	19.9	18.4

Table 4.27 Antifungal activity of benzothiazole derivative

Fungal	Diameter of zone of inhibition (mm)	
	FAS65	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	11.6	23.9
Aspergillus clavatus RCMB 02593	10.7	22.4
Mucor circinelloides RCMB 07328	13.2	17.9
Absidia corymbifera RCMB 09635	0	19.8
Penicillium marneffei RCMB 01267	11.4	20.6
Syncephalastrum racemosum RCMB 05922	12.2	19.7
Candida albicans RCMB 05035	13.8	21.9
Candida tropicalis RCMB 05049	17.2	25.4
Candida krusei RCMB 05051	0	19.4
Candida parapsilosis RCMB 05065	10.7	18.4

The results are the average of three independent readings
Zone diameters ≥ 15 mm are highlighted

4.3.1.13 Conclusion

The well diffusion tests of the 67 compounds revealed very interesting results:

Failmentous fungi

- 30 had some antifungal activity; and were active against *Aspergillus clavatus* RCMB 2593;
- 36 had some activity against *Aspergillus fumigatus* RCMB 02564;
- 31 compounds were active against *Penicillium marneffei* RCMB 01267;
- 29 compounds were active against *Mucor circinelloides* RCMB 07328;
- 23 compounds were active against *Absidia corymbifera* RCMB 09635;
- 29 compounds were active against *Syncephalastrum racemosum* RCMB 05922

Unicellular fungi

- 36 compounds were active against *Candida albicans* RCMB 05035;
- 38 compounds were active against *Candida tropicalis* RCMB 05049;
- 19 compounds were active against *Candida parapsilosis* RCMB 05065 and
- 18 compounds had some activity against *Candida krusei* RCMB 05051.

The most active compounds overall were selected on the basis of a broad spectrum of activity, and / or wide zone of inhibition, or novel chemical structure. These compounds are summerised in Table 4.28, and were investigated further in minimum inhibitory concentration (MIC) assays to quantify their activity against the reference isolates.

4.3.2 MICs of antifungal of compounds

The MIC of the compounds was determined by the microdilution assay (see section **4.3.2)**. Wells of Microtiter trays were prepared in the dilution: 0.003, 0.007, 0.015, 0.03, 0.06, 0.12, 0.24, 0.49, 0.98, 1.95, 3.9, 7.8, 15.63, 31.25, 62.5, 125, 250, and 500 µg/ml and the method is described in section **2.4.2.6**.

Table 4.28 The most active compounds which were taken forwardfor MIC determination

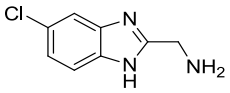
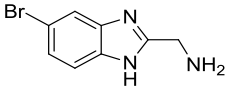
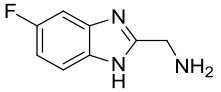
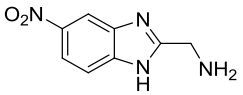
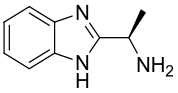
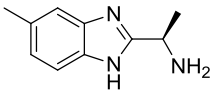
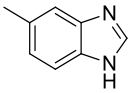
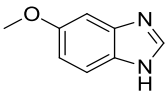
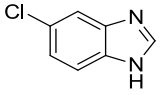
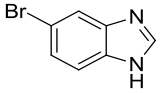
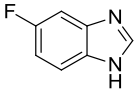
Compound code	Chemical structure	Note
FAS4		➢ Active against multiple strains with zone diameter of 14 – 20.6 mm. ➢ Antifungal activity more than the control drug against *Candida parapsilosis* RCMB 05065, with zone diameter of 19.3 mm.
FAS5		➢ Weak activity against multiple strains with zone diameter of 12.3 – 14.5 mm. ➢ To study the vairation in the antifungal activity on the compounds of this series.
FAS6		➢ Active against all strains with zone diameter of 11.7 – 22.1 mm. ➢ Antifungal activity more than the control drug against *Candida parapsilosis* RCMB 05065, with zone diameter of 22.1 mm.
FAS7		➢ Active against multiple strains with zone diameter of 17.3 – 23.9 mm. ➢ *parapsilosis* RCMB 05065, with zone diameter of 23.9 mm. ➢ Antifungal activity more than the control drug against *Absidia corymbifera* RCMB 09635, with zone diameter of 20.9 mm. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 20.8 mm.
FAS8		➢ Active against multiple strains with zone diameter of 13.6– 19.4 mm. ➢ Antifungal activity more than the control drug against *Candida parapsilosis* RCMB 05065, with zone diameter of 18.9 mm.
FAS9		➢ Active against multiple strains with zone diameter of 13.1– 16 mm.
FAS16		➢ Active against all strains with zone diameter of 16.8– 22.1 mm. ➢ Antifungal activity more than the control drug against *Absidia corymbifera* RCMB 09635, with zone diameter of 20.8 mm. ➢ Antifungal activity more than the control drug against *Candida parapsilosis* RCMB 05065, with zone diameter of 21.4 mm.
FAS17		➢ Active against all strains with zone diameter of 16.4– 21.4 mm. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 20.7 mm. ➢ Antifungal activity more than the control drug against *Absidia corymbifera* RCMB 09635, with zone diameter of 20.7 mm. ➢ Antifungal activity more than the control drug against *Candida parapsilosis* RCMB 05065, with zone diameter of 21.3 mm.
FAS18		➢ Active against all strains with zone diameter of 18.9– 24.9 mm. ➢ Active against all strains more than the control drug, except against *Candida tropicalis* RCMB 05049 and *Candida krusei* RCMB 05051.
FAS19		➢ Active against all strains with zone diameter of 19.2– 26 mm. ➢ Active against all strains more than the control drug, except against *Candida krusei* RCMB 05051
FAS20		➢ Active against all strains with zone diameter of 17.6– 25 mm. ➢ Active against all strains more than the control drug, except against *Aspergillus fumigatus* RCMB 02564, *Candida tropicalis* RCMB 05049 and *Candida krusei* RCMB 05051.

Table 4.28 (continued

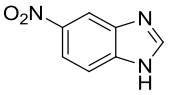
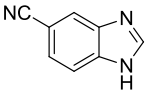
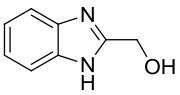
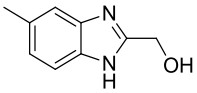
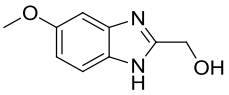
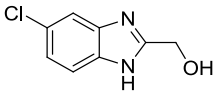
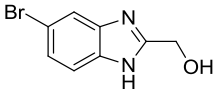
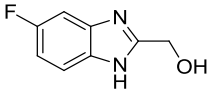
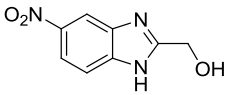
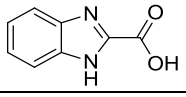
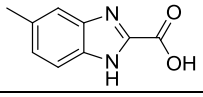
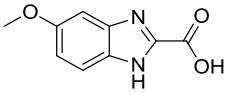
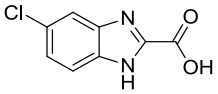
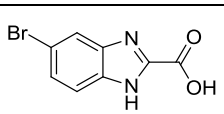
Compound code	Chemical structure	Note
FAS 21		➢ Active against multiple strains with zone diameter of 12.9–20.6 mm. ➢ Antifungal activity more than the control drug against *Candida parapsilosis* RCMB 05065, with zone diameter of 19.3 mm.
FAS51		➢ Active against multiple strains with zone diameter of 17.4–24.6 mm. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 24.6 mm. ➢ Antifungal activity more than the control drug against *Syncephalastrum racemosum* RCMB 05922, with zone diameter of 20.6 mm. ➢ Antifungal activity more than the control drug against *Candida krusei* RCMB 05051, with zone diameter of 20.2 mm
FAS22		➢ Weak active against multiple strains compounds with zone diameter of 10.3 – 12.9 mm. ➢ To study the variety in the antifungal activity on the compounds of this series.
FAS23		➢ Weak activity against multiple strains with zone diameter of 12.9 – 15 mm. ➢ To study the vairation in the antifungal activity on the compounds of this series.
FAS24		➢ Weak activity against multiple strains with zone diameter of 10.4 – 13.6 mm. ➢ To study the vairation in the antifungal activity on the compounds of this series.
FAS25		➢ Weak activity against multiple strains with zone diameter of 12.9 – 15.6 mm. ➢ To study the vairation in the antifungal activity on the compounds of this series.
FAS26		➢ Active against multiple strains with zone diameter of 14.9–21.3 mm. ➢ Antifungal activity more than the control drug against *Syncephalastrum racemosum* RCMB 05922, with zone diameter of 20 mm. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 20.6 mm.
FAS27		➢ Active against multiple strains with zone diameter of 13.8–19.1 mm. ➢ Antifungal activity more than the control drug against *Candida parapsilosis* RCMB 05065, with zone diameter of 19.1 mm.
FAS28		➢ Active against multiple strains with zone diameter of 12.7–19.4 mm.
FAS29		➢ Active against multiple strains with zone diameter of 11.6–17 mm.
FAS30		➢ Active against multiple strains with zone diameter of 12.1–17.7 mm.
FAS31		➢ Active against multiple strains with zone diameter of 12.2–18.4 mm.
FAS32		➢ To study the viration in the antifungal activity on the compounds of this series.
FAS33		➢ Active against multiple strains with zone diameter of 13.6–19.6 mm. ➢ Antifungal activity more than the control drug against *Candida parapsilosis* RCMB 05065, with zone diameter of 19.6 mm.

Table 4.28 (continued

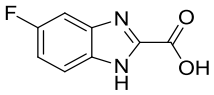
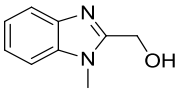
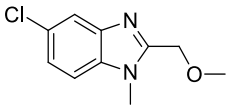
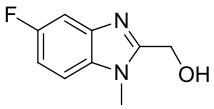
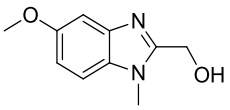
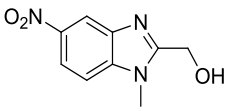
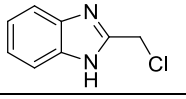
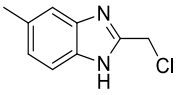
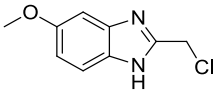
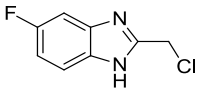
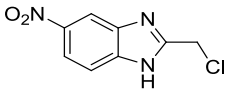
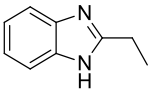
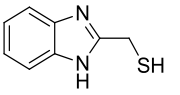
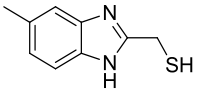
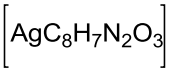
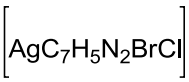
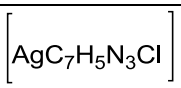
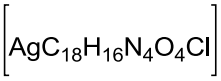
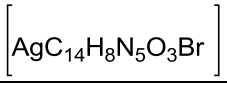
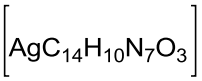
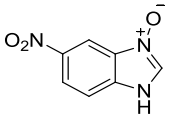
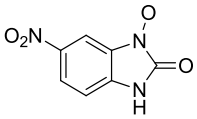
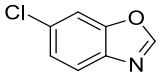
Compound code	Chemical structure	Note
FAS34		➢ Active against multiple strains with zone diameter of 12.5–16.9 mm.
FAS36		➢ Active against multiple strains with zone diameter of 11.3–16.7 mm.
FAS39		➢ Active against multiple strains with zone diameter of 11.4–17.4 mm.
FAS41		➢ Active against multiple strains with zone diameter of 16.3–20.3 mm. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 19.8 mm. ➢ Antifungal activity more than the control drug against *Absidia corymbifera* RCMB 09635, with zone diameter of 20.1 mm.
FAS42		➢ Active against all strains with zone diameter of 17.9– 23.4 mm, except against *Absidia corymbifera* RCMB 09635.
FAS50		➢ Active against all strains with zone diameter of 18.3- 24.5 mm, except against *Absidia corymbifera* RCMB 09635.
FAS43		➢ Active against multiple strains with zone diameter of 12.6–17.9 mm.
FAS44		➢ Active against multiple strains with zone diameter of 14.73– 21.2 mm.
FAS45		➢ Active against filamentous strains with zone diameter of 17.7– 19.9 mm. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 19.9 mm.
FAS48		➢ Active against multiple strains with zone diameter of 13.6–18.2 mm.
FAS49		➢ Active against multiple strains with zone diameter of 18.1-22.3 mm. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 18.1 mm. ➢ Antifungal activity more than the control drug against *Absidia corymbifera* RCMB 09635, with zone diameter of 20 mm.
FAS54		➢ Active against all strains with zone diameter of 19.9– 23.9 mm, except against *Absidia corymbifera* RCMB 09635. ➢ Antifungal activity more than the control drug against *Aspergillus clavatus* RCMB 02593, with zone diameter of 23.2 mm. ➢ Antifungal activity more than the control drug against all strains except against *Aspergillus fumigatus* RCMB 02564, *Absidia corymbifera* RCMB 09635, and *Candida tropicalis* RCMB 05049 with rang of zone diameter of 19.9-23.9 mm.
FAS56		➢ Active against all strains with zone diameter of 14.5– 20.9 mm, except against *Absidia corymbifera* RCMB 09635. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 19.8 mm. ➢ Antifungal activity more than the control drug against *Syncephalastrum racemosum* RCMB 05922, with zone diameter of 20.9 mm.

Table 4.28 (continued

Compound code	Chemical structure	Note
FAS57		➢ Active against multiple strains with zone diameter of 11.2-17.4 mm. ➢ To study the vairation in the antifungal activity on the compounds of this series.
FAS69		➢ Active against multiple strains with zone diameter of 10.5-17.4 mm.
FAS70		➢ Active against all strains with zone diameter of 15.7– 20.8 mm, except against *Absidia corymbifera* RCMB 09635. ➢ Antifungal activity more than the control drug against *Syncephalastrum racemosum* RCMB 05922, with zone diameter of 20.8 mm.
FAS71		➢ Active against all strains with zone diameter of 13.6– 19.3 mm, except against *Absidia corymbifera* RCMB 09635. ➢
FAS72		➢ Active against all strains with zone diameter of 16.36– 22.6 mm, except against *Candida krusei* RCMB 05051. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 18.9 mm. ➢ Antifungal activity more than the control drug against *Syncephalastrum racemosum* RCMB 05922, with zone diameter of 22.6 mm.
FAS73		➢ Active against all strains with zone diameter of 10– 16.4 mm, except against *Absidia corymbifera* RCMB 09635, and *Candida krusei* RCMB 05051.
FAS74		➢ Active against all strains with zone diameter of 13– 21 mm, except against *Absidia corymbifera* RCMB 09635, and *Candida krusei* RCMB 05051
FAS59		➢ Active against all strains with zone diameter of 13.6– 19.8 mm, except against *Absidia corymbifera* RCMB 09635, and *Candida krusei* RCMB 05051. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 19.8 mm. ➢ Antifungal activity more than the control drug against *Syncephalastrum racemosum* RCMB 05922, with zone diameter of 19.8 mm.
FAS60		➢ Active against all strains with zone diameter of 20.3– 25.8 mm, except against *Absidia corymbifera* RCMB 09635.
FAS63		➢ Active against all strains with zone diameter of 19.9– 23.7 mm, except against *Absidia corymbifera* RCMB 09635. ➢ Antifungal activity more than the control drug against *Mucor circinelloides* RCMB 07328, with zone diameter of 21.9 mm. ➢ Antifungal activity more than the control drug against *Syncephalastrum racemosum* RCMB 05922, with zone diameter of 23.7 mm. ➢ Antifungal activity more than the control drug against *Candida krusei* RCMB 05051, with zone diameter of 20.1 mm. ➢ Antifungal activity more than the control drug against *Candida parapsilosis* RCMB 05065, with zone diameter of 19.9 mm.

