ISOLATION, CHARACTERIZATION
AND DERIVATIZATION OF SELECTED
FATTY ACIDS

ABSTRACT
THESIS
SUBMITTED FOR THE AWARD OF THE DEGREE OF
Doctor of Philosophy
IN
CHEMISTRY

BY
NIDA NAYYAR FARSHORI

UNDER THE SUPERVISION OF
Dr. ABDUL RAUF

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH, INDIA
2010
Abstract

The thesis entitled, "Isolation, Characterization and Derivatization of Selected Fatty Acids" consist of five chapters. The work is based on the isolation and characterization of hydroxy fatty acids from natural source and synthesis of various fatty acid derivatives. The synthesized compounds have also been investigated for their in vitro antibacterial and antifungal activities. The preparation of such derivatives is a valuable addition to the fatty acid and medicinal chemistry.

1. Synthesis, Antibacterial and Antifungal Activity of Some New 2,5-Disubstituted-1,3,4-oxadiazoles

A series of novel 2,5-disubstituted-1,3,4-oxadiazoles (3a-d) (Scheme 1) have been synthesized from long chain alkenoic acid hydrazides (1a-d) via the formation of semicarbazides (2a-d). The reactions occurred under reflux conditions and afforded the desired product in good yields. The structures of these compounds have been elucidated by elemental and spectral (IR, $^1$H NMR, $^{13}$C NMR, Mass) analyses.

Further, these compounds were screened for their in vitro antibacterial activity against the representative panel of two Gram-positive (Streptococcus pyogenes and Staphylococcus aureus (ATCC-25923)) and three Gram-negative (Pseudomonas aeruginosa (ATCC-27853), Klebsiella pneumoniae (Clinical isolate) and Escherichia coli (ATCC-25922)) bacterial strains.

All the synthesized compounds were also tested for their inhibitory action against four strains of fungus viz. *Candida albicans*, *Aspergillus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured). The various compounds show potent inhibitory action against test organisms. The compounds 3c and 3d were found to be the most active antimicrobials whereas the compound 3b was the most potent antifungal agent.

![Synthesis of 2, 5-disubstituted-1,3,4-oxadiazoles 3a-d](image)

**Scheme 1.** Synthesis of 2, 5-disubstituted-1,3,4-oxadiazoles 3a-d

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
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<tbody>
<tr>
<td>1a, 2a, 3a</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>1b, 2b, 3b</td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>1c, 2c, 3c</td>
<td><img src="image" alt="Structure" /></td>
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<tr>
<td>1d, 2d, 3d</td>
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</tr>
</tbody>
</table>
Abstract

2. A Facile One-pot Synthesis of Novel 2,5-Disubstituted-1,3,4-oxadiazoles Under Conventional and Microwave Conditions and Evaluation of their in vitro Antimicrobial Activities

Fatty acid derivatives containing hetero atoms are regarded as potential antimicrobial agents. Thus a rapid and efficient solvent-free synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (3a-l) (Scheme 2) from fatty acid hydrazides (1a-f) under microwave irradiation is described. The procedure constitute a simple, practical and green synthetic protocol for the synthesis of pharmaceutically important 2,5-disubstituted-1,3,4-oxadiazoles (3a-l). The one-pot synthesis on solid inorganic support provided the products in good yields. The structural elucidation of these compounds is based on their spectral data (IR, $^1$H NMR, $^{13}$C NMR and MS).

All the compounds (3a-l) were also screened for their antibacterial and antifungal activities against various Gram positive and Gram negative bacterial strains and selected strains of fungus by disk diffusion method using Ciprofloxacin and Greseofulvin as a standard drugs respectively.

\[
\begin{align*}
  &\text{Scheme 2. Conventional synthesis of 2,5-disubstituted-1,3,4-oxadiazoles 3a-l.}
\end{align*}
\]
<table>
<thead>
<tr>
<th>Entry</th>
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<td>3l</td>
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</table>
Abstract

3. Synthesis, Antibacterial and Antifungal Activity of Some New 2,5-Disubstituted-1,3,4-thiadiazoles

The long chain alkenoic acid hydrazides (1a-d) on reaction with phenylthiocyanate gave their corresponding thiosemicarbazides (2a-d) (Scheme 3), which on further refluxing with Ac₂O underwent dehydrative cyclization to give the corresponding 2,5-disubstituted-1,3,4-thiadiazoles (3a-d) in good yields. The structure elucidation of synthesized compounds is based on the elemental analysis and spectral data (IR, ¹H NMR, ¹³C NMR and MS).

