The Synthesis and antimicrobial activity of nitropropenyl arenes and related compounds

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BSc. (Medicinal Chemistry)

A thesis submitted in fulfillment of the requirements for the degree of Master of Applied Science

School of Applied Sciences
RMIT University
September 2011
Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

King Hei Lo
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Abstract

This history of antimicrobials is marked with impressive discoveries, the majority of which have their origin in natural products. However in this work, the antimicrobial activity of β-nitrostyrenes and related compounds of synthetic origin is reviewed and investigated with a particular focus on the influence of fluorine functionality.

![Substituents](image)

Various fluorinated β-methyl-β-nitrostyrene compounds were prepared, their minimum inhibition concentration in cultures of Gram positive, Gram negative and a fungus and their lipophilicity was determined.

Consequently, 1-fluoro-4-(nitroprop-1-enyl) benzene [12c] was found to have the highest activity against *E. coli*, whereas more lipophilic compounds were more effective against Gram positive bacteria. However compound lipophilicity did not correlate with antimicrobial activity, highlighting the importance of the structure of the antibiotic activity towards the microorganisms studied.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-HEAF</td>
<td>2-hydroxyethylammonium formate</td>
</tr>
<tr>
<td>α</td>
<td>Alpha</td>
</tr>
<tr>
<td>A</td>
<td>Ampere</td>
</tr>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>Ar</td>
<td>Aromatic ring</td>
</tr>
<tr>
<td>β</td>
<td>Beta</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>BDMI</td>
<td>4-[(E)-2-nitroprop-1-enyl]-1,3-benzodioxole</td>
</tr>
<tr>
<td>13C</td>
<td>Carbon-13 NMR</td>
</tr>
<tr>
<td>C. albicans</td>
<td>Candida albicans</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation spectroscopy</td>
</tr>
<tr>
<td>CFU/mL</td>
<td>Colony forming units per mL</td>
</tr>
<tr>
<td>CH$_3$COONH$_4$</td>
<td>Ammonium acetate</td>
</tr>
<tr>
<td>Cu(OTf)$_2$</td>
<td>Copper(II) triflate</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>Deuterated Chlorofrom</td>
</tr>
<tr>
<td>CH$_3$OK</td>
<td>Potassium methoxide</td>
</tr>
<tr>
<td>CH$_3$ONa</td>
<td>Sodium methoxide</td>
</tr>
<tr>
<td>CH$_3$SiNa</td>
<td>Sodium trimethylsilanethiolate</td>
</tr>
<tr>
<td>(CH$_3$)$_3$SiCF$_3$</td>
<td>Trimethyl (trifluoromethyl)silane</td>
</tr>
<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>dd</td>
<td>Doublet of doublet</td>
</tr>
<tr>
<td>DAST</td>
<td>(diethylamino)sulfur trifluoride</td>
</tr>
<tr>
<td>DABCO</td>
<td>1,4-diazabicyclo[2.2.2]octane</td>
</tr>
<tr>
<td>DBN</td>
<td>1,5-diazabicyclo[5.4.0]nonene-5</td>
</tr>
<tr>
<td>DBU</td>
<td>1,5-diazabicyclo[5.4.0]undec-ene-5</td>
</tr>
<tr>
<td>DEPT 45</td>
<td>Distortionless Enhancement by Polarization Transfer 45° angle</td>
</tr>
<tr>
<td>DEPT 90</td>
<td>Distortionless Enhancement by Polarization Transfer 90° angle</td>
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<tr>
<td>DEPT 135</td>
<td>Distortionless Enhancement by Polarization Transfer 135° angle</td>
</tr>
<tr>
<td>Deoxo – Fluor®</td>
<td>Bis (2-methoxyethyl) amino sulfurrifluoride</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPP-4</td>
<td>dipeptidyl peptidase-4</td>
</tr>
<tr>
<td>E</td>
<td>E configuration</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>E. faecalis</td>
<td><em>Enterococcus faecalis</em></td>
</tr>
<tr>
<td>EI</td>
<td>Electron Impact</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>gCOSY</td>
<td>Correlation spectroscopy with gradient</td>
</tr>
<tr>
<td>GC/MS</td>
<td>Gas chromatography coupled to mass spectrometry</td>
</tr>
<tr>
<td>GHz</td>
<td>Giga hertz</td>
</tr>
<tr>
<td>hr</td>
<td>Hour(s)</td>
</tr>
<tr>
<td>¹H</td>
<td>Proton-¹ NMR</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear multiple-bond correlation spectroscopy</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear single-quantum correlation spectroscopy</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>kbar</td>
<td>Kilobar</td>
</tr>
<tr>
<td>K₂CO₂</td>
<td>Potassium carbonate</td>
</tr>
<tr>
<td>Kₐ</td>
<td>Partition coefficients</td>
</tr>
<tr>
<td>KF</td>
<td>Potassium fluoride</td>
</tr>
<tr>
<td>KOH</td>
<td>Potassium hydroxide</td>
</tr>
<tr>
<td>LDA</td>
<td>Lithium diisopropylamide</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>mmol</td>
<td>Millimole</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mPTPB</td>
<td>Mycobacterium protein tyrosine phosphatase B</td>
</tr>
<tr>
<td>ml/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>M⁺</td>
<td>Molecular ion</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>Magnesium sulfate</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>Mtb</td>
<td><em>Mycobacterium tuberculosis</em></td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>Sodium carbonate</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NK-1</td>
<td>Neurokinin-1</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
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<td>Neurokinin-1</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
</tbody>
</table>
p  Pentet
ppm  Parts per million

*P. aeruginosa*  *Pseudomonas aeruginosa*

PTPs  Protein tyrosine phosphatases
q  Quartet

QSAR  Quantitative structure-activity relationship

\( r^2 \)  Coefficient of determination

R  Variable group

\( \delta \)  Delta
\( \delta_H \)  Chemical shifts for proton -1 NMR
\( \delta_C \)  Chemical shifts for carbon -13 NMR
s  Singlet

*S. aureus*  *Staphylococcus aureus*

SARS  Structure-activity relationships

SPAR  Structure-property-activity-relationship

t  triplet
t-BuOK  Potassium tert-butoxide
THF  Tetrahydrofuran

\( \mu g/mL \)  Microgram per millilitre
\( \mu L \)  Microlitre
UV  Ultra Violet
V  Volt

Z  Z Configuration
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Chapter 1

1 Introduction

1.1 Resistance of micro-organisms to antibiotics

1.1.1 Background of antimicrobial agents

Antibiotics are chemicals secreted by bacteria and fungi, they can also be synthetic and unnatural to kill or inhibit competitor microbes in the microenvironment and thus are part of microbial self protection\(^1,2\). At present, many secondary metabolites of bacteria and semi-synthetic antimicrobial agents from natural products have been made even though drug resistant strains has necessitated continuing modifications to the original antibiotic parent compound. From these natural scaffolds medicinal chemists modify structures to create semi-synthetic derivatives with improved properties. Some naturally occurring antibiotics (such as cephalosporins and macrolides) have much more complex chemical structures compared to the synthetic antibiotics (such as sulfa drugs and quinolones).

The first discovery of a successful anti-infective compound was made by a German physician Paul Ehrlich. Ehrlich suggested that to be suitable for therapeutic use, a chemical should be selectively toxic, i.e. show greater toxicity to the target microorganism than to host cells. In 1904 he discovered that the dye trypan red was active against the trypanosome causing African sleeping sickness, and suggested that it could be used therapeutically. Later, Ehrlich successfully synthesized the drug, arsphenamine (Salvarsan) which was used to treat the protozoal disease. Ehrlich, with a Japanese scientist, Sahachiro Hata also found that arsphenamine was active against the syphilis spirochete.
The progress in finding new antimicrobial agents in the next 20 years was slow until the aminoacridine, Proflavine, was introduced in 1934. This drug unfortunately was too toxic to be used against bacterial infections and was used as a disinfectant and antiseptic.\(^3\)

The sulfonamide drugs became the first and the only effective antibacterial agents against systemic bacterial infections until the advent of penicillin G\(^4,5\). Gerhard Domagk in the 1930s, showed that the red dye sulfonamidochrysoidine, synthesized by Bayer, completely protected mice against bacterial infections. Later workers at the Pasteur Institute showed that the dye was metabolized by, intestinal micro-organisms to the active form, sulfanilamide. Sulfanilamide (Prontosil), commercialised by Bayer, was the first sulfonamide and it was quickly followed by sulphonamide drugs and the sulfonamides were successfully used to treat *streptococcal* infections.

In 1928 Penicillin was accidently discovered by Alexander Fleming\(^1,5\) when he noted its inhibitory effect on *Staphylococcus aureus*. Penicillin was the first natural product isolated from the fungus, *Penicillium notatum*. Howard Florey, Ernst Chain and Norman Heatley developed Penicillin as the first antibiotic drug. It was first used therapeutically in 1941\(^6\). It is active against Gram positive bacteria and the spirochaetes causing syphilis. Howard Florey and his coworkers showed that Penicillin G was more effective in controlling *staphylococcal*, *streptococcal* and *pneumococcal* infections and syphilis. Unfortunately, clinically significant resistance to Penicillin appeared in 1947, but it is still a widely used drug. Many successful semi-synthetic beta-lactam derivatives have been developed from this scaffold. Cephalosporin, which also contains a β-lactam ring, was isolated from a fungus, *Cephalosporium*, in 1948. Similarly, many semi-synthetic derivatives have been developed from this parent compound.

The discovery of the first of the aminoglycoside antibiotics, streptomycin, in the 1940’s, extended the anti-bacterial spectrum to include Gram negative bacteria and the tubercle bacillus. More antibiotics were quickly discovered, e.g. the tetracyclines, erythromycin and other marolides.
Overall semi-synthetic derivatives have been developed e.g. sulfa drugs, quinolones, azoles etc, with reference to medicinal chemistry to improve drug scaffolds.

The fluoroquinolones, are a class of synthetic antibacterial agents first synthesized in the early 1960s. Several generations of fluoroquinolones have since been synthesized. They interfere with bacterial DNA gyrase, eventually inhibiting nucleic acid synthesis.

The major classes of antibacterial agents (e.g. β-lactams and tetracyclines group, synthetic sulfonamides and fluoroquinolones¹−⁴) are lead compounds for the synthesis of a new generation of drugs with improved stability, pharmacokinetics and spectrum of activity⁷.

Despite the large number of effective antibacterial agents that have been developed, drug resistance occurred quickly for all the major classes of anti-infectives, so there is an urgent need to develop new classes of antimicrobial compounds with diverse microbial targets.

1.1.2 The problems of drug resistance in bacteria

Antimicrobial drugs play an important role in assisting humans in overcoming infections due to pathogenic microorganisms, thus providing successful treatments for microbial diseases. The rapidly increasing emergence of microbial strains resistant to existing antimicrobial agents is a global problem and a serious limitation on the use of antimicrobial chemotherapy and this significant threat particularly is relevant to hospitals. Outbreak infections due to methicillin-resistant *S. aureus*, vancomycin-resistant enterococci and multidrug resistant *Pseudomonas aeruginosa* are increasingly being reported worldwide⁸,⁹.

Antimicrobial drug resistance is the ability that microorganisms gain to resist biological attack from anti-infective chemotherapeutic agents⁴. Antimicrobial agents are used for medical and veterinary therapy, as disinfectants, antiseptics, agricultural biocides and
food and animal feed additives. The massive amounts of antimicrobial agents being used for many industrial purposes are causing the emergence and spread of drug resistance and giving rise to a rapidly increasing number of pathogenic strains that are resisting treatment with anti-infective agents\textsuperscript{4, 5, 10}.

### 1.1.3 Mechanisms of drug resistance

The resistance of bacteria to antibiotics is a natural phenomenon\textsuperscript{4, 6}. In nature microorganisms develop mutations or acquire resistance genes to common metabolites from other microorganisms in the environment. Resistance genes are readily transmitted horizontally between related species, and even between unrelated species, so they can spread through microbial habitats, particularly if there is a selection pressure from the presence of industrial antibiotics.

There are a few common mechanisms of drug resistance in bacteria.

1. Lowered penetration or permeability of drugs into the cell membrane of pathogens. For example Penicillin G is not effective against enteric and related gram negative bacteria as it cannot penetrate the outer membrane\textsuperscript{1, 4, 5}.

2. Alteration in a drug’s target receptors lowers the binding efficacy of the drug. Vancomycin is no longer effective against enterococci because the target for the vancomycin terminal D-alanine-D-alanine in enterococcal peptidoglycan has been changed to D-alanine-D-lactate.

3. Drugs may also be expelled by the pathogen’s plasma membrane translocases. Efflux pumps can be specific to one drug as in tetracycline resistance. Multidrug-resistant pumps are relatively nonspecific and can pump many different unrelated drugs out of the cell. Gram negative bacteria such as \textit{E. coli} and \textit{Pseudomonas aeruginosa} contain this type of efflux system\textsuperscript{4, 5, 8}.

4. Bacteria can secrete enzymes that modify drugs thereby inactivating them\textsuperscript{1, 4, 5}. For example, secretion of bacterial beta lactamases which hydrolyze the \(\beta\)-lactam antibiotics
(e.g. penicillin, cephalosporin) makes them clinically ineffective\textsuperscript{5, 10} as the β-lactam ring is the key structural component of these antibiotics.

(5) Bacteria can develop an alternative metabolic/biochemical pathway to make their products\textsuperscript{1, 4, 5} and they take up folic acid from their surroundings, therefore they do not need to synthesize folic acid when the pathway to make folic acid is blocked by sulfonamide drugs.

(6) Some antimicrobial agents may not be able to inhibit bacteria if they do not have the structure that antimicrobial agents can target. In other words, the bacteria are naturally resistant to some antimicrobial agents. Mycoplasmas bacteria are naturally resistant to penicillins because they do not have a cell wall.

### 1.1.4 The necessity for new antimicrobial agents

The number of new anti-infective drugs brought to market in the last 20 years has been very low\textsuperscript{11, 12}. No new major class antibiotics since the fluoroquinolones have been discovered between 1962 and 2000\textsuperscript{1}. The newest naturally occurring antibiotics which have been put into clinical practice were the oxazolidinones in 2000\textsuperscript{1}. All the other new agents have narrow spectrum of activity effective only against a few pathogens or of only one type. Another reason is that the development of new antibacterial agents is a costly and time-consuming process before a new drug can be brought to market. For this reason many pharmaceutical companies have stopped or limited their efforts to develop new antimicrobial agents. Only a few pharmaceutical companies are currently active in this field\textsuperscript{10}. Scientists have already discovered some major antibiotic cellular targets for drugs to kill or inhibit the growth of microorganisms. Those major cellular targets include: the bacterial cell wall; the bacterial plasma membrane; synthesis of bacterial proteins, bacterial nucleic acids and bacterial metabolism.\textsuperscript{1}
Barker has reviewed recent antibacterial drug discovery and structure-based design for the development of new antibacterial compounds\textsuperscript{2}. It has been proposed that structure-based design is an excellent tool for designing compounds with increased potency and selectivity. As well as this, a molecular approach can focus chemistry on regions suitable for modification, improving stability or bulk properties such as solubility, without affecting potency. Barker,\textsuperscript{2} Bush \textit{et al.}\textsuperscript{13} and Projan and Bradford\textsuperscript{14} have suggested that new drug development should focus on some new targets for inhibition such as fatty acid synthesis. There is evidence that protein tyrosine phosphatase could be a possible cellular target\textsuperscript{7,15}.

### 1.1.5 Protein tyrosine phosphatase

Protein tyrosine phosphatases (PTPs) exist in both eukaryotic and prokaryotic cells. Scientists have investigated the role of protein tyrosine phosphatase in eukaryotes and have found that many important cell functions such as, cell growth and differentiation, cell motility, metabolism and the immune system. However the discovery of Protein tyrosine phosphatases in bacteria occurred much later, the tyrosine phosphorylation in bacteria is less common and less well investigated. Zhou \textit{et al.}\textsuperscript{15} recently investigated protein tyrosine phosphatase B in \textit{Mycobacterium tuberculosis} (\textit{Mtb}). They suggested that mycobacterium protein tyrosine phosphatase B (mPTPB) secreted by \textit{Mtb} might mediate \textit{Mtb} survival in marcophages in the host cell. Thus specific mPTPB inhibitors may help the host cell to enlarge the intrinsic host signaling pathways to eliminate the tuberculosis infection.

White\textsuperscript{7} tested the ability of 4-\{(\textit{E})-2-nitroprop-1-enyl\}-1,3-benzodioxole (Compound 7) to inhibit human and bacterial tyrosine phosphatases by enzymic assay. She stated that nitroalkene compounds related to 7 have been shown to be a competitive, slow and reversible inhibitor of protein tyrosine phosphatase. It was found that 7 showed less inhibitive ability to protein tyrosine phosphatase, which was consistent with the results reported by Park and Pei\textsuperscript{16} for related benzyl nitropropene compounds. Both of these
results have suggested that 7 is a less potent inhibitor of tyrosine phosphatases. Thus, White suggested 7 as a potential lead compound to develop anti-infective agents based on PTP inhibition. In other words, the nitroalkenes like nitrostyrene and β-methyl-β-nitrostyrene which have similar chemical structure to 7 are potential compounds for the development of new inhibitors of protein tyrosine phosphatase in bacteria.

1.2 Antimicrobial history of nitrostyrene

1.2.1 Early reports of the antibacterial activity of β-nitrostyrene

Substituted β-nitrostyrenes [structure 1a] are members of the class of nitroalkenes, and their biological activities have been studied previously for a few strains of bacteria or fungi\textsuperscript{17-22}.

\[
\begin{align*}
R & \quad NO_2 \\
\text{1a} & 
\end{align*}
\]

Reports have shown that β-nitrostyrene [1a] derivatives have a toxic effect on insects\textsuperscript{23, 24} and inhibit the growth of fungi\textsuperscript{20, 23, 25}. For example, one of the frequently used fungicides, β-bromo-β-nitrostyrene, has a wide-spectrum of activity against fungi\textsuperscript{26}. It may therefore be possible to use this type of compound for the protective treatment of organic materials such as leather. Based on the biological properties of β-nitrostyrene, it could also be used as an antibacterial agent.

Large numbers of derivatives of β-nitrostyrene [1a] were investigated for activity against bacteria by Schales and Graefe\textsuperscript{19}. In 1952, they synthesized 55 compounds including 20 new arylnitroalkenes using the Henry reaction and tested their antibacterial properties against the Gram positive bacterium, \textit{Micrococcus pyogenes} var. \textit{aureus}, and the Gram negative bacterium, \textit{Escherichia coli}. The β-nitrostyrene derivatives were synthesized from benzaldehyde and its derivatives having different substituents on the aromatic ring.
by reaction with nitromethane using various catalysts. It was shown that, β-nitrostyrene derivatives had activity against both the Gram positive and Gram negative bacteria. The data showed the effectiveness of selected compounds against both types of bacteria especially when substituents such as the methoxy group (-OCH$_3$) were present on the aromatic ring\textsuperscript{19}. However, some compounds were less effective than the parent compound, β-nitrostyrene. They showed that the effectiveness of each compound against \textit{M. pyogenes} was slightly decreased or not affected (for compound 2) by introducing albumin into the culture medium.

![Chemical Structure](image)

2

Early work by Schales and Graefe indicated that the presence of plasma proteins reduced the biological activity of antibacterial agents\textsuperscript{27}, but this was not always the case, as they showed that addition of albumin to the culture medium actually enhanced the antimicrobial activity of compounds 3 and 4.

![Chemical Structures](image)

3
4

Additional work done by them showed that chlorine substituents at the position 4 (para to the vinyl group) (compounds 5 and 6) of the ring showed improved biological activity compared with positions 2 and 3 (data not shown).

![Chemical Structures](image)

5
6

Nitroethane was used to form the β-methyl-β-nitrostyrene compounds. The nitropropene compounds were found to be more effective against \textit{Micrococcus pyogenes} var. \textit{aureus}, but were not as effective against \textit{E. coli}. From the published biological activity of β-
nitrostyrene, and other research on nitrostyrene derivatives, it could be concluded that β-nitrostyrene derivatives have potential as antibacterial agents.

1.2.2 Nitrostyrene derivatives as antimicrobial agents

Compound [7], is broadly active against a wide range of Gram positive bacteria, Gram negative bacteria, filamentous fungi and yeast. It is a yellow-colored crystalline compound with melting point of 96°C, is insoluble in water but is soluble in organic solvents such as acetone and ethanol and is stable at room temperature, and to heat, but unstable to UV light on long time exposure. It strongly and reversibly binds to serum albumin. In 1997, Denisenko et al. made a series of known nitrostyrene compounds by the Henry reaction in which nitro alkene compounds were synthesized from aromatic aldehydes. Denisenko et al. tested these compounds for antimicrobial activity and showed that many compounds had biological activity against the bacterial strains. Further studies on 7 by White confirmed its broad antimicrobial activity. She also showed that 7 does not alter the function of major bacterial targets such as DNA replication, ribosomal function, cell wall synthesis or cell membrane integrity or the synthesis of major fungal targets of cell membrane and cell walls. However, 7 did inhibit protein tyrosine phosphatases in bacteria. The broad spectrum of activity against resistant bacteria, the metabolic targets of 7 in both prokaryotic and eukaryotic microorganisms is sufficiently selective to allow for differential toxicity between microbial and mammalian cells.
1.2.3 Recent work on antibacterial activity

Milhazes et al., in 2006, synthesized analogues of β-nitrostyrene and β-methyl-β-nitrostyrene derivatives with substituents on the aromatic ring such as hydroxy groups (-OH, compound 8c), methoxy groups (-OCH₃, compound 9b) and the methylene dioxy group (-OCH₂O-, 7) and studied the influence of aromatic substitution patterns on antibacterial activity.