4.3.2.1 MIC results of the 2-amino-alkylbenzimidazole derivatives (series 1 and 2)

The results of the six compounds tested from the series 2- amino-alkylbenzimidazole derivatives showed activity for FAS4 and FAS7 especially against *Mucor circinelloides* RCMB 07328 with low MICs of, 3.9 and 0.49µg/ml, respectively. Compounds FAS4, FAS6, FAS7, and FAS8 showed good activity against *Candida parapsilosis* RCMB 05065; with MICs in the range of 1.95 - 0.06µg/ml, which is less or equivalent to the MIC of the amphotericin B. The antifungal activity of these compounds against the rest of the selected strains was on the range of 500 - 0.06µg/ml (Table 4.29).

4.3.2.2 MIC results of the 5-substituted benzimidazole derivatives (series 3)

The results of the seven compounds tested from the series 5-substituted benzimidazole derivatives showed activity for six of them especially for compounds FAS18, FAS19, and FAS20 with MICs range of MIC ≥ 0.06 µg/ml, which were thus as active as the amphotericin B or less in some cases. All the compounds were active against *Mucor circinelloides* RCMB 07328 better than the control drug by three to six folds, except for FAS 21. Moreover, all compounds were active against *Candida parapsilosis* RCMB 05065, better than the control drug by two to nine folds, except FAS21. The antifungal activity of these compounds against the rest of the strains was in the range of 0.98 - 003µg/ml (Table 4.30).

Table 4.29 MIC results of the2-aminoalkylbenzimidazole derivatives

Fungal	MIC µg/ml						
	FAS4	**FAS5**	**FAS6**	**FAS7**	**FAS8**	**FAS9**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	1.95	125	0.98	0.12	1.95	15.63	0.06
Aspergillus clavatus RCMB 02593	1.95	125	1.95	0.24	3.9	31.25	0.12
Mucor circinelloides RCMB 07328	3.9	125	7.8	0.49	15.63	125	3.9
Absidia corymbifera RCMB 09635	31.25	125	1.95	0.49	62.5	31.25	0.98
Penicillium marneffei RCMB 01267	15.63	125	62.5	0.98	15.63	125	0.49
Syncephalastrum racemosum RCMB 05922	3.9	>500	3.9	0.98	3.9	>500	0.98
Candida albicans RCMB 05035	0.49	125	0.49	0.24	1.95	15.63	0.12
Candida tropicalis RCMB 05049	0.98	62.5	0.24	0.12	0.98	7.8	0.015
Candida krusei RCMB 05051	62.5	>500	31.25	7.8	62.5	>500	0.98
Candida parapsilosis RCMB 05065	1.95	31.25	0.24	0.06	1.95	62.5	1.95

Table 4.30 MIC results of the 5-substituted-benzimidazole derivatives

Fungal	MIC µg/ml							
	FAS16	**FAS17**	**FAS18**	**FAS19**	**FAS20**	**FAS21**	**FAS51**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	0.24	0.24	0.06	0.015	0.06	1.95	3.9	0.06
Aspergillus clavatus RCMB 02593	0.49	0.98	0.12	0.06	0.12	3.9	0.98	0.12
Mucor circinelloides RCMB 07328	0.49	0.49	0.24	0.12	0.12	7.8	0.06	3.9
Absidia corymbifera RCMB 09635	0.24	0.24	0.12	0.03	0.06	15.63	>500	0.98
Penicillium marneffei RCMB 01267	1.95	0.98	0.12	0.03	0.03	7.8	3.9	0.49
Syncephalastrum racemosum RCMB 05922	1.95	1.95	0.98	0.98	0.98	125	3.9	0.98
Candida albicans RCMB 05035	0.49	0.98	0.03	0.007	0.03	0.49	3.9	0.12
Candida tropicalis RCMB 05049	0.24	0.24	0.015	0.007	0.06	0.49	1.95	0.015
Candida krusei RCMB 05051	7.8	15.63	1.95	0.98	0.98	31.25	1.95	0.98
Candida parapsilosis RCMB 05065	0.24	0.12	0.03	0.003	0.06	0.98	15.63	1.95

MICs < MICs of Amphotericin B are highlighted

4.3.2.3 MIC results of the 2-methanolbenzimidazole derivatives (series 4)

The results for the seven compounds tested from the series of 2-methaol-benzimidazole derivatives showed good activity for only one compound FAS26 against some species namely; *Absidia corymbifera* RCMB 09635, *Syncephalastrum racemosum* RCMB 05922, *Candida krusei* RCMB 05051, and *Candida parapsilosis* RCMB 05065, with MICs in the range of 0.49-0.98 µg/ml, which was active as the control drug or more active by two folds. The antifungal activity of this compound against the rest of the selected strains was on the range of 31.25 to 0.49 µg/ml (Table 4.31)

4.3.2.4 MIC results of the2-carboxylic acid benzimidazole derivatives (series 5)

The results for the six compounds tested from the series of 2-carboxylic acid benzimidazole derivatives showed activity for three compounds FAS31,FAS32 and FAS33, with low MICs of 0.98 µg/ml against *Candida parapsilosis* RCMB 05065, which is one fold more potent than the control drug or equal. The antifungal activity of these compounds against the rest of the selected strains was in the range of MIC 0.98- ≥ 500µg/ml (Table 4.32).

Table 4.31 MIC results for the 2-methanolbenzimidazole derivatives.

Fungal	MIC µg/ml							
	FAS22	**FAS23**	**FAS24**	**FAS25**	**FAS26**	**FAS27**	**FAS28**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	500	62.5	62.5	31.25	3.9	15.63	15.63	0.06
Aspergillus clavatus RCMB 02593	125	31.25	125	15.63	1.95	62.5	125	0.12
Mucor circinelloides RCMB 07328	>500	>500	>500	31.25	31.25	7.8	7.8	3.9
Absidia corymbifera RCMB 09635	250	62.5	500	62.5	0.98	3.9	15.63	0.98
Penicillium marneffei RCMB 01267	125	125	125	125	3.9	1.95	3.9	0.49
Syncephalastrum racemosum RCMB 05922	>500	>500	>500	>500	0.98	>500	>500	0.98
Candida albicans RCMB 05035	>500	62.5	>500	31.25	0.98	3.9	1.95	0.12
Candida tropicalis RCMB 05049	>500	31.25	>500	31.25	0.49	1.95	0.98	0.015
Candida krusei RCMB 05051	>500	>500	>500	>500	0.98	>500	>500	0.98
Candida parapsilosis RCMB 05065	>500	15.63	>500	15.63	0.49	1.95	7.8	1.95

Table 4.32 MIC results for the 2-carboxylic acid-benzimidazole derivatives

Fungal	MIC µg/ml						
	FAS29	**FAS30**	**FAS31**	**FAS32**	**FAS33**	**FAS34**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	31.25	15.63	15.63	7.8	7.8	7.8	0.06
Aspergillus clavatus RCMB 02593	125	31.25	62.5	15.63	31.25	15.63	0.12
Mucor circinelloides RCMB 07328	250	125	125	62.5	62.5	31.25	3.9
Absidia corymbifera RCMB 09635	62.5	31.25	7.8	7.8	3.9	7.8	0.98
Penicillium marneffei RCMB 01267	15.63	7.8	15.63	15.63	7.8	62.5	0.49
Syncephalastrum racemosum RCMB 05922	>500	>500	>500	>500	>500	>500	0.98
Candida albicans RCMB 05035	3.9	3.9	7.8	0.98	0.98	7.8	0.12
Candida tropicalis RCMB 05049	3.9	7.8	3.9	1.95	1.95	7.8	0.015
Candida krusei RCMB 05051	>500	>500	>500	>500	>500	>500	0.98
Candida parapsilosis RCMB 05065	62.5	3.9	1.95	0.98	0.98	12.5	1.95

MICs < MICs of Amphotericin B are highlighted

4.3.2.5 MIC results of the *N* – methyl-2- methanol-benzimidazole derivatives (series 6)

The results of the five compounds tested from the of series *N*–methyl-2-methanolbenzimidazole derivatives showed activity for three compound, FAS41, FAS42, and FAS50 against *Mucor circinelloides* RCMB 07328 with MICs in the range of 0.98 - 0.12-µg/ml, which were more potent than the control drug by two, four, and five folds respectively. The antifungal activity of these compounds against the rest of the selected strains was in the range of ≤ 500 to 0.12 µg/ml (Table 4.33). FAS42 and FAS50 were the most active compounds against; *Syncephalastrum racemosum* RCMB 05922, and *Candida krusei* RCMB 05051 with MICs in the range of 0.98 - 0.12µg/ml, which were more active than the amphotericin B by one, two, and three folds potent or equivalent.

4.3.2.6 MIC results of the 2-chloromethylbenzimidazole derivatives (series 7)

The results for the four compounds tested from the of series 2-chloromethylbenzimidazole derivatives showed activity for two of them, FAS44 and FAS49, with MICs of 1.95 µg/ml against *Mucor circinelloides* RCMB 07328, which is one fold potent than the amphotericin B. Moreover, the MICs for those compounds were more potent than the control drug by three to four folds against *Candida parapsilosis* RCMB 05065, wheras FAS48 was as potent as the control drug against the selected strain.The antifungal activity of these compounds against the rest of the selected strains was in the range of ≤ 500 to 0.12- µg/ml (Table 4.34).

Table 4.33 MIC results for the *N*-methyl-2- methanolbenzimidazole derivatives

Fungal	MIC µg/ml					
	FAS36	**FAS39**	**FAS41**	**FAS42**	**FAS50**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	500	62.5	1.95	1.95	0.98	0.06
Aspergillus clavatus RCMB 02593	125	500	3.9	0.49	0.24	0.12
Mucor circinelloides RCMB 07328	31.25	62.5	0.98	0.24	0.12	3.9
Absidia corymbifera RCMB 09635	7.8	15.63	0.98	>500	>500	0.98
Penicillium marneffei RCMB 01267	15.63	3.9	1.95	0.98	0.49	0.49
Syncephalastrum racemosum RCMB 05922	>500	>500	>500	0.24	0.12	0.98
Candida albicans RCMB 05035	31.25	15.63	0.98	0.98	0.98	0.12
Candida tropicalis RCMB 05049	31.25	7.8	0.49	0.49	0.12	0.015
Candida krusei RCMB 05051	>500	>500	>500	0.98	0.49	0.98
Candida parapsilosis RCMB 05065	15.63	3.9	15.63	7.8	7.8	1.95

Table 4.34 MIC results for the 2-chloromethylbenzimidazole derivatives

Fungal	MIC µg/ml					
	FAS43	**FAS44**	**FAS45**	**FAS48**	**FAS49**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	125	7.8	>500	62.5	0.49	0.06
Aspergillus clavatus RCMB 02593	62.5	31.25	>500	31.25	0.98	0.12
Mucor circinelloides RCMB 07328	31.25	1.95	>500	62.5	1.95	3.9
Absidia corymbifera RCMB 09635	3.9	1.95	>500	7.8	0.98	0.98
Penicillium marneffei RCMB 01267	1.95	3.9	>500	1.95	0.98	0.49
Syncephalastrum racemosum RCMB 05922	>500	>500	>500	>500	1.95	0.98
Candida albicans RCMB 05035	31.25	1.95	>500	7.8	0.24	0.12
Candida tropicalis RCMB 05049	15.63	0.49	>500	3.9	0.12	0.015
Candida krusei RCMB 05051	>500	>500	>500	>500	1.95	0.98
Candida parapsilosis RCMB 05065	7.8	0.24	>500	1.95	0.12	1.95

MICs < MICs of Amphotericin B are highlighted

4.3.2.7 MIC results of the 2-ethanebenzimidazole derivatives (series 48)

The results of the single compound tested from the series of 2-ethanebenzimidazole derivatives showed activity for this compound, FAS54 against *Mucor circinelloides* RCMB 07328, *Syncephalastrum racemosum* RCMB 05922, and *Candida parapsilosis* RCMB 05065, with MICs of 0.12, 0.24, and 1.95 µg/ml respectively, which is five, two, and equivalent folds potent than the amphotericin B.The antifungal activity of this compound against the rest of the selected strains was in the range of 500 - 0.12 µg/ml (Table 4.35)

Table 4.35 MIC results of the 2-ethanebenzimidazole derivative

Fungal	MIC µg/ml	
	FAS54	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	0.49	0.06
Aspergillus clavatus RCMB 02593	0.24	0.12
Mucor circinelloides RCMB 07328	0.12	3.9
Absidia corymbifera RCMB 09635	>500	0.98
Penicillium marneffei RCMB 01267	1.95	0.49
Syncephalastrum racemosum RCMB 05922	0.24	0.98
Candida albicans RCMB 05035	0.24	0.12
Candida tropicalis RCMB 05049	1.95	0.015
Candida krusei RCMB 05051	1.95	0.98
Candida parapsilosis RCMB 05065	1.95	1.95

MICs < MICs of Amphotericin B are highlighted

4.3.2.8 MIC results of the silver complexes (series 13)

The results of the two compounds tested from the series of silver complexes showed activity for FAS70 and FAS72 with MICs of 0.98, and 0.49 µg/ml respectively, against *Syncephalastrum racemosum* RCMB 05922, which were equal to the control drug or more potent by one fold.The antifungal activity of these compounds against the rest of the selected strains was in the range of 500 - 0.49 µg/ml (Table 4.36).

Table 4.36 MIC results of the silver complexes

Fungal	MIC µg/ml		
	FAS70	**FAS72**	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	3.9	1.95	0.06
Aspergillus clavatus RCMB 02593	1.95	0.98	0.12
Mucor circinelloides RCMB 07328	15.63	7.8	3.9
Absidia corymbifera RCMB 09635	>500	62.5	0.98
Penicillium marneffei RCMB 01267	3.9	62.5	0.49
Syncephalastrum racemosum RCMB 05922	0.98	0.49	0.98
Candida albicans RCMB 05035	7.8	1.95	0.12
Candida tropicalis RCMB 05049	1.95	0.49	0.015
Candida krusei RCMB 05051	7.8	>500	0.98
Candida parapsilosis RCMB 05065	31.25	7.8	1.95

4.3.2.9 MIC results of the 2-methanthiolbenzimidazole derivatives (series 9)

The results of the only compound tested from the of series 2-methanthiolbenzimidazole derivatives showed activity for this compound, FAS56 with low MICs of 1.95 µg/ml against *Mucor circinelloides* RCMB 07328, which is one fold more potent than the amphotericin B. Furthermore, the compound showed good activity against *Syncephalastrum racemosum* RCMB 05922 with an MIC of 0.98 µg/ml, which is equal to the MIC of the control drug .The antifungal activity of this compound against the rest of the selected strains was on the range of 500 - 0.98 µg/ml (Table 4.37).

4.3.2.10 MIC results of the *N*-oxidebenzimdazole derivatives (series 10)

The results of the only compound tested from the series of *N*-oxidebenzimdazole derivatives showed activity for FAS60 against some of the filamentous strains, with

MICs of 0.03, 0.24, and 0.12 µg/ml; *Mucor circinelloides* RCMB 07328, *Penicillium marneffei* RCMB 01267, and *Syncephalastrum racemosum* RCMB 05922 respectively. As a result of this, FAS60 is seven, three, and one folds more potent than the amphotericin B. Furthermore, the compound recorded good activity against two strains of the unicellular fungi; *Candida krusei* RCMB 05051, *Candida parapsilosis* RCMB 05065, with MICs more potent than the control drug by two, and one folds (MICs of 0.24, and 0.98 µg/ml, respectively). The antifungal activity of this compound against the rest of the selected strains was on the range of 500 - 0.03 µg/ml (Table 4.38).