The synthesized 2,5-disubstituted-1,3,4-thiadiazoles have been screened for antibacterial and antifungal activities against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains and *Candida albicans, Aspergillus fumigatus, Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) fungal strains by disk diffusion method. The investigation of antimicrobial screening revealed that compounds 3c, 3d and 3b showed good antibacterial and antifungal activities respectively.

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Abstract

Scheme 3. Synthesis of 2,5-disubstituted-1,3,4-thiadiazoles 3a-d

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
</tr>
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<tbody>
<tr>
<td>1a, 2a, 3a</td>
<td><img src="#" alt="R" /></td>
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<td>1b, 2b, 3b</td>
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<td><img src="#" alt="R" /></td>
</tr>
<tr>
<td>1d, 2d, 3d</td>
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4. Facile One-pot Synthesis and in vitro Antimicrobial Activity of Novel 3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones.

A series of 3-substituted-1,6-dihydro-1,2,4-triazin-5(2H)-ones (3a-f) (Scheme 4), have been synthesized using the cyclocondensation reaction of fatty acid hydrazides (1a-f) with 2-chloroacetamide (2) in N,N-dimethylformamide medium. The structural
elucidation of the synthesized compounds is based on IR, $^1$H NMR, mass spectral data and elemental analysis.

![Chemical Reaction](attachment:image.png)

Scheme 4. Synthesis of 3-substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones 3a-f

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
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<tr>
<td>1</td>
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<tr>
<td>3</td>
<td>1c, 3c</td>
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</tr>
<tr>
<td>4</td>
<td>1d, 3d</td>
<td><img src="" alt="Structure" /></td>
</tr>
<tr>
<td>5</td>
<td>1e, 3e</td>
<td><img src="" alt="Structure" /></td>
</tr>
<tr>
<td>6</td>
<td>1f, 3f</td>
<td><img src="" alt="Structure" /></td>
</tr>
</tbody>
</table>

The newly synthesized compounds have been tested for their in vitro antibacterial and antifungal activities. The MIC, MBC and MFC were also determined. The data pertaining to antibacterial studies showed that the compounds 3c, 3d and 3f have a significant influence on the antibacterial profile of S. pyogenes, S. aureus and E. coli.
species at 6.25 µg/ml concentrations. The antifungal screening data revealed the compounds 3d and 3f to be the most potent antifungal agents.

5. Fatty acid alkenoates

A. Synthesis, Characterization and Preliminary Antimicrobial Activity of 7-Hydroxy-coumarin Derivatives

A new series of 7-O-coumarinyl alkenoates (6-9) (Scheme 5) were synthesized in a single step from 7-hydroxy-coumarin (5) and fatty acids (1-4) using DCC and DMAP as catalyst. The synthesized compounds were characterized on the basis of their spectral data (IR, $^1$H NMR, $^{13}$C NMR, COSY and MS).

All the target compounds were evaluated for their in vitro antimicrobial activity against Bacillus subtilis (ATCC 6501), Staphylococcus aureus (ATCC 25923), Streptococcus pyogenes (recultured), Pseudomonas aeruginosa (ATCC 27853), Salmonella typhimurium (recultured) and Escherichia coli (ATCC 25922) bacterial strains and fungal cultures of Candida albicans (ATCC 24433), Candida krusei (ATCC 6528), Candida parapsilosis (ATCC 22019) and Cryptococcus neoformans (recultured). The minimum inhibitory concentration (MIC) was determined for the test compounds as well as for reference standards.

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Among the tested compounds, 7-O-coumarinyl (9Z,12R)-12-hydroxyoctadec-9-enoate (8) and 7-O-coumarinyl(12Z,9R)-9-hydroxyoctadec-12-enoate (9) showed the most potent antifungal as well as antibacterial activities.