They proposed that these substituents would provide different electronic environments, possibly affecting the antimicrobial activity of these nitrostyrene derivatives. Milhazes et al. also mentioned the development of new antimicrobial drugs generally based on the structure-activity relationship (SAR), structure-property-activity relationship (SPAR) and quantitative structure-activity relationship (QSAR) studies. In their investigation of the antimicrobial activity, Gram positive bacteria (Staphylococcus aureus and Enterococcus faecalis) and Gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa) were used to test their nitrostyrene derivatives. It was found that a methyl group on the β-carbon leads to an increase of the inhibitory effect and the potency was in the range of 2 to 8 fold greater than the parent compound, β-nitrostyrene. The enhancement of activity by the methyl group on the β-carbon was most pronounced on the Gram positive bacteria (e.g. S. aureus). The compound 3-hydroxy-4methoxy-β-methyl-β-nitrostyrene [10a] gave the best results (MIC 16) against all Gram positive bacteria while the 3,4-dihydroxy-β-methyl-β-nitrostyrene [8c] was the most effective (MIC 64) against all the Gram negative bacteria except for P. aeruginosa. (Table 1)
Table 1: Antimicrobial activity of the β-methyl-β-nitrostyrene derivatives against several bacteria

<table>
<thead>
<tr>
<th>Strain</th>
<th>Minimum Inhibitory Concentration, MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8c</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>64</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>256</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>64</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>64</td>
</tr>
</tbody>
</table>

Furthermore, Milhazes *et al.* found that antibacterial activity did not increase even though changing the polarity of the compounds with aromatic substituents would also change the electronic characteristics and lipophilicity of the compound (they only tested *S. aureus*).

They also concluded that β-methyl-β-nitrostyrene derivatives could be potential antimicrobial agents for clinical use.

Recent work related to the synthesis and antimicrobial activity of nitrostyrene derivatives was reported by Nicoletti *et al.*[^29^]. They focused mainly on synthesizing β-methyl-β-nitrostyrene derivatives[^11^], and evaluated their antimicrobial activity against a panel of Gram positive bacteria, Gram negative bacteria and fungi.

In the nitrostyrene compounds, there were different substituents on the aromatic ring and other novel reactions were carried out to produce pyridine, imidazole, benzoxazole, thiazole and other analogues. According to their results, most compounds had significantly high activity against Gram positive bacteria and fungi. The compound which had the highest activity against Gram negative bacteria was the 4-fluoro derivative, 4-(2-nitroprop-1-enyl)-1-fluorobenzene, 12c.
Based on these discoveries, new designs for the synthesis and development of new antibacterial compounds for SAR were continued in this investigation (Masters Program). The Henry reaction was used and compounds were prepared under two different conditions: Method A and Method B (See Chapter 3). The lipophilicity of each compound was measured by determination of octanol-water partition coefficients. The methods of evaluation of antibacterial activity were determined by minimum inhibitory concentration (MIC).

1.3 Fluorine as a substituent

This project, in part, investigated the antimicrobial activity of fluorine substituted nitrostyrene compounds. The 1906 Nobel Prize in Chemistry was awarded to Henri Moissan for his discovery and isolation of the element fluorine. Neil Barrett never received the Nobel Prize, however, in 1962 he was the first to produce xenon fluoride thereby discovering the reactivity of noble gases in forming fluorides. Organic compounds with very stable covalent carbon-fluorine bonds are produced when fluorine atoms or groups are substituted for hydrogen or oxygen. An example is the ubiquitous use of Teflon (polytetrafluoroethylene) coatings in non-stick frying pans. Two fluorine compounds, highlights of medicinal chemistry research in the 1950s, are the anti-inflammatory drug 9α-fluoro-hydrocortisone acetate and the anticancer drug 5-fluorouracil. In recent times, drugs for the treatment of high cholesterol levels include atorvastatin calcium (Lipitor®, Pfizer), rosuvastatin calcium (Crestor®, AstraZeneca), ezetimibe (Zetia®, Merck/Schering-Plough) and fluvastatin sodium (Lescol®, Novartis), and all contain one or more fluorine atoms and are amongst the highest selling prescription drugs developed to date. As well as this, compounds with fluorine substitution have been widely used in the manufacture of medicines, agrochemicals and polymers in recent years32-35.
Some examples of fluorine-containing drugs

Reports have shown that not more than 40 organofluorine compounds from natural products have been isolated, but none of them contained an aryl fluoride in their structure. Previous reviews have shown that the fluorine atom itself is not sterically demanding and has a very small van der Waals radius, which is slightly larger in size to the hydrogen atom. Carbon-fluorine bonds are considered the strongest covalent bonds. Fluorine always increases hydrogen bond acidity, and the strength of carbon-fluorine bond is high, 439.6 kJ/mol. It can also go to higher bond energy of 485.7 kJ/mol. Table 2 shows some properties of hydrogen, fluorine, chlorine, oxygen and carbon. The thermal stability could be enhanced due to this bonding energy. When hydrogen is replaced by fluorine, the lipid solubility/lipophilicity or hydrophobicity would be expected to increase biological absorption, although this was not the case for the fluorination of alkanes.
Fluorine has the highest electronegativity of all the elements, which means it has a high ionization potential. Therefore it has a higher ability to withdraw electrons from other atoms in molecules towards fluorine, and that modifies the reactivity of compounds containing fluorine (refer to Chapter 2). Fluorine has a notebale leaving group ability, offering the possibility to design mechanism-based enzyme inhibitors and the small covalent radius can facilitate docking with drug receptor(s). Because of the low F-F bond energy (36.6 kcal/mol or 153 kJ/mol), the strong repulsion of its lone pair electrons, when reacted with other compounds to form high energy bonds, makes reactions of F₂ with other elements or compounds extremely exothermic and often explosive. Other concerns are, for example high reactivity, lack of selectivity and potential toxicity (due to the inability of fluorinated compounds to be metabolized) as well as the risk of free radical initiation during reaction. All these properties make working with elemental fluorine a challenge.

### 1.4 Fluorine in organic chemistry

Petrik and Cahard and other workers indicated that organic compounds of fluorine are rare in nature whereas laboratory synthesized fluorinated compounds have readily been prepared and research related to fluorine is widespread in chemical sciences. Introduction
of fluorine into an organic molecule can be achieved by using nucleophilic fluorine reagents \(^{(Scheme ~1)}\)\(^{46,47}\) and electrophilic fluorine reagents \(^{(Scheme ~2)}\)\(^{48,49}\).

\[
\begin{align*}
\text{SCH}_3\text{OEt} & \xrightarrow{1) \text{BrF}_3 \atop 2) \text{aq. HCl, HF}} \text{OCH}_3 \text{OF} \quad \text{70\% yield} \\
\text{Ref. 46}
\end{align*}
\]

\[
\begin{align*}
\text{SCH}_3\text{OEt} & \xrightarrow{\text{Ba}_2\text{NEF}_3} \text{OCH}_3 \text{OF} \quad \text{71\% yield} \\
\text{Ref. 47}
\end{align*}
\]

**Scheme 1:** Examples of introduction of fluorine via nucleophilic reagents

\[
\begin{align*}
\text{OCH}_3 & \xrightarrow{1) \text{RLi} \atop 2) (\text{CF}_3\text{SO}_2)\text{NF}} \text{OF} \quad \text{81\% yield} \\
\text{Ref. 48}
\end{align*}
\]

\[
\begin{align*}
\text{Selectfluor}^{TM} & \xrightarrow{\text{71\% yield}} \text{OF} \\
\text{Ref. 49}
\end{align*}
\]

**Scheme 2:** Examples of introduction of fluorine aromatic rings via electrophilic reagents

Fluoroorganic chemistry is becoming important in science as it has many applications in chemistry and medicinal chemistry. Fluorine-containing compounds are well known antibacterial agents; for example, a fluorine atom improves the hydrogen bond donor acidity which makes a hydrogen bond with protein and lipid component of biosystem easily to elicit bioactivity in bacteria\(^{33}\). In another example Giménez *et al.*\(^{50}\) showed that a fluorine atom improved drug activity due to the increase in hydrophobicity of the drug. Ismail\(^{51}\) mentioned that **Linezolid** can be used to treat infections caused by serious Gram positive bacteria and Purser *et al.*\(^{52}\) gave some examples about recent effective fluorinated antibacterial agents now on the market. Moreover, substitution with fluorine in nitrostyrene in a previous study\(^{53}\) was shown to enhance the antimicrobial properties of these compounds.
The van der Waals radius of fluorine is approximately 1.47 Å and is between oxygen (1.52 Å) and hydrogen (1.20 Å). It enables fluorine to have the ability to form hydrogen bond. The strong C-F bond enables organofluoride compounds to kill pests harmful to agriculture. There are examples of organofluorides being used as insecticides [13, 14], as arthropodicides [15], as herbicides [16], as fungicides [17, 18, 19], as pesticides [20], as agrochemicals [21, 22] and pharmaceuticals [23, 24] uses. (Full name of compounds in Appendix)
Examples of organofluorides as insecticides, arthropodicides, herbicides, fungicides and pesticides

Examples of fluorine containing agrochemicals

Examples of organofluoride pharmaceuticals

28
Other examples of biologically activity fluorine substituted compounds are the important phosphodiesterase inhibitors **Roflumilast** and **N-[1-(2-chloro-phenyl)ethyl]-2-(4-fluoro-phenoxy)benzamide** [25].

**Two examples of phosphodiesterase inhibitors**

![Roflumilast](image)

Previous discoveries of fluorine-containing compounds that showed effective antimicrobial properties are exemplified by the fluoro-quinolones and the fluorininated quinazoline derivatives [68, 69]. Fluoroquinazoline has been widely used as a fungicide [70] herbicide [71] and additionally as an antitumor agent [72]. Fluoro-quinolones, first approved in the 1960s, had emerged as a significant class of chemotherapeutic agents [73]. Clairefond *et al.* [73] showed the effect of fluorine at carbon-5, 6 or 8 in a series of compounds that were tested against *E. coli* DNA-gyrase.

**Fluoro-quinolone compound with C-6 fluorine substitution**

![Fluoro-quinolone compound with C-6 fluorine substitution](image)

They found that fluorine substitution at carbon-6 [26] or -8 showed enhancements of antimicrobial activity against both Gram positive and Gram negative bacteria. However, fluorine substitution at carbon -5 had markedly decreased activity due to compensatory electronic effects. Koga *et al.* carried out similar experiments but obtained markedly different results in the potency of the compound substituted at carbon-8 *in vitro* (10 fold
The oxazolidinones were other examples given by Barbachyn et al.\textsuperscript{75} that showed fluorine substitution enhanced antibacterial activity. He referred to the structure activity-relationships of Gregory et al.\textsuperscript{76} who postulated that reducing the electron density in the phenyl ring of phenyloxazolidinones by substitution of electron withdrawing groups in the \textit{para} position might increase the potency of the compound. Barbachyn et al. introduced a stronger electron withdrawing group (e.g. fluorine) in their test compound \textsuperscript{27} to determine its antibacterial activity. Eventually, they proved that fluorine substitutions had significantly enhanced the potency better than that with chlorine substitution.

\[ \text{(R}^1 = \text{alkoxy, amino; R}^2 = \text{H, F; R}^3 = \text{F, Cl, CF}_3) \]

\subsection{1.4.1 Introduction of fluorine into heterocyclic and aromatic compounds}

Many reviews have illustrated that special fluorinating reagents are commonly used to introduce elemental fluorine into heterocycles (Scheme 3)\textsuperscript{77-81}. Further fluorination of heterocycles [compound \textsuperscript{28 – 30}] can also be carried out by using the Balz-Schiemann reaction\textsuperscript{82} to convert \textit{–NH}_2 to \textit{–F}, or halogen exchange methods\textsuperscript{83}, or reaction with high-valency metal fluorides\textsuperscript{84}.
Another typical example is that of the fluoroquinolones, which have been mentioned previously. Early organometallic fluorinating reagents, because of their limited thermal stability, caused the incorporation of fluorine into organic molecules to be less developed than in the case of early work with hydrocarbons\textsuperscript{85}. Later, fluorinated organometallic reagents were developed with good thermal stability and so more fluorinated organometallic compounds were synthesized. An example of the effect of aryl ring fluorination on the antimicrobial activities was the compound 12c\textsuperscript{53} which showed significant enhancement of activity on Gram negative bacteria.

1.4.2 Trifluoromethyl substitution in organic compounds

\textit{Bis (2 – methoxyethyl) amino sulfur trifluoride (Deoxo – Fluor\textregistered)} discovered by Lal and co-workers\textsuperscript{86, 87} is a very versatile fluorinating reagent in organic synthesis serving as a thermally stable alternative to (diethylamino)sulfur trifluoride (DAST) and can transform carboxylic acids to acid fluorides or trifluoromethyl derivatives. (Scheme 4)
Scheme 4: Trifluoromethylation of carboxylic acids via Deoxo – Fluor®

Trimethyl(trifluoromethyl)silane, \((\text{CH}_3)_3\text{SiCF}_3\), (Ruppert – Prakash reagent) is widely used for nucleophilic trifluoromethylation. (Scheme 5)\(^{88,89}\)

Scheme 5: Nucleophilic trifluoromethylation by means of Ruppert – Prakash reagent

1,3-Dihydro-3,3-dimethyl-1-(trifluoromethyl)-1,2-benzodioxole known as the Togni reagent is an electrophilic trifluoromethylation reagent based on hypervalent iodine. (Scheme 6)\(^{90}\)

Scheme 6: Electrophilic trifluoromethylation via Togni reagent
The bis aryl thiotrifluoromethyl reagents shown below are also electrophilic trifluoromethylating reagents [compound 31, 32].

![Chemical structures of compounds 31 and 32]

Trifluoromethylated aromatics can also be prepared by converting benzotrichloride into benzotrifluoride [compound 33] (Scheme 7). Hydrogen fluoride could also be used.

![Scheme 7: Conversion of benzotrichloride into benzotrifluoride]

The trifluoromethyl group (-CF₃) itself has a high electron withdrawing effect similar to that of oxygen and has a size slightly larger than an isopropyl group. The effect of substitution of –CF₃ on organic compounds is to assist the changing of regioselectivity (from an –(S) or –(R) enantiomer transformed into a chiral compound) and reactivity (by producing different compounds by the same chemical reaction (compared to –CH₃) of the compounds. McClinton and McClinton have commented that –CF₃ causes minimal disruption to an enzyme substrate complex due to only slight effect of the bond length when a trifluoromethyl group replaces a methyl group attached to a carbon. As well as this, Maier et al. and Reynolds et al. pointed out the high lipophilicity of -CF₃ in pharmaceutical and agrochemical compounds. When present showed an improvement in membrane transport characteristics in vivo, thereby facilitating lower dosage. Muller suggested lipophilicity is strongly dependent on the position of the fluorine within the molecule.
The trifluoromethyl containing compound 35 showed at least 3-fold potency of inhibition over the non-trifluoro substituted compound [34].

1.4.3 Trifluoromethoxy substitution in organic compounds

The trifluoromethoxy group (-OCF₃), should impart physical properties consistent with its higher electron withdrawing ability and may alter lipophilicity compared with its methoxy analogue. Due to its unusual stability, the -OCF₃ group is strongly resistant to strong acids, strong bases and strong oxidizing and reducing conditions. Trifluoromethoxy compounds [36] (or trifluoromethyl ethers) can be prepared by reacting a variety of substituted phenols with hydrogen fluoride in excess carbon tetrachloride in a closed pressure vessel under autogeneous pressure. (pressure generated from the reaction) (Scheme 8)

\[
\text{Scheme 8: Conversion of substituted phenol to trifluoromethyl ether derivative}
\]
The deactivation of the aromatic system occurs when there is substitution of the trifluoromethoxy group on an aromatic ring, even though a trifluoromethoxy group could exhibit electron withdrawing behavior similar to the halogens\textsuperscript{107}. It has been shown that the following substituted trifluoromethoxyphenyl compound [37] has valuable pharmacological activity (as a hypoglycemic agent to lower blood glucose level)\textsuperscript{108}.

\[
\text{Ref. 108}
\]

1.5 The roles of fluorine in medicinal chemistry

Drugs containing fluorine atoms have constituted around 5 to 15\% of the total number of drugs on the world market over the past 50 years\textsuperscript{39}. Medicinal chemists will have much more success in synthesizing and designing new fluorinated drugs now that new fluorinating methodologies and fluorinated commercial intermediates continue to be made available. Recent reviews by Kirk\textsuperscript{44} and Hagmann\textsuperscript{39} highlighted recent developments of fluorine containing drugs.

Some examples of fluorine in medicinal chemistry:

3-(((2S,3S)-2-(3,5-bis(trifluoromethyl)benzyloxy)-3-phenylmorpholino)methyl)-1H-1,2,4-triazol-5(4H)-one [compound 38]\textsuperscript{109}

\[
\text{Ref. 108}
\]

NK-1 Antagonists, in vivo potency being improved by fluorine substitution. Removal of any –CF\textsubscript{3} group from the compound results in a 3-fold decrease in receptor affinity. This drug is for treatment of chemotherapy induced nausea and vomiting. Also NK-1 antagonists contain a bis-trifluoromethylphenyl group would help for central nervous system penetration.
Covalent binding of cannabinoid-1 receptor was improved by introduction of fluorine atoms. The covalent protein binding and bioavailability were 2-fold improved by two additional of fluorine atoms on the phenoxy ring.

Enhanced potency of a DPP-4. Treatment for Type 2 Diabetes, this 2,5-difluorophenyl derivative was almost 5-fold more potent than the fluorine free dipeptidyl peptidase-4 inhibitors (DPP-4). It showed that fluorine substitutions on the phenyl ring of triazolopiperazine of DPP-4 played an important role in the improvement in potency and pharmacokinetics.

D₂ Prostaglandin Receptor Antagonists. Treatment of allergic rhinitis, the parent compound containing methylsulfone group was replaced by a fluorine atom and improved the biliary properties, plasma clearance properties and lengthened the plasma half-life of the drug.
These successful biochemical outcomes became the lead compounds for later rational drug design.

### 1.6 Significance of the project

The increasing microbial resistance of clinically important bacteria to current antibiotics is of great concern for public health suggesting an increased need for new, more effective and safer antibacterial agents. The aim of this project is to develop highly efficient $\beta$-nitrostyrene derivatives as antibacterial agents. Previous research on the structure activity relationships (SAR) of these compounds for antibacterial activity revealed that a substance with a fluorine substituent on the benzene ring showed the highest activity against Gram negative bacteria\textsuperscript{53}. Furthermore, the number of new effective antimicrobial agents with selective toxicity brought to the market in the past 20 years has been very low. It is necessary to synthesize and design more similar or novel compounds based on the fluorinated nitrostyrene compound \textsuperscript{[12c]} as well as identifying their antibacterial properties.
1.7 Chemistry of β-nitrostyrenes

1.7.1 The Chemical properties of β-nitrostyrene

\[ \text{NO}_2 \]

\( \text{β-nitrostyrene, 1a} \)

β-Nitrostyrene [1a] is the parent member of the aryl-nitroalkene family, and is a yellow crystalline solid of molecular weight 149 g/mol. It dissolves in organic solvents such as acetone, ethanol and dichloromethane, but not in water. It has a partition coefficient (octanol/water) of 59 and a melting point range of 58-59°C\textsuperscript{114, 115}. 

1.7.2 The nature of nitroalkenes and their applications in chemistry

Nitroalkenes are, generally prepared by the aldol condensation between carbonyl compounds and nitroalkanes via the β-nitroalcohol intermediate (nitroalcohol) and this is known as the Henry reaction (Scheme 9). This reaction has found widespread application in synthetic organic chemistry\textsuperscript{116-123}.

![Scheme 9: General synthesis of nitroalkenes](image)

Dehydration of the β-nitroalcohol intermediate forms nitroalkenes and these compounds have found a variety of applications such as Michael acceptors\textsuperscript{117, 124-131}, dienophiles\textsuperscript{118, 132-141}, domino reactions\textsuperscript{142}, masked ketones\textsuperscript{143-145}, pyrrole formation\textsuperscript{146, 147}, Friedel-Crafts alkylation\textsuperscript{148, 149}.
The Henry reaction is characterized by:

a) Reaction products that are usually formed as diastereomeric syn- and anti-mixtures. The modification of the experimental conditions can result in the isolation of β-nitro alcohols with high diastereoselectivity.

b) A variety of ionic and non-ionic bases can be used including: sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium methoxide (CH₃ONa), potassium methoxide (CH₃OK), potassium tert-butoxide (t-BuOK), ammonium acetate (CH₃COONH₄), sodium carbonate (Na₂CO₃), potassium carbonate (K₂CO₃), potassium fluoride (KF), solid supported bases, amines, 1,5-diazabicyclo[5.4.0]undec-ene-5 (DBU) and 1,5-diazabicyclo[5.4.0]nonene-5 (DBN).

c) Only catalytic quantities of base are required and mildly basic conditions are necessary for the dehydration.

d) Typical reported yields are in the range 11 – 95%\textsuperscript{120, 133} (depending on types of catalysts and solvents used).
1.7.3 Some synthetic applications of nitroalkenes

Diels-Alder or cycloaddition reactions

Nitroalkenes can act as powerful electron withdrawing substituents which makes nitroalkene derivatives potent dienophiles, undergoing the Diels-Alder or cycloaddition reactions\(^ {132}\) (Scheme 10).