Table 4.37 MIC results of the 2-methanthiolbenzimidazole derivative

Fungal	MIC µg/ml	
	FAS56	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	7.8	0.06
Aspergillus clavatus RCMB 02593	3.9	0.12
Mucor circinelloides RCMB 07328	1.95	3.9
Absidia corymbifera RCMB 09635	>500	0.98
Penicillium marneffei RCMB 01267	31.25	0.49
Syncephalastrum racemosum RCMB 05922	0.98	0.98
Candida albicans RCMB 05035	31.25	0.12
Candida tropicalis RCMB 05049	62.5	0.015
Candida krusei RCMB 05051	15.63	0.98
Candida parapsilosis RCMB 05065	125	1.95

Table 4.38 MIC results of the *N*-oxidebenzimdazole derivative

Fungal	MIC µg/ml	
	FAS60	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	0.49	0.06
Aspergillus clavatus RCMB 02593	1.95	0.12
Mucor circinelloides RCMB 07328	0.03	3.9
Absidia corymbifera RCMB 09635	>500	0.98
Penicillium marneffei RCMB 01267	0.24	0.49
Syncephalastrum racemosum RCMB 05922	0.12	0.98
Candida albicans RCMB 05035	0.49	0.12
Candida tropicalis RCMB 05049	0.49	0.015
Candida krusei RCMB 05051	0.24	0.98
Candida parapsilosis RCMB 05065	0.98	1.95

MICs < MICs of Amphotericin B are highlighted

4.3.2.11 MIC result of the benzoxazole derivative (series 11)

The results of the only single compound tested from the series of benzoxazole derivatives showed activity against *Mucor circinelloides* RCMB 07328, and *Syncephalastrum racemosum* RCMB 05922 respectively for FAS63 with low MICs of 0.49 and 0.12 µg/ml,. As a result of this, FAS63 appears three folds more potent than the Amphotericin B. Moreover, the most activity recorded against unicellular fungi selected for this study was against *Candida parapsilosis* RCMB 05065, with MIC equivalent to the MIC of the control drug. The antifungal activity of this compound against the rest of the selected strains was in the range of 500 - 0.12 µg/ml (Table 4.39).

Table 4.39 MIC results of the benzoxazole derivative

Fungal	MIC µg/ml	
	FAS63	**Amphotericin B**
Aspergillus fumigatus RCMB 02564	1.95	0.06
Aspergillus clavatus RCMB 02593	0.98	0.12
Mucor circinelloides RCMB 07328	0.49	3.9
Absidia corymbifera RCMB 09635	>500	0.98
Penicillium marneffei RCMB 01267	1.95	0.49
Syncephalastrum racemosum RCMB 05922	0.12	0.98
Candida albicans RCMB 05035	0.49	0.12
Candida tropicalis RCMB 05049	0.98	0.015
Candida krusei RCMB 05051	1.95	0.98
Candida parapsilosis RCMB 05065	1.95	1.95

MICs < MICs of Amphotericin B are highlighted

4.3.2.12 Conclusion

The microdilution tests of the most active 41 compounds of the 65 compounds revealed that; FAS18 and FAS20 had some antifungal activity against *Aspergillus fumigatus* RCMB 02564 with MIC equal of the control (0.06 µg/ml). Three compounds showed good antifungal activity against *Aspergillus clavatus* RCMB

02593, with MIC range 0.06-0.12 µg/ml. The rest of the compounds were unlike this and therefore were not better than the amphotericin B (control drug). 18 compounds had activity against *Mucor circinelloides* RCMB 07328 with MIC range 3.9 - 0.06µg/ml. Nine compounds showed good activity against *Absidia corymbifera* RCMB 09635 with MIC in range 0.98-0.03 µg/ml. Five compounds showed good activity against *Penicillium marneffei* RCMB 01267 with MIC in range 0.49-0.03 µg/ml. 13 compounds showed good activity against *Penicillium marneffei* RCMB 01267 with MICs in the range of 0.98 - 0.12µg/ml. only two compounds, FAS18 and FAS20 showed good activity against *Candida albicans* RCMB 05035 with MIC 0.03 µg/ml equivalent to the MIC of the control drug. Moreover, FAS18 and FAS19 are the only two compounds that showed good activity against *Candida tropicalis* RCMB 05049 with MICs of 0.015 and 0.007 µg/ml respectively. Six compounds showed good activity against *Candida krusei* RCMB 05051 with MIC of 0.98 µg/ml which equal, to the MIC of the control drug. 20 compounds showed good activity against *Candida parapsilosis* RCMB 05065 with MIC in range 0.98 - 0.003µg/ml.

5 DISCUSSION AND FUTURE WORK

5.1 Discussion

A total of 70 compounds were synthesised belonging to three different classes; these include 66 benzimidazoles, three benzoxazoles, and one benzothiazole derivative. These compounds were prepared according to literature procedures or by adapting conventional methods (Phillips, 1928, Podunavac-Kuzmanovic *et al.*, 2004, Meyers *et al.*, 2005, Harisha *et al.*, 2009). The latter two classes were included to help evaluate the importance of the benzimidazole framework. The compounds were screened to see if they had any antibacterial or antifungal activities. Unfortunately, not all of the compounds synthesised could be used in the biological studies. One compound; FAS 14, which could not be purified to acceptable standards was excluded. In addition, two of the compounds (FAS67 and FAS68), which were synthesised and isolated in extremely low yield from a mixture containing many by-products, proved to be insufficient for the biological evaluation.

From the literature review (Table 2.4), it can be seen that eleven compounds are novel (have not been previously synthesised) and some compounds were found to have incomplete analytical data. The majority of compounds (62) were evaluated for their antibacterial and antifungal properties for first time in this project and the results are considered to be novel.

5.1.1 Factors affecting the yield

Figure 5.1 displays the range of yields obtained for the benzimidazole derivatives. The yield of these compounds was affected by several factors such as difficulties in purification where the R_f of the desired products were too close to the R_f of the impurities and some starting materials were reluctant to undergo cyclisation.

One of the most difficult series to synthesise was the *N*-bromoalkyl-2-chloromethyl-benzimidazole derivatives, where the R_f of the desired product was very close to the R_f of the other by-products. Therefore, multi runs of column chromatography were performed in attempts to isolate the desired product. However, this resulted in very poor yield (3-10%). Additionally, recrystallisation did not resolve the issue as there was no selectivity between the desired product and the by-products.

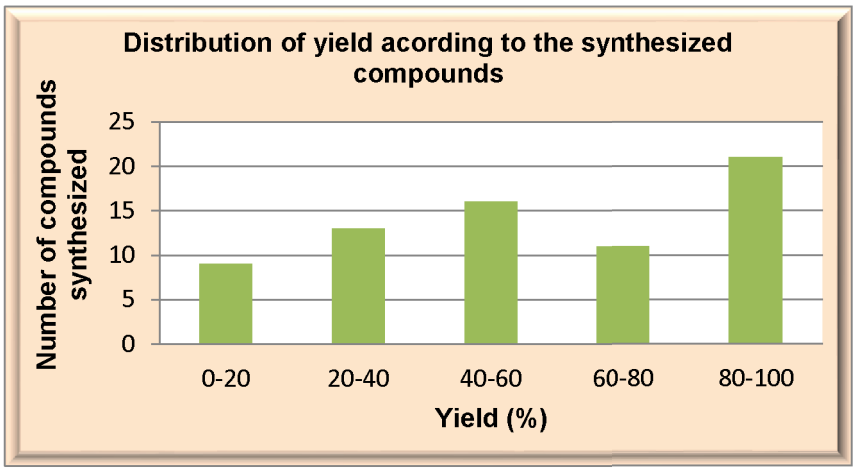


Figure 5.1 Distribution of yield according to the number of compounds

Purification was especially difficult for the methylation reaction at *N*-1 of the 5-substituted-2-methanolbenzimidazole derivatives FAS22-28. The reaction yielded a mixture of the desired product and the unreacted starting materials. The purification of the 5-substituted-*N*-methylbenzimidazole derivatives FAS36-42,50 required multiple column chromatography attempts (3-4 attempts) which gave the products in poor to satisfactorily yield range (9-53%).

Severe difficulties were encountered in the synthesis of the (1*H*-benzimidazole-2-yl)alkylamines as amino acids are extremely reluctant to undergo ring closure with 1,2-phenylenediamines to produce the corresponding benzimidazoles, and this is reflected in the reaction times which were extremely long (e.g. 336 hours).

Furthermore, some early literature reports support this by stating that no reaction was observed even with the extended reaction times, and concluded that the reaction was not feasible (Hughes and Lions, 1938). However, other researchers have found that the reaction with amino acids, where the amino group is at the β-carbon had been proved to be more reactive than when the amino group is on the α-carbon. Moreover, increasing the substituents on the α-carbon of the amino acid also reduced the reactivity of the amino acids and, as expected, glycine was more reactive than alanine (Cescon and Day, 1962). For example, (*S*)-1-(5-nitro-1*H*-benzoimidazol-2yl)ethanamine FAS14, after 336 hours reaction time still contained 50% starting material (see section **3.3.3**).

In addition, the yields were influenced by the reactivity of the 4-substituted 1,2-phenylenediames. This is dictated by the nature of the substituent. The effect of the substituent in position 4 of 1,2-phenylenediamine could either enhance or diminish the reaction rate. From the proposed mechanism for the formation of these derivatives, it can be seen that the reaction starts with the nucleophilic attack by the electron pair of the nitrogen of an amino group on the 1,2-phenylenediamine. When there is no substituent at position 4, or there is a donating substituent, the reaction tends to be faster and the yield higher. In contrast, when the substituent is an electron withdrawing type, the reaction tends to be slower and the yield is lower. This phenomenon could be explained by the engagement of the electron pair in the resonance of the aromatic ring and is observed when an electron withdrawing group, such as the nitro group, is involved. So the existence of the nitro group removes the electrons from the aromatic ring by π-π resonance and hinders the reaction. Therefore the electron pair of the amino group will be engaged in this resonance which reduces its reactivity (Figure 5.2).

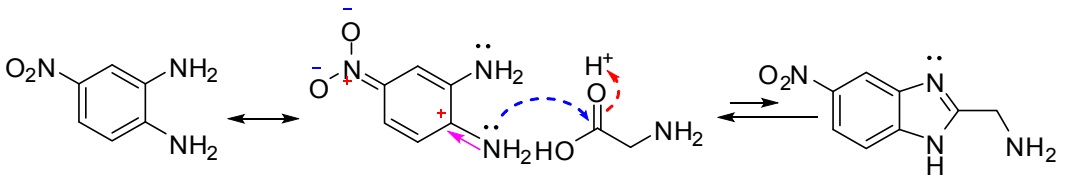


Figure 5.2 Resonance structures of 4-nitro-1,2-phenylenediamine

Excellent yields were obtained for 21 compounds (80-100%) and good yields were obtained for 11 compounds (60-80%). Only eight compounds were isolated in yields ≤ 20% (Figure 5.1).

5.1.2 Attempts to improve the yield

Researchers have used several strategies to improve the reaction and the yield of benzimidazole derivatives. In this project one method was chosen which had the possibility to enhance the synthesis of the desired product. This method involved the use of ionic liquids as solvents. The ionic liquids are fluid at ambient temperature, have low viscosity and are easily handled (Sheldon, 2001). The reactions in ionic liquids are often quicker than in conventional organic solvents, and they are considered as green solvents because of their lack of vapor pressure (Martyn and Seddon, 2000). There is also the possibility of recycling the ionic liquid after its use in the reaction (Hu, 2006). An example of the advantage of using ionic liquids is the multistep synthesis of the benzimidazole linked pyrrolo[1,2-a]benzimidazolones, pyrido[1,2-a]benzimidazolones and isoindolo[1,2-a]benzimidazoles. These reactions could also be conducted under microwave conditions, in a short time with high yields and this was supported by many studies (Sapkal *et al.*, 2009, Thummanagoti *et al.*, 2011). The products were also easy to isolate.

The Phillips method is well known as the reaction of 1,2-phenylenediamines with organic acids to give benzimidazoles in the presence of hydrochloric acid. The reaction produces the desired product in low yields (Wright, 1951). However, the imidazolium-based ionic liquid has been used in an attempt to improve the reaction (Martyn and Seddon, 2000, Thummanagoti *et al.*, 2011). Also, it had been reported that the Phillips method did not work for the synthesis of benzimidazoles from amino acids and 1,2-phenylenediamines, although Cescon and Day (1962) found that the method could be successful when the reaction period was greatly extended (Cescon and Day, 1962).

In the present work, improvement to the Phillips' method was also attempted by using a series of phosphonium-based ionic liquids (Bu_4P^+ Cl^-, Oct_4P^+ Br^-, *i*-Bu_3P^+Me OTs^-, Bu_3P^+Et Br^-, Bu_3P^+Oct Br^-, $^-$) at RT, 40ºC and 80ºC for 24h. The reaction at 80ºC gave the best yields which are displayed in Table 5.1. Bu_3P^+Oct Br was the only ionic liquid to give the product for glycine (29%) while two ionic liquids, *i*-Bu_3P^+Me OTs^- and Bu_3P^+Et Br^-, afforded the product in 37% and 51% yield respectively. Not surprisingly, the results with β-alanine were more promising; three ionic liquids yielded the product in yields of 13%, 37% and 75%. The highest yield was obtained in Bu_4P^+ Cl^-.

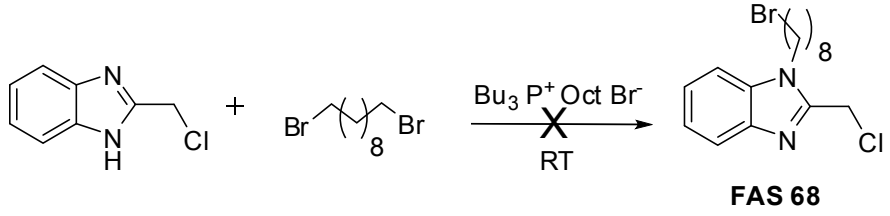
Additionally, microwave-mediated synthesis in the ionic liquids (after just 5 minutes reaction at 80ºC) was also successful. The reactions with gycine and β-alanine gave the desired benzimidazoles in 27% and 25% yield in Bu_3P^+Oct Br and Oct_4P^+ Br^- respectively. The conventional Phillips method for glycine resulted in 89% yield after heating under reflux for 13 days, followed by extremely slow evaporation over several days. The benzimidazole derivative from alanine was obtained after 11 days in 67% Yield. β-Alanine required eight days reaction time and gave 66% yield (Donkor, 2007).

The results obtained are very encouraging as there are currently no alternatives to the Phillips method for the construction of benzimidazoles from amino acids. However, it is difficult at this stage to understand why some ionic liquids gave the product and others were unsuccessful. Alternative reaction conditions using different ionic liquids (varying the anions and cations), including the use of the imidazolium-based ionic liquids need to be considered in future modifications. In addition, the application of ultrasound at different temperatures could be investigated.