**Scheme 5. Synthesis of 7-O-coumarinyl alkenoates (6-9).**

<table>
<thead>
<tr>
<th>Compound</th>
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<td>1, 6</td>
<td>![Diagram of R1 and R6]</td>
</tr>
<tr>
<td>2, 7</td>
<td>![Diagram of R2 and R7]</td>
</tr>
<tr>
<td>3, 8</td>
<td>![Diagram of R3 and R8]</td>
</tr>
<tr>
<td>4, 9</td>
<td>![Diagram of R4 and R9]</td>
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</tbody>
</table>
B. Synthesis and in vitro Antimicrobial Activity of β-Sitosteryl Esters of Hydroxy and Non-hydroxy Olefinic Fatty Acids

A new series of β-sitosteryl alkenoates (11-14) (Scheme 6) were synthesized in quantitative yields using an appropriate synthetic route involving DCC and DMAP as catalyst. The reactions were carried out at room temperature. The synthesized compounds were characterized by their spectral data (IR, ¹H NMR, ¹³C NMR and MS).


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Abstract

All the synthesized compounds were evaluated for their *in vitro* antimicrobial activity by disk diffusion method. The minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC) and minimum fungicidal concentration (MFC) was determined for test compounds as well as for reference standards. Among the compounds tested, compounds having hydroxy group at the fatty acid chain (13 and 14) showed the most potent antibacterial as well as antifungal activities.
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Dedicated
To
My Family
Certificate

This is to certify that work embodied in this thesis entitled "Isolation, Characterization and Derivatization of Selected Fatty Acids", is the original work carried out by Ms. Nida Nayyar Farshori under my supervision. The thesis is suitable for submission for the award of Ph.D. degree in Chemistry.

Dr. Abdul Rauf
Acknowledgements

First of all, I thank Almighty Allah for giving me the courage and ability to work on this thesis.

I express my heartfelt gratitude to my supervisor Dr. Abdul Rauf for his innumerable advises and invaluable training that encouraged me to work on my endeavor. His unrelenting support and blessings made me achieve my goals. I attribute all the good things of this humble endeavor to his critical acumen and scientific temperament.

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I owe this achievement of my life to my aunt, Mrs. Nighat Javed, who could not see my study as she is no more. I find myself short of words to mention my gratitude to her. She has always been a source of encouragement to me. May Allah bless her soul.

My work is a tribute to my father Mr. Nayyar Farshori, my mother Mrs. Nuzhat Nayyar and my brother Mr. Noman Nayyar Farshori. I express my indebtedness to them for their best wishes, affection, loving support and encouragement in the completion of this work. My deepest thanks are due to my aunt Dr. Iffat Asghar, Reader, Hindi Department, Aligarh Muslim University for her constant love and support.

Nida Nayyar Farshori
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<td>Results and Discussion</td>
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<td>Experimental</td>
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Introduction
In the next century the availability of fossil organic feedstock—both as energy sources and for the production of organic chemical raw materials will gradually decrease. Hence it is important to look for alternatives. These can be found in renewable resources both for the energy purposes as well as for raw materials for the chemical industry. In the later case, much attention has already been given to the use of natural fats and oils of vegetable and plant origin in the oleochemical (oils/fats derived chemicals) industry.

Abundant fatty acids from natural sources are recognized as a versatile group of chemicals in the oleochemical industry. About 15% of the world production of fats and oils is used in the oleochemical industry as starting materials for a wide range of chemical products. The utilization of fatty acids as agrochemicals finds their way into industries not only in a simple acid form but also as esters or other derivatives. The reactions of fatty acids are readily accepted to lead towards general perfection of the development and progress of organic chemistry.

The recent trends in technology of fats and fatty acids have given importance to industrial processes such as polymerization, oxidation and metathesis. Consequently fatty acids have interestingly been found usable as specific and characteristic base materials for the emerging organic chemical industry.

For quite sometime now, the attention of chemist has been diverted to synthesize oleochemicals. These oleochemicals by virtue of their economic and ecological advantages are now competing with petrochemicals. These fat-derived chemicals are essential to a variety of industrial uses such as in coatings, surfactants, plasticizers, lubricant additives, cosmetics, pharmaceuticals and organic pesticides. In esterified form
the fatty acids are used as antifogging agents and found very useful for plasticizer in biodegradable plastic materials and also known to be good alternative fuel (biodiesel). Fatty acid monoesters are widely used in industry due to their lubricating and softening applications. Some fatty esters are found very effective for the treatment of dermatitis, cardiovascular, hepatic and renal diseases.