![Scheme 10: Novel class of di-N-oxy-β-lactam compounds by cycloaddition reactions](image)

Scheme 10 shows β-nitrostyrene has been used (4 equivalents in the reaction) to form regioisomeric products 43 and 44. The functions of β-nitrostyrene in this reaction are that they firstly act as an electron-poor diene in an inverse electron demand in the Diels-Alder reaction with enol ether, which is an electron rich compound. After that, the electron poor intermediate 45a (dipolarophile) reacts with another β-nitrostyrene to form 1,3-dipolar cycloaddition compounds [43 and 44]. Both first and second step reactions were done under high pressure (15 kbar); compound 45b, which is the β-lactam compound, was surprisingly simple to purify from compound 43 by using silica gel chromatography (eluted by an eluent containing triethylamine).
**Michael addition reactions**

In addition, nitroalkenes are powerful electrophiles and form highly stable carbanions\(^{150}\) that readily undergo asymmetric conjugate addition reactions with nucleophiles or radicals\(^{134, 151}\). β-Nitrostyrenes are also good acceptors in Michael addition reactions applied to some natural products\(^{130}\), shown in Scheme 11.

![Diagram of Michael addition reactions](image)

**Scheme 11**: Novel Formation of isoxazoline N-oxide with Michael adduct compound.

Whereby 11, a\(^′\) is added to the polarized nitroalkene derivative [b\(^′\)] to form the adduct c. The Michael adduct [compound 46] could be made from c by electron and proton transfer. Alternatively, compound c could also form the isoxazoline N-oxide compound by ring formation. Itoh and Kishimoto\(^{129}\) discovered an interesting mechanistic feature that the isoxazoline N-oxide ring is generated by an induction of intramolecular nucleophilic attack by the nitronate anion to the carbon-oxygen bond fission of the furan ring.
**Domino Reaction**

β-Nitrostyrene can be used in triple cascade organocatalytic reactions\(^\text{152}\) (domino reactions; Scheme 12) to prepare highly substituted nitro cyclohexene derivatives.

**Scheme 12: Triple cascade organocatalytic reactions**

This reaction produced 47, and ent-47 with multiple stereogenic centres. The advantages of this reaction are low reaction time (16-24 hours at room temperature), and cost, including purification of intermediates and steps avoiding the protection and deprotection of functional groups. It is greener chemistry, as the reaction is environmentally friendly in that organocatalysts are used that are non toxic, the reaction is highly efficient, starting
materials are readily available and are metal-free and compounds with excellent stereoselectivities are often obtained\cite{142}.

Scheme 12 shows that the catalyst makes the enamine to be formed, which made the aldehyde to be selectively added to the nitrostyrene in Michael – type reaction. The hydrolysis process liberated the catalyst, which causing them to be able to form the iminium ion of α,β-unsaturated aldehyde to complete the conjugate addition with compound A. In the third step, the enamine activation of intermediate B makes it possible for an intramolecular aldol condensation to form compound C. Further hydrolysis occurred to recycle the catalyst and release the desired product compound 47.

**Friedel-Crafts alkylation**
Another recent application of nitrostyrene compounds in chemistry is the enantioselective Friedel-Crafts alkylation of indoles with nitoalkenes catalyzed by different copper(II) triflate (Cu(OTf)$_2$) bisoxazoline complexes (Scheme 13)\cite{149}.

![Scheme 13: Enantioselective Friedel-Crafts alkylation of indoles with trans- β-nitrostyrene](image-url)
Nef reaction

The Nef reaction provides protocol to form masked ketone \([48]\) compounds from nitroalkenes (Scheme 14)

\[
\text{Ar} = \text{NO}_2
\]

1. Diels - Alder reaction
   - Butadiene, heat

2. Nef reaction

\[
\begin{align*}
\text{Ar} & \quad \text{Nef reaction} \\
\text{Ar} & \quad \text{Diels - Alder reaction}
\end{align*}
\]

Scheme 14: Formation of ketones via Diels – Alder and Nef reactions

In summary, β-nitrostyrenes are versatile precursors for the synthesis of diverse chemical functionalities which are very reactive 1,3-dipolar reagents that can be converted into nitrile oxides, nitrones and nitronates\(^{117-119, 124, 125, 129, 133, 134, 142, 153}\). Therefore, β-nitrostyrenes (and nitroalkenes) are excellent C – C bond forming agents and are used widely in organic synthesis to prepare novel compounds.
1.7.4 Other modern methods to synthesize β-nitrostyrenes

Apart from the methods presented in Chapter 3, there are newer methods that recently have been used to make β-nitrostyrenes and a few of the methods provided a shorter reaction time, environmental friendly and excellent yield of the product. Alizadeh et al.\textsuperscript{132,154} developed a green method to synthesize β-nitrostyrenes using a cost-effective ionic liquid, 2-hydroxyethylammonium formate (2-HEAF), in the reaction. (Scheme 15)

They concluded the advantages of this reaction are this is a very clean and high yielding processing with no acid, base or metal catalyst required in the reaction. No side products were formed and all products were a crystalline forms which were easily characterized by
their melting points and spectroscopic data. As well as this one pot synthesis procedure avoided using hazardous organic volatile solvents and toxic catalyst, the reaction was done under room temperature and the use of cost-effective ionic liquids. The ionic liquids will be recovered in the procedure and can be used again. Overall commercially available and low-cost with high conductivity, great solvating ability and low melting point ionic liquids can be potentially used in other organic synthesis method.

Yoshida et al.\textsuperscript{155} found the addition of dehydrating reagent, MgSO\textsubscript{4}, to the reaction can improve the yield (33\% up to 81\%) of β-nitrostyrenes when using excessive nitroalkane (5 equivalents). Scheme 16

Scheme 16: Recent method of synthesizing β-nitrostyrenes

The advantages of this method are good yields obtained and only a one step synthesis of the nitroalkene. As well as this the catalyst, \textit{O-tert}-butyldiphenylsilyl L-tyrosine lithium salt, can play two different roles in the reaction: it helps to form the nitroalkene, and the catalyst can be used in the next step of reaction. This can help reducing the use of catalysts, in other words cost effective. However the longer reaction time (two days) it takes to synthesize the nitroalkene and organic solvent was used in the reaction become the disadvantages of this method.

1.8 Microwave assisted Henry reactions

Microwaves were used to assist the Henry Reaction in this project. Microwave irradiation (MWI) relies on the dielectric heating properties\textsuperscript{156-158}. All dedicated microwave reactors for chemical synthesis generally operate with frequency at 2.45 GHz\textsuperscript{156, 159}. This thermal
effect is dependent on the polar nature of specific solvent or reagents. There are two mechanisms which make solvents absorb microwave energy and convert it into heat. The dipolar rotation mechanisms, which results from dipolar polarization as a consequence of dipole-dipole interactions between polar molecules and the electromagnetic field\textsuperscript{158}, or ionic conduction mechanisms which result when ions cluster in solution. The ions will circulate in solution by an electric field, the and collision rate will increase due to this movement in solution causing an expenditure of energy. The resulting kinetic energy is converted into heat\textsuperscript{157}. Overall, the effectiveness of microwave irradiation is associated with the polarity of a molecule.

**Examples of microwave assisted Henry Reactions** (Scheme 17)\textsuperscript{115, 160, 161}

\begin{center}
\textbf{Scheme 17: Microwave assisted Henry Reactions}
\end{center}
Microwave irradiation

β-Nitrostyrene reacts with a carbonyl compound and an amine on alumina with microwave irradiation which is an efficient method of forming pyrrole compounds (Scheme 18).\(^{147}\)

![Scheme 18: Formation and reaction of β-methyl-β-nitrostyrene using microwave irradiation](image)

1.9 Partition coefficients

It is important to gain some idea of the permeability of a drug to pass through living cellular membranes. For this purpose, the partition coefficients (K\(_D\)) of drugs need to be determined. Partition coefficients are used to determine the lipophilic nature of a compound. Solubility, permeability, oral absorption, cell uptake, blood-brain barrier penetration and metabolism of a compound are influenced by its degree of lipophilicity.\(^{162}\)

There are five key techniques for the determination of lipophilicity: solvent/water partitioning, chromatographic approaches, artificial membranes, electrokinetic approaches and partitioning between lipid/water phases.\(^{162}\) A widely used technique to define the lipophilicity of a drug is the octanol/water partitioning and this technique was used to determine the lipophilicity of each nitrostyrene derivative prepared in this work.
Chapter 2

2 Results and Discussion

2.1 Introduction
This chapter contains discussion of the synthesis and biological activity of nitroarenes, with emphasis on substituted 2-nitroprop-1-enyl benzenes. The antibacterial efficacy of the products was assessed by their activity against a panel of bacteria and a fungus (Candida albicans). The results appear as the minimum inhibitory concentration (MIC) for each compound. The partition coefficient ($K_D$) between octanol and water for each compound, representing its degree of lipophilicity, was also determined in order to access the extent of its interaction with the surface of the microorganism. Compounds with the incorporation of fluorine were of considerable interest in these studies, particularly as earlier studies had indicated an improvement of antibacterial activity from this approach. Results are discussed in terms of structure-activity relationships that are important for activity against the microorganisms studied.

2.2 Synthesis of 2-nitroprop-1-enyl benzene derivatives
The Henry reaction was utilized for the preparation of most of the compounds. Two different conditions, referred to as Method A, Method B and other variations from the literature were used.

Method A used methylamine as catalyst and was carried out at room temperature under mild alkaline conditions using sodium carbonate. Method B was performed in glacial acetic acid with ammonium acetate at 100°C or higher (see details in Chapter 3). In some cases Method A gave better yields than Method B, while in other cases, the reverse applied. Generally, the NMR data and mass spectrum of each compound were sufficient
as a guide for purity especially if the final melting point after recrystallization was sharp (range 1 - 2°C).

### 2.3 The Henry reaction

The Henry Reaction (refer to *Chapter 1*) can be used for the preparation of nitroalkenones and this reaction was the dominant reaction used in this project. The condensation was performed under various conditions. One technique was based on the method of Knoevenagel and Walter\(^{166}\). In this condensation reaction, the aldehyde and nitro compound react in the presence of potassium carbonate and methylamine in a solvent (ethanol) at room temperature. According to Crowell and Ramirez\(^{167}\), and Crowell and Kim\(^{168}\) the key reagent for this reaction is the amine, which acts as a catalyst in the reaction. The amine catalyst reacts with benzaldehyde to form an imine and water as a by-product. The water produced in the reaction, in fact, was found to have an appreciable effect on the yield, as it will shift the equilibrium of the reaction to the left, hence reducing the yield of the product\(^{167, 168}\). For this reason ethanol was introduced to reduce the influence of water and so optimize the yield\(^{168}\).

A table below (Table 3) showed the structure, yield and melting points of compounds that have been synthesized in this research project. Synthesis methods and conditions can refer to *Chapter 3* the experimental section.
Table 3: Synthesized compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>Yield (%)</th>
<th>M.P. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>H</td>
<td>H</td>
<td>82</td>
<td>58-59</td>
</tr>
<tr>
<td>1b</td>
<td>4-F</td>
<td>H</td>
<td>57</td>
<td>99-100</td>
</tr>
<tr>
<td>8b</td>
<td>4-OH</td>
<td>CH₃</td>
<td>50</td>
<td>121-122</td>
</tr>
<tr>
<td>9b</td>
<td>3,4-dimethoxy</td>
<td>CH₃</td>
<td>27</td>
<td>71-72</td>
</tr>
<tr>
<td>11</td>
<td>H</td>
<td>CH₃</td>
<td>22</td>
<td>60-62</td>
</tr>
<tr>
<td>12a</td>
<td>2-F</td>
<td>CH₃</td>
<td>34</td>
<td>45-47</td>
</tr>
<tr>
<td>12b</td>
<td>3-F</td>
<td>CH₃</td>
<td>52</td>
<td>NA</td>
</tr>
<tr>
<td>12C</td>
<td>4-F</td>
<td>CH₃</td>
<td>30</td>
<td>65-66</td>
</tr>
<tr>
<td>12d</td>
<td>2,4-difluoro</td>
<td>CH₃</td>
<td>43</td>
<td>48-49</td>
</tr>
<tr>
<td>49a</td>
<td>2-CF₃</td>
<td>CH₃</td>
<td>46</td>
<td>NA</td>
</tr>
<tr>
<td>49b</td>
<td>3-CF₃</td>
<td>CH₃</td>
<td>46</td>
<td>NA</td>
</tr>
<tr>
<td>49c</td>
<td>4-CF₃</td>
<td>CH₃</td>
<td>42</td>
<td>96-98</td>
</tr>
<tr>
<td>50a</td>
<td>3-OCF₃</td>
<td>CH₃</td>
<td>51</td>
<td>NA</td>
</tr>
<tr>
<td>50b</td>
<td>4-OCF₃</td>
<td>CH₃</td>
<td>73</td>
<td>47-48</td>
</tr>
<tr>
<td>51</td>
<td></td>
<td>CH₃</td>
<td>18</td>
<td>99-101</td>
</tr>
<tr>
<td>52</td>
<td>4-CH₃</td>
<td>CH₃</td>
<td>80</td>
<td>54-55</td>
</tr>
<tr>
<td>53a</td>
<td>1-naphth</td>
<td>CH₃</td>
<td>49</td>
<td>62-64</td>
</tr>
<tr>
<td>53b</td>
<td>2-naphth</td>
<td>CH₃</td>
<td>41</td>
<td>90-91</td>
</tr>
<tr>
<td>54a</td>
<td>H</td>
<td>phenyl-benzpyran</td>
<td>43</td>
<td>88-90</td>
</tr>
<tr>
<td>54b</td>
<td>H</td>
<td>4’ fluorophenyl-benzpyran</td>
<td>55</td>
<td>88-89</td>
</tr>
<tr>
<td>55</td>
<td>H</td>
<td>CH₂CH₃</td>
<td>60</td>
<td>NA</td>
</tr>
</tbody>
</table>
2.4 Structure-activity relationships (SARs)

The important structural features studied were:

1. An aromatic ring (most compounds were based on β-nitrostyrene)
2. A nitro group on alkenyl side chain
3. Other substituents on the aromatic ring
4. Variations in the structure of the side chain

Most compounds tested for microbiological activity possessed an aromatic ring. Previous studies likewise have been carried out on aromatic compounds as it was thought that the flat surface of the aromatic ring could facilitate van der Waals bonding to other flat structures in the microorganism or other molecules that interfere with metabolism within the cell, such as enzyme inhibitors. Likewise, the nitro group was treated as a fundamental group for investigation. The only variation being that one compound \[51\] (page 71) possessed two nitro groups. The side chain to which the nitro group was attached was unsaturated and was varied from two to four carbon atoms. The compound with three carbon atoms (propenyl group) has been shown in previous studies\[22\] to give superior activity to those with two carbon atoms.

Other aromatic ring substituents included: –OH, -OCH\(_3\), -O-CH\(_2\)-O-, -CH\(_3\), -F, -CF\(_3\), -OCF\(_3\), including multiple substituents or otherwise varied according to the position on the ring. Two unsubstituted compounds \[1a and 11\] were included as controls for these substitution effects.

2.5 The importance of previous work

The previous experimental results by Nicoletti \textit{et al.} (unpublished work)\[29\] influenced the direction of this project. Comparisons were made of activity against the bacteria and the fungus common to both studies. The Nicoletti \textit{et al} studies showed that:
a) *E. coli* (Gram negative bacterium) was suppressed effectively by chloro or fluoro substituents at the 4-position relative to the side chain of β-methyl-β-nitrostyrene; β-methyl-β-nitrostyrene with a methylene-dioxy ring substitution at positions 3- and 4- on the aromatic ring was not as effective.

b) *S. aureus* (Gram positive bacterium) was suppressed effectively by a wide range of nitropropenyl arenes including β-methyl-β-nitrostyrene, the 4-fluoro and 4-chloro substituted derivatives of β-methyl-β-nitrostyrene. Imidazolyl, 3,4-dihydroxy and benzothiazole derivatives and the 3,4-methylene dioxy derivative were also very active. An important finding was that with two hydroxy groups in the 2- and 4- positions or the 2- or 5- positions, activity against this microorganism was noticeably reduced. The fact that this also occurred with substitution by \(N,N\)-dimethyl and \(N,N\)-diethyl groups indicated that the more polar nature of these derivatives was detrimental to activity. This was supported by the \(K_D\) values of the latter compounds being relatively low compared with the unsubstituted and halogenated-substituted compounds.

c) *B. subtilis* (Gram positive bacterium) was suppressed by a wide range of compounds in a similar way to *S. aureus* and the dihydroxy substituted compounds [2,4- and 2,5-isomers] derivatives were unsatisfactory as chemical agents against this bacterium. However, 3,4-dihydroxy substitution gave high activity.

d) *C. albicans* was suppressed by the 4-chloro and 4-fluoro derivatives, as well as the 3,4-dichloro derivative. However 4-fluoro and the benzothiazole derivatives were also very active as well as 3,4-dihydroxy substituted compound.

Previous results indicated that 3,4-methylenedioxy-β-methyl-β-nitrostyrene and many of the aromatic nitro compounds were not very effective against *E. coli*. The methylene dioxy group was not quite as effective as a simple –OH at position 3 relative to the side chain (3-hydroxy-β-methyl-β-nitrostyrene) or dihydroxy (positions 3 and 4 to the side chain). The 4-fluoro substituent was superior to all other substitutions and also to the β-methyl-β-
nitrostyrene. No improvement in activity was favored by the use of a combination of –OH and –OCH₃ (3-hydroxy-4-methoxy-β-methyl-β-nitrostyrene and 2-methoxy-3-hydroxy-β-methyl-β-nitrostyrene) or by 3,4-dimethoxy groups, although not all possible substituted positions were tested. These results suggested that compounds having some degree of hydrophilicity were the most effective against E. coli and this is borne out by the relatively low Kᵯ values of the most effective compounds (Kᵯ 65 – 150) compared with ineffective ones such as 3,4-methylenedioxy-β-methyl-β-nitrostyrene (Kᵯ 362). These compounds correspond to log₁₀ Kᵯ values of 1.8 – 2.2, is often referred to as optimal Log P values for antibacterial activity.²² It could be speculated that for many Gram negative bacteria (such as E. coli) which are known to have polysaccharide structures, there would be greater affinity for hydrophilic compounds and hence passage through cell walls of these types of bacteria would be facilitated. For our results, the only Log P value for that can be cited for an effective fluorinated compound on E. coli (Gram negative) is 2.00 [¹²c]. For the Gram positive bacteria, a range of Log P values of 1.15 – 2.19 appeared to be related to efficacy.

For the non-fluorinated compounds, optimal Log P values were over a much wider range from 1.61 – 3.41, and if C. albicans is included the range is even wider, from 1.15 – 3.41.

2.6 Initial experiments

The initial experiments were performed on key compounds that would be expected to provide acceptable standards for high activity. In this respect, 4-fluoro-β-methyl-β-nitrostyrene was chosen as the most promising against E. coli and was compared against the 2-fluoro and 2,4-difluoro compounds. The 3,4-dimethoxy derivative of β-methyl-β-nitrostyrene was also compared against the 3,4-methylene-dioxy compound. The unsubstituted compound was also tested in the main series of experiments. It was important to show that Method A (base catalysed reaction, which usually been used by Professor Hugh Cornell in this project) produced compounds of equal activity to those
prepared by Method B (ammonium acetate – acetic acid). Finally, the wide range of partition coefficients ($K_D$ values) of the compounds tested was wide (65 - 362) and therefore ideal for testing correlations with MIC values for both Gram positive and Gram negative bacteria. The results of these experiments are shown in Table 4, which lists the MIC values for each compound and their $K_D$ values. Three Gram positive bacteria (Staphylococcus aureus, Bacillus subtilis and Enterococcus faecalis), one Gram negative bacterium (Escherichia coli) and a fungus (Candida albicans) were tested in this program.
Table 4: Geometric mean MIC values in μg/mL and $K_D$ values for initial compounds tested against a fungus and a panel of bacteria.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Compounds and MIC values (μg/mL)</th>
<th>7</th>
<th>9b</th>
<th>12a</th>
<th>12c</th>
<th>12d</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td></td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>B. subtilis</td>
<td></td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>E. faecalis</td>
<td></td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5.5</td>
<td>6</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td>256</td>
<td>128</td>
<td>42</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td>C. albicans</td>
<td></td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Partition Coefficient</td>
<td></td>
<td>362</td>
<td>250</td>
<td>138</td>
<td>101</td>
<td>65</td>
</tr>
</tbody>
</table>

Figure 1: The compounds tested for antibacterial activity

7: 3,4-methylenedioxy-β-methyl-β-nitrostyrene
9b: 2-dimethoxy-4-(2-nitroprop-1-enyl)benzene (3,4-dimethoxy-β-methyl-β-nitrostyrene)
12a: 1-fluoro-2-(2-nitroprop-1-enyl)benzene (2-fluoro-β-methyl-β-nitrostyrene)
12c: 1-fluoro-4-(2-nitroprop-1-enyl)benzene (4-fluoro-β-methyl-β-nitrostyrene)
12d: 1,3-difluoro-4-(2-nitroprop-1-enyl)benzene (2,4-difluoro-β-methyl-β-nitrostyrene)

The results in Table 3 were tested for correlation between MIC value and $K_D$ value for $E. coli$ and $E. faecalis$. 
Figure 2: Correlation between MIC value and $K_D$ value for *E. coli*  

![Graph showing the correlation between MIC value and $K_D$ for E. coli with the equation $y = 1.2172x + 62.087$ and $r^2 = 0.9237$.]