Table 5.1 Reactions conducted in ionic liquids and in a microwave

Entry	Ionic liquid	Amino acid	Microwave method yield (%)	Conventional method yield (%)
1	$Bu_4P^+Cl^-$	glycine	0	0
2	$Oct_4P^+Br^-$	glycine	0	0
3	*i*-$Bu_3P^+Me\ OTs^-$	glycine	0	0
4	$Bu_3P^+Et\ Br$	glycine	0	0
5	**$Bu_3P^+Oct\ Br$**	**glycine**	**27**	**29**
6	$Bu_4P^+Cl^-$	alanine	0	0
7	$Oct_4P^+\ Br^-$	alanine	0	0
8	***i*-$Bu_3P^+Me\ OTs^-$**	**alanine**	**0**	**37**
9	$Bu_3P^+\ Et\ Br$	alanine	0	51
10	$Bu_3P^+Oct\ Br$	alanine	0	0
11	**$Bu_4P^+\ Cl^-$**	**β-alanine**	**0**	**75**
12	**$Oct_4P^+\ Br^-$**	**β-alanine**	**25**	**39**
13	***i*-$Bu_3P^+Me\ OTs^-$**	**β-alanine**	**0**	**13**
14	$Bu_3P^+Et\ Br$	β-alanine	0	0
15	$Bu_3P^+Oct\ Br$	β-alanine	0	0

The ionic liquid, $Bu_3P^+Oct\ Br^-$, was used specifically in the synthesis of *N*-bromodecyl-2-chloromethyl-benzimidazole FAS68 (Figure 5.3), but even with the extended reaction times, no product was detected (see section 3.7).



FAS 68

Figure 5.3 Synthesis of *N*-bromodecyl-2-chloromethylbenzimidazole FAS75

5.2 Methodology for biological evaluation

Sections **2.4.1** and **2.4.2** describe the methods which were used in this project to assess the antibacterial and antifungal activity of the compounds. However, the experiments were done *in vitro*, and the results could be different if they are tested *in vivo*. This depends on the factors that must be taken into account, such as absorption of the drug or the level of the toxicity.

Overall, 67 benzimidazole derivatives were screened for their biological activity against a set of reference bacteria and fungi by using a disc (well) diffusion and MICs test (see section 2.4.3) (Table 2.4).

The following methods were used for screening the biological activity of the compounds: The first method used was the disc (well) diffusion test, which measures the diameter of the zone of inhibition of a compound. This can vary and depends on many factors, including local moisture levels, solubility, diffusibility, stability of the compound, and stability of concentration gradients that can form across the surface of agar. Moisturising can affect the inhibition zone by sometimes showing a margined zone. In this method, it was hard to pick a single amount to help in studying the activity of the compounds against the selected strains. If only 100 µg had been used on the disc for studying the antibacterial activity, this could have displayed no activity for some compounds. Therefore, three different amounts (10, 100, and 200 µg) were selected to study any activity of the compounds. Furthermore, there is a need to be careful in screening the compounds at fix concentration as this could have resulted with a distorted set of data due to the occurrence of Eagle's phenomena. Sometimes this rare situation is observed when the antibiotics effect is paradox lower at higher concentration of the drug, whereas the drug is more active against the selected strains at lower concentration (Eagle and Musselman, 1948). Moreover, more than one level of concentration was required for this study which gave a broad range of activity, and it would also give a

reliable investigation to determine the MICs of each compound. So, for all above mentioned reasons, a three fixed concentrations approach was selected for the disc diffusion assay. In addition to this, for economic reasons this approach was selected instead of using one concentration which may have led to many tests on the compound at different concentration levels. Furthermore, reproducibly, quick, and easy means for screening compounds for any inhibitory results of replicate tests were generally in good agreement with each other. The second method used was the agar dilution test and broth dilution test, which provides a convenient method for determining minimum inhibitory concentration (MICs) for a variety of antimicrobial agents as a quantitative measurement of antimicrobial sensitivity. Both methods gave good results and were easy to implement, but the MIC test was better in identifying the level of resistance in less sensitive strains (Bala *et al.*, 2005, Erfani *et al.*, 2011).

In conclusion, five derivatives showed good activity against some of the Gram +ve and Gram –ve strains, and especially against the ciprofloxacin resistant strains (see Table 4.9). For the antifungal studies, 26 compounds were as active as amphotericin B, or more potent against some of the selected fungi (see Table 4.28).

5.3 Antimicrobial activity in relation to molarity

In the previous section, the activity of different compounds was compared on the basis of their zone diameters or MIC values recorded as µg/ml. However, such comparisons are by necessity over simplifying the situation because they are based on the same weight of each compound being used in the assay. This does not necessarily mean that the same amount i.e. number of moles was present, because some of the compounds have widely different molecular weights.

In the case of the antibacterial screening, the compounds tested were inoculated on the discs in 10 µg, 100 µg, and 200 µg, which vary in what amounts are present. So,

when the amount of the compound increases on the disc, the molarity increases too, and this is reflected on the amount of activity recorded against the selected strains, which also increases. None of the compounds tested in this study showed appreciable activity against the selected Gram –ve strains, except some compounds which revealed activity against *Burkholderia cepacia* NCTC 10744, and only FAS42 (series 6) was active against *Escherichia coli* NCTC 10418, *Serratia marcescens* NCTC 1377 (Table 5.2).

While the molecular weights for compounds FAS39 (series 6) and FAS42 (series 6) (FAS39 is 210.66 g/mol and FAS42 is 192.21 g/mol) (Table 5.2), are not very different, the compounds behave differently against the Gram –ve strains. Their biological properties must arise from the different groups on the benzimidazole which affects their movement through the cell wall. As mentioned earlier (section **1.1.1**), the cell wall structure of the Gram –ve strains have a thin layer of peptidoglycan and another outer membrane which functions as a permeability barrier.

The most potent compound against Gram +ve species were two compounds from series 2 (FAS11 (5-Cl), FAS12 (5-Br), one compound from series 6 FAS42 (5-OMe), one compound from series 7, FAS47 (5-Br), one compound from series 9, FAS56 (5-H), and one compound from series 11, FAS61 (5-H). However, the most potent compounds against Gram –ve species were one compound from series 2 FAS12 (5-Br), one compound from series 6, FAS42 (5-OMe), one compound from series 7, FAS47 (5-Br), and one compound from series 9, FAS56 (5-H), So compounds FAS12 (series 2), FAS42 (series 6), FAS 47 (series 7) and FAS56 (series 9) were the most active compounds against both Gram +ve and Gram –ve species.

In the case of antifungal screening, the compounds were inoculated on the discs in 250 µg amounts. Most of the compounds tested were active against all the filamentous strains, although the activity against *Absidia corymbifera* RCMB 09635, *Syncephalastrum racemosum* RCMB 05922, *Mucor circinelloides* RCMB 07328,

and *Aspergillus clavatus RCMB 02593,* was not at the same level to the other filamentous strains (Table 5.3).

The activity of the compounds against the unicellular strains selected for this study was superior against two strains; *Candida albicans* RCMB 05035 and *Candida tropicalis* RCMB 05049. Moreover, not all the compounds tested were active against *Candida parapsilosis* RCMB 05065. In addition to this, only 25 compounds were active against *Candida krusei* RCMB 05051. The differences observed in activity against the filamentous and the unicellular strains could be explained by the difference type of cell structure of each strain (section 1.1.2).

Table 5.2 Summary of the relationship of activity of most active compound tested with their molarity against the bacterial strains

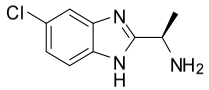
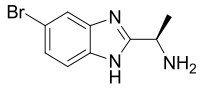
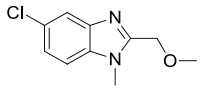
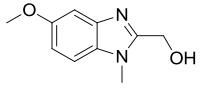
Series	Code	Structure	Mw g/mol	Molarity in 10 µg disc(mol/ml)	Molarity in 100 µg disc(mol/ml)	Molarity in 200 µg disc(mol /ml)	Gram +ve	Gram -ve
Series 2: 2-ethanamine benzimidazole	FAS11		195.65	0.00005	0.0005	0.001	➢ Active against three species of *Staphylococcus* at 0.001 moles (Table 4.1).	NA
Series 2: 2-ethanamine benzimidazole	FAS12		240.10	0.00004	0.0004	0.0008	➢ Active against three species of MRSA at 0.00004 moles. ➢ Most activity recorded at 0.0004 moles (Table 4.1).	➢ Active against *B. cepacia* NCTC 10744 at 0.0004 moles (Table 4.1).
Series 6: *N*-methyl-2-methanol-benzimidazole derivatives	FAS39		210.66	0.000047	0.00047	0.0009	NA	NA
Series 6: *N*-methyl-2-methanol-benzimidazole derivatives	FAS42		192.21	0.00005	0.0005	0.001	➢ Active against multiple strains at 0.0005 and 0.001 moles with zone of inhibition of 12-17 mm (Table 4.4).	➢ At 0.0005 moles only Active against *B.cepacia* NCTC 10744 (Table 4.4). ➢ At 0.001 moles active against *E. coli* NCTC 10418, *S. marcescens* NCTC 1377, and *B. cepacia* NCTC 10744 (Table 4.4).

Table 5.2 (continued

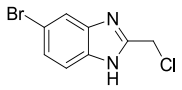
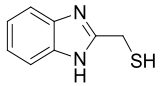
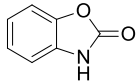
Series	Code	Structure	Mw g/mol	Molarity in 10 µg disc(mol/ml)	Molarity in 100 µg disc(mol/ml)	Molarity in 200 µg disc(mol /ml)	Gram +ve	Gram -ve
Series 7: 2-chloromethylbenzimidazole derivatives	FAS47		245.50	0.00004	0.0004	0.0008	➢ Active at 0.0004 moles against two species of MRSA (Table 4.5). ➢ Active against some of Gram + ve strains at 0.0008 moles with zone of inhibition 13-15 mm (Table 4.5).	➢ *Active at 0.004 and 0.0008 moles against B. cepacia* NCTC 10744 (Table 4.5).
Series 9: 2-methanethiolbenzimidazole derivatives	FAS56		164.23	0.00006	0.0006	0.0012	➢ Active against multiple species at 0.0006 and 0.0012 moles (Table 4.5).	➢ *Active at* 0.0006 and 0.0012 moles *against B.cepacia* NCTC 10744 (Table 4.5).
Series 11: benzoxazole derivatives	FAS61		135.12	0.00007	0.0007	0.0014	➢ Active against *S. aureus* (Oxford) NCTC 6571, at 0.00007 moles (Table 4.7).	NA

Table 5.3 Summary of the relationship of activity of most active compound tested with their molarity against the fungal strains

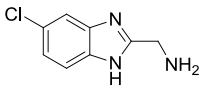
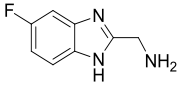
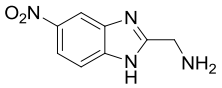
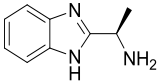
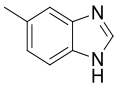
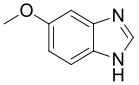
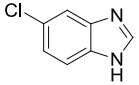
Series	Code	Structure	Mwt g/mol	Molarity in 250 µg disc(mole/ml)	Unicellular	Filamentous
Series 1: 2-aminomethylbenzimidazole derivatives	FAS4		181.62	0.0013	➢ Active against all species except agaisnst *C. krusei* RCMB 05051 (Table 4.16).	➢ Active against all species except agaisnst *A. corymbifera* RCMB 09635 (Table 4.16).
Series 1: 2-aminomethylbenzimidazole derivatives	FAS6		165.17	0.0015	➢ Active against all species except agaisnst *C. krusei* RCMB 05051 (Table 4.16).	➢ Active against all species except agaisnst *P.marneffei* RCMB 01267 (Table 4.16).
Series 1: 2-aminomethylbenzimidazole derivatives	FAS7		192.17	0.0013	➢ Active against all strains.	➢ Active against all strains.
Series 2: 2-ethanamine benzimidazole derivatives	FAS8		161.20	0.0015	➢ Active against all species except agaisnst *C. krusei* RCMB 05051 (Table 4.16).	➢ Active against all species except agaisnst *A. corymbifera* RCMB 09635 (Table 4.16).
Series 3: 5-substituted benzimidazole derivatives	FAS16		132.16	0.0018	➢ Active against all strains (Table 4.17).	➢ Active against all strains (Table 4.17).
Series 3: 5-substituted benzimidazole derivatives	FAS17		148.16	0.0016	➢ Active against all strains (Table 4.17).	➢ Active against all strains (Table 4.17).
Series 3: 5-substituted benzimidazole derivatives	FAS18		152.58	0.0016	➢ Active against all strains (Table 4.17).	➢ Active against all strains (Table 4.17).

Table 5.3 (continued

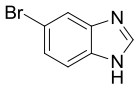
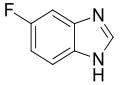
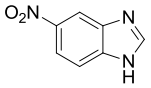
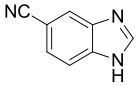
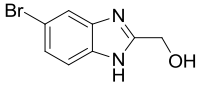
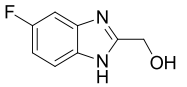
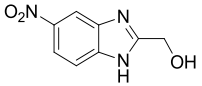
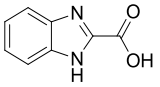
Series	Code	Structure	Mwt g/mol	Molarity in 250 µg disc(mole/ml)	Unicellular	Filamentous
Series 3: 5-substituted benzimidazole derivatives	FAS19		197.03	0.0012	➢ Active against all strains (Table 4.17).	➢ Active against all strains (Table 4.17).
Series 3: 5-substituted benzimidazole derivatives	FAS20		136.13	0.0018	➢ Active against all strains (Table 4.17).	➢ Active against all strains (Table 4.17).
Series 3: 5-substituted benzimidazole derivatives	FAS21		163.13	0.0015	➢ Active against all species except against *C. krusei* RCMB 05051 (Table 4.17).	➢ Active against all species except against *A. corymbifera* RCMB 09635 and *S. racemosum* RCMB 05922 (Table 4.17).
Series 3: 5-substituted benzimidazole derivatives	FAS51		143.15	0.0017	➢ Active against all strains (Table 4.17).	➢ Active against all species except against *A.corymbifera* RCMB 09635 (Table 4.17).
Series 4: 2-Methanol-benzimidazole derivatives	FAS26		227.06	0.0011	➢ Active against all strains (Table 4.18).	➢ Active against all species except against *M. circinelloides* RCMB 07328 (Table 4.18).
Series 4: 2-Methanol-benzimidazole derivatives	FAS27		166.15	0.0015	➢ Active against all species except against *C. krusei* RCMB 05051 (Table 4.18).	➢ Active against all species except against *A.clavatus RCMB 02593* and *S. racemosum* RCMB 05922 (Table 4.18).
Series 4: 2-Methanol-benzimidazole derivatives	FAS28		193.16	0.0012	➢ Active against all species except against *C. krusei* RCMB 05051 (Table 4.18).	➢ Active against all species except against *A. clavatus RCMB 02593* and *S. racemosum* RCMB 05922 (Table 4.18).
Series 5: 2-carboxylic acid benzimidazole derivatives	FAS29		162.15	0.0015	➢ Active against *C. albicans* RCMB 05035 and *C. tropicalis* RCMB 05049 (Table 4.19).	NA

Table 5.3 (continued

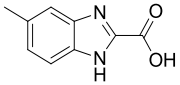
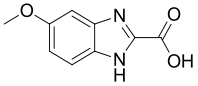
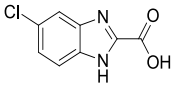
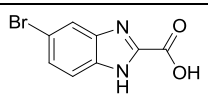
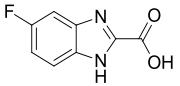
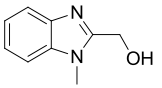
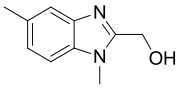
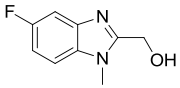
Series	Code	Structure	Mwt g/mol	Molarity in 250 µg disc(mole/ml)	Unicellular	Filamentous
Series 5: 2-carboxylic acid benzimidazole derivatives	FAS30		176.17	0.0014	➢ Active against all species except against *C. krusei* RCMB 05051 (Table 4.19).	➢ Active against *A. fumigatus* RCMB 02564(Table 4.19).
Series 5: 2-carboxylic acid benzimidazole derivatives	FAS31		192.17	0.0013	➢ Active against all species except against *C. krusei* RCMB 05051 (Table 4.19).	➢ Active against *A. fumigatus* RCMB 02564 and *A. corymbifera* RCMB 09635 (Table 4.19).
Series 5: 2-carboxylic acid benzimidazole derivatives	FAS32		196.59	0.0012	NA	NA
Series 5: 2-carboxylic acid benzimidazole derivatives	FAS33		241.04	0.001	➢ Active against all species except against *C. krusei* RCMB 05051 (Table 4.19).	➢ Active against *A. fumigatus* RCMB 02564 and *A.corymbifera* RCMB 09635 (Table 4.19).
Series 5: 2-carboxylic acid benzimidazole derivatives	FAS34		180.14	0.0013	➢ Active against all species except against *C.krusei* RCMB 05051 and *C. parapsilosis* RCMB 05065 (Table 4.19).	➢ Active against all species except against *M. circinelloides* RCMB 07328, *P. marneffei* RCMB 01267, and *S. racemosum* RCMB 05922 (Table 4.19).
Series 6: N-methyl-2-methanol-benzimidazole derivatives	FAS36		162.19	0.0015	NA	➢ Active against *A.corymbifera* RCMB 09635 and *P. marneffei* RCMB 01267 (Table 4.20).
Series 6: N-methyl-2-methanol-benzimidazole derivatives	FAS37		176.22	0.0014	➢ Active against all species except against *C. krusei* RCMB 05051 and *C. parapsilosis* RCMB 05065 (Table 4.20).	➢ Active against *P. marneffei* RCMB 01267 (Table 4.20).
Series 6: N-methyl-2-methanol-benzimidazole derivatives	FAS41		180.18	0.0013	➢ Active against all species except against *C. krusei* RCMB 05051 (Table 4.20).	➢ Active against all species except against *S. racemosum* RCMB 05922 (Table 4.20).