Heterocycles form by far the largest of the classical divisions of organic chemistry and are of immense importance biologically, industrially, and indeed to the functioning of any developed human society. The majority of pharmaceuticals and biologically active agrochemicals are heterocyclic, as are countless additives and modifiers used in industries as varied as cosmetics, reprography, information storage, and plastics. Many natural drugs such as quinine, papaverine, emetine, theophylline, atropine, procaine, codeine, morphine and reserpine are heterocycles. Almost all the compounds we know as synthetic drugs such as diazepam, chlorpromazine, isoniazid, metronidazole, azidothymidine, barbiturates, antipyrine, captopril and methotrexate are also heterocycles. Some dyes (e.g. mauveine), luminophores, (e.g. acridine orange), pesticides (e.g. diazinon) and herbicides (e.g. paraquat) are also heterocyclic in nature.

Furthermore, it has been found that the incorporation of a heterocyclic moiety into drug molecules can enhance the biological activity of the drug for eg. Red sulfanilamide and the less toxic white sulfanilamide (second generation) were the first sulfa drugs, and these contained no heterocyclic fragments. However, the intensive research work that followed their discovery demonstrated that modification of the p-aminobenzenesulfonamide structure by the introduction of heterocyclic substituent into the amide markedly enhanced their biological activity.
Fatty acids are a privileged class of organic chemistry having distinctive feature of undergoing classical and non-classical reactions. Fatty acids have two dynamic sites which can be employed in chemical reactions for the synthesis of derivatives displaying diverse activities: (i) double bond (terminal and internal) (ii) carboxylic acid end. Along with the classical reactions of organic chemistry, fatty acids also act as a potential substrate to undergo microwave assisted reactions. The microwave assisted organic synthesis (MAOS) continues to affect synthetic chemistry significantly by enabling rapid, reproducible and scalable chemistry development. Microwave heating has attracted the attention of investigators as it makes it possible to shorten the reaction time significantly, to increase their selectivity, and to increase the product yields, which is particularly important in the case of high-temperature processes that takes a long time.

Further, the development of antimicrobial agents (antibacterials, antifungals, antivirals and antiparasitics) to treat infections has been one of the most notable medical achievements of the past century. These advances in medical care are threatened, however, by a natural phenomenon known as “antimicrobial resistance.” The increased use of antibacterial and antifungal agents in recent years has resulted in the development of resistance to these drugs with important implications for morbidity, mortality and health care costs. In spite of a large number of antibiotics and chemotherapeutics available for medical use, the antimicrobial resistance created a substantial medical need for new classes of antimicrobial agents in the last decades. In view of the above, the design and synthesis of newer antimicrobials will always remain an area of immense significance.
A variety of new industrially and biologically useful long-alkyl and alkenyl/hydroxyalkenyl chain heterocyclic moieties can thus be prepared by taking advantage of the inherently present functional groups in the fatty acids. These aspects created an active interest to undertake the present work which involves the isolation and characterization of the hydroxy fatty acids from natural source and the synthesis and antimicrobial activity screening of the various fatty acid derivatives.
CHAPTER 1

2,5-Disubstituted-1,3,4-oxadiazoles
1.1. Theoretical

1,3,4-Oxadiazoles are five membered aromatic heterocycles with great utility in synthetic, medicinal, polymer and material chemistry. The widespread use of 1,3,4-oxadiazoles as a scaffold in medicinal chemistry has established this moiety as a member of the privileged structural class. In a study of new pharmaceuticals, the application of these heterocycles is warranted to improve their biological profile. These molecules are used as pharmacophores because of their favorable metabolic profile and ability to engage in hydrogen bonding. Presently there are a number of drugs used clinically, which comprise oxadiazole moiety in association with various heterocyclic rings. In particular marketed antihypertensive agents such as tiodazosin and nesapidil and antibiotics, such as furamizole contain oxadiazole nucleus.