Figure 3: Correlation between MIC value and $K_D$ value for *E. faecalis*  

![Graph showing the correlation between MIC value and $K_D$ for E. faecalis with the equation $y = -153.68x + 966.98$ and $r^2 = 0.8777$.]

The results suggest that $K_D$ values in the lowest range (65 – 138) are associated with high activity against *E. coli* whilst the opposite is the case for *E. faecalis*. Note the opposite gradients for each bacterium shown in Figures 4 and 5.
There were no correlations between MIC value and $K_D$ value for *S. aureus*, *B. subtilis*, and *C. albicans* (graphs not shown).

### 2.6.1 Effects of substituents

**Compound 12c**

Of the five different compounds tested for antimicrobial activity, the most active compound was the one prepared from 4-fluorobenzaldehyde and nitroethane [1-fluoro-4-(2-nitroprop-1-enyl)benzene] [12c]. This confirmed the results obtained by Nicoletti et al.\textsuperscript{29} using a panel of Gram positive bacteria, enteric and non-enteric Gram negative bacteria and fungi, in which a large number of compounds with different types of substitution on the aromatic ring were tested. There was no significant difference in activity between the two samples of the above compound, one prepared by Method A, the other by Method B. The lowest MIC values against *E. coli* (27µg/mL) were obtained with 12c (Refer to Table 4). MIC values against the other microorganisms were all less than 8µg/mL, hence all the compounds tested would be described as very effective against the three Gram positive bacteria and *C. albicans*.

**Compound 7**

With regard to the *E. coli* results, the least effective compound was compound 7. The compounds prepared from reaction of 2-fluorobenzaldehyde [12a] and 2,4-difluorobenzaldehyde [12d] with nitroethane had slightly lower activity against *E. coli* than 12c. The compound [9b] prepared from reaction of 3,4-dimethoxybenzaldehyde with nitroethane gave excellent results against all microorganisms except *E. coli*. However, the latter preparation gave results with *E. coli* that were comparable to commercial compound 7. Nicoletti et al.\textsuperscript{29} (unpublished work) also evaluated some aromatic nitropropene compounds with –OH and –OCH$_3$ substitution and found that those with one –OH and one -OCH$_3$ were the most promising. The compound with dimethoxy substitution [9b] had high activity against the three Gram positive bacteria and the fungus common to both
experiments but was inferior to 12c against the gram negative E. coli. It did, however, show relative higher activity than compound 7 (Table 4).

There was a good correlation (Figure 2) between the effectiveness against E. coli and the partition coefficients of the compounds tested ($r^2 = 0.9237$). Thus for high effectiveness against E. coli, compounds with low K<sub>D</sub> values are indicated to be the most effective, i.e. compounds with low lipophilicity. In Figure 3, there is also a good correlation ($r^2 = 0.8777$) between the effectiveness against E. faecalis and the partition coefficients of the compound tested. The results show that for high effectiveness against E. faecalis, compounds with high K<sub>D</sub> values are the most effective, i.e. compounds with high lipophilicity. E. faecalis is an enteric bacterium and its cell wall structure governs penetration of antibacterial compounds. High K<sub>D</sub> values appeared to be favoured for high activity. No conclusions could be drawn concerning correlations with other Gram positive bacteria as the MIC values were all in the range 2-3. Likewise MIC values for C. albicans were also very low. Milhazes et al.<sup>22</sup> used E. coli, E. faecalis and S. aureus for test on various nitrostyrene derivatives, but found no correlation between MIC values and lipophilicity on E. faecalis and S. aureus.

The reason for the effectiveness of fluorine substitution on the aromatic ring is probably connected with the high electronegativity of fluorine, although size factors could also be important. Perhaps the electronegativity of fluorine could affect binding affinity to the binding site of the bacteria, thus causing inhibition of the enzyme.

The results indicate that further experiments with fluorine substitution would be valuable as a way of determining the structural features required for the optimal anti-bacterial activity of nitro compounds of this type. Fluorine substitutions made on both the ring and side chain will be attempted in order to present a clearer picture of structure-activity relationships and possible mechanisms of action.
2.7 Discussion of structure – activity relationships (SARs)

This section will focus on the effectiveness against the chosen bacteria and fungi of the main series of compounds investigated. For chemical structures of compounds refer to Table 5 page 69 and 70.

2.7.1 Hydroxy and methoxy substituted compounds

See 8a, 8b, 8c, 9a, 9b, 10a, 10b against 11 (no substitution) and refer to Table 5

1. One substituent –OH group

One –OH in 3-position [8a] to the side chain made marginal changes to the activity compare to the unsubstituted compound [11] and a small increase in K₀ values (113 to 145) was not consistent with the slightly better result against E. coli caused by the substitution. The compound with substitution on the ring in 4-position [8b] with slightly higher in K₀ values than 8a showed better results against tested bacteria except result against E. coli which both of them had obtained the same MIC values (64).

2. Two –OH groups

Two –OH groups, at position 3 and 4 relative to the side chain [8c] made a small improvement with the Gram positive bacteria relative to 8a, but there was no change in activity against E. coli, the latter observation being consistent with virtually the same K₀ value. The result with C. albicans was excellent (MIC 4).

3. –OH and –OCH₃ together

10a with –OH at position 3 and –OCH₃ at position 4 to the side chain failed against C. albicans, as did 10b with these positions reversed. This suggested that, as both of these compounds were reduced in effectiveness against C. albicans compared with the unsubstituted compound [11], high polarity effects of these groups reduced the activity against this fungal material. Both still maintained reasonably high activity against the Gram positive bacteria but the activity of 10a against E.
coli was somewhat lower than 10b, the latter being about the same as the unsubstituted compound.

4. One substituent –OCH$_3$ group

A substitution of a –OCH$_3$ group in 4-position [9a] relative the side chain also changes the activity compared to the unsubstituted compound [11] giving a much more active compound against the chosen bacteria (same results obtained as compound 8b) although a huge increase in $K_D$ values (113 to 479) had occurred. These results, suggest that substitution in 4-position of the ring could optimize the activity of the parent compound [11] against the Gram positive, Gram negative bacteria and the fungus.

5. Two –OCH$_3$ groups

Compound 9b with –OCH$_3$ groups at positions 3 and 4 to the side chain gave excellent results on C. albicans and the Gram positive bacteria, in complete contrast to 10a and 10b with adjacent –OH and –OCH$_3$ groups. It could be argued that this was due to optimal polarity effects, but it does not explain why compound 8c with its two hydroxy groups is also of excellent activity against C. albicans whereas the compounds with one of each type of substituent [10a and 10b] are much inferior.

Activity of 9b against E. coli was slightly less than 10b and similar to that of 10a.

For these –OH and –OCH$_3$ substitutions it appears that the main achievements were improved activity against Gram positive bacteria and Candida albicans, seen with substitution at position 3 and 4 with –OH groups [8c] and –OCH$_3$ groups [9b]. However, it must be pointed out that most of these substitutions only marginally improved the activity and in two cases [10a and 10b] with one of each group as a substituent, there was a large decrease in activity against C. albicans. Milhazes et al.$^{22}$ found a dihydroxy derivative to be the most active against Gram negative bacteria, which agrees with the results obtained on E. coli.
Several other compounds also showed high antibacterial activity, particularly compounds \textbf{8b} or \textbf{9a}, which compared favourably with compounds with –OH and –OCH$_3$, substitutions previously tested against \textit{E. coli}. Compounds \textbf{8a} and \textbf{8c} (Nicoletti \textit{et al.}\textsuperscript{29}) were more active than \textbf{7} and are marginally better than the non-substituted compound \textbf{11} and compound \textbf{10b} [1-hydroxy-2-methoxy-4-(2-nitroprop-1-enyl)benzene]. These results indicate that one –OH group gave some enhancement of activity against \textit{E. coli}, (e.g. compound \textbf{8a}) as well as compound \textbf{8c} (with two OH groups on adjacent carbon atoms). However, compounds \textbf{10a} and \textbf{10b} each with one hydroxy group and one methoxy group were not as active and compound \textbf{9b} with adjacent methoxy groups was also less active.

With compound \textbf{8b} (4-hydroxy), two comparisons can be made against \textbf{9a} (4-methoxy) with –OCH$_3$ instead of –OH, and against compound \textbf{11}, which has no ring substitutions. The activities of \textbf{9a} and \textbf{8b} are almost identical yet \textbf{9a} has much higher K$_D$ than \textbf{8b} (479 against 150). All results except those with \textit{E. coli} are excellent.

Results were generally better with compound \textbf{8b} (4-hydroxy) than for Compound \textbf{11} against the chosen Gram positive bacteria, but were the same for \textit{E. coli} and \textit{C. albicans}, suggesting the substitution of –OH \textbf{[8b]} and –OCH$_3$ \textbf{[9a]} had improved the potency against Gram positive bacteria. K$_D$ values of 479 \textbf{[9a]}, 150 \textbf{[8b]} and 113 \textbf{[11]}, suggested that a more lipophilic nature is tolerated for antibacterial properties in the case of Gram positive bacteria.

The compounds \textbf{8a} (3-hydroxy), \textbf{8c} (dihydroxy) \textbf{10a} and \textbf{10b} (Table 5) are included to offer further comparisons with the work of Nicoletti \textit{et al.}\textsuperscript{29} They have similar good activity against the Gram positive bacteria, but the activity of Compounds \textbf{10a} and \textbf{8c} is inferior to the others against \textit{E. coli} and \textbf{10a} and \textbf{10b} have the lowest activity of all compounds in Table 5 against the fungus. The reasons are possibly related to the presence of the polar hydroxy group in \textbf{8a}, \textbf{10a} and \textbf{10b}. Compound \textbf{8c}, with two methoxy groups, is the best of
this series of compounds. Referring again to Table 4, it is seen that Compound 9b (3,4-dimethoxy substitution) is very active against Gram positive bacteria, but lacks activity against *E. coli*.

### 2.7.2 Fluorine substitution on the ring

The effects of different fluorine substitutions on the aromatic ring on biological activity were explored further in order to determine which groups enhanced the activity of β-methyl-β-nitrostyrene [11], which was the main parent compound investigated. Previous results of MIC determinations on twenty different selected compounds, showed that 4-fluoro-β-methyl-β-nitrostyrene had the highest antimicrobial activity across the range of microorganisms tested (Nicoletti *et al.*). It was pointed out that tests were performed using a large panel of Gram positive bacteria, Gram negative bacteria and fungi. In the present series of tests, a smaller panel of bacteria and a fungus were used to test the activities of compounds (see Fig 4). The results are shown in Table 5, the most active compounds against *E. coli* were compounds 8a, 8b, 8c, 9a and 12c; all with MIC values of 64 µg/mL. Results with compound 11 (no substitution) indicated that the basic structure of β-methyl-β-nitrostyrene already has slightly lower activity against this bacterium compare to compounds 8 (a, b, and c) 9a and 12c. Substitution on the ring with –OCH₃, -OH, -CF₃ and -F only marginally improved this activity. However –OCF₃ substitution has caused a large reduction in activity, this maybe due to an opposing electronic effect of the oxygen atom, as this effect was not noticed with –CF₃. Results against the Gram positive bacteria and the fungus *Candida albicans* were generally good to excellent. More detailed analysis and comparisons follow.

The results again showed that the halogenated derivatives had enhanced potency, with 12c having lower MIC values than 7, the difference being seen clearly with the Gram negative bacterium *E. coli*. The early results (Table 4) again indicated that there was a
relationship between the MIC value and the $K_D$ value for the compounds studied ($r^2 = 0.5066$, graph not shown). The interpretation of this relationship is that the low $K_D$ value, representing a lower degree of lipophilic character, is necessary for disruption of the polysaccharide-rich membrane of the membranes of Gram negative bacteria. 12c has a much lower $K_D$ value than 7 (Refer to Table 4). The lower value of $r^2$ for this batch of results (Table 5) compared with results in Table 4 was due to the greater diversity of structures and $K_D$ values (e.g. $\text{CF}_3$, $\text{OCF}_3$, OH, OCH$_3$, dinitro compound) and side chain.

Compound 12c gave better results (64 MIC) against *E. coli* than 7 (MIC 128). The partition coefficient ($K_D$) of 12c is lower than that of 7 and further studies of $K_D$ values against MIC values were carried out to investigate the value of this test. Fluorine substitution shows effective enhancement of activity, with excellent results not just on *E. coli*, but also against all bacteria except *E. faecalis*. Compounds 8a (3-hydroxy), 8b (4-hydroxy), 8c (3,4-dihydroxy), 9a (4-methoxy) and 12c (refer to Table 5) all gave the same good results against *E. coli*. However, as noted in Table 4, compound 9a has a larger $K_D$ compared to 7. This suggested that lipophilicity of the compounds would not be the only factor or the dominant factor in activity against the chosen bacteria and may not apply to all the Gram positive bacteria.

Compound 9a (4-methoxy substitution) gave excellent results against Gram positive bacteria as did 12c yet 9a and has much higher $K_D$ than 12c (479 against 101). This suggests that low $K_D$ values are not required for high activity against Gram positive bacteria. Interestingly, this is opposite to what was generally observed for the Gram negative bacterium *E. coli*. Compounds 8b (4-hydroxy) and 49c (4-trifluoromethyl) have identical results to 9a with all the microorganisms tested excepted *E. coli* (49c has MIC values of 96). Compound 9a gave much better results (MIC 64) against *E. coli* than compound 50a (4-trifluoromethoxy) (MIC 512). Fluorine substitution of this type has detracted from activity with other bacteria and the fungus. Comparisons of activity with
Gram positive bacteria demonstrated that the $K_D$ is not the main factor involved in activity against the bacteria as $50b$ has a lower $K_D$ than $9a$. The structures are quite different and will govern $K_D$ values.

No improvement in antibacterial efficacy was found by the use of other fluorine containing substituents such as $-CF_3$ and $-OCF_3$. In fact, the latter group was greatly detrimental when substitution at the 4 position to the side chain was effected (compare $49c$ with $50b$). At positions C-2 and C-3 the $-CF_3$ detracted from activity against *E. coli*, indicating that this type of substitution may be interfering with the antimicrobial effect, e.g. by blocking access of the reacting species.

Compound $49c$ (4-trifluoromethyl) has a similar structure that of $50b$ (4-trifluoromethoxy), except for the extra oxygen atom attached to the $-CF_3$. They both gave similar antibacterial results, except for *E. coli*. $50b$ has a much higher MIC against *E. coli* (MIC $>512$) than $49c$, which is expected due to the $K_D$ for $50b$ being 155 and being 68 for $49c$. Compound $49c$ has quite good activity (MIC 96) and similar to the compounds with fluorine on the aromatic ring.

### 2.7.3 Other non-β-methyl-β-nitrostyrene based compounds

The introduction of a second nitro group by the use of terephthaldehyde [51] was also seen to be an important factor for study. Comparisons were made against the standard, unsubstituted aromatic compound [11]. The main series of microbiological tests included compounds with the naphthalene [53a, 53b] instead of the benzene ring of β-methyl-β-nitrostyrene. A nitrochromene compound [54a] was also tested, along with the fluorine derivative [54b]. All the compounds tested, except compounds $10a$ and $10b$, showed good to excellent activity against the fungus *Candida albicans*. With regard to the Gram positive bacteria, all the compounds tested showed good to excellent activity with several
[9a, 8b, 49c, 50b, 51a, 52 and 11] being comparable to 12c, in accordance with the results of previous studies (Nicoletti et al.29). Hence, so far, it can be concluded that all of these nitrocompounds appear to belong to a class of compounds which are quite effective as antimicrobial agents.

The two nitropropenyl groups of compound 51, compared to compound 11, proved to be detrimental in activity against \(\text{E. coli}\). A considerably higher \(K_D\) was also observed with 51; however, results for the Gram positive bacteria and the \(C. albicans\) all were excellent.

All these compounds have large \(K_D\) values. Nitrochromene [54a] was not active against \(E. coli\) to any appreciable extent and there was no improvement with fluorine substitution [54b]. Generally for \(E. coli\) inhibition the more hydrophilic compounds, with lower \(K_D\) values, performed better than those with higher \(K_D\) values.

In summary, fluorine substitution on the ring at position 4 [12c] was slightly better than substitution with –OH [8b] and –OCH\(_3\) [10b], –CF\(_3\) [49c] and no ring substitution [11] against \(E. coli\). The initial series indicated 1-fluoro-4-(2-nitroprop-1-enyl)benzene as the most active, and all compounds had very good activity (MIC 2-27) against \(S. aureus\), \(B. subtilis\) and \(E. faecalis\).

2.8 Results with \(E. faecalis\) and \(E. coli\)

By considering \(E. faecalis\) (Gram positive) and \(E. coli\) (Gram negative) the influence of substituents is apparent as the MIC values are higher than for other bacteria and the fungus.

\(E. faecalis\)

- \(-\text{OCH}_3\), \(-\text{OH}\), \(-\text{CF}_3\) at 4-position, \(-\text{OCF}_3\) at 4-position, the dinitropropenyl compound prepared from terephthaldehyde [51], 7 and 12c, and unsubstituted \(\beta\)-methyl-\(\beta\)-
nitrostyrene [11] all had high activity. There was no obvious benefit of any substitution.

- The best performance (MIC 4) was seen with the 2-naphthyl derivative [53b]. The 1-naphthyl derivative [53a] was distinctly less active but a good result (MIC 32) was obtained against this Gram positive bacterium.

- 3-fluoro and 4-fluoro substitution on the ring [12b, 12c] gave very good results respectively (MIC 16), akin to the results with 7 results and no substitution [11].

- Good results (MIC 32) were also obtained with compound 52 (CH$_3$ at position 4), the 1-naphthyl derivative [53a].

- The nitrochromenes (MIC both 128) were only moderately active.

- The β-nitrostyrene [1a] compound made from nitromethane was inferior (MIC 128) to the β-methyl- β-nitrostyrene derivative [11] (MIC 16). Activity was improved by substitution at position 4 with fluorine [1b], but not to the extent as with 12c. A methyl group at position 4 [52] showed no antibacterial enhancement (MIC 32 against 16 for the unsubstituted compound, 11).

_E. coli_

- Compound 7 was not significantly active against _E. coli_ (MIC 128).

- Fluorine substitution at position -4 [12c] enhanced activity but fairly good results were obtained without any substitution [11] (MIC 92).

- An –OH group at position -3 or -4, two –OH groups at position 3 and 4, an -OCH$_3$ at position 4, and –CF$_3$ at position 4 gave compounds that were all fairly active (MIC 64).

- An –OCF$_3$ at position 4 [50b] caused a sharp drop in activity compared to the same substituent at position 3 [50a].

- The terephthaldehyde product [51] had extremely poor activity (MIC > 512) in complete contrast with the result against _E. faecalis_ (MIC 2).
The product with 2-OCH₃ groups or one with an –OH and -OCH₃ were only of moderate activity [10a and 9c].

Best results (MIC 64) were obtained with 3-OH [8a], 4-OH [8b], 3,4-dihydroxy [8c], 4-OCH₃ [9a], 3,4-dimethoxy [9b], 4-fluoro [12c], 4-CF₃ [49c] and parent compound [11].


Substitution with –CF₃ at position 2 [49a] gave a very unsatisfactory result (MIC 512).

In contrast to the *E. faecalis* results, the naphthyl derivatives were both very poor and the 2-naphthyl derivative [53b] (best with *E. faecalis*) gave the worst result of all (MIC>512).

The nitrochromene derivatives [54a and 54b] were both poor (MIC 256) and both were worse than the unsubstituted compound [11] made from nitroethane. Substitution with fluorine was of no consequence.

Against *E. coli*, the most active compounds were compounds 8a, 8b, 8c, 9a and 12c; all with MIC values of 64 µg/mL. Results with compound 11 (no substitution) indicated that the basic structure of β-methyl-β-nitrostyrene already has a high activity against this bacterium. Substitution on the ring with –OCH₃, -OH, -CF₃ and –F have only marginally improved this activity.

Results against the Gram positive bacteria and the fungus *Candida albicans* were generally good to excellent. More detailed analysis and comparisons follow.
Table 5: Microbiological evaluation of nitropropenyl arenes. Figures are MIC values in μg/mL

<table>
<thead>
<tr>
<th>Strain</th>
<th>Compounds and MIC values (μg/mL)</th>
<th>1a</th>
<th>1b</th>
<th>7</th>
<th>8a</th>
<th>8b</th>
<th>8c</th>
<th>9a</th>
<th>9b</th>
<th>10a</th>
<th>10b</th>
<th>11</th>
<th>12b</th>
<th>12c</th>
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<td>8</td>
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<tr>
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<td>256</td>
<td>8</td>
<td>6</td>
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<td>4</td>
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![Chemical structures](image)
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<th>Strain</th>
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<th>49b</th>
<th>49c</th>
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<td><em>Escherichia coli</em></td>
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<td>&gt;512</td>
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<tr>
<td><em>Candida albicans</em></td>
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<td>2561</td>
<td>280</td>
<td>429</td>
<td>70</td>
</tr>
</tbody>
</table>

![Chemical structures](image-url)
Figure 4: The correlations between MIC and $K_D$ values for each organism

**S. aureus**

- $y = -1.6937x + 377.79$
- $R^2 = 0.0281$

**B. subtilis**

- $y = -1.1383x + 369.46$
- $R^2 = 0.0214$
\[ y = -1.6676x + 423.16 \]
\[ R^2 = 0.0139 \]

\[ y = 1.6686x - 54.032 \]
\[ R^2 = 0.2379 \]
Please note that in C. albicans $K_D$-MIC correlations a reversal of the slope/gradient was observed.