Table 5.3 (continued

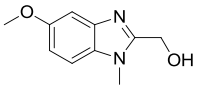
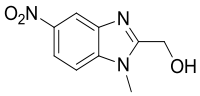
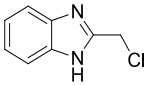
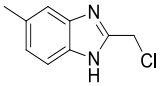
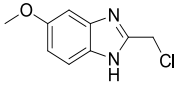
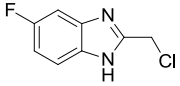
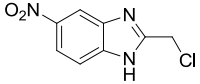
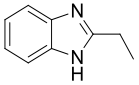
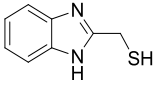
Series	Code	Structure	Mwt g/mol	Molarity in 250 µg disc(mole/ml)	Unicellular	Filamentous
Series 6: N-methyl-2-methanol-benzimidazole derivatives	FAS42		192.21	0.0013	➢ Active against all strains (Table 4.20).	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.20).
Series 6: N-methyl-2-methanol-benzimidazole derivatives	FAS50		207.19	0.0012	➢ Active against all strains (Table 4.20).	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.20).
Series 7: 2-Chloromethylbenzimidazole derivatives	FAS43		166.61	0.0015	➢ Active against *C. parapsilosis* RCMB 05065 (Table 4.21).	➢ Active against *A. corymbifera* RCMB 09635 and *P. marneffei* RCMB 01267 (Table 4.21).
Series 7: 2-Chloromethylbenzimidazole derivatives	FAS44		180.63	0.0013	➢ Active against all species except agaisnst *C. krusei* RCMB 05051 (Table 4.21).	➢ Active against all species except against *A. clavatus RCMB 02593* and *S. racemosum* RCMB 05922.
Series 7: 2-Chloromethylbenzimidazole derivatives	FAS45		196.63	0.0012	NA	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.21).
Series 7: 2-Chloromethylbenzimidazole derivatives	FAS48		184.60	0.0013	➢ Active against all species except against *C. krusei* RCMB 05051 (Table 4.21).	➢ Active against *Absidia corymbifera* RCMB 09635 and *P. marneffei* RCMB 01267 (Table 4.21).
Series 7: 2-Chloromethylbenzimidazole derivatives	FAS49		211.61	0.0011	➢ Active against all strains (Table 4.21).	➢ Active against all strains (Table 4.21).
Series 8: 2-ethylbenzimidazole derivatives	FAS54		146.19	0.0017	➢ Active against all strains (Table 4.22).	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.22).
Series 9:2-methanethiolbenzimidazole derivatives	FAS56		164.23	0.0015	➢ Active against all species except against *C. parapsilosis* RCMB 05065 (Table 4.22).	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.22).

Table 5.3 (continued

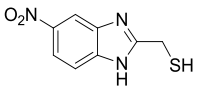
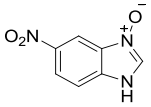
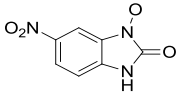
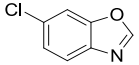
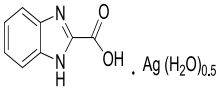
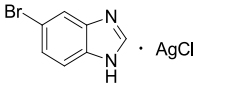
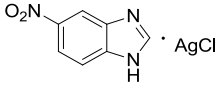
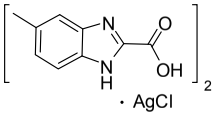
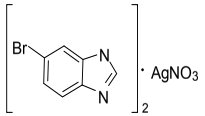
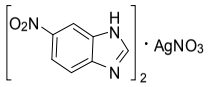
Series	Code	Structure	Mwt g/mol	Molarity in 250 µg disc(mole/ml)	Unicellular	Filamentous
Series 9:2-methanethiolbenzimidazole derivatives	FAS57		209.23	0.0011	NA	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.22).
Series 10: 1-oxide-benzimidazole derivatives	FAS59		195.13	0.0012	➢ Active against *C. tropicalis* RCMB 05049 and *C. krusei* RCMB 05051 (Table 4.25).	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.25).
Series 10: 1-oxide-benzimidazole derivatives	FAS60		195.13	0.0012	➢ Active against all strains (Table 4.25).	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.25).
Series 11: benzoxazole derivatives	FAS63		153.57	0.0016	➢ Active against all strains (Table 4.26).	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.26).
Series 13: silver complex of 2-carboxylic acid-benzimidazole derivatives	FAS69		279.02	0.0008	NA	➢ Active against all species except against *M. circinelloides* RCMB 07328 and *A. corymbifera* RCMB 09635 (Table 4.24).
Series 13: silver complex of 2-carboxylic acid-benzimidazole derivatives	FAS70		340.35	0.0007	➢ Active against all strains (Table 4.24).	➢ Active against all species except against *A. corymbifera* RCMB 09635 (Table 4.24).
Series 13: silver complex of 2-carboxylic acid-benzimidazole derivatives	FAS71		306.45	0.0008	➢ Active against all species except against *C. parapsilosis* RCMB 05065 (Table 4.24).	➢ Active against all species except against *A.corymbifera* RCMB 09635 (Table 4.24).
Series 13: silver complex of 2-carboxylic acid-benzimidazole derivatives	FAS72		465.76	0.0005	➢ Active against all species except against *C. krusei* RCMB 05051 (Table 4.24).	➢ Active against all strains (Table 4.24).

Table 5.3 (continued

Series	Code	Structure	Mwt g/mol	Molarity in 250 µg disc(mole/ml)	Unicellular	Filamentous
Series 13: silver complex of 2-carboxylic acid-benzimidazole derivatives	FAS73		561.92	0.0004	NA	➢ Active against *A. clavatus RCMB 02593, P. marneffei* RCMB 01267, and *S. racemosum* RCMB 05922, (Table 4.24).
Series 13: silver complex of 2-carboxylic acid-benzimidazole derivatives	FAS74		496.14	0.0005	➢ Active against *C. tropicalis* RCMB (Table 4.24).	➢ Active against all species except against *A. corymbifera* RCMB 09635 and *P. marneffei* RCMB 01267 (Table 4.21).

5.4 Structure Activity Relationships (SAR)

5.4.1 Effect of substituents on the structure

An investigation into the antimicrobial activity of the series of substituted benzimidazoles depends on the substituents attached to the bicyclic aromatic heterocycle. Therefore, in order to study the significance of the substituents and the SAR, i.e., the importance of the substituent attached at positions 1, 2, and 5, the effect of each substituent on the benzimidazole ring, is initially described (Table 5.4).

When the substituent at position-5 of the benzimidazole system is a methyl group, it will act as an activating group through the inductive effect (Table 2.4 and Table 5.4). Podunavac-Kuzmanovic and Cvetkovic (2007) observed that the unsubstituted analogue was more potent than the methyl analogues of the 2-aminobenzimidazoles (Figure 1.27). The methoxy group will also activate the aromatic system, and this is through resonance. The literature suggests that this results in increased antimicrobial activity. For example, the study by Sambanthamoorthy (2011) determined that 5-methoxy-2-[(4-methylbenzyl)sulfanyl]-1*H*-benzimidazole, Figure 5.4, inhibited the biofilm formation by multiple bacterial pathogens (Sambanthamoorthy *et al.*, 2011). Furthermore, the study by Vidaillac *et al.* (2007) developed some analogues of omeprazole in restoring the bactericidal activity of norfloxacin over a prolonged period and concluded that the compound, shown in Figure 1.30, was more effective than omeprazole. They also found that when the methoxy substituent was present on either the benzimidazole or the pyrrolo[1,2-*a*]quinoxaline nucleus, the compounds were more potent than the unsubstituted compounds (Vidaillac *et al.*, 2007).

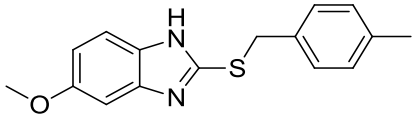


Figure 5.4 Structure of 5-methoxy-2-[(4-methylbenzyl)sulfanyl]-1*H*-benzimidazole

(Sambanthamoorthy *et al.*, 2011)

When the halogen substituent is present, overall it acts as a deactivating group (Table 5.4).

When the halogen substituent is chloro or bromo, this has the potential of significantly improving the biological activity. Also, increasing the acidity of the compound could increase the biological activity. Tavman's study (2009) showed that the chloro derivative of 2-(5-substituted-1*H*-benzo[*d*]imidazol-2-yl)-6-methoxyphenol (Figure 5.5) was the most active compound against selected strains; *Staphylococcus epidermidis* and *Staphylococcus aureus* (Tavman *et al.*, 2009). However, they did not study the fluoro and bromo analogues. Some benzimidazole ribonucleoside derivatives possessing dichloro substituents (Figure 1.24) appear to be more biologically stable and orally active (Stedman and Barclay, 2000)

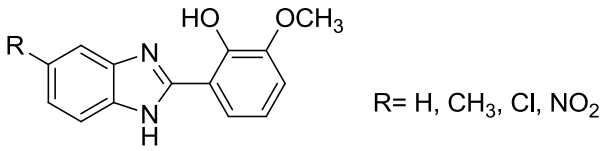
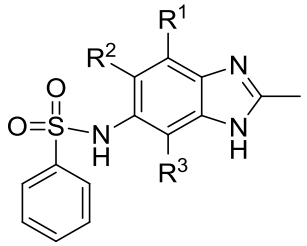


Figure 5.5 Structure of 2-(5-substituted-*1H*-benzo[*d*]imidazol-2-yl)-6-methoxyphenols.

(Tavman *et al.*, 2009)

The nitro group will deactivate the aromatic system, by the electron withdrawing effect through the conjugation effect (Table 5.4). A SAR study by González-Chávez *et al.*, (2011) suggested that the presence of an electron withdrawing group on the benzimidazole ring increases the antimicrobial activity, especially, for compounds

possessing a nitro group in either the 5- or 7-position of *N*-(2-methyl-4,5,7-substituted-1*H*-benzo[*d*]imidazol-6-yl)benzenesulfonamide (Figure 5.6) against *Staphylococcus aureus,* MRSA and *Bacillus subtilis* (González-Chávez *et al.*, 2011). Clearly the sulfonamide group is well known to enhance antimicrobial activity (Kalidhar and Kaur, 2011).



R^1	R^2	R^3
H,	H,	NO_2
H,	NO_2,	H
H,	F,	NO_2
Br,	NO_2,	H
Cl,	NO_2,	H
H,	Cl,	NO_2
H,	OCH_3	NO_2

Figure 5.6 Structure of *N*-(2-methyl-4,5,7-substituted-1*H*-benzo[*d*]imidazol-6-yl)benzenesulfonamides (González-Chávez *et al.*, 2011)

Substitution at position 2 of the benzimidazole ring also has a significant role in determining the biological activity. Therefore, different groups have been used for the SAR. The effect of the groups is described according to their nucleophilicity and is summarised in Table 5.4. There is a large amount of research published on the effect of different groups on biological activity (Puratchikody *et al.*, 2008, Tuncbilek *et al.*, 2009). For example, the 2-amino, 2-(6-fluorochromyl), 2-methyl, 2-aminomethyl, 2-thiomethyl, 2-phenoxymethyl, and 2-chloromethyl substituents have been studied by many researchers (Kumar *et al.*, 2006, Madkour *et al.*, 2006, Podunavac-Kuzmanovic and Cvetkovic, 2007, Vaidehi and Deepika, 2012). The 2-chloromethyl group has been selected for this study, although it had been previously studied by other researchers for the antimicrobial activity against helminthes, bacterial, and fungal strains (Table 2.4). Some of the strains which were studied

with this compound were the same as the strains selected for this project, but were of a different species (Different species of *Staphylococcus aureus* (≥ 512 µg/ml), *Escherichia coli* (> 512 µg/ml), *Pseudomonas aeruginosa* (≥ 512 µg/ml), *Aspergillus fumigatus* (125 µg/ml), and *Penicillium marneffei* (17.9 mm) as mentioned in Table 2.4). Therefore, there was interest in studying this compound with a variety substituents at position 5 of the 2-chloromethyl derivatives (FAS43-49,53, Table 2.4) of the benzimidazole to investigate the SAR further (Karuvalam *et al.*, 2012, Vaidehi and Deepika, 2012, Zhang *et al.*, 2012b).

The substitution of the methyl group at position 1 will increase the electron density on the aromatic system through the inductive effect. The presence of the methyl group at this position has biological importance, and this has been proved by many studies. A study by Pawar *et al.*, (2004) found that the *N*-methyl derivative of 2-(4-thiazolyl)-1*H*-benzimidazole (Figure 1.31) showed lower antifungal activity, and no antibacterial activity compared to the unmethylated derivative(Pawar *et al.*, 2004).

In the *N*-oxide derivative, the electrons of the oxygen do not interfere with the π orbitals of the benzimidazole ring (Table 5.4). A study by Boiani *et al.,* (2009), which selected an *N*-oxide moiety and developed new 2*H*-benzimidazole-1,3-dioxides, found they were less toxic and more selective antichagasic drugs. Another study also found that some *N*-oxide benzimidazole derivatives (Figure 5.7) were more potent DNA topoisomerase I inhibitors, than their analogues without *N*-oxide bond (Boiani *et al.*, 2009, Blaszczak-Swiatkiewicz and Mikiciuk-Olasik, 2013).