1,3,4-Oxadiazoles is a popular bioisostere for improving the pharmacological profile of biologically active amides, esters and ureas. They are used as surrogates of carboxylic acids, esters and carboxamides. Symmetrical and unsymmetrical 1,3,4-oxadiazoles are biologically versatile compounds displaying a variety of biological effects which include antipheripheral vasmotility, anti-inflammatory, antimicrobial, antiparasitic, insecticidal, herbicidal, hypoglycemic and anti-tumor activity. A number of biologically relevant entities containing 1,3,4-oxadiazole motif are used as HIV integrase (i) and angiogenesis (ii) inhibitors.
Some C-(β-D-glucopyranosyl)-1,3,4-oxadiazoles (iii) were synthesized as potential glycogen phosphorylase inhibitors. 2-Amino-1,3,4-oxadiazoles were shown to possess antidiabetic, antiarthritic and anti-inflammatory activities. 2-Hydroxyphenyl-1,3,4-oxadiazole acts as a hypnotic and as a sedative. Analgesic, anti-inflammatory, anticonvulsive, diuretic and antiemetic properties are exhibited by 5-aryl-2-hydroxymethyl-1,3,4-oxadiazoles.

In addition oxadiazoles were used as models for studying intramolecular interactions and structural chemical design of new molecules with improved parameters. 2-Amino-1,3,4-oxadiazoles are also used as functional tools for chemical synthesis, for eg. Ishikawa et al. have exploited them as azadiones in [4+2] cycloaddition reactions for the synthesis of vinca alkaloids. Certain oxadiazole derivatives also have interesting photochemical properties and are becoming an important
2,5-Disubstituted-1,3,4-oxadiazoles

A member of heterocyclic family because of their wide usage in dyes and electrical materials.

2,5-Disubstituted-1,3,4-oxadiazoles containing 2-benzothiazolythiomethyl moiety (vi) were synthesized by condensation of (2-benzothiazolylthio)acetic acid imino ester dihydrochloride (iv) with hydrazides of various carboxylic acids (v)\(^{23}\) (Scheme 1.1.1).

![Scheme 1.1.1]

The treatment of a suspension of salicylic hydrazide (X=O)/thiosalicylic hydrazide (X=S) (vii) in toluene with acetic anhydride or acid chloride in presence of an equimolar amount of methansulfonic acid at room temperature, and then heating to reflux temperature gave 1,3,4-oxadiazoles (ix)\(^{24}\) (Scheme 1.1.2).

![Scheme 1.1.2]

A series of substituted 5-[4-(benzyloxy)-phenyl]-3-(2-cyanoethyl)-1,3,4-oxadiazol-2(3H)-one (xii) were synthesized from substituted 1-[4-(benzyloxy)benzoyl]-2-
2,5-Disubstituted-1,3,4-oxadiazoles

(2-cyanoethyl) hydrazine, (x), by a cyclization reaction using bis(trichloromethyl)carbonate(triphosgene)\(^{25}\) (Scheme 1.1.3).

\[
\begin{align*}
X &= \text{H, 2-I, 3-I, 4-I} \\
Y &= \text{H, I}
\end{align*}
\]

Scheme 1.1.3

5,5'-Dimercapto-bis-[1,3,4-oxadiazol-2-yl]octane and 5,5'-dibenzylthio-bis-[1,3,4-oxadiazol-2-yl]-butane were synthesized\(^{26}\) by the reaction of alkanedioic acid hydrazides (xiii) with CS\(_2\) in alcoholic KOH solution to give (xiv) which yielded (xv) with the addition of benzyl bromide (Scheme 1.1.4).

\[
\begin{align*}
x & \quad \text{xi} \quad \text{xii} \\
\text{xiii} & \quad \text{xiv} \quad \text{xv}
\end{align*}
\]

Scheme 1.1.4

5-Aryl-3-(4-hydroxyphenyl)-1,3,4-oxadiazole-2(3\(H\))-thiones (xvii), inhibiting the function of P-glycoprotein, were prepared by the cyclocondensation of 1-(4-hydroxyphenyl)-2-arylpbenzylhydrazines (xvi) with thiophosgene\(^{27}\) (Scheme 1.1.5).
2-Chloro-5-(5-aryl-1,3,4-oxadiazole-2-yl-methyl)-pyridine (xix) were synthesized from 2-chloropyridine-5-acetic acid hydrazide (xviii) by refluxing with phosphorus oxychloride for 8 hrs\textsuperscript{28} (Scheme 1.1.6).