2.8.1 Results with Gram positive bacteria

Results against the Gram positive bacteria (including the previous data of Nicoletti et al.\cite{29}) indicated that the growth of this group of bacteria is more readily suppressed by β-methyl-β-nitrostyrene than in the case of E. coli. Structure activity relationships were only able to be followed on Enterococcus faecalis as nearly all results on S. aureus and B. subtilis were excellent. In the case of some compounds notably 7, the compound with –OCF$_3$ substitution at position 4 [50b], the terephthaldehyde product [51] and a naphthalene derivative [53b] there was a complete turnaround from extremely poor results with E. coli to extremely good results with E. faecalis (MIC values 8 or less). In contrast to the E. coli results, the compounds with high $K_D$ values performed much better than those with low $K_D$ values suggesting that the more hydrophobic the compound was the better it performed against E. faecalis. Performance of compounds of this type was excellent against the other Gram positive species, S. aureus and B. subtilis with MIC values all in the range 2-16. Correlation between effectiveness against Gram positive bacteria were only able to be tested against E. faecalis. Figure 4 shows that a good correlation was obtained for the
particular compounds tested with negative gradient, the opposite to those with *E. coli*.

### 2.8.2 Results with *Candida albicans*

For *Candida albicans*, all compounds, irrespective of their activity against the Gram positive or Gram negative microorganisms, performed extremely well with MIC values in the range 2-16, the only exceptions being the following:

- Two hydroxy – methoxy substituted compounds from previous studies [10a] and [10b] (both MIC 128)

The reason for the lowering of activity of these compounds against *C. albicans*, seen strikingly in the two hydroxy – methoxy compounds [10a and 10b] compared with the two unsubstituted compounds (β-nitrostyrene [1a] and β-methyl-β-nitrostyrene [11]) is unknown. However, the results of these compounds [10a and 10b] with *E. coli* are also unimpressive and may be due to the effects of two polar groups causing significant blocking of an otherwise interactive site. The 3,4-dihydroxy derivative [8c] gave an excellent result and highlights the importance of the positions of substitution in interactions between the compounds and receptor sites on the microorganism.

Substitution of β-methyl-β-nitrostyrene with fluorine at the 4-position [12c] resulted in a slight increase in activity against *Candida albicans* (12c against 11). The results of substitution with –CF₃ at the 4-position produced no change in activity, but –OCF₃ at this position may have attenuated the activity (compare 49c, 50b and 11). The unsatisfactory results with both nitrochromenes [54a and 54b] may be due to blocking by groups in the vicinity of the nitro group (oxygen atom and aromatic ring). These compounds likewise did not perform well against the bacteria.
2.9 Summary of SARs results

2.9.1 Substitutions on aromatic ring

1. –OH vs. –OCH$_3$ (8b vs. 9a)

   The activities were identical with excellent results against all microorganisms except *E. coli*, which gave an MIC of 64 in each case. The result for the methoxy substituted compound is surprisingly good, especially considering its high $K_D$ value (479). The results are somewhat better than the parent compound [11].

2. –OCH$_3$ vs. –OCF$_3$ (9a vs. 50b)

   The methoxy substitution proved vastly superior to the trifluoromethoxy group in tests against *E. coli* (MIC 64 against 512). However, in the tests against other bacteria and *C. albicans*, both substitutions were observed to be slightly better than no substitution [11], the major difference being that activity against *E. coli* was adversely affected as mentioned above. For this group of compounds, the $K_D$ values were not reliable indicators of activity against *E. coli*.

3. –CF$_3$ vs. –OCF$_3$ (49c vs. 50b)

   The results with –CF$_3$ were excellent against all the microorganisms tested except *E. coli*. The compound [49c] still had fairly high activity (MIC 96) making it comparable to one without substitution, but the –OCF$_3$ substitution destroyed the activity against *E. coli*. The $K_D$ value of compound 49c was only 68 and indicative of its superiority to 50b ($K_D$ 155) against *E. coli*.
4. –CH₃ vs. –CF₃ (52 vs. 49c)

The methyl group at position 4 gave similar results to the –CF₃, suggesting that the fluorine substitution for hydrogen had no significant effect on activity across the range of microorganisms tested despite the huge differences in Kᵱ values.

5. –F vs. –CF₃ (12c against 49c)

The excellent results reported for –F substitution at position 4 was only marginally better than those for –CF₃ at the same position. Nevertheless, -F substitution remains the best type for overall high activity against the Gram negative, Gram positive and fungal microorganisms tested.
The Gram positive bacteria tested were most effectively inhibited by the following substitutions shown in Table 6.

**Table 6: Most effective compounds against the Gram positive bacteria**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Bacteria (MIC)</th>
</tr>
</thead>
<tbody>
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<td><img src="image" alt="Structure 8b" /></td>
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<tr>
<td></td>
<td><strong>B. subtilis</strong> (2)</td>
</tr>
<tr>
<td></td>
<td><strong>E. farcalis</strong> (4)</td>
</tr>
<tr>
<td><img src="image" alt="Structure 9a" /></td>
<td><strong>S. aureus</strong> (2)</td>
</tr>
<tr>
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<td><strong>B. subtilis</strong> (2)</td>
</tr>
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<td></td>
<td><strong>E. farcalis</strong> (4)</td>
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<tr>
<td><img src="image" alt="Structure 9b" /></td>
<td><strong>S. aureus</strong> (2)</td>
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<tr>
<td></td>
<td><strong>B. subtilis</strong> (2)</td>
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<td></td>
<td><strong>E. farcalis</strong> (5)</td>
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</tr>
</tbody>
</table>

These products were also the most effective against the fungus *Candida albicans* and were likewise superior to the unsubstituted parent compound.
Relative activities of compounds
The relative activities of some compounds are summarized Table 7.

Table 7: Relative activities of some compounds

<table>
<thead>
<tr>
<th>Relative activities</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has the best activity against <em>E. coli</em>, and was also excellent against <em>C. albicans</em> and very good against <em>S. aureus</em> and <em>B. subtilis</em>.</td>
<td>12c</td>
</tr>
<tr>
<td>It does not show results for <em>E. coli</em> as good as 12c, but it is comparable on Gram positive bacteria and fungus.</td>
<td>7</td>
</tr>
<tr>
<td>Compounds showed excellent activity against the Gram positive microorganisms and the fungus</td>
<td>8b, 8c, 9a, 9b, 49c, 50b, 51 and 53b*</td>
</tr>
<tr>
<td>These compounds have relatively high activity against <em>E. coli</em></td>
<td>8a, 8b, 8c, 9a, 9b, 10b, 11, 12c and 49c</td>
</tr>
<tr>
<td>Very poor activity against <em>E. coli</em> (MIC &gt; 512) in contrast to their high activity against the Gram positive bacteria and the fungus</td>
<td>49a, 50b, 51 and 53b</td>
</tr>
<tr>
<td>There was very good agreement on the compounds tested by Nicoletti <em>et al.</em></td>
<td>7, 12c, 8a, 8c, 10a and 10b</td>
</tr>
</tbody>
</table>

#Note: The $K_D$ values of 556 [51] and 2561 [53b] were quite the opposite of the low $K_D$ values associated with activity against *E. coli*. 
2.10 The effect of different substitutions on lipophilicity

In Chapter 1 lipophilicity was discussed as an indicator of the permeability of a drug to pass through living cellular membranes. In this section, the results of the effect of different substituents on lipophilicity are presented. Substitutions of –F, -CF<sub>3</sub>, -OCF<sub>3</sub>, -OH, -OCH<sub>3</sub> and the combination of the latter two were studied and are shown in 2.9.1. Figure 5. *E. coli* gave better correlations than the other tested bacteria and fungus even though they were not as good as the correlations shown in Fig 2. It still shows that low K<sub>D</sub> values generally work better against *E. coli* than the compounds with higher K<sub>D</sub> values.

Although compound activity again did not correlate with K<sub>D</sub> values, the Gram positive bacteria did not display good correlations. Compounds with high K<sub>D</sub> values are more compatible/effective against the Gram positive bacteria. This is also the case with *C. albicans*.
2.10.1 Summary of tested substitutions on β-methyl-β-nitrostyrene

Figure 5: Various substitutions on β-methyl-β-nitrostyrene

1. Fluorine atom(s) substitution, -F

![Chemical structures](image1)

2. Trifluoromethyl substitution, -CF$_3$

![Chemical structures](image2)

3. Trifluoromethoxy substitution, -OCF$_3$

![Chemical structures](image3)

4. Hydroxy substitution, -OH

![Chemical structures](image4)

5. Methoxy substitution, -OCH$_3$

![Chemical structures](image5)

6. Hydroxy and methoxy substitution, -OH and -OCH$_3$

![Chemical structures](image6)
2.10.2 Summary of results of lipophilicity studies

The results obtained showed, surprisingly, that changing the position (-2, -3, -4) of the fluorine atom on the aromatic ring (12a, 12b, and 12c, Table 8) altered the lipophilicity of the parent compound [11] by either increasing \( K_D \) slightly or decreasing \( K_D \). This was especially noted with 12b, in which the 3-position of substitution gave a very low \( K_D \) (14). Compound 12d with two fluorine atoms substituted on the ring also decreased the lipophilicity of compound 11 by half (\( K_D \) 65). However, only 12a gave a larger \( K_D \) value (246, refer to Table 4) than compound 11. (\( K_D \) 113)

The lipophilicity results of compounds 49a, 49b and 49c showed that substitution of a trifluoromethyl group on the ring decreased the \( K_D \) value (repetive \( K_D \) values 60, 30, 68) of the parent compound [11] by about a half. Substitution on position 3 gave the lowest \( K_D \) value out of the group.

Substitution on position 3 of the ring of a methoxy group [50a] again showed an extremely low \( K_D \) value (\( K_D \) 18), this being lower than other derivatives. Compound 50b was similar to 12c both compounds having higher \( K_D \) values (\( K_D \) 155 and 101) than the parent compound [11].

It can be seen by reference to Table 9 that hydroxy group substitutions gave similar results to the fluorine atom but substitution on position 3 [8a] still results in a lower \( K_D \) value than substitution on position 4 [8b] even though the difference of 8a and 8b in \( K_D \) is not large. The dihydroxy compound remains the lowest \( K_D \) (111) of the other two derivatives and has a slightly lower \( K_D \) than compound 11, while 8a and 8b did not have lower \( K_D \) values than compound 11.

Substitution on the 4-position showed the same result as previously; a large \( K_D \) was obtained that was significantly larger than the parent compound [11]. The dimethoxy
substitution [9b] gave a product having $K_D$ about half that of the monosubstituted compound [9a], and the result is similar to the other two di-substituted compounds (8c and 12d). However, the $K_D$ of 9b is still greater than compound 11.

A small $K_D$ (41) was obtained on compound 10a, in which a hydroxyl group is located at the 4 position of the ring. The value of $K_D$ increased to back over 150 when the –OH group was moved to position 4, which also happened with compound 8b.

In summary, low $K_D$ values compounds usually mean high efficiency against *E. coli* (Gram negative bacterium), while high $K_D$ values compounds generally are more effective against Gram positive bacteria. For the fungus, all compounds generally have good antibacterial activity even with high or low $K_D$ values. However, the $K_D$ values can only be used as a guide to estimate whether a compound is likely to be effective against the bacteria being tested. Some compounds, for example 12b and 12d, have lower $K_D$ values than 12c, but they do not have comparable activity to 12c against *E. coli*. 
Table 8: $K_D$ values of fluorine-substituted derivatives of β-methyl-β-nitrostyrene

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substitution</th>
<th>$K_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>12a</td>
<td>2-fluoro</td>
<td>138</td>
</tr>
<tr>
<td>12b (8a and 10a)</td>
<td>3-fluoro (3-hydroxy and 3-hydroxy-4-methoxy)</td>
<td>14 (145 and 41)</td>
</tr>
<tr>
<td>12c (8b)</td>
<td>4-fluoro (4-hydroxy)</td>
<td>101 (150)</td>
</tr>
<tr>
<td>12d</td>
<td>2,4-difluoro</td>
<td>65</td>
</tr>
<tr>
<td>49a</td>
<td>2-trifluoromethyl</td>
<td>60</td>
</tr>
<tr>
<td>49b</td>
<td>3-trifluoromethyl</td>
<td>30</td>
</tr>
<tr>
<td>49c</td>
<td>4-trifluoromethyl</td>
<td>68</td>
</tr>
<tr>
<td>53</td>
<td>4-methyl</td>
<td>60</td>
</tr>
<tr>
<td>50a (10b)</td>
<td>3-trifluoromethoxy (3-methoxy-4-hydroxy)</td>
<td>18 (186)</td>
</tr>
<tr>
<td>50b (10a)</td>
<td>4-trifluoromethoxy (4-methoxy)</td>
<td>155 (479)</td>
</tr>
<tr>
<td>1a</td>
<td>No ring substitution, no methyl substitution</td>
<td>51</td>
</tr>
<tr>
<td>11</td>
<td>Parent compound</td>
<td>113</td>
</tr>
</tbody>
</table>

Table 8 shows $K_D$ values of the various fluoro derivatives tested. Note the low values of $K_D$ as the result of substitution at the 3-position by –F, -CF$_3$, -OCF$_3$. Correlation between activity and $K_D$ values were not suggestive of a relationship. Results in brackets are intended for comparison.

Table 9: $K_D$ values of hydroxy and methoxy derivatives of β-methyl-β-nitrostyrene

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substitution</th>
<th>$K_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>3,4-methylene dioxy</td>
<td>362</td>
</tr>
<tr>
<td>8a</td>
<td>3-hydroxy</td>
<td>145</td>
</tr>
<tr>
<td>8b</td>
<td>4-hydroxy</td>
<td>150</td>
</tr>
<tr>
<td>8c</td>
<td>3,4-dihydroxy</td>
<td>111</td>
</tr>
<tr>
<td>9a</td>
<td>3-hydroxy-4-methoxy</td>
<td>41</td>
</tr>
<tr>
<td>9b</td>
<td>3-methoxy-4-hydroxy</td>
<td>186</td>
</tr>
<tr>
<td>10a</td>
<td>4-methoxy</td>
<td>479</td>
</tr>
<tr>
<td>10b</td>
<td>3,4-dimethoxy</td>
<td>250</td>
</tr>
<tr>
<td>11</td>
<td>None</td>
<td>113</td>
</tr>
</tbody>
</table>

Table 9 lists the $K_D$ values of compounds with hydroxy and methoxy substitutions showing the general tendency for $K_D$ to be raised by methoxy, dimethoxy and methylene dioxy substitution. A notable exception is in the case of the 3-hydroxy-4-methoxy derivative. For these compounds, correlations between activity and $K_D$ values were not as strong as previously.
Table 10 provides a summary of the effects of –F, -CF₃ and –OCF₃ substitutions of β-methyl-β-nitrostyrene. The 4-fluoro derivative had the highest activity against *E. coli*, while the 2-fluoro derivative was also of good activity. The activity against the Gram positive bacteria and the fungus were all high except for 49a, where a –CF₃ group was substituted at the 2-position. Further details can be found in section 2.8.

**Table 10**: MIC values (μg/mL) of β-methyl-β-nitrostyrenes with fluorine-containing substitutions

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substitution</th>
<th>K_D</th>
<th><em>E. coli</em></th>
<th><em>S. aureus</em></th>
<th><em>B. subtilis</em></th>
<th><em>E. faecalis</em></th>
<th><em>C. albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>12a</td>
<td>2-fluoro</td>
<td>138</td>
<td>42</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>12b</td>
<td>3-fluoro</td>
<td>14</td>
<td>256</td>
<td>8</td>
<td>16</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>12c</td>
<td>4-fluoro</td>
<td>101</td>
<td>27</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12d</td>
<td>2,4-difluoro</td>
<td>65</td>
<td>45</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>49a</td>
<td>2-CF₃</td>
<td>60</td>
<td>512</td>
<td>16</td>
<td>32</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>49b</td>
<td>3-CF₃</td>
<td>30</td>
<td>256</td>
<td>16</td>
<td>8</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>49c</td>
<td>4-CF₃</td>
<td>68</td>
<td>96</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>50a</td>
<td>3-OCF₃</td>
<td>18</td>
<td>256</td>
<td>16</td>
<td>8</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>50b</td>
<td>4-OCF₃</td>
<td>155</td>
<td>512</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

2.10.3 The optimal K_D values for activity of β-methyl-β-nitrostyrene derivatives

In this project lipophilicity was used as a guide to identify the optimal K_D values of tested compounds against the chosen bacteria. The results (Table 11), showed that the lower K_D values for fluorinated compounds normally are effective against Gram positive bacteria and fungus. However, only 12c gave the best result against the Gram negative bacterium *E. coli*. Conversely, the Log *P* range for non-fluorinated compounds was very broad against the Gram positive and fungus. For *E. coli* the range has been narrowed.
significantly, possibly due to the structure of each compound being similar to the β-methyl-β-nitrostyrene derivatives. With this we discovered that the lipophilicity of β-methyl-β-nitrostyrene derivatives is not the dominant factor affecting the potency against the chosen bacteria. The structure activity relationships (SARs) would still indicate the major factors that affects the potency.

Table 11: The range of optimal $K_D$ values for activity of β-methyl-β-nitrostyrene derivatives

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Non-fluorinated compounds</th>
<th>Fluorinated compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effective $K_D$ range (MIC ≤ 16)</td>
<td>Log $P$ range</td>
</tr>
<tr>
<td><strong>E. faecalis</strong></td>
<td>70-2561</td>
<td>1.85-3.41</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td>41-2561</td>
<td>1.61-3.41</td>
</tr>
<tr>
<td><strong>B. subtilis</strong></td>
<td>111-2561</td>
<td>2.05-3.41</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>111-479</td>
<td>2.05-2.68</td>
</tr>
<tr>
<td><strong>C. albicans</strong></td>
<td>14-2561</td>
<td>1.15-3.41</td>
</tr>
</tbody>
</table>

2.11 Trends with Gram positive bacteria

The correlations between MIC and $K_D$ values were not straightforward with the Gram positive bacteria, but generally large $K_D$ values were associated with higher activity against these microorganisms. This was in contrast to $K_D$ values of compounds tested against *E. coli*, where lower $K_D$ values were required for high activity. The trends of results in detail are as follows.

2.11.1 Trends with *E. faecalis*

There was no correlation between MIC values and $K_D$ values for *E. faecalis*, even when the compounds were grouped as fluorine-containing and non-fluorine containing types. The $r^2$ values were 0.0624 and 0.0261 respectively. However, it was apparent that for
each group (excluding those compounds that were not based on β-nitrostyrene alone) the
$K_D$ values were higher (mean = 530) for the most active (MIC 2 – 6) compounds. For
those compounds with less activity (MIC $\geq$ 8) the mean $K_D$ was 229.

The compounds with high activity, despite low $K_D$ values (< 70) was 49c (-CF$_3$ at position
4), 12d (2 fluorine atoms at position 2 and 4), 12a (fluorine at 2 position) were partly due
to the influence of fluorine substitution on activity and $K_D$. All other compounds with high
activity had $K_D$ values of $\geq$ 150. However, the poorest activity (MIC 64) was observed
with compound 1a, without the β-methyl group and the same result was obtained with 1b,
with no β-methyl group. Despite fluorine substitutions, compound 12c with $K_D$ 101 and
MIC 16, was not among the best performers and had about the same activity as the
unsubstituted β-methyl-β-nitrostyrene with $K_D$ 113. Generally, substitution with fluorine
reduced the $K_D$ values but the extent of this reduction depended upon the position of
substitution. For example in compounds 12a, 12b and 12c, substitution at position 3 gave
the lowest $K_D$ of 14. However, the highest activity was seen in compound 12a (position 2
substitution) with MIC 5 and $K_D$ 139 (the highest $K_D$ of the three).

The difference between –CF$_3$ and –CH$_3$ was seen with compounds 49c and 52 both with
substitution at the 4 position. The –CF$_3$ substituted compound was highly active with MIC
4 compared with MIC 32. Substitution of –CF$_3$ at position 2 [49a] resulted in a compound
of low activity (MIC 45). With –OCF$_3$ substitution, compound 50b (position 4) with $K_D$ 155
and MIC 8 was not quite as active as the methoxy compound with $K_D$ 479 and MIC 4. The
best results of all were with compound 51 with two nitro groups having $K_D$ 556 and MIC of
only 2.
2.11.2 Trends with *S. aureus*

For –OH and –OCH$_3$ substitution, the most active compounds against *S. aureus* were the 4-hydroxy [8b] substituted compounds of β-methyl-β-nitrostyrene together with the 3,4-dihydroxy [8c], 4-methoxy [9a] and the 3,4-dimethoxy [9b] derivatives. All of these compounds were more active than the unsubstituted parent compound but there was no significant correlation between the MIC and K$_D$ values. Substitution at the 3-position (compound 8a) was not as effective as substitution of –OH at the 4-position and was of no advantage over the unsubstituted compound. For all non-fluorine substitutions, the only compound of comparable activity to those above was compound 53b, the 2-naphthyl derivative with K$_D$ 2561 and MIC 4. For the fluorine-containing compounds, the most active were 12d (2,4-difluoro), 49c (4-trifluoromethyl) and 50b (4-trifluoromethoxy), closely followed by 12a (2-fluoro). However, no correlation between K$_D$ and MIC values was observed. The worst result was with 1b, the 4-fluoro-compound without β-methyl substitution.