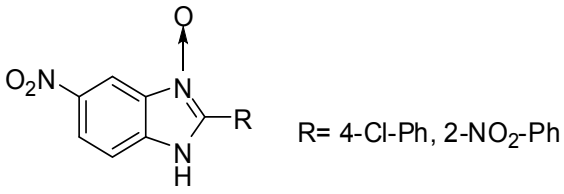
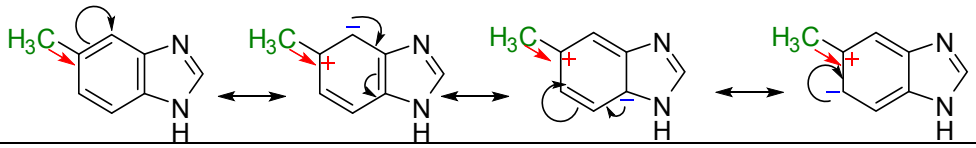
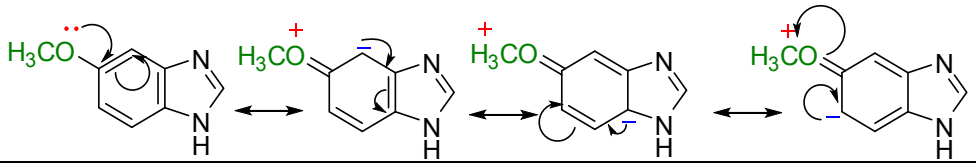
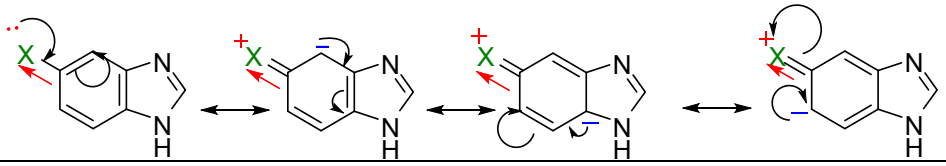
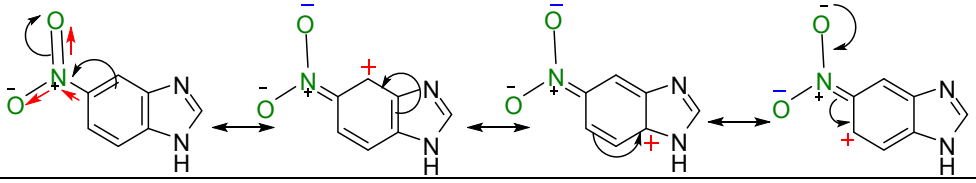
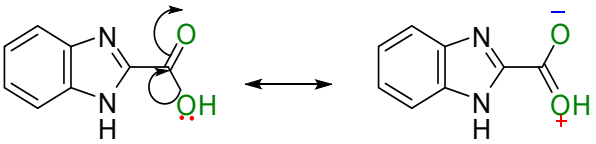
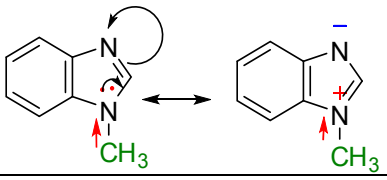
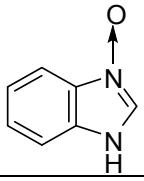


Figure 5.7 Structure of 5-nitro-1*H*-benzimidazole-*N*-oxides

Table 5.4 The effect of each substituent on the benzimidazole ring

Substituent	Effect
5-CH_3	Electron releasing through the inductive effect 
5-OCH_3	Electron releasing through conjugation effect 
5-Cl, Br, F	Electron releasing through conjugation effect and electron withdrawal by inductive effect 
5-NO_2	Electron withdrawal through conjugation effect 
2-CH_2X X: NH_2, OH, SH, OMe, Cl,	The nucleophilicity of sulfur is much greater than other atoms and its lone pairs of electrons are readily accessible **SH>NH_2>OMe>OH>Cl**,
2-COOH	
N-CH_3	
***N*-Oxide**	

5.4.2 Antibacterial evaluation

5.4.2.1 The effect of substituent at position 5

From the series of 2-ethanamine benzimidazole derivatives (series 2), only two compounds; FAS11 (5-chlorobenzimidazole) and FAS12 (5-bromobenzimidazole) showed activity against two MRSA strains; Methicillin-resistant *Staphylococcus aureus* (HG-1), and MRSA BIG 0050; with MIC of 32 µg/ml, which is equivalent to the MIC of ciprofloxacin (control drug). This suggests that the higher electronegativity of bromine and chlorine, increased the antibacterial activity and this was also observed by Tavman (Table 2.4 and Table 5.4) (Tavman *et al.*, 2009).

FAS39 (5-chloro) from the series of *N*-methyl-2-methoxymethyl-benzimidazole (series 6) showed activity only against three species of MRSA (Table 4.11), but this was not as good as the control drug, and hence cannot be a competitor to ciprofloxacin.This is in contrast to the observation by Vidaillac (2007), where the presence of the methoxy group increases the biological activity. However, this also agreed with another study done by Reddy *et al.* (2009), which revealed that the 2-phenoxymethylbenzimidazole derivatives have potential antitubercular activity and may be worth further study. Furthermore, some researchers have also found that the presence of halogen and methoxy substituents on fluoroquinolone (well-known drug) increased the activity against Gram +ve cocci including the resistant strains (Peterson, 2001, Vidaillac *et al.*, 2007, Reddy *et al.*, 2009).

In the 2-chloromethylbenzimidazole derivatives (series 7), six compounds were active, and therefore their MICs were determined. Unfortunately, no promising results were observed for all the compounds tested. This observation agrees with the finding by others for the unsubstituted derivative (Table 2.4). In spite of this, as shown in Table 4.12, FAS47, the brominated derivative, was the most active compound followed by FAS46 which is the chlorinated derivative. This could support

the hypothesis that there is a direct relationship between biological activity and the electron withdrawing effect (Table 5.4) (Tavman *et al.*, 2009).

For the 2-methanthiol benzimidazole derivatives (series 9), one compound was active, the unsubstituted derivative at position 5 FAS56, which was selectively active against most of the Gram +ve strains with MIC of 64 µg/ml, except against *Staphylococcus haemolyticus* NCTC 11042, the MIC value was 32 µg/ml. The activity recorded against Gram -ve strains, was only against *Burkholderia cepacia* with MIC of 64 µg/ml (Table 4.13). The inhibition activity decreases when position 5 is methylated as in compound FAS57. This was also observed by Podunavac-Kuzmanovic and Cvetkovic (2007), who found that the unsubstituted analogue was more potent than the methyl analogues of the 2-aminobenzimidazole (Table 5.4).

The $-CH_2NH_2$, $-CH_2OH$, and -COOH substituents at position 2, did not impart any activity as seen in the determination of the zone inhibition. Therefore, their MICs were not considered.

5.4.2.2 The effect of the substituent at position 2

Only two compounds from the series derived from 1,2-phenylenediamine displayed activity according to their zones of inhibition; these were compounds FAS43 (series 7) and FAS56 (series 9). However, FAS56 containing CH_2SH, was the most active compound in terms of MICs, which was three to four folds more potent than FAS43 which possesses the CH_2Cl substiuent. This may indicate that the thiol at position 2 (FAS56) is the main reason for the activity. This has also been observed by others who reported a similar impact on the antimycobacterial activity on some 2-alkylsulphanylbenzimidazoles derivatives, which showed considerable activity (Table 5.4) (Klimesˇova´ *et al.*, 2002). Another review by Goswami and Singh (2012) reported that 2-substituted benzimidazole derivatives possess biological activity,

and the 2-thiolakyl and 2-thioaryl exhibited good antiprotozoal and antibacterial activity.

Additionally, a series of 2-[(2-nitro-1-phenylalkyl)thiomethyl]benzimidazole derivatives (with R=Br, $N(CH_3)_2$) (Figure 5.8) synthesised by Utku *et al.,* (2008) were found to be active as ampicillin against two strains *Staphylococcus aureus* and *Streptococcus faecalis*. Interestingly, the unsubstituted compound showed antifungal activity close to the control drug (Ketoconazole) (Utku *et al.*, 2008).

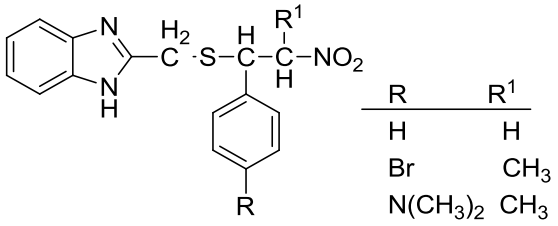


Figure 5.8 Structure of 2-[(2-nitro-1-phenylalkyl)thiomethyl]benzimidazole derivatives

From the 5-chloro substituted benzimidazoles, only three derivatives showed activity against the tested strains, $-CH_2NH_2$, $-CH_2Cl$ and $-CH_2OMe$ derivatives, (FAS11 (series 2), FAS46 (series 7), and FAS39 (series 6) respectively). FAS11 from series 2 was selectively the most active against three species of *Staphylococcus aureus*; (Oxford) NCTC 6571, Methicillin-resistant *Staphylococcus aureus* (HG-1) (equivalent to MIC of ciprofloxacin 32 µg/ml), and MRSA BIG 005 (equivalent to MIC of ciprofloxacin 32 µg/ml), and the most active against one strain of Gram –ve; *Burkholderia cepacia* NCTC 10744. Other researchers found that the presence of an amino group at position 2 of the benzimidazole ring increased the antibacterial activity (Figure 1.27) (Podunavac-Kuzmanovic and Cvetkovic, 2007).

In the 5-bromobenzimidazole series, only two derivatives showed activity against the tested strains, $-CH_2NH_2$, $-CH_2Cl$ (FAS12 (series 2), FAS47 (series 7) respectively). FAS12 was selectively the most active (the same as FAS11), against three species of *Staphylococcus aureus*, and the most active against one strain of

Gram –ve; *Burkholderia cepacia* NCTC 10744. Although FAS47 showed the most activity amongst the rest of the Gram +ve strains with MIC of 32,64 µg/ml. The results proved that the presence of a halogen in both positions (2 and 5) increased the antibacterial activity. An early study was done by Hughes and Lions on 2-chloromethylbenzimidazole (where all the other substituents at position 1 and 5, are H). FAS43 did not show an appreciable activity against some bacteria strains, which agrees with this study (see section **5.1.1**). Therefore the activity of the 5-bromo derivatives can be attributed to the presence of the bromine (See section **2.4.3**) (Table 2.4 and 5.5) (Hughes and Lions, 1938, Tavman *et al.*, 2009).

Only compound, FAS42 (series 6), from the 5-methoxy substituted benzimidazole series which has $-CH_2OH$ group at position 2, showed activity against multiple strains according to its zones of inhibition The studies by Sambanthamoorthy and Vidaillac found that the methoxy group increases the biological activity, but in terms of the MIC values for this compound, it is not active (Vidaillac *et al.*, 2007, Tavman *et al.*, 2009, Sambanthamoorthy *et al.*, 2011).

From the 5-methyl derivatives, compound FAS44 (series 7), which had a $-CH_2Cl$ group attached at position 2, demonstrated good activity against the selected strains (which was more than the other methyl derivatives) according to its zones of inhibition, but was not as active according to the MIC values. However, the activity of this compound could be due to the existence of the chlorine atom at position 2, whereas the presence of the 5-methyl group was not as effective compared to the rest of the substituents. This is in agreement with the results obtained by Podunavac-Kuzmanovic and Cvetkovic (2007) (Table 5.4) (Podunavac-Kuzmanovic and Cvetkovic, 2007, Tavman *et al.*, 2009).

The compounds having a nitro group at position 5 of the benzimidazole were disappointing; except for one derivative, with $-CH_2SH$ group at position 2 (FAS57 from series 9), which exhibited activity against only *Staphylococcus epidermidis* NCTC 11047 according to the zone of inhibition. This result could be either due to

the presence of the sulfur atom (-CH_2SH) at position 2 and this agreed with many studies, or with the presence of the nitro group at position 5 which helped in deactivating the aromatic ring. When comparing the antibacterial activity of FAS57 (5-NO_2, 2-CH_2SH) from series 9 with FAS21 (5-NO_2, 2-H) from series 3, there was no activity for FAS21. Therefore, the activity observed for FAS57, was due to the presence of the –CH_2SH group at position 2 (not active as FAS56 (5-H)) (Table 5.4) (Klimesˇova´ *et al.*, 2002, Utku *et al.*, 2008, González-Chávez *et al.*, 2011).

5.4.2.3 The effect of the substituent at position 1

Only *N*-methylation was studied. In this series (series 6) only two compounds, FAS39 (5-Cl) and FAS42 (5-OMe), were active according to their zones of inhibition. But according to their MIC values, FAS39 was the most active compound against three species of MRSA, with MIC of 128 µg/ml. This result contradict the results of the Pawar study, which found that the unmethylated derivatives were more active (Pawar *et al.*, 2004).

Two *N*-oxide derivatives, FAS59 and FAS60 from series 10, were synthesised to assess their activity against the selected strains; unfortunately none of them were active. This was a little surprising since studies done by Blaszczak-Swiatkiewicz and Mikiciuk-Olasik, and Boiani *et al.,* (2009) found different derivatives of benzimidazole *N*-oxides to be potent (See Section **5.1.1**) (Boiani *et al.*, 2009, Blaszczak-Swiatkiewicz and Mikiciuk-Olasik, 2013).

5.4.2.4 The antibacterial activity of silver complexes

Silver complexes were synthesised using some benzimidazole derivatives as ligands (series13). These ligands (series 3 and 5) were selected because they exhibited no activity themseleves. Therefore any activity of the complexes would be attributed to the presence of the silver. Unfortunately, none of the complexes

showed any activity. A study done by Podunavac-Kuzmanovic also supported this observation. The silver ion, which has ten electrons in a d valence shell, which make it a polarizable metal, is almost saturated. Therefore, if there is an increase in the polarizability of the metal, this could diminish the lipophilicity of the complexes, leading to decreasing or lack of the antimicrobial activity (Shoeib *et al.*, 2001, Podunavac-Kuzmanovic *et al.*, 2004)

5.4.2.5 The effect of different heterocycles

A small series of benzoxazole (series 11) and one benzothiazole (series 12) were assessed against the selected strains to compare their activity to the benzimidazole derivatives. The benzothiazole derivative FAS65 (benzo[*d*]thiazol-2-ylmethanol) according to the zone of inhibition, showed slightly more activity against most of the Gram +ve strains except against *Staphylococcus aureus* (Oxford) NCTC 6571, and *Staphylococcus epidermidis* NCTC 2749. From the entire Gram –ve strains selected for this project, the only activity was recorded against *Burkholderia cepacia* NCTC 10744. FAS65 was more active than the other derivatives, although there were no promising results in terms of their MICs in comparison to the MIC of ciprofloxacin. FAS61 (benzo[*d*]oxazol-2(3*H*)-one) from series 11 was the most active compound according to MIC values against multiple strains as in Table 4.14. Some benzothiazole derivatives have been found to be more potent antimycobacterial agents compared to benzimidazole and benzoxazole derivatives (Pytela and Klimešová, 2011).

5.4.3 Antifungal evaluation

5.4.3.1 The effect of the substituent at position 5

Six compounds from the series of 5-substituted benzimidazole derivatives (series 3) showed promising results, especially compounds FAS18 (5-Cl), FAS19 (5-Br), and FAS20 (5-F) with MICs in the range of MIC ≥ 0.06 µg/ml. All the compounds were more active against *Mucor circinelloides* RCMB 07328 than the control drug by three to six folds. This suggests that when there is no substituent at position 2 and an electron withdrawing substituent is present at position 5, the antifungal activity is increased (Table 5.4). This confirms the hypothesis that there is a direct relationship between the antifungal activity and the electron withdrawing effect of substituents. Only FAS18 (5-Cl) has previously been studied for its antifungal activity (Karuvalam *et al.,* 2012), against different species of *Aspergillus fumigatus* NCIM No. 902, and *Penicillium marneffei*, but no activity was recorded. This is in contrasts to the results obtained in this project which showed that FAS18 was highly active against the selected strains with MIC equivalent to amphotericin B, and two folds potent (Karuvalam *et al*., 2012).