\[ \text{R} = \text{H, Cl, F, CH}_3, \text{OCH}_3 \]

The treatment of 3-chloro-2-(N-formylacid hydrazide)-benzo[\textit{b}] thiophene (xx) under reflux with phosphorus pentoxide in xylene yielded corresponding 2-(3-chloro-1-benzothien-2-yl)-1,3,4-oxadiazoles (xxi)\textsuperscript{29} (Scheme 1.1.7).
The reaction of 2-\{[[3-alkyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]-methyl]-1,3,4-thiadiazol-2-yl}thio\}-acetohydrazide derivatives (xxii) with carbon disulfite in presence of KOH afforded 5-alkyl-2-[[5-\{[(5-mercapto-1,3,4-oxadiazol-2-yl)-methyl]thio\}-1,3,4-thiadiazol-2-yl]-methyl]-2,4-dihydro-3H-1,2,4-triazol-3-ones (xxiii)\textsuperscript{30} (Scheme 1.1.8).

\[ \text{Scheme 1.1.8} \]

The synthesis of 5-furan-2-yl-[1,3,4]oxadiazole-2-thiol (xxv) from furan-2-carboxylic acid hydrazide (xxiv) was reported by Koparir \textit{et al.}\textsuperscript{31} (Scheme 1.1.9).

\[ \text{Scheme 1.1.9} \]

When terephthalic acid hydrazide (xxvi) was refluxed with phenyl isocyanate (xxvii) then bis-semicarbazide (xxviii) was formed. The compound (xxviii) on treatment with phosphorus oxy chloride underwent cyclization to corresponding 5,5'-(1,4-phenylene)-bis-(2-phenylamino-1,3,4-oxadiazole) (xxix)\textsuperscript{32} (Scheme 1.1.10). The compound (xxix) showed antibacterial activity.
A effective one-pot acylation/cyclization approach for conversion of acylhydrazides to 1,3,4-oxadiazoles is described by James et al. Tetrasubstituted alkenyl-1,3,4-oxadiazoles (xxxi) were synthesized in excellent yield, under mild conditions and in presence of sensitive functional groups, via the cyclization of diacylhydrazides (xxx) using triphenyl phosphine and hexachloroethane in presence of Hunig’s base (Scheme 1.1.11).
2,5-Disubstituted-1,3,4-oxadiazoles

\[
\begin{align*}
\text{HN} & \quad \text{NH} \\
\text{R} & = \text{c-Pr, CH}_2\text{Br, OMe, CH}_2\text{OTBS, CO}_2\text{Et, H}
\end{align*}
\]

Scheme 1.1.11

4-(5-Amino-[1,3,4]oxadiazole-2-yl-methylsulphonyl)chromen-2-ones (xxxiii) were prepared by refluxing (2-oxo-2H-chromen-4-methylsulphonyl)acetic acid hydrazide (xxxii) with cyanogen bromide in methanol for 3 h\(^3^4\) (Scheme 1.1.12).

\[
\begin{align*}
\text{R} & = 6\text{-CH}_3, 7\text{-CH}_3, 7,8\text{-Beno}
\end{align*}
\]

Scheme 1.1.12

The cyclo-condensation of (7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid hydrazide (xxx) with carbon disulfide afforded 7-hydroxy-4-[(5-mercapto-1,3,4-oxadiazol-2-yl)methyl]-2H-chromen-2-one (xxxv) (Scheme 1.1.13), which were found to possess high antimicrobial activity against *Staphylococcus pneumoniae*\(^3^5\).
A series of new 2-[(4-alkylthio/alkylsulfonyl phenoxy)methyl]-5-substituted-1,3,4-oxadiazoles (xxxvii) exhibiting promising antimicrobial activity, have been synthesized from 2-(4-alkylthio/alkylsulfonyl phenoxy)-acetohydrazides (xxxvi)\(^{36}\) (Scheme 1.1.14).