2.11.3 Trends with *B. subtilis*

For –OH and –OCH$_3$ substitution, the 3,4-dimethoxy derivative, with the highest K$_D$ of 250 was the most active compound. Also of high activity were 8a (3-hydroxy) and 8c (3,4-dihydroxy). For all the non-fluorine containing substitutions the results were similar to those of *S. aureus*, with 53b (2-naphthyl derivative) again showing high activity. For the fluorine-containing derivatives, the results were very similar to those for *S. aureus*, the worst result being with 1b (4-fluoro derivative but without β-methyl substitution), as it was with *S. aureus*.

In summary, the results for the Gram positive bacteria are quite different from those with the Gram negative bacterium, *E. coli*, and indicate that high K$_D$ values are more likely to be preferable to low K$_D$ values for optimal activity. An example of this is the 2-naphthyl
derivative, (compound 53b, Kd 2561) which is highly active against all these of the Gram positive bacteria (MIC 4) yet is inactive against E. coli (MIC > 512). Compound 12c is highly active against E. coli, but is not among the highest activities against any of the Gram positive bacteria.

Compounds 8b, 9a, 9b, 12a, 12d and 53b are compounds that are highly active against all Gram positive bacteria tested. Compounds 12a, 12d and 49c are the fluorine derivatives that are highly active against these bacteria.

2.12 Trends with Fungus

2.12.1 Trends with C. albicans

For –OH and –OCH₃ substitution, the most active compounds were again the 3,4-dimethoxy and 3,4-dihydroxy derivatives, as was the case for the Gram positive microorganisms. Both showed greater activity than the unsubstituted compound [11]. Importantly, the worst compounds were 10a and 10b with MICs of 128, yet each of these compounds had one methoxy group and one hydroxy group. The compound without β-methyl group (1a) had less activity than compound 11, but was superior to compounds 10a and 10b. For all non-fluorine substitutions, many of the compounds performed well with MIC values of 4. The outstanding compounds (because of poor activity, MIC 128) were 10a and 10b as mentioned previously (see last paragraph) 1a without the β-methyl group (MIC 32) and a nitrochromene (compound 54a) with MIC 64. There was no significant correlation between MIC and Kd values. For all fluorine-containing compounds, the two of highest activity were compound 12a (2-fluoro derivative) and 12d (2,4-difluoro derivative). Fluorination of the nitrochromene made little improvement (54b compared with 54a). Comparison of different positions of the fluorine atom indicated that position 2 may result in better activity than at positions 3 and 4. With regard to the –CF₃ group, substitution at position 4 appeared to be the most favorable for activity (MIC 4). The –
OCF₃ group at position 4 gave a product with MIC 2, but this was little different to an –OCH₃ group (MIC 4). The best –fluoro, -trifluoromethyl and –trifluoromethoxy substitutions proved to be more active than the one without substitution [11]. No significant correlation was obtained between MIC and $K_D$ values.

In summary, the results for the fungus, *C. albicans*, are similar to those for the Gram positive bacteria. However, the following points of difference were observed. Compound 8c (3,4-dihydroxy derivative) was highly active against *C. albicans*, and also highly active against *S. aureus*, but had less activity against *B. subtilis*. Otherwise, all of the compounds which were highly active against the Gram positive bacteria were also highly active against *C. albicans*.

### 2.13 Mechanism of action

One possible mechanism for activity of these compounds was suggested by Park and Pei¹⁶ who showed that β-nitro-ethenyl benzene (β-nitrostyrene) is a reversible inhibitor of the tyrosine phosphatases PTP1B by means of the formation of a covalent complex with cysteine at the catalytic site. In the absence of free thiol they pictured the selective nucleophilic attack by cysteine on the nitrogroup of β-nitro-ethenyl benzene as the following:

Their mechanistic studies provided the basis for their reasoning. The rationale proposed was that the positive charge on the nitrogen atom, should be particularly reactive to nucleophilic attack, forming a reversible adduct that inhibited PTP1B. However, exactly
the opposite is found in the literature. The conjugate addition to nitroalkenes reflects the high reactivity of nitroalkenes towards nucleophiles\textsuperscript{169,170} and it is widely recognized that nitro group olefins undergo rapid conjugate addition with thiol-type nucleophiles\textsuperscript{171-174}. A literature search on the reaction of thiols with nitroarenes found the investigation by Hwu and co-workers\textsuperscript{175} who reported that at 185°C for 24hr CH\textsubscript{3}SiSNa was able to reduce various aromatic nitro compounds to amines. The susceptibility of the double bond to act as a highly active Michael acceptor for the cysteine nucleophile is for greater than the selective of direct nucleopholic reaction with the nitro group. It is proposed that the following mechanisms could apply\textsuperscript{176}. However it does not account for the greater antibacterial potencies of the β-methyl-β-nitrostyrene compounds.

\[
\begin{align*}
\text{AH} &+ \text{O}_2\text{N} & \text{AH} &+ \text{O}_2\text{N} \\
S^- &+ \text{R}^- & S^- &+ \text{R}^- \\
\text{H} &+ & & \text{H} &+ \\
\end{align*}
\]

The difference between the cell wall of the Gram positive and Gram negative bacteria is that Gram negative bacteria have an extra layer called the lipopolysaccharide layer\textsuperscript{1,4-6,9}. The reason why high lipophilicity compounds (e.g. 53b) can not penetrate into a Gram negative bacterium like \textit{E. coli} is because this high density lipopolysaccharide act as an effective barrier to prevent highly lipophilic agents penetrating the membrane to the interior of the cell\textsuperscript{6,9}. This is a possible rationale why compounds like 53b work effectively
against the Gram positive bacteria as they do not have this layer and high lipophilicity compounds and are able to penetrate the cell walls of these bacteria.

2.14 Conclusions

The performance of β-nitrostyrene derivatives is governed by the type of substitution on the aromatic ring, as well as by the length of the side chain. According to the tests performed the following conclusions could be drawn:

1. β-nitrostyrene has antibacterial and antifungal activity, but activity against *E. coli* is unsatisfactory.

2. β-methyl-β-nitrostyrene is superior to β-nitrostyrene, showing greater activity to all the microorganisms except *S. aureus*. The β-methyl group confers optimum activity, but a further increase in the size of the side chain results in lower activity.

3. Compound [7], with a methylene dioxy ring bridging positions 3- and 4- on the aromatic ring with commercial potential, BDM-I (Biodiem Pty Ltd), showed high activity against the Gram positive bacteria and fungus (*C. albicans*) but was unsatisfactory against *E. coli*.

4. Further experiments to investigate the value of substitutions on the aromatic ring with fluorine and fluorine-containing groups such as –CF₃ and –OCF₃ indicated that fluorine substitution at the 4-position provided the most active derivative (compound 12c). A general improvement in activity was noted compared with β-methyl-β-nitrostyrene (parent compound).

5. The substitution on the aromatic ring of β-methyl-β-nitrostyrene by hydroxy and methoxy groups produced many compounds with high activity against the Gram positive bacteria and *C. albicans*. However, many of these did not have high activity against *E. coli*. Compounds with comparable activity to the 4-fluoro compound [12c] across the range of microorganisms tested were the 4-methoxy
and 3,4-dimethoxy derivatives. Combinations of hydroxy and methoxy were not quite as effective and the positions of substitution were important factors.

6. Compound 53b, featuring a naphthalene substitution was interesting in that it gave excellent results against all the Gram positive bacteria and C. albicans, but failed badly against E. coli. It had a high $K_D$ value (2561), which seems to be desirable for activity against Gram positive bacteria.

7. Compounds with lowest $K_D$ values appear to be more effective against E. coli than those with high $K_D$ values and high degrees of correlation were obtained in most cases.

8. Other compounds which performed well against Gram positive bacteria and C. albicans was compound 51, with two nitropropenyl groups. However, it was completely ineffective against E. coli.

9. Other fluorine derivatives which also performed well against Gram positive bacteria and C. albicans were compound 49c with substitution of –CF$_3$ at position 4 and compound 50b, with substitution of –OCF$_3$ at the same position. The latter compound was completely ineffective against E. coli.

10. The requirements for high activity against E. coli are vastly different from those against the Gram positive bacteria. Differences are also seen with C. albicans, but the results against this fungus are much closer to those against the Gram positive bacteria than the E. coli.

Compounds that were not simple derivatives of β-methyl-β-nitrostyrene, i.e. compounds, 51, 54a, 54b were of interest, but only compound 51 was in the higher activity bracket. The compound with two nitropropenyl groups is extremely interesting as it had high activity against all microorganisms tested except E. coli. It had a $K_D$ of 556, again reinforcing the view that higher $K_D$ values are often associated with higher activity against the Gram positive bacteria. Further work will evaluate compounds with other type of
structures such as β-lactams, macrolides, and so on... Therefore it will then be possible to compare the β-nitro arenes with the antibiotics already in use.

2.15 *E/Z* configurations of the tested compounds

The *E* and *Z* configurations of a compound is always important in Medicinal Chemistry as either of them can be the key conformation to enhance the drugs efficiency. Most of the compounds in this project are based on β-methyl-β-nitrostyrene except 1a, 1b, 55a and 55b.

The definition of *E* and *Z* configurations depends upon the assignment of priorities to double bond substituents based on Cahn-Ingold-Prelog, priority rules\(^\text{177}\). For example consider an alkene which the two high-priority groups are on the opposite side of the double bond, the compound will be assigned as *E* configuration. The *Z* configuration is when high priority groups are on the same side of the double bond. According to the NMR obtained for each β-methyl-β-nitrostyrene derivatives the *E* configuration was the dominant conformation. The ratio of *E* to *Z* for example in 49c is 14:1 and the ratio of *Z* configuration would decrease after recrystallization, as the *Z* compound is more soluble in the solvent (95% alcohol). However, an exceptional case was obtained in 49a where the *Z* configuration is the dominant structure (approx. 1:1.8). As 49a existed as a liquid, recrystallization can not apply to this compound to minimize the ratio between the *E/Z* configurations unless submitting the sample to other analytical instruments (e.g. HPLC) to separate the two isomers. According to the structure of most of the major compounds, the *E* configuration domination is due to two high-priority groups, which are the phenol and nitro group (take 49c as an example), are on the opposite side of the double bond except in 49a.
2.16 Substrate for nitrostyrene formation via Henry reaction protocol

Two compounds that have been synthesized, but not yet been tested were 1c and 56b (See Chapter 3). They certainly can be tested in the future; however, the aim for making them is they are precursors for future synthesis of other novel nitrostyrene compounds.

2.17 Future work

There are some compounds that should be investigated for biological testing. Some of them are fluorinated compounds, some of them are the novel compounds and some of them would be designed to compare their biological activity with the existing compounds. (Compounds with * indicates new substances)

2.17.1 The fluorinated compounds

Additionally 19e should be made for biological evaluation. Compound 50c* could be used to complete the comparison of antimicrobial activity with substitution series of –F, -CF₃ and -OCF₃.
Compound $58^*$ and $62^*$ could be used to test whether replacing the proton by fluorine atom(s) would still affect the biological activity.

### 2.17.2 Chain extension compounds

![Diagram of 58a and 58b](image)

These two chain extended compounds can be used to prove the chain length of the parent compounds are the ideal length for biological activity supporting Milhazes et al.\textsuperscript{22} theory.

### 2.17.3 New compounds for comparison purposes

![Diagram of 62*](image)

Although a similar compound to the above structure has been made before, such a compound with saturated six-membered ring needs to be tested.

### 2.17.4 Existing compounds for comparison purposes

![Diagram of 63, 64, 65a, and 65b](image)

These known compounds can be found in the literature and again obvious results could be seen from them and we can use them to show that β-methyl-β-nitrostyrene derivatives are one of the most effective types of antibacterial agents.
Chapter 3

3 Experimental

3.1 General Methods and Conditions

3.1.1 Octanol-water Partition Coefficients

The lipophilicity level of each compound was determined by octanol-water partition coefficients. The buffer solution used in the determination of $K_D$ was made by mixing sodium chloride (3.78g, 65mmol), disodium hydrogen orthophosphate (2.14g, 18mmol) and sodium dihydrogen orthophosphate (0.78g, 5.5mmol) in 500mL water at room temperature (~22°C) and had a pH of 7.5. Each compound (10mg) was dissolved in octanol (2mL) in a stoppered test tube, followed with the addition of the buffer solution (2mL) and the tube was shaken over 48 hours and finally the mixtures were allowed to separate into two layers. The top layers were removed and absorbance measured after dilution 1:20, 1:50 or 1:200 to 3mL with octanol in a 1 cm path length apart cuvette at 370nm for each diluted sample. The aqueous bottom layers were removed and the absorbance measured without dilution.

$K_D$ measurements were according to the equation:

$$K_D = \frac{[\text{Octanol}]}{[\text{Water}]}$$

As the absorbance of the octanol and water layers is directly proportional to the concentration in each layer, the $K_D$ value can be calculated from the relative absorbance of each layer.

The calculated log P values (C log P) were obtained using Marvin Sketch (ChemAxon); and a table of all structures with measured and calculated log P values can be referred in Appendix section.
3.1.2 Analysis and instruments

Gas Chromatography and Mass Spectroscopy (GC/MS) spectra were obtained in either electron ionization (EI) or positive/negative electrospray (ESI) modes with the Varian Saturn 2200 GC/MS/MS (ion-trap) coupled to a Varian CP-3800 GC (FactorFour – Capillary Column; Stationary Phase: VF-5ms; L(m) ID(mm) x OD (mm): 30 x 0.25 x 0.39) or Micromass Platform II ESI/MS (240 V, 10 A).

Melting points of the products were determined on a Gallenkamp melting point apparatus and are not corrected.

$^1$H and $^{13}$C NMR spectra were determined using a Bruker Advance 300 MHz or Bruker Avance 300 III MHz spectrometer. All spectra were obtained and interpreted using TopSpin v2.0. Some FIDs from Bruker Advance 300 MHz were processed using Mestrec23. All samples, except 8b which was dissolved in DMSO, were dissolved CDCl$_3$. Proton ($^1$H) chemical shifts were recorded as δ values in parts per million (ppm) downfield shifts; the reference peak is a singlet at 2.78 ppm for DMSO and singlet at 7.26 for CDCl$_3$. Chemicals shifts are presented in multiplicity, coupling constant(s) (J in Hz), integration and assignments. Abbreviations are: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = doublet of doublet, m = multiplet. Carbon ($^{13}$C) chemical shifts were $^1$H decoupled, and recorded as δ values in parts per million (ppm). Reference peak for DMSO is a massive multiplet at 40.0 ppm and triplet at 77.0 for CDCl$_3$. Additional information to assist assignments of peaks are from CH COSY spectra, gCOSY, DEPT 45, 90, 135, HMBC and HSQC.
3.2 Materials

Organic reagents, solvents and purification reagents were purchased from Ajax Finechem Pty Ltd, BDH, Chem Supply, Merck and Sigma Aldrich and all were of AR quality or better than 99% purity. Results of compounds 7, 8a, 8b, 10a and 10b were from Nicoletti et al. and for comparison and completeness. Compounds 7, 8b, 8c, 9a, and 10b were prepared by HIGH FORCE Research Ltd U.K.

Microbial stocks

The strains used for biological tests were: Staphylococcus aureus ATCC 29213, Bacillus subtilis ATCC 6633, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922 and Candida albicans ATCC 10231. The microbial stocks were kept under -80°C in MHB (Oxoid, Cambridge, UK). The antibiotics used as a control for biological testing were erythromycin, tetracycline (Sigma Aldrich, St-Louis, Mo, USA) and ciprofloxacin (MP Biomedicals, Irvine, CA, USA). The nitrostyrene derivatives were diluted and stored in the dark room at room temperature for a month and maintained inhibitory potency when assayed by measurement of the MIC in bacterial species.

3.3 Minimum inhibitory concentrations

The microbiology testing and dilution was based on the National Committee for Clinical Laboratory Standards methods in MHB (Oxoid) for bacteria or Sabouraud Liquid Medium (SLM, Oxoid) for the fungus. C. albicans. Microplates assays were performed in clear, round-bottomed, 96-well plates (Sarstedt Australia, SA, AUS) with a total volume of 200 μL per well. The densities of inoculums were estimated by suspension turbidity using McF0.5 standard. Standard inoculums densities for bacteria were approximately 1 x 10^5 Colony Forming Units per mL (CFU/mL) and 1 x 10^4 CFU/mL for C. albicans.
Inoculum densities were confirmed by serial dilution plating onto NA and incubation aerobically at 37°C for 24 hrs. The tested nitrostyrene derivatives were added to plates at two times tested concentrations in 100 μL media. Ciprofloxacin was used as an internal positive control for bacteria and Miconazole for *C. albicans*. Microplates were incubated 18-24 hrs at 37°C aerobically before reading wells visually for turbidity. All assays included duplicated wells and were at least twice replicated. The geometric MIC (μg/mL) for each strain was adjusted to the nearest log₂ dilution tested. MIC results were reported as MIC (μg/mL) for standards.

### 3.4 Synthesis of nitroprop-1-enyl-benzene series

The standard Henry reaction has been used as Method A and B as well as other methods from previous work. Each method had a different reaction time as well as having used different reagents in the reaction, but nitroethane was common to all, except for β-nitrostyrene, where nitromethane was used.

#### Standard Henry Reaction (Method A)

The first method used was by Knoevenagel where condensation was carried at room temperature in the presence of aliphatic amines such as methylamine. The reaction time required was much longer than for Method B, but no heating was required. Overall yield was often lower than from Method B, therefore most of the compounds were synthesized using Method B. Method B was based on Gairaud and Lappin’s method of making nitrostyrene compounds.
3.4.1 Synthesis of β-Nitrostyrene

β-Nitrostyrene/2-nitroeth-1-enylbenzene [1a]

\[
\begin{align*}
\text{\(\text{CHO} \quad \text{CH}_3\text{NO}_2\)} & \quad \text{\(\rightarrow\)} & \quad \text{\(\text{NO}_2\)} \\
\text{\(\text{CH}_3\text{COONH}_4\), reflux} & \quad & \text{\(\text{Ia}\)}
\end{align*}
\]

The synthesis procedure was based on Black et al. The benzaldehyde (1.04g, 9.8mmol) was added to a stirring solution containing ammonium acetate (0.20g, 2.6mmol) and nitromethane (5.68g, 93.44mmol). The mixture was heated at reflux (90°C) for 6 hours, poured to water (100mL) and extracted with diethyl ether (3 x 30mL). The organic extract was washed with saturated brine solution (25%, 100mL), dried over magnesium sulphate (MgSO\(_4\)), filtered and concentrated under high vacuum. The residue was purified by recrystallization from ethanol (95%) to give yellow needles of compound 1a with a yield of 82% and melting point 58-59°C (Lit. 58-59°C)

\(^1\text{H NMR}\) (300 MHz, CDCl\(_3\)): \(\delta_H\) (ppm): 7.96-7.91 (d, \(J = 13.7\) Hz, 1H, C=αC-H), 7.54-7.49 (d, \(J = 13.7\) Hz, 1H, C=βC-H), 7.43-7.38 (m, 5H, Ar-H).

\(^{13}\text{C NMR}\) (75 MHz, CDCl\(_3\)): \(\delta_C\) (ppm): 139.1 (C=C, \(\alpha\) carbon), 137.1 (Ar), 132.2 (C=C, \(\beta\) carbon), 130.1 (Ar), 129.4 (Ar), 127.1 (Ar).

\text{GC/MS} \ m/z (M^+) 149.

4-Fluoro-β-nitrostyrene [1b]

The synthesis method used was that by Côté et al. which was modified from Andrey et al. A stirred solution of acetic acid (33.5mL) and ammonium acetate (4.4g, 57.1mmol) was added to nitromethane (10g, 164.0mmol) followed by 4-fluorobenzaldehyde (2.94g, 23.7mmol) and the solution was refluxed in an oil bath at 100°C for 5 hours and 30 minutes. The dark orange mixture was then cooled to room temperature and poured into water (100mL). The pH of the mixture was then regulated to 7 using adding sodium hydroxide solution (2M), after which the product was extracted with ethyl acetate (3 x
100mL). The combined organic extracts were dried over MgSO$_4$ and filtered under high vacuum. Further purification was done by recrystallizing the compound from 95% ethanol to remove the brown oily impurities, resulting in obtaining pale yellow needles with yield 57%, and melting point 99-100°C (Lit. 98-100°C).$^{182}$

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$H (ppm): 7.94-7.89 (d, $J = 13.8$ Hz, 1H, αC-H), 7.52-7.44 (m, multiplet occurred due to the peaks have overlapped with peaks in the ring, 1H, βC-H), 7.52-7.44 (m, multiplet occurred due to the peaks have overlapped with peaks in the β carbon, 2H, Ar-H), 7.11-7.05 (t, $J = 8.4$, 2H, Ar-H).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$C (ppm): 164.9 (d, $J = 255.1$ Hz, C-F), 137.8 (C=C, β carbon), 136.8 (C=C, β carbon), 131.2 (Ar), 126.3 (Ar), 116.7 (Ar).

GC/MS m/z (M$^+$) 167.