From the series of 2-alkylamine benzimidazole derivatives (series 1 and 2), promising results were observed for FAS4 (5-Cl) and FAS7 (5-NO_2), especially against *Mucor circinelloides* RCMB 07328 with MICs of 3.9 and 0.49 µg/ml, respectively. However, no activity was observed for the 5-bromo analogue (FAS5). Both FAS4 and FAS7 were active and their activity was equivalent to the control drug, and FAS7 was three folds potent than the control. This suggests that the more electron withdrawing the substituent is, the higher the antifungal activity (Table 5.4). Furthermore, compounds FAS4 (5-Cl), FAS6 (5-F), FAS7 (5-NO_2), and FAS8 (5-H) showed good activity against *Candida parapsilosis* RCMB 05065; with a MIC range of 1.95-0.06 µg/ml for compounds FAS4 and FAS8, which is equivalent to the MIC of amphotericin B (control drug). FAS6, the 5-fluoro analogue, was 3 times more

potent, while FAS7 (5-NO_2) was fivefold more potent than the control drug. This suggests that when the electron withdrawing effect increases, the antifungal effect also increases. When comparing FAS7 (5-NO_2, 2-CH_2NH_2) with FAS21 (5-NO_2, 2-H) in terms of their antifungal activity, FAS7 was more potent and this may be due to the existence of the –CH_2NH_2 group at position 2 (Table 5.5), and hence this result agrees with the study by Dhua and Biswas (2011) (Tavman *et al.*, 2009, Dhua and Biswas, 2011, González-Chávez *et al.*, 2011).

From the series of 2-methanol benzimidazole derivatives (series 4), compound FAS26 (5-Br) exhibited promising results against *Absidia corymbifera* RCMB 09635, *Syncephalastrum racemosum* RCMB 05922, *Candida krusei* RCMB 05051 with MICs equivalent to the control, but two folds more potent against *Candida parapsilosis* RCMB 05065 than the control. This suggests that the more electronegative bromine increases the antifungal activity (Table 5.4) (Tavman *et al.*, 2009).

In the 2-carboxylic acid benzimidazole series (series 5), promising results were obtained for two compounds, FAS32 (5-Cl) and FAS33 (5-Br), with MICs of 0.98 µg/ml only against *Candida parapsilosis* RCMB 05065, which is one fold more potent than the control drug. But when comparing these results with the results of their unsubstituted (at position 2) analogues from series 3 (FAS18, 5-Cl and FAS19, 5-Br), the latter compounds were more active antifungals. Therefore, this result confirmed that the activity is dependent on the halogen substituents at position 5 (Table 5.4) (Tavman *et al.*, 2009).

Promising results were obtained for two compounds in the 2-chloromethyl benzimidazole series (series 7). FAS44 (5-Me) and FAS49 (5-NO_2), showed MICs of 1.95 µg/ml against *Mucor circinelloides* RCMB 07328, which is one fold more potent than the amphotericin B. Also, the MICs for these compounds were higher than the control drug by three to four folds against *Candida parapsilosis* RCMB 05065. This can be explained, in case of FAS49 (5-NO_2), which was more active

than the methyl derivative FAS44, by the presence of the highly electron withdrawing substituent. As a result of this, FAS49 (5-NO_2) is almost two folds more active than FAS44 (5-Me) (Table 5.4) (González-Chávez *et al.*, 2011).

From the 2-ethylbenzimidazole series (series 8), good activity was exhibited by compound FAS54 (5-H) with MICs of 0.12 µg/ml against *Mucor circinelloides* RCMB 07328 and against *Syncephalastrum racemosum* RCMB 05922, with MIC of 0.24 µg/ml, which was five and three folds potent than the amphotericin B, respectively. Interestingly, FAS54 which is unsubstituted at position 5, showed promising activity, while the nitro derivative FAS55 showed no activity. Surprisingly, the inhibition activity decreases when the electron withdrawing effect of the substituent on position 5 increases; this is in contrast with previous results (Table 5.4) (González-Chávez *et al.*, 2011).

Table 5.5 Comparisons between different compounds in term of their antifungal activity

Compound	Substituents at position			Antifungal activity
	1	2	5	
FAS18	H	H	Cl	Active as amphotericin B, or more potent by two to six folds against all species except against *Candida krusei* RCMB 05051.
FAS4	H	CH_2NH_2	Cl	Active as amphotericin B against two species; *Mucor circinelloides* RCMB 07328, and *Candida parapsilosis* RCMB 05065 with MICs of, 3.9, 1.95 µg/ml, respectively.
↑ FAS18 was the highest antifungal activity				↓ FAS4 was the lowest antifungal activity
FAS19	H	H	Br	Active as amphotericin B, or by one to nine folds against all strains.
FAS5	H	CH_2NH_2	Br	Not active against all species.
↑ FAS19 was the highest antifungal activity				↓ FAS5 was not displayed antifungal activity
FAS20	H	H	F	Active against all strains with MICs of two to five folds or equivalent, except against *Candida tropicalis* RCMB 05049, was less by two folds.
FAS6	H	CH_2NH_2	F	Active only against *Candida tropicalis* RCMB 05049, with MIC of three folds potent than the control drug (0.24 µg/ml).
↑ FAS20 was higher antifungal activity				↓ FAS6 was lower antifungal activity
FAS21	H	H	NO_2	The only activity recorded more than the control drug was against *Candida parapsilosis* RCMB 05065 with one folds.
FAS7	H	CH_2NH_2	NO_2	Active as amphotericin B against *Syncephalastrum racemosum* RCMB 05922, and more active by one to five folds against *Mucor circinelloides* RCMB 07328, and *Absidia corymbifera* RCMB 09635 *Candida parapsilosis* RCMB 05065. .
↑ FAS7 was higher antifungal activity				↓ FAS21 was the lower antifungal activity

5.4.3.2 The effect of the substituent at position 2

Only two of the unsubstituted compounds at position 2, FAS54 (series 8), and FAS56 (series 9) displayed a broad spectrum of activity against many of the tested strains. Compound FAS54 from series 8, was active with MICs of 0.12-1.95 µg/ml, which was almost two to seven folds more potent than FAS56 from series 9, against all strains except against *Absidia corymbifera* RCMB 09635. This may indicate that the presence of the ethyl group at position 2 (in FAS54) is the reason for this activity. When comparing this result with the result for the unsubstituted analogue (FAS15, 2-H) at position 2, no promising activity was recorded for FAS15. However, the MICs for FAS56 were in the range 0.98 -500 µg/ml. It is the thiol which contributes to the antibacterial activity since FAS22 (2-CH_2OH) from series 4, was not as active. It should be noted that no antibacterial activity was recorded for compound FAS54, whereas FAS56 was active. This suggests that the presence of the thiol group is required for antibacterial activity, but decreases the antifungal activity. Other studies have highlighted the biological importance of the thiol group (Table 5.4) (Klimesˇova´ *et al.*, 2002, Utku *et al.*, 2008, Goswami and Singh, 2012).

Two 5-methyl derivatives FAS16 (2-H) from series 3 and FAS44 (2-CH_2Cl) from series 7, showed antifungal activity against all strains. While both compounds exhibited the same activity (MIC 0.24 µg/ml) against one strain of unicellular fungal, *Candida parapsilosis* RCMB 0506, FAS16 (2-H), was more active (by one to eight folds) than FAS44 (2-CH_2Cl).

In the 5-methoxy series, two compounds FAS17 (2-H) from series 3 and FAS42 (*N*-Me, 2-CH_2OH) from series 6, were active. FAS42 was selectively more active than FAS17 by two to four folds against three strains of the filamentous fungal; *Aspergillus clavatus* RCMB 02593, *Mucor circinelloides* RCMB 07328, and

Syncephalastrum racemosum RCMB 05922, and only one strain of the unicellular fungal; *Candida tropicalis* RCMB 05049.

FAS18 (5-Cl) from series 3 was the only active compound in the 5-chloro series. It was more active than Amphotericin B by two to six, or in equivalent folds potent. The antifungal activity can be attributed to the presence of the halogen atom. In the 5-bromo series, only FAS19 showed promising activity. FAS19 (5-Br) was more active than Amphotericin B by one to nine, or in equivalent folds against the fungal strains. FAS20 (5-F) was the most active fluorinated derivatives, and it was more active than amphotericin B by two to five, or in equivalent folds against the fungal strains. In conclusion, FAS19 (5-Br) was the derivative which displayed the best antifungal activity out of the other halogenated derivatives (Table 5.4).

In the 5-nitro series, FAS7 from series 1 was the only derivative which showed good activity against some of the selected strains. It was more active than Amphotericin B by one to five, or in equivalent folds. This can be due to presence of $–CH_2NH_2$ at position 2. Dhua and Biswas (2011) studied other 2-aminomethylbenzimidazole drivatives and found that they were active against *Staphylococcus aureus, Bacillus subtilis,* and *Salmonella typhyi.*

5.4.3.3 The effect of the substituent at position 1

From the *N*-methyl-2-methanol benzimidazole series (series 6), three compounds were active. FAS41 (5-F), FAS42 (5-OMe), and FAS50 (5-NO_2) showed MIC values of MIC ≤ 0.12 µg/ml. This can be explain in case of FAS42 (5-OMe) more active than the fluorinated derivative FAS41 (5-F), due to the methoxy substituent which is the most potent electron donating substituents in all the substituents. Regardless of the activity of compound FAS42 (5-OMe) against many of the tested strains which inhibited the fungi strains with MIC ≤ 0.24 µg/m, compound FAS50 (5-NO_2) almost

double potent against the selected strains, and this keep the nitro group on position 5, the most active compounds against the tested fungal (Table 5.6) (Vidaillac *et al.*, 2007, González-Chávez *et al.*, 2011, Sambanthamoorthy *et al.*, 2011).

From *N*-oxide derivatives (sereis 10)only one compound recorded antifungal activity against some of the selected strains. FAS60 (5-NO_2), was more active than Amphotericin B by one to seven folds potent. The existence of –C=O at position 2 increases the antifungal activity (Table 5.4). Literature review supported that the presence of the carbonyl group at position 2 of the benzimidazolin, showed some fungicide activity in case of benzoimidazol-2-one (Kadyrov and Khalikov, 1983).

Table 5.6 Comparisons between different compounds in term of their antifungal activity

Compound code	Substituents at position			Antifungal activity
	1	2	5	
FAS41	Me	CH_2OH	F	More active than Amphotericin B by tow folds against *Mucor circinelloides* RCMB 07328, and with equivalent potent against *Absidia corymbifera* RCMB 09635.
FAS27	H	CH_2OH	F	Only active as the control drug against *Candida parapsilosis* RCMB 05065.
FAS20	H	H	F	Active against all strains with MICs of two to five folds or equivalent, except against *Candida tropicalis* RCMB 05049, was less by two folds.
↑ FAS20 was the highest antifungal activity				↓ FAS27 was the lowest antifungal activity
FAS42	Me	CH_2OH	OMe	More active than Amphotericin B by four folds against *Mucor circinelloides* RCMB 07328, with two folds potent against *Syncephalastrum racemosum* RCMB 05922, and equivalent fold *Candida krusei* RCMB 05051.
FAS24	H	CH_2OH	OMe	No activity recorded as the control drug
FAS17	H	H	OMe	More active than Amphotericin B by three folds against *Mucor circinelloides* RCMB 07328, with two fold potent against *Absidia corymbifera* RCMB 09635, and four folds potent against *Candida parapsilosis* RCMB 05065.
↑ FAS17 and FAS42 were the highest antifungal activity				↓ FAS24 was the lowest antifungal activity
FAS50	Me	CH_2OH	NO_2	More active than Amphotericin B by five folds against *Mucor circinelloides* RCMB 07328, with three folds potent against *Syncephalastrum racemosum* RCMB 05922, two folds *Candida krusei* RCMB 05051, and equivalent fold potent against *Penicillium marneffei* RCMB 01267.
FAS28	H	CH_2OH	NO_2	No activity recorded as the control drug
FAS21	H	H	NO_2	The only activity recorded more than the control drug was against *Candida parapsilosis* RCMB 05065 with one folds.
↑ FAS50 was the highest antifungal activity				↓ FAS21 and FAS28 were the lowest antifungal activity

5.4.3.4 The activity of the silver complexes

The antifungal screening test was done against selected strains, and showed promising activity, which were recorded for two complexes against *Syncephalastrum racemosum* RCMB 05922. FAS70 (ligand = 5-bromobenzimidazole) was as active as the control drug, while FAS72 (ligand = 5-methyl-1*H*-benzo[*d*]imidazole-2-carboxylic acid) was more active by one fold. When comparing the antifungal activity of the complexes, with the activity of the ligand, it can be seen in case of FAS70 that the complex showed equivalent activity as the ligand against *Syncephalastrum racemosum* RCMB 05922, and less activity against the remainder of the strains. FAS72 was more active than the ligand against all the selected strains except against *Candida parapsilosis* RCMB 05065, *Absidia corymbifera* RCMB 09635, and *Penicillium marneffei* RCMB 01267. This provides evidence that silver improved the antifungal activity. Abu-Youssef (2007) tested some newly prepared silver (I) compounds and found that they possessed antimicrobial activity.

5.4.3.5 The effect of different heterocycles

Benzoxazole (series 11) and benzothiazole (series 12) were synthesised and were assessed against the selected strains to compare their activity to the benzimidazole derivatives (series 3, 4). As a result of this, only benzimidazole derivative FAS18 (5-Cl) from series 3 showed the most activity against some of the selected strains than Amphotericin B by two to six or equivalent folds potent MIC. However, the benzimidazole compound FAS18 (5-Cl) from series 3 was more active than the benzoxazole FAS63 (5-Cl) from series 11, with MICs of one to twelve, or equivalent folds more potent, against all the strains except against *Syncephalastrum racemosum* RCMB 05922. FAS63 (5-Cl) from series 11, was more active by three

folds with MIC value of 0.12 µg/ml. Moreover, the benzimidazole derivative FAS22 (5-H) from series 4, was more active than the benzothiazole FAS65 (5-H) from series 12. So it can be concluded, that the most activity were recorded, when the bezimidazole core was present. A previous study found that benzimidazoles were on of the most important class of compounds which are fungicides (Kaplancikli *et al.*, 2004).

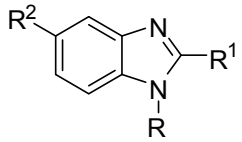
5.5 Next generation of antimicrobial agents of benzimidazole and future work

In this section, suggestions for the next generation of optimized antimicrobial agents are made. This is based on the results of the MICs (Section 4.3.2**)** and the SAR study (Section 5.4). In each case, the five most promising antibacterial and 24 antifungal molecules have been selected.

Alternative reaction conditions were explored in attempts to address the sluggish reaction of 1,2-phenylenediamine with amino acids. A marked improvement of the Phillips method was found during the microwave mediated synthesis of benzimidazoles in ionic liquids as solvents. Although, this was only a preliminary study and a further more extensive study is required to establish the optimum reaction conditions.