### 3.4.2 Synthesis of β-methyl-β-nitrostyrene

2-Nitroprop-1-enyl benzene $^{[11]}$

The synthesis of this compound was performed by Professor Hugh Cornell who used Method A. To benzaldehyde (2.12g, 20mmol), was added nitroethane (1.8g, 24mmol), anhydrous sodium carbonate (0.3g, 3mmol), methylamine hydrochloride (0.15g, 2.2mmol) with potassium hydroxide (0.05g, 0.9mmol, dissolved in 1.0 mL ethanol) and 2.5mL ethanol. The mixture was reacted at room temperature with sufficient stirring for 44 hours. After that, the mixture was dissolved in hot ethanol (95%) and the hot solution decanted and cooled to 5°C for 2 hours. The dried crude product was filtered and air dried to give yellow crystals produced with yield 22% and melting point from 60-62°C. The crude product was then recrystallized from 3mL ethanol (95%), washed with 2 x 0.5 portions of chilled ethanol. Purified yellow crystals were obtained in 9% yield with melting point 63-64°C (Lit. 64°C).$^{183}$
\(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta_H\) (ppm): 8.10 (s, 1H, H-C=C), 7.46 (s, 5H, Ar-H), 2.47 (s, 3H, CH\(_3\)).

\(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta_C\) (ppm): 147.7 (C=C, \(\beta\) carbon), 133.5 (C=C, \(\alpha\) carbon), 132.4 (s, C=C-C in Ar), 129.9 (Ar), 129.7 (Ar), 128.9 (Ar), 14.0 (CH\(_3\)).

GC/MS \(m/z\) (M\(^+\)) 149.

### 3.4.3 Synthesis of monofluoro substitution product of \(\beta\)-methyl-\(\beta\)-nitrostyrene

1-Fluoro-2-(nitroprop-1-enyl) benzene [12a]

![Reaction Scheme](image)

Method B was used for this reaction. 2-Fluoro-benzaldehyde (4.81g, 38.8mmol) was dissolved in nitroethane (4.01g, 53.5mmol, 20% excess), ammonium acetate (4.00g, 52mmol) and glacial acetic acid (5mL) were added and the mixture was refluxed for 2 hours in an oil bath at 100°C. The orange coloured mixture was then chilled and de-ionized water (6mL) was then added. A small portion of the crude orange crystalline product obtained by filtration was taken for determination of melting point. The rest of the product was dissolved in hot ethanol (95%, 2ml) and chilled for an hour to obtain the recrystallized product. The recrystallization process was repeated and light yellow crystals were obtained (Compound 12a; 2.38g, 34% of theoretical yield). Melting points were 1\(^{st}\) crude: 42- 43°C, 2\(^{nd}\) crude: 43- 44°C and final product 45-47°C.

\(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta_H\) (ppm): 7.98 (s, 1H, H-C=C), 7.39-7.33 (t, \(J = 8.7 \text{ Hz}, 1\text{H, Ar-H}\)), 7.19 (d, \(J = 8.7 \text{ Hz}, 1\text{H, Ar-H}\)), 7.12 (d, \(J = 8.6 \text{ Hz}, 1\text{H, Ar-H}\)), 7.04 (d, \(J = 8.8 \text{ Hz}, 1\text{H, Ar-H}\)), 2.36 (s. 3H, C-H, \(E\)), 1.59 (s, 3H, C-H, \(Z\)).

\(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta_C\) (ppm): 165.1 (d, \(J = 252.2 \text{ Hz, C-F}\)), 147.5 (C=C, \(\beta\) carbon), 132.5 (C=C, \(\alpha\) carbon), 132.2 (Ar), 132.0 (Ar), 128.7 (Ar), 116.4 (Ar), 116.2 (Ar), 14.0 (CH\(_3\)); GC/MS \(m/z\) (M\(^+\)) 181.
Similar procedures were repeated as described above to obtain compounds. 12c, 12d, 53a and 53b.

1-Fluoro-3-(nitroprop-1-enyl) benzene [12b]

This compound was prepared with a method similar to of Werbal, L. M. et al.\textsuperscript{184} by reacting 3-fluorobenzaldehyde (1g, 8.05mmol) with ammonium acetate (0.19g, 2.4mmol) in nitroethane (4.98g, 66.4mmol), under reflux overnight (approximately 17 hours) in an oil bath at 125°C. The compound was identified by thin layer chromatography (TLC). The mixture was then concentrated under high vacuum to remove the excess nitroethane and then the yellow mixture was dissolved in chloroform (20ml), washed with water (3 x 20mL) and with sodium chloride solution (25%, 20mL). The mixture was dried (MgSO\textsubscript{4}) and concentrated under high vacuum. A yellow liquid was obtained (0.75g) this being 52% of theoretical yield.

\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta\) (ppm): 8.04 (s, 1H, C=C-H), 7.46-7.39 (m, multiplet due to proton peaks in the ring coupled with other peaks in the ring, 1H, Ar-H), 7.46-7.39 (m, multiplet due to proton peaks in the ring overlapped with other peaks in the ring and with a F atom, 1H, Ar-H), 7.22-7.19 (d, \(J = 7.8\) Hz, 1H, Ar-H), 7.13-7.09 (d, \(J = 9.3\) Hz, 1H, Ar-H coupled with F), 2.42 (s, 3H, CH\textsubscript{3}).

\textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): \(\delta\) (ppm): 164.3 (d, \(J = 246.8\) Hz, 1C, C-F), 148.7 (C=C, \(\beta\) carbon), 134.6 (Ar), 132.0 (Ar), 130.6 (C=C, \(\alpha\) carbon), 130.5 (Ar), 125.9 (Ar), 125.7 (Ar), 13.9 (CH\textsubscript{3}).

GC/MS m/z (M\textsuperscript{+}) 181.

Similar procedures were repeated as described above to obtain compounds: 12e, 49a, 49b, 50a and 50b.
1-Fluoro-4-(nitroprop-1-enyl) benzene [12c]

The product was obtained as yellow crystals with 30% of theoretical (2.13g). Melting points: 1st crude: 38°C, 2nd crude: 45°C and final product: 65-66°C\textsuperscript{154}.

\textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta_H\) (ppm): 8.06 (s, 1H, H-C=C), 7.46 (dd, \(J = 3.3, 5.4\) Hz, 2H, Ar-H), 7.17 (t, \(J = 8.7\) Hz, 2H, Ar-H), 2.46 (s, 3H, C-H, \(E\)), 1.59 (s, 3H, C-H, \(Z\)).

\textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}): \(\delta_C\) (ppm): 165.2 (d, \(J = 252.2\) Hz, 1C, C-F), 147.5 (C=C, \(\beta\) carbon), 132.5 (C=C, \(\alpha\) carbon), 132.2 (Ar), 128.5 (Ar), 128.5 (Ar), 116.3 (Ar), 116.0 (Ar), 14.0 (CH\textsubscript{3})

GC/MS m/z (M\textsuperscript{+}) 181.

1,3-Difluoro-4-(nitroprop-1-enyl) benzene [12d]

This compound was prepared by the same method as 12d (Method B) making use of the Henry reaction. Quantities of chemicals used were: 2,4-difluorobenzaldehyde (0.78g, 5.4mmol), nitroethane (0.46g, 5.2mmol, 20% excess), ammonium acetate (0.80g, 10.0mmol and glacial acetic acid (1mL). The mixture was refluxed as before for 2 hours in an oil bath at 100°C. 12d was obtained as yellow crystals with a melting point of 48-49°C (0.47g, 43% of theoretical yield). \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta_H\) (ppm): 8.06 (s, 1H, C=C-H), 7.45 – 7.35 (dd, \(J = 8.3, 8.4\)Hz, coupling due to two fluorine atoms were coupling with this proton. 1H, Ar-H); 7.04 – 6.96 (dd, for the proton at the middle of two fluorine atoms it should split to a quartet as two fluorine atoms were coupling with it. \(J = 8.5, 8.5\) Hz. 1H Ar-H. The other proton at position 6 should be a triplet as there is only fluorine atom coupling with it. t, \(J = 8.5\) Hz, 1H, Ar-H), 2.38 (s 3H, CH\textsubscript{3}, \(E\)), 1.57 (s 3H, CH\textsubscript{3}, \(Z\)).
\(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta_C\) (ppm): 163 – 162.3 (quartet due to two fluorine atoms coupling with the carbon. \(J = 222.1, 233.6\)Hz. 1C, F-\(\text{C}=\text{C}-\text{C}=\text{F}\)), 162.2 – 159 (q, \(J = 234.4\)Hz. 1C, F-\(\text{C}=\text{C}-\text{C}=\text{F}\)), 149.5 (s, \(\text{C}=\text{C} \alpha\) carbon), 134.1 – 131.1 (quartet due to two fluorine atoms coupling with the carbon. \(J = 234.6\)Hz, 1C, F-\(\text{C}=\text{C}-\text{C}=\text{C}=\text{F}\)), 125.7 – 125.4 (s, \(\text{C}=\text{C} \beta\) carbon), 117.0 – 116.5 (d, \(J = 9.9\)Hz, 1C, F-\(\text{C}=\text{C}\)), 112.3 – 111.7 (q, \(J = 3.6, 21.7, 24.9\)Hz, 1C, F-\(\text{C}=\text{C}-\text{C}=\text{F}\)), 104.8 – 104.4 (d, \(J = 25.8\), 1C, F-\(\text{C}=\text{C}\)), 14.2 – 14.0 (CH\(_3\)).

**GC/MS** \(m/z\) (\(M^+\)) 199.

**Pentafluoro-2-(nitroprop-1-etyl) benzene [12e]**

![12e](image)

The scale of chemicals used in the reaction was 1/50 to what Werbal, L. M. et al.\(^{184}\) used. A mixture of pentafluorobenzaldehyde (1g, 5.1mmol) and ammonium acetate (0.12g, 1.53mmol) in nitroethane (3.15g, 42mmol) was heated (120°C) under reflux for 17 hours. The large excess of nitroethane was removed under high vacuum. The residue was dissolved in chloroform (10mL) and the mixture was then washed with water (4 x 20mL) and with saturated brine solution (25%, 2 x 20mL). The chloroform solution was dried over MgSO\(_4\), filtered and concentrated under high vacuum. The product was purified by flash column chromatography on silica gel with 30% ethyl acetate in hexane (v/v) to obtain a yellow liquid in yield of 2.5%.

**GC/MS** \(m/z\) (\(M^+\)) 253; (the amount of compound was only enough for GC/MS characterization).
3.4.4 Synthesis of trifluoromethyl substitution of β-nitrostyrene

1-Trifluoromethyl-4-(nitroprop-1-enyl) benzene [49c]

Compound 49c was synthesized by a method similar to that of Bergner and Opatz. 4-Trifluoromethylbenzaldehyde (1g, 5.7mmol) and ammonium acetate (0.38g, 5.0mmol) were dissolved in nitroethane (20mL, 280mmol), heated to 100°C and refluxed overnight. The excess nitroethane was removed under high vacuum and the yellow coloured mixture was poured into water (20mL) and extracted with ethyl acetate (3 x 20mL). The combined organic extracts were washed with water (3 x 20mL) and sodium chloride solution (25%, 20mL) and then dried over anhydrous MgSO$_4$. The solvent was removed under high vacuum (enhanced with liquid nitrogen) to yield a yellow solid (1.16g, 87% yield). The material was purified by washing with cold ethanol (95%) to yield 49c (0.748g, 42%), a yellow solid which had a melting point of 96-98°C (Lit. 36-38.5°C).

$^1$H NMR (300 MHz, CDCl$_3$): δ$_{H}$ (ppm): 8.02 (s, 1H, C=C-H), 7.65 (d, $J = 8.3$ Hz, 2H, Ar-H), 7.46 (d, $J = 8.2$ Hz, 2H, Ar-H), 2.37 (s, 3H, C-H, $E$), 1.59 (s, 3H, C-H, $Z$).

$^{13}$C NMR (75 MHz, CDCl$_3$): δ$_{C}$ (ppm): 148.9 (s, C=C, β carbon), 135.6 (Ar), 132.6 (s, C=C, α carbon), 131.4 (s, Ar-C=CF$_3$), 129.8 (Ar), 128.5 (Ar), 123.7 (q, $J = 273.6$ Hz, CF$_3$), 13.6 (CH$_3$).

GC/MS m/z (M$^+$) 231.
1-Trifluoromethyl-2-(nitroprop-1-enyl) benzene [49a]

![Chemical Structure](attachment:49a.png)

The method of synthesis was similar to compound 12b. The quantities of chemicals used were: 2-trifluoromethylbenzaldehyde (1.0g, 5.7mmol), ammonium acetate (0.13g, 1.7mmol) and nitroethane (3.52g, 47mmol) were refluxed as before for 19 hours in an oil bath at 125°C. The compound was purified by flash column chromatography on silica gel with hexane/ethyl acetate (20/1) to obtain a yellow liquid in a yield of 46%. For this compound the relative amount of the cis and trans isomers are 60% and 40% respectively.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta_H$ (ppm): 8.16 (s, 1H, C=C-H), 7.69-7.67 (d, $J = 7.8$ Hz, 1H, Ar-H, trans-compound), 7.61-7.53 (m, multiplets due to overlap of the cis and trans-compounds, 1H, Ar-H), 7.48-7.42 (m, multiplets due to overlapped with the cis and trans-compounds, 1H, Ar-H), 7.39-7.34 (t, $J = 6.6$ Hz, cis-compound, 1H, Ar-H), 7.28-7.26 (d, $J = 7.5$ Hz, cis-compound, 1H, Ar-H), 7.17-7.15 (d, $J = 6.3$ Hz, trans-compound, 1H, Ar-H), 2.31 (s, trans-compound, 3H, CH$_3$), 2.17 (s, cis-compound, 3H, CH$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C$ (ppm): 149.9 (s, 1C, C=C, $\beta$ carbon), 148.9 (Ar), 132.0 (Ar), 130.2 (s, C=C, $\alpha$ carbon), 130.0 (cis Ar), 129.3 (Ar), 129.0 (trans Ar), 128.4 (cis Ar), 127.2 (q, $J = 264.5$ Hz, 1C, CF$_3$), 126.4 (trans Ar), 125.9 (Ar), 124.7 (Ar), 122.0 (Ar), 19.3 (s, 1C, trans CH$_3$), 13.7 (s, 1C, cis CH$_3$).

GC/MS m/z (M$^+$) 231.

1-Trifluoromethyl-3-(nitroprop-1-enyl) benzene [49b]

![Chemical Structure](attachment:49b.png)

The quantities of chemicals used were the same as for compound 49a. However, the mixture was refluxed for 17 hours in an oil bath at 140°C. The compound was purified by
flash column chromatography on silica gel with hexane/ethyl acetate (20/1) to obtain a yellow liquid in a yield of 46%.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ (ppm): 8.07 (s, 1H, C=C-H), 7.44 - 4.38 (t, $J = 8.7$ Hz, 1H Ar-H), 7.27 (d, $J = 7.9$ Hz, 1H, Ar-H), 7.19 (d, overlapped with other peaks, 1H, Ar-H), 7.17 (s, 1H, r-H), 2.34 (s, 3H, CH$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ (ppm): 149.1 (s, C=C, $\beta$ carbon), 133.3 (Ar), 132.8 (Ar), 131.6 (Ar), 131.2 (Ar), 130.3 (Ar), 129.5 (Ar), 126.3 (s, C=C, $\alpha$ carbon), 123.6 (q, $J = 272.4$ Hz, 1C, CF$_3$), 13.8 (s, CH$_3$).

GC/MS $m/z$ (M$^+$) 231.

3.4.5 Synthesis of trifluoromethoxy derivative of β-nitrostyrene

1-Trifluoromethoxy-4-(nitroprop-1-enyl) benzene [50b]

Compound 50b was prepared using the same method as 12b. 4-Trifluoromethoxybenzaldehyde (1g, 5.3mmol), ammonium acetate (0.12g, 1.6mmol) and nitroethane (3.3g, 43.3mmol) were heated at 115°C for 5 hours. The yellow mixture was placed under high vacuum to remove excessive nitroethane and then dissolved in chloroform (10mL), washed with water (3 x 20mL) and washed again with saturated brine solution (25%, 2 x 20mL). The washed mixture was then dried over MgSO$_4$, and then concentrated under high vacuum (liquid nitrogen assisted). Yellow crystals were obtained in yield of 73% with melting point 47-48°C.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ (ppm): 7.98 (s, 1H, C=C-H), 7.42 (d, $J = 8.7$ Hz, 2H, Ar-H), 7.24 (d, $J = 8.3$ Hz, 2H, Ar-H), 2.37 (s, 3H, CH$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ (ppm): 150.0 (s, O-C in Ar), 148.3 (s, C=C, $\beta$ carbon), 131.9 (s, C=C, $\alpha$ carbon), 131.5 (Ar), 130.9 (Ar), 121.1 (Ar), 120.3 (q, $J = 258.2$ Hz, 1C, CF$_3$), 13.9 (s, CH$_3$).
The quantities of chemicals used were the same as for compound 50b. In the case, the mixture was refluxed for 24 hours immersed in an oil bath at 100°C. The orange yellow mixture was then extracted with ethyl acetate (20mL) and the extract washed with sodium bicarbonate (NaHCO₃) (3 x 20mL) and saturated brine solution (25%, 20mL). The mixture was dried over MgSO₄ and concentrated under high vacuum (liquid nitrogen assisted). A yellow liquid was obtained in yield of 51%. For this compound the relative amount of the cis and trans isomers are 31% and 69% respectively.

**1H NMR** (300 MHz, CDCl₃): δₗ (ppm): 8.05 (s, 1H, C=C-H), 7.45-7.49 (t, J = 8.7 Hz, 1H, Ar-H, trans compound), 7.40-7.36 (t, J = 6.6 Hz, 1H, Ar-H, cis compound), 7.31-7.28 (d, J = 7.2 Hz, 1H, Ar-H, trans compound), 7.19-7.17 (d, J = 7.8 Hz, 1H, Ar-H, cis compound), 6.49 (s, 1H, Ar-H), 2.45 (s, 1H, CH₃, trans compound), 2.38 (s, 1H, CH₃, cis compound).

**13C NMR** (75 MHz, CDCl₃): δₓ (ppm): 149.3 (s, C=OCF₃), 149.2 (s, C=C, β carbon), 134.4 (Ar), 131.7 (s, C=C, α carbon), 130.4 (trans Ar), 130.0 (Ar), 128.1 (cis Ar), 126.2 (trans Ar), 124.1 (Ar), 122.6 (q, J = 258.1 Hz, 1C, OCF₃), 122.1 (Ar), 121.2 (trans Ar), 120.6 (cis Ar), 19.9 (s, 1C, cis CH₃), 13.9 (s, 1C, trans CH₃).

**GC/MS** m/z (M⁺) 247.
1-Methyl-4-(nitroprop-1-enyl) benzene [52]

Method B was used to synthesize this compound. 4-methyl-benzaldehyde (4.00g, 33.3mmol) was dissolved in nitroethane (3.12g, 33.3mmol, 25% excess), ammonium acetate (5.13g, 66.6mmol) and glacial acetic acid (10mL) added and the mixture refluxed for 3 hours in an oil bath at 110°C. The green brown coloured mixture was then chilled and de-ionized water (15mL) was then added. The product was then dissolved in hot ethanol (95%, 2ml) and chilled for an hour to obtain the recrystallized product as light yellow crystals afterward Compound 52 (4.72g, 80% of theoretical yield). The melting point was 54-55 °C.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta_H$ (ppm): 7.97 (s, 1H, H-C=C), 7.46 (d, $J = 8.1$ Hz, 2H, Ar-H), 7.17 (d, $J = 8.0$ Hz, 2H, Ar-H), 2.37 (s, 3H, C-H)$_3$, 2.31 (s, 3H, C-H, $Z$).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C$ (ppm): 146.5 (C=C, $\beta$ carbon), 140.9 (C=C-CH$_3$, carbon in the ring), 133.8 (C=C, $\alpha$ carbon), 130.2 (Ar), 129.7 (Ar), 129.5 (Ar), 21.3 (CH$_3$, methyl carbon connected to the ring ), 14.1 (CH$_3$, connected$\beta$ carbon)

GC/MS m/z (M$^+$) 177.

3.4.6 Synthesis of 3-nitrochromene derivatives

3-Nitrochromene [54a]

The synthesis method was based on that of Yan et al.$^{187}$ A mixture of compound 1a (0.5g, 3.4mmol) and salicylaldehyde (4.1g, 33.6mmol,) was swirled until the solution became
homogeneous, then a catalytic amount of DABCO (0.38g, 3.4mmol) was added to the mixed solution. The mixture was refluxed (under 40°C) for 1.5 hours and then 20mL of 5% hydrochloric acid (HCl) was added to the solution. Organic material was extracted using dichloromethane (3 x 60mL) and then dried over MgSO₄, and filtered. Dichloromethane was removed under high vacuum. Purification of the compound was done by silica gel flash column chromatography (ethyl acetate: hexane = 1: 50). A yellow-orange solid was obtained in yield of 43% and which had a melting point of 88-90°C (Lit. 88-89°C)\(^{187}\). \(^1\)H NMR and \(^{13}\)C NMR spectra are consistent with Yan et al. GC/MS m/z (M⁺) 253.

3-Nitrochromene with \emph{para} substitution of fluorine on phenyl ring [54b]

\[
\begin{align*}
\text{54b} \\
\text{NO}_2 \\
\text{F}
\end{align*}
\]

A similar procedure was used to obtain compound 54b. The quantities of chemicals used were: compound 1b (0.23g, 1.4mmol), salicylaldehyde (1.68g, 13.8mmol) and DABCO (0.15g, 1.4mmol). The reaction was carried out under the same reaction conditions and the purification was as for 54a. A yellow solid was obtained in yield of 55% and which had melting a point of 88-89°C.

\(^1\)H NMR (300 MHz, CDCl₃): δ\(_H\) (ppm): 7.98 (s, 1H, C=αC-H), 7.30-7.24 (m, due to peaks are overlapping each other, 4H, Ar-H), 6.96-6.89 (due to peaks are overlapping each other, 3H, Ar-H), 6.80-6.77 (d, \(J = 8.4\) Hz, 1H, Ar-H), 6.47 (s, 1H, O-C-H).

\(^{13}\)C NMR (75 MHz, CDCl₃): δ\(_C\) (ppm): 164.9-161.6 (d, \(J = 249.1\) Hz, 1C, Ar-C-F), 153.3 (Ar), 141.0 (s, C=C=NO₂), 134.4 (s, 2C, Ar-C), 132.7 (s, 1C, C-Ar-F), 130.4 (Ar), 129.3 (Ar), 129.0 (Ar), 122.6 (Ar), 117.8 (C=C-C in between of two Ar), 117.3 (Ar), 115.8 (Ar), 73.5 (s, 1C, O=C-C=Ar-F).

GC/MS m/z (M⁺) 271.
3.4.7 Synthesis of β-ethyl- β-nitrostyrene

2-Nitrobut-1-enyl- benzene [55]

The method of Kawai et al.\textsuperscript{188} was used. A mixture of benzaldehyde (2.12g, 20mmol), ammonium acetate (1.54g, 20mmol) and 1-nitropropane (39.9g, 448.5mmol) was refluxed at 110°C overnight (18 hours). The excess 1-nitropropane was removed under high vacuum and after addition of water (30mL), the organic materials were extracted with ethyl acetate (3 x 30mL) and the extract then dried over MgSO\(_4\). The combined extracts were filtered and concentrated under high vacuum. The product was purified by silica gel column chromatography with hexane/ethyl acetate (20: 1) to obtain a yellow liquid [55] in yield of 60%.

\(^1\)H NMR (300 MHz, CDCl\(_3\)): δ\(_H\) (ppm): 8.03 (s, 1H, C=C-H), 7.47-7.45 (m, 5H, Ar-H), 2.92-2.85 (q, J = 7.5 Hz, 2H, CH\(_2\)), 1.32-1.27 (t, J = 7.5 Hz, 3H, CH\(_3\)). \textsuperscript{188}

\(^13\)C NMR (75 MHz, CDCl\(_3\)): δ\(_C\) (ppm): 153.3 (s, C=C, β carbon), 133.1 (s, C=C, α carbon), 132.3, (Ar), 129.9 (Ar), 129.6 (2C, Ar), 129.0 (2C, Ar), 20.7 (s, CH\(_2\)), 12.5 (s, CH\(_3\)).

GC/MS m/z (M\(^+\)) 177.

3.4.8 Synthesis of nitro-naphthalene derivatives

1-(Nitroprop-2-enyl) naphthalene [53a]

Method B was applied for this reaction and likewise for compound 53b. A mixture of 1-naphthaldehyde (1g, 6.4mmol), ammonium acetate (0.99g, 12.8mmol) and glacial acetic acid (3mL) in nitroethane (0.6g, 8.0mmol) was refluxed for 2 hours in an oil bath at 100°C. The orange coloured mixture was then chilled and de-ionized water (6mL) was then
added to the orange mixture and the product which precipitated was then recrystallized from 95% ethanol. After washing with cold 95% ethanol a light yellow solid, 53a, was obtained in yield 49% and which had a melting point of 62-64°C\(^{189}\).

\(^1H\) NMR (300 MHz, CDCl\(_3\)): \(\delta_H\) (ppm): Due to multiplets occurred in the spectra because of the protons in both aromatic ring overlapped to each other, the integration of the proton will be used to identify the peaks. 8.62 (Integration of \(^1H\): 1, \(\alpha\)C-H), 7.98-7.87 (Integration of \(^1H\): 3, Ar-H), 7.64-7.51 (Integration of \(^1H\): 4, Ar-H), 2.39 (Integration of \(^1H\): 3, CH\(_3\)).

\(^{13}C\) NMR (75 MHz, CDCl\(_3\)): \(\delta_C\) (ppm): 149.3 135.7 (s, C=C, \(\beta\) carbon), 133.5 (Ar), 131.9 (Ar), 131.4 (s, C in between two rings), 130.2 (s, C=C, \(\alpha\) carbon), 129.7 (s, C in between two rings), 128.8 (Ar), 127.1 (s, 2C, Ar), 126.6 (Ar), 125.1 (Ar), 124.1 (Ar), 14.1 (s, 1C, CH\(_3\)).

GC/MS m/z (M\(^+\)) 213.

2-(Nitroprop-2-enyl) naphthalene [53b]

\[\text{53b} \]

Quantities of chemicals used were: 2-naphthaldehyde (0.5g, 3.2mmol), nitroethane (0.3g, 4.0mmol), ammonium acetate (0.49g, 6.4mmol) and glacial acetic acid (3mL). An orange-yellow solid was obtained in yield of 41% and melting point 90-91°C\(^{190}\).

\(^1H\) NMR (300 MHz, CDCl\(_3\)): \(\delta_H\) (ppm): Same as 53a, the integration of the proton will be used to identify the peaks. 8.20 (Integration of \(^1H\): 1, \(\alpha\)C-H), 7.88-7.79 (Integration of \(^1H\): 4, Ar-H), 7.49-7.43 (Integration of \(^1H\): 3, Ar-H), 2.79 (Integration of \(^1H\): 3, CH\(_3\)).

\(^{13}C\) NMR (75 MHz, CDCl\(_3\)): \(\delta_C\) (ppm): 147.8 (s, C=C, \(\beta\) carbon), 133.7 (Ar), 133.5 (Ar), 133.0 (s, C in between two rings), 130.5 (s, C=C, \(\alpha\) carbon), 129.8 (s, C in between two rings), 128.6 (Ar), 128.4 (Ar), 127.7 (Ar), 127.6 (Ar), 126.9 (Ar), 126.3 (Ar), 14.2 (s, 1C, CH\(_3\)).

GC/MS m/z (M\(^+\)) 213.
3.4.9 Materials to synthesize the novel compound

2,4-Dimethoxy- β-nitrostyrene [1c]

The method to make this compound was based on Fierro et al.\textsuperscript{191} and also for the other dinitro compound [56b]. A mixture of 2,4-dimethoxybenzaldehyde (20g, 120.4mmol), n-butylamine (12.1 mL) and glacial acetic acid (120 mL) in nitromethane (14.7g, 240.8mmol) was refluxed for 1.5 hours at 110°C. The dark brown mixture was evaporated by high vacuum to remove the acid and water (more than 20 mL) was added to the neutralized mixture which became a yellow colour. Compound 1c was then filtered and recrystallized in methanol to form yellow solids obtained in yield 64% and melting point of 101-104°C\textsuperscript{192}.

\textbf{\textsuperscript{1}H NMR} (300 MHz, CDCl$_3$): $\delta$ (ppm): 8.10-8.06 (d, $J = 13.5$ Hz, 1H, C=αC-H), 7.85-7.80 (d, $J = 13.5$ Hz, 1H, C=βC-H), 7.39-7.37 (d, $J = 8.4$ Hz, 1H, Ar-H), 6.57-6.54 (d, $J = 8.6$ Hz, 1H, Ar-H), 6.49 (s, 1H, Ar-H), 3.93 (s, 6H, O-CH$_3$).

\textbf{\textsuperscript{13}C NMR} (75 MHz, CDCl$_3$): $\delta$ (ppm): 164.2 (s, 4-Ç-O in Ar), 161.2 (s, 2-Ç-O in Ar), 136.0 (s, Ç=C, α carbon), 135.7 (s, Ç=C, β carbon), 134.3 (Ar), 112.4 (Ar), 105.9 (Ar), 98.6 (Ar), 55.6 (s, ÇH$_3$).

\textbf{GC/MS} m/z (M$^+$) 209.

2-[2´,4´-Dimethoxybenzene]-1,3-dinitropropanes [56b]

The method to synthesize this compound was also based on Fierro et al.\textsuperscript{191}, the starting material 1c (9g, 43mmol) and a base, potassium fluoride (3.2538g, 51.6mmol) were added in nitromethane (5340mmol) and refluxed with stirring at 110°C for 1.5 hours. The orange brown mixture was then evaporated under high vacuum to remove the excess
nitromethane and the solid dissolved in ethyl acetate (100 mL). After washing with water (50 mL) and then with diethyl ether (2 x 50 mL). The combined organic extract was washed again with water (3 x 40 mL). The organic extract was dried over MgSO$_4$ the diethyl ether was removed under high vacuum to obtain a light brown solid in yield 88% with melting point of 53-56°C.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta_H$ (ppm): 7.06-7.02 (d, $J = 8.1$ Hz, 1H, C=C-H the ortho hydrogen in the ring), 6.49-6.41(broad peaks due to chemical shifting and coupling of the para proton and ortho hydrogen and little coupling occurred with meta hydrogen in the ring), 4.84-4.82 (d, $J = 6.90$ Hz, 4H, H-H-C next to NO$_2$), 4.42-4.34 (p, $J = 14.1$, 7.2, 6.9 Hz, C=C-C-H), 3.87-3.79 (s, 6H, O-CH$_3$).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C$ (ppm): 161.4 (s, 1C, C-OCH$_3$), 158.1 (s, 1C, C-OCH$_3$), 130.6 (Ar), 114.2 (Ar), 104.8 (Ar), 99.3 (Ar), 75.6 (s, 2C, CH$_2$), 55.5 (s, 1C, OCH$_3$), 55.4 (s, 1C, OCH$_3$), 38.9 (s, 1C, NO$_2$-C-C-C-NO$_2$).

GC/MS m/z (M$^+$) 270.

3.5 Attempted synthesis of other nitro compounds

There were a few additional compounds required to be made for antimicrobial tests [56a, 57, 58a and 58b] and also as starting materials [59 and 60] required for making novel compounds. All of them, except 57, are mentioned in the literature, but none were able to be synthesized successfully. They are described below:
3.5.1 Compound with fluorine substitution on α-carbon

α-Fluoro-β-methyl-β-nitrostyrene [57]

![Chemical structure](image)

The synthesis procedure was based on Yusubov et al. To a mixture of solution of 1-phenyl-1-propyne (0.7g, 6.0mmol) and sodium nitrate (1.02g, 12.0mmol) in acetic acid (20mL), potassium fluoride (0.35g, 6.0mmol) was added when the solution was at 85°C. The orange mixture was refluxed overnight (18 hours) and the product formation was monitored by TLC. The reaction mixture was then poured into water (60mL) and extracted with diethyl ether (3 x 100mL). The organic extracts were washed with water (3 x100mL) and saturated brine solution (25%. 60mL) and dried over MgSO₄. Diethyl ether was removed by high vacuum. However, the orange coloured compound with some white crystals was still not pure and it could not be purified by column chromatography.

3.5.2 Compounds mentioned in literature

(4-Nitrobuta-1,3-dienyl)benzene [58a]

![Chemical reaction](image)

Attempts were made to synthesize this compound using Method A, Method B and a literature method from Dockendorff et al., but none of them was successful.

(4-Nitropenta-1,3-dienyl)benzene [58b]

![Chemical structure](image)

Methods A, B and the method from Dockendorff et al. were used for this compound. Neither of them succeeded as well as described. However, method from Rodríguez and Dolors Pujol suggested another possibility to synthesize 58a and 58b.
(1,3-Dinitroprop-2-enyl)benzene [56a]

\[
\begin{align*}
\text{CHO} + \text{CH}_3\text{NO}_2 & \rightarrow \text{NO}_2-\text{CH}_2\text{NO}_2 \\
\text{56a} & \rightarrow \text{1a} (78\%)
\end{align*}
\]

Attempts were made to synthesize this compound using the methods from Ballini et al.\textsuperscript{196} and Iturriaga-Vásquez et al.\textsuperscript{197}. However, neither method yielded the dinitro-compound. The method of Fierro et al.\textsuperscript{191} to make compound 56b may be the way to synthesize this compound.

3.5.3 Attempted synthesis of starting materials

3,3,3-Trifluoro-1-phenylpropyne [59]

\[
\text{59}
\]

The literature method was according to Bunch and Bumgardner\textsuperscript{198}. However, due to limited material available for the reaction, the compound could not be obtained.
Alkyl Phosphonate [60]

The method of synthesis was from Kandil et al.\textsuperscript{199} on a 50% scale. A solution of lithium diisopropylamide (LDA, 1.6mL, 11mmol diisopropylamine reacted with 1.0mL, 11mmol of 2.5M n-butyllithium in hexane) in dry tetrahydrofuran (THF, 25mL) was prepared under nitrogen at -78°C. Nitroethane (0.412g, 5.5mmol) in dry THF (50mL) was then added dropwise over half an hour. The mixture was stirred for another half hour and a solution of diethyl chlorophosphate (0.95g, 5.5mmol) in dry THF (5mL) was added dropwise over 15 minutes and the mixture was stirred continuously for an additional 3 hours. The solution was warmed to -30°C and stirred for another 2 hours. After that the mixture was cooled back to -78°C and acetic acid (1.32g, 22mmol) in dry THF was added dropwise to quench the mixture and stirring was maintained for one hour at -78°C. The mixture was then gradually warmed to room temperature. The mixture was diluted with water (50mL) and the organic materials were extracted with ethyl acetate (3 x 25mL), washed with saturated brine solution (25%, 2 x 14mL) and dried with MgSO\textsubscript{4}. The combined extracts were filtered and concentrated under high vacuum. Further purifications were achieved by dissolving the compound in ether (20mL) and then extracting with the saturated sodium carbonate solution (3 x 20mL). The combined aqueous extracts were washed with diethyl ether (2 x 20mL) and acidified to pH 7 by glacial acetic acid and to pH 2 with 10% aqueous hydrochloric acid. The remaining compound was then extracted with diethyl ether (3 x 50mL), filtered and dried over MgSO\textsubscript{4}. The solvent was removed in high vacuum, but the desired product could not be obtained, which was shown by \textsuperscript{1}H NMR and GC/MS.
3.5.4 Other compounds synthesized by Professor Hugh Cornell

1,2-Dimethoxy-4-(2-nitroprop-1-enyl)benzene [9b]

\[ \text{Compound } 9b \text{ was prepared by Method A. A mixture of 3,4-dimethoxybenzaldehyde (1.66g, 10mmol), nitroethane (1g, 13.3mmol), anhydrous sodium carbonate (0.2g, 2mmol), methylamine hydrochloride (0.11g, 1.5mmol) potassium hydroxide (0.05g, 0.9mmol, dissolved in 1.0 mL ethanol) and ethanol (2.5mL) was reacted at room temperature with sufficient stirring for 24 hours. After that, the mixture was diluted with water (3mL) and chilled to 5°C for 3 hours. The crude product was filtered and air dried, recrystallized with 95% ethanol and air dried. The final product was yellow and crystalline with yield of 27% and a melting point of 71-72°C.} \]

\[ ^{1} \text{H NMR (300 MHz, CDCl}_3\text{): } \delta_H (ppm): 7.99 (s, 1H, C=C-H), 6.94 (s, 1H, Ar-H), 7.03-7.00 (d, J = 8.1 Hz, 1H, Ar-H), 6.88-6.85 (d, J = 9.3 Hz, 1H, Ar-H), 3.85 (s, 6H, OCH}_3\text{).} \]

\[ ^{13}\text{C NMR (75 MHz, CDCl}_3\text{): } \delta_C (ppm): 150.7 (s, C3 of C-OCH}_3\text{ in the ring), 149.1 (s, C4 of C-OCH}_3\text{ in the ring), 145.9 (s, C=C, } \beta \text{ carbon), 133.8 (Ar), 125.0 (s, C=C, } \alpha \text{ carbon), 124.0 (Ar), 113.0 (Ar), 111.2 (Ar), 56.0 (s, 2C, OCH}_3\text{), 14.1 (CH}_3\text{).} \]

\[ \text{GC/MS } m/z (M^+) 223. \]

1-Hydroxy-4-(2-nitroprop-1-enyl)benzene [8b]

Method A was also used for preparation 8b. A mixture of 4-hydroxybenzaldehyde (2.44g, 20mmol) dissolved in absolute ethanol (3mL), nitroethane (2g, 26.6mmol), anhydrous sodium carbonate (0.3g, 3mmol) and methylamine hydrochloride (0.15g, 2.2mmol), was prepared and a solution of potassium hydroxide (0.05g, 0.9mmol, dissolved in 1.0 mL ethanol) added with thorough mixing. The mixture was reacted at room temperature with
sufficient stirring for 48 hours. Yellow crystals were obtained after chilling to 5°C for 2 hours. The crude product was filtered and recrystallized from 95% ethanol to yield 1g of product (50% of the theoretical yield) and melting point 121-122°C.

\(^1\)H NMR (300 MHz, DMSO): \(\delta_H (ppm): 8.03 (s, 1H, C=C-H), 7.50-7.47 (d, J = 8.7 Hz, 1H, Ar-H), 6.90-6.87 (d, 8.7 Hz, 1H, Ar-H), 2.40 (s, 3H, CH\textsubscript{3}). \)

\(^{13}\)C NMR (75 MHz, DMSO): \(\delta_C (ppm): 159.7 (s, C-OH), 144.4 (s, C=O, \alpha carbon), 133.7 (s, C=O, \beta carbon), 132.8 (Ar), 122.5 (Ar), 115.9 (Ar), 13.9 (CH\textsubscript{3}). \)

GC/MS \(m/z (M^+) 179. \)

1,4-Bis-(2-nitro-propenyl)-benzene [51]

\[
\begin{align*}
\text{OHC} & \quad \text{CHO} \\
\text{CH\textsubscript{3}COONH\textsubscript{4}, CH\textsubscript{3}COOH} & \quad \text{Reflux} \\
\begin{array}{c}
\text{O}_2\text{N} \\
\text{NO}_2
\end{array} & \quad \text{NO}_2\text{O}_2\text{NO}_2
\end{align*}
\]

Method B was applied to produce this compound. A stirred mixture of terephthaldicarboxaldehyde (1.34g, 10mmol), nitroethane (1.8g, 24mmol) and ammonium acetate (2g, 26mmol) in glacial acetic acid (3mL) was refluxed for 1 hour in an oil bath at 100°C. A yellow precipitate formed which was washed with water and extracted with ethyl acetate (2 x 15mL). The orange solution was dried over MgSO\textsubscript{4} and then concentrated under high vacuum. A semi-solid was formed with melting point 90-95°C. The semi-solid was then recrystallized with ethanol (95%) and produced a yellow solid in yield 18% with melting point 99-101°C. The method from Fierro et al.\textsuperscript{191} is potentially another good method to synthesize this compound.

\(^1\)H NMR (300 MHz, CDCl\textsubscript{3}): \(\delta_H (ppm): 8.10 (s, 2H, C=C-H), 7.54 (s, 4H, Ar-H), 2.50 (s, 6H, CH\textsubscript{3}). \)

\(^{13}\)C NMR (75 MHz, CDCl\textsubscript{3}): \(\delta_C (ppm): 148.7 (s, C=C, \beta carbon), 133.8 (Ar), 132.4 (s, C=C, \alpha carbon), 130.3 (s, 4C in Ar), 14.1 (s, 2C, CH\textsubscript{3}). \)

GC/MS \(m/z (M^+) 248 \)

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References

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### Appendix

**Compound no.:** Name

25: 4-fluorobut-2-ynyl 2-(3-fluoro-4-methylphenyl)-3,3-dimethylbutanoate
26: 4-(2,3-difluorophenyl)-6-(4-fluorobut-2-ynyloxy)pyrimidine
27: 3-(4-fluorobut-2-ynyloxy)-5-phenyl-1,2,4-thiadiazole
28: 1-(4-(3-chlorophenyl)-4-fluorobut-2-ynyloxy)-4-fluoro-2-methoxybenzene
29: 6-(4-chlorophenyl)-2-(4-fluoropent-2-ynyl)-4,5-dihydropyridazin-3(2H)-one
30: methyl-2-chloro-5-(3-fluoro-3,4-dimethylpent-1-ynyl)benzylcarbamate
31: (E)-methyl-3-methoxy-2-(2-methyl-5-(3,4,4-tetrafluoro-3-methylbut-1-ynyl)phenoxy)acrylate
32: 4′-(3-fluorobut-1-ynyl)biphenyl-2-amine
33: methyl 2-(7-(3-fluoro-3-methylbut-1-ynyl)naphthalen-1-yl)-3-methoxypropanoate
34: 2-((5-(3-fluorobut-1-ynyl)thiophen-2-yl)ethynyl)benzo[b]thiophene
35: 6-chloro-4-cyclopropyl-4-(3-fluoroprop-1-ynyl)-3,4-dihydroquinazolin-2(1H)-one
36: N-(4-chlorobenzyl)-8-(3-fluoroprop-1-ynyl)-1-methyl-6-(morpholinomethyl)-4-oxy1,4-dihydroquinoline-3-carboxamide
Table of all structures with measured logP and calculated logP

<table>
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<th>Structure</th>
<th>Measured logP</th>
<th>Calculated logP</th>
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</thead>
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<td>2.13</td>
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<tr>
<td>1b</td>
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