5.5.1 Next generation antibacterial

The MRSA species which causes serious infection was of particular interest from all the bacteria strains selected for this study. Therefore, if any compound was active against this strain, it would be considered to be promising lead. Figure 5.9 shows the molecules which have been identified as the most effective antibacterial compounds during the biological assays against bacteria, especially MRSA species (see Table 5.2).



Code	R	R^1	R^2
FAS11	H	$CH(CH_3)NH_2$	Cl
FAS12	H	$CH(CH_3)NH_2$	Br
FAS39	CH_3	CH_2OCH_3	Cl
FAS47	H	CH_2Cl	Br
FAS56	H	CH_2SH	H

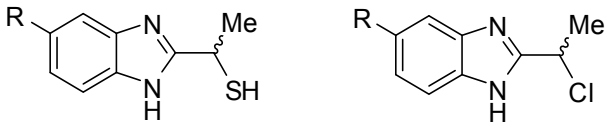
Figure 5.9 Structures of the most active compounds against MRSA bacteria species

In terms of the SAR, the key factor in this case is the presence of the chlorine or bromine substituent at position 5 of the benzimidazole ring, and the presence of –$CH(CH_3)NH_2$ or -CH_2Cl at position 2. Moreover, the presence of –CH_2SH at position 2 (H at position 5), proved effective for antimicrobial activity. Derivatives with –CH_2NH_2 were not active against bacteria, but when a branched methyl group is added (–$CH(CH_3)NH_2$), the antibacterial activity improved. The latter compounds were prepared from *S*-alanine. Therefore the product, which is also chiral and has the *S*-stereochemistry, showed selectively active against the MRSA species. Chirality is a feature for most drugs, with one of the stereoisomers to be active biologically and the other inactive or even toxic. Therefore, the chirality has a significant impact on enhancing the biological activity of the compound (Sekhon, 2013, Shen *et al.*, 2013).

Future work should concentrate on further modifications on these compounds, including testing the *R*-isomer and racemic mixture, (Figure 5.10) which may be beneficial in increasing the antibacterial activity. Therefore, the next generation compounds proposed for further development are based on the chirality at position 2 as shown in Figure 5.10. Thus, based on the activity of the 2-methanthiol benzimidazole derivative, further work could study the chiral derivative prepared by the condensation of *(S)* and *(R)*-2-mercaptopropanoic acid and 5-substituted (H, Cl, Br)-1,2-phenylendiamines (Chimirri *et al.*, 2000).

Similarly, based on activity of the 2-chloromethyl derivative (Figure 5.10), new research could focus on reacting (*S*) and (*R*)-2-chloro-propionic acid with 5-substituted (H, Cl, Br)-1,2-phenylendiamines (Yadav and Pal, 1996). Moreover,

work needs to be carried out to complete the series of compounds to fully investigate the structure activity relationships.



Both *(R)* and *(S)* and racemic mixture R= H, Cl, Br

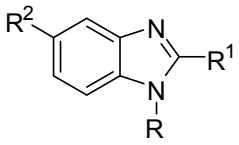
Figure 5.10 Proposed next generation antibacterial agents

5.5.2 Next generation antifungal

There were more compounds which were potent fungicides against selected fungal strains as shown in Figure 5.11. This includes two silver complexes; FAS70 (ligand =FAS19), and FAS72 (ligand =FAS30). These compounds were the most effective antifungal compounds during the biological assays against fungi species with equivalent or greater potentency in their MIC values than amphotericin B. In particular, compounds FAS18, 19, and 20 showed broad spectrum activity.

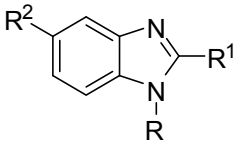
According to the SAR study, an extremely important factor is the nature of the substituent attached to the aromatic ring. The presence of the halogen atom (Cl, Br, F) at position 5, increased the antifungal activity. For the 5-nitro derivative, the presence of $–CH_2NH_2$ at position 2 increased the activity. The *N*-methylated-2-methanolbenzimidazole derivatives (series 4) were more active than the unmethylated analogues and this is observed when the substituent at position 5 was F, OMe, or NO_2.

Future work should focus on further modifications on these compounds as presented in Figure 5.12, which may improve the antifungal activity. Moreover, future work could explore the silver complexes of different ligands of benzimidazole such as the proposed next generation compounds which are shown in Figure 5.12.



Code	R	R^1	R^2	Code	R	R^1	R^2
FAS4	H	CH_2NH_2	Cl	FAS31	H	COOH	OMe
FAS7	H	CH_2NH_2	NO_2	FAS32	H	COOH	Cl
FAS8	H	$CH(CH_3)NH_2$	H	FAS33	H	COOH	Br
FAS16	H	H	CH_3	FAS41	CH_3	CH_2OH	F
FAS17	H	H	OCH_3	FAS42	CH_3	CH_2OH	OMe
FAS18	H	H	Cl	FAS50	CH_3	CH_2OH	NO_2
FAS19	H	H	Br	FAS44	H	CH_2Cl	CH_3
FAS20	H	H	F	FAS48	H	CH_2Cl	F
FAS21	H	H	NO_2	FAS49	H	CH_2Cl	NO_2
FAS51	H	H	CN	FAS54	H	CH_2CH_3	H
FAS26	H	CH_2OH	Br	FAS56	H	CH_2SH	H
FAS27	H	CH_2OH	F	FAS60	O	H	NO_2

Figure 5.11 Structures of the most active compounds against fungal species



R = alkyl, aryl, alkylhalide, arylhalide
R^1 = alkylamine
R^2 = OMe, OH, Cl, Br, F, NO_2

Figure 5.12 Proposed of some of the next generation of antifungal agents

5.5.3 Biological future work

One of the most important steps in the further biological work is the study of the toxicity of the compounds in order to see if they can be used clinically. Therefore, the toxicity assay will also be required to explore the potential of these compounds as selective antimicrobial drugs which target the microbe cells rather the human cells. In addition, the mechanism of the antibacterial and antifungal action of the benzimidazoles needs to be understood so that more effective antimicrobial inhibitors can be designed and synthesised.

A study done by Sambanthamoorthy *et al*. identified a novel benzimidazole compound (see section **5.4.1**) which does not inhibit the growth of bacteria, but they found it prevented bacterial biofilm formation in multiple Gram +ve and Gram -ve

bacterial pathogens (Sambanthamoorthy *et al.*, 2011). Therefore there is still hope that the compounds prepared in these compounds could exhibit antibiofilm activity by targeting the biofilm formation between the microbe cells. Therefore, further biological studies on behaviour of the benzimidazoles on the biofilm of bacteria strains is required in order to identify the antibiofilm activity of the synthesised compounds.

Despite the promising results obtained in this project, this does not mean that these compounds will replace the well-known drugs, such as fluoroquinolones, which have the required potential to be used clinically. The duration for the development of a new drug is in the range of 10-15 years, starting from the stage of determining the biological activity down to the stage where the drug come to the market (Department of Health, 2013b). In this period, i.e. drug development duration, there are several stages, which includes assessing the toxicity of the compound, and if the compound is highly toxic, it will be eliminated from the rest of the stages of development. Several researchers had reported that even when their preliminary results were promising, but unfortunately, these compounds could not be developed into new drugs because of their high toxicity towards the normal cells. An example of this is aziridinyl substituted benzimidazolequinones (Donkor, 2007, Fahey *et al.*, 2010). In addition, the toxicity issue is more problematic for antifungal agents. As mentioned earlier in chapter 1.5, human cells and fungi cells are all eukaryotic. Thus, it is challenging to design drugs which target the fungi cells without affecting the human cells. For example, amphotericin B is a clinically antifungal drug which targets the yeast and it can also be a major cause of toxicity the human cells (Wilcock *et al.*, 2013). Despite this, the investigation continues to find new drugs with low toxicity and high therapeutic effect. However, there are more challenges which hinder the development strategies in order to achieve the optimal drugs.

One of the most important of these challenges is the increasing resistance of the microbes against the well-known drugs. This is an extremely challenging issue

which researchers in this field are focusing on in order to find strategies to resolve the problem through the development of new drugs. One of the solutions would be through cooperation between several research organizations, as is the case in the campaign 10/20, which was launched in 2010 and which aimed to get 10 new drugs by 2020. This was agreed by U.S. President Barack Obama, who signed an agreement with the representative of the European Union (the Swedish Prime Minister Fredrik Reinfeldt) to establish a transatlantic task force to focus on solving the problem of the antibacterial drugs pipeline (IDSA, 2010). Thus, it is clear that the issue has escalated and has reached the stage which required political intervention. This is issue of antibacterial resistance is now acknowledged as a worldwide problem.

It is worth mentioning how resistance occurs and then spreads. Two figures, taken from the CDC report (2013) illustrate this. Figure 5.13 shows how resistance occurs, and Figure 5.14 shows how it spreads. The CDC report also highlighted the estimated minimum number of illnesses and deaths caused by antibiotic resistance, which is at least 2,049,442 illnesses, and 23,000 deaths, respectively. For *clostridium difficile* infections in particular, the annual mortality rate in United States is no less than 14,000 people, whereas the morbidity rate is almost 250,000 every year. However, the healthcare-associated infection, is the most serious Gram –ve infection which is produced by common pathogens such as Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter*. Moreover, these pathogens reached the acute levels of concern because they are "pan resistant pathogens" (resistant to almost all therapeutic drugs). Therefore, these pathogens are difficult to treat. Besides the mentioned challenges which hinder the development of new drugs, there are several factors which may also contribute to the dwindling “antimicrobial agents pipeline” (see section **1.6, 1.7,** and **1.8**), including economic impact incidence, transmissibility, availability of effective antibiotics, and barriers to prevention (Centers for Disease Control and Prevention, 2013).

To combat the antibiotic resistance, the CDC report approved four actions to be follow in the following order: preventing infections, tracking resistant, prudent use of today's antibiotics, and then development of new antibiotics and diagnostic tests as well. This is consistent also with what is emphasised by the UK AMR strategy (Department of Health, 2013b).

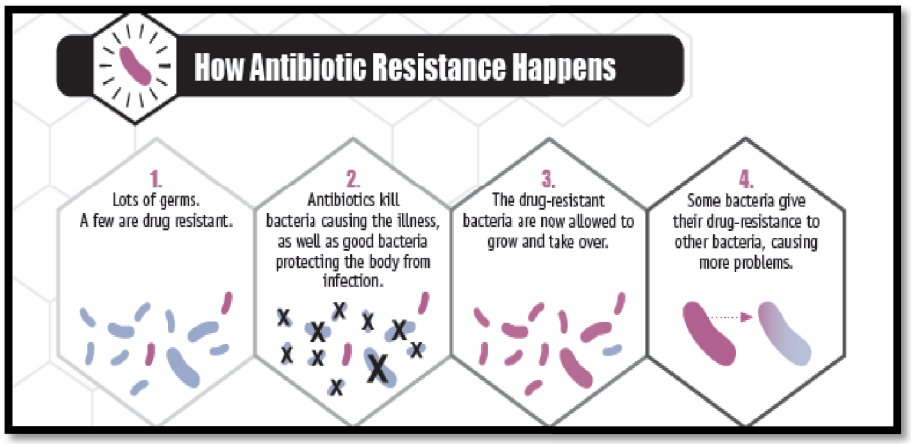


Figure 5.13 How antibiotic resistance happens. *Source:* (Centers for Disease Control and Prevention, 2013)

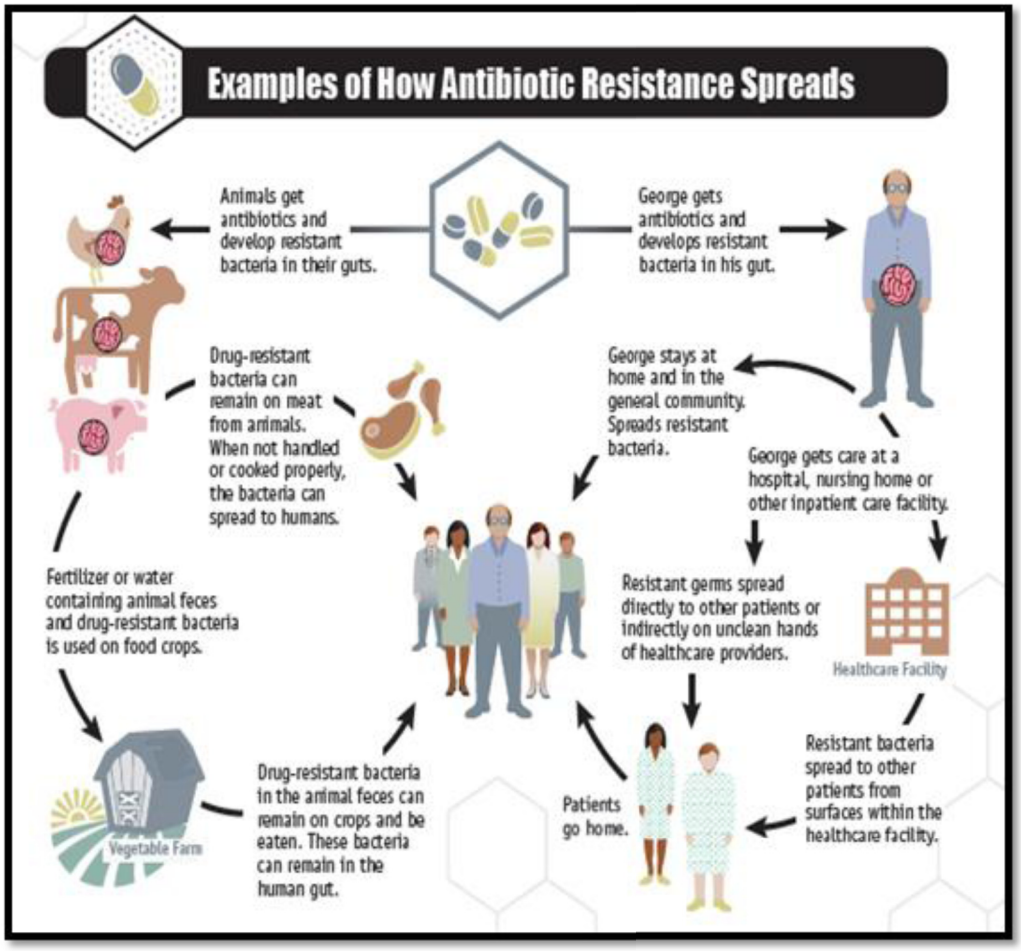


Figure 5.14 Examples of how antibiotic resistance spreads. *Source:* (Centers for Disease Control and Prevention, 2013)

Therefore, one new strategy is to reuse an old (known) drug for new therapeutic applications such as screening for antivirulence activity rather than spending large sums in designing and synthesising a new drug. A study by Imperi *et al.* (2013) found that flucytosine, which is a well-known antimycotic drug, inhibited the production of the major P. *aeruginosa* virulence factors; pyoverdine, PrpL protease, and exotoxin.

5.6 Conclusions

The aims of this project were to synthesise benzimidazole derivatives and to evaluate them *in vitro* as inhibitors against selected bacteria and fungi of medical

importance. In order to achieve this, 66 compounds were synthesised based on the 2-benzimidazole derivatives and compared to other heterocycles, benzoxazole and benzothaizole. Eleven compounds were novel and more than 50 compounds were tested for the first time against a library of bacteria and fungi strains. Moreover, the Phillips method was improved in this project by using ionic liquids and microwave assisted synthesis. Some silver complexes of benzimidazole derivatives were also screened against the selected strains to evaluate the importance of the silver metal.

According to the SAR analysis, it can be concluded that the derivatives of benzimidazole offer enormous possibilities for the development of new broad spectrum antimicrobials. Through appropriate modification and fine-tuning of substituents at positions 1, 2, and 5, new microbial inhibitors are possible.

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