The reaction of 1-adamantanecarbonyl chloride (xxxviii) with various carboxylic acid hydrazides (xxxix) yielded the corresponding \(N\)-acyl adamantine-1-carbohydrazide derivatives (xL), which were cyclized to the corresponding 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles (xLi) via heating with phosphorus oxy chloride\(^{37}\) (Scheme 1.1.15). The synthesized compounds showed anti-inflammatory activity.
2,5-Disubstituted-1,3,4-oxadiazoles

\[
\begin{align*}
\text{xL} & \quad \text{xLi} \\
\end{align*}
\]

\( R = C_6H_5, 4-FC_6H_4, 4-ClC_6H_4, 4-BrC_6H_4, 4-NO_2C_6H_4, 3,5-(NO_2)_2C_6H_3, 1-\text{Adamantyl} \)

**Scheme 1.1.15**

2,5-Disubstituted-1,3,4-oxadiazoles (xLiv) have been synthesized by the condensation of 4-methoxybenzohydrazide (xLii), by refluxing with different aromatic acids (xLiii) in presence of phosphorus oxychloride\(^*\) (Scheme 1.1.16). The \textit{in vitro} growth inhibiting activity against different strains of bacteria and fungi was assessed and encouraging results were obtained.

\[
\begin{align*}
\text{xLii} & \quad \text{xLiii} & \quad \text{xLiv} \\
\end{align*}
\]

\( \text{Ar} = C_6H_5, 4-\text{CH}_3C_6H_4, 4-\text{NO}_2C_6H_4, 4-\text{NH}_2C_6H_4, 4-\text{OHC}_6H_4, C_5H_4N \)

**Scheme 1.1.16**

New derivatives of 2-aminomethyl-1,3,4-oxadiazoles (xLvi) were synthesized by the reaction of \( N \)-protected phenylglycine hydrazide (xLv) and triethyl orthoesters in presence of glacial acetic acid\(^{39} \) (Scheme 1.1.17).
2,5-Disubstituted-1,3,4-oxadiazoles

2,5-Disubstituted-1,3,4-oxadiazoles (xLvi) have been conveniently prepared by oxidative cyclization of N-acyl-N'-aryldien-hydrazines (xLvii) promoted by an excess of Dess-Martin periodinane under mild conditions \(^{40}\) (Scheme 1.1.18).

A new class of 1,3,4-oxadiazoles were prepared from acid hydrazides on treatment with different carboxylic acids in presence of phosphorus oxychloride. The arylsulfonylacetic acid/arylmethanesulfonylacetic acid hydrazides (xLix) reacted with aromatic carboxylic acids to give 2-(arylsulfonylmethyl)-5-aryl-1,3,4-oxadiazoles/2-(arylmethanesulfonylmethyl)-5-aryl-1,3,4-oxadiazoles (L) in good yields \(^{41}\) (Scheme 1.1.19).

\[ R_1 = \text{Ph, 4-ClC}_6\text{H}_4, 4-\text{NO}_2\text{C}_6\text{H}_4, 2-\text{Furyl, 4-Pyridyl} \]

\[ R_2 = \text{Ph, 4-MeOC}_6\text{H}_4, 4-\text{BrC}_6\text{H}_4, 2-\text{Furyl, 4-Pyridyl, 2-Thienyl, Pr} \]
2,5-Disubstituted-1,3,4-oxadiazoles

\[ \text{R} \backslash \text{C} \backslash \text{H} \backslash \text{COOH} \]

\[ \text{POCl}_3 \]

\( n=1,2 \)

\( R= \text{H, 4-Cl} \)

\( R_1= \text{H, 2-Cl} \)

**Scheme 1.1.19**

The reaction of ethyl 2,6-dimethyl-5-(N-methylsulfanylthiocarbohydrazino-carbonyl) nicotinate (Li) with a two fold excess of hydrazine hydrate in 2-propanal leads to the formation of 2,6-dimethyl-5-(5-methylsulfanyl [1,3,4]oxadiazol-2-yl)nicotinic acid hydrazide (Lii)\(^{42}\) (**Scheme 1.1.20**).

\[ \text{H}_3\text{C} \backslash \text{N} \backslash \text{CH}_3 \]

\[ \text{CH}_3 \]

\[ \text{Li} \]

\[ 2\text{NH}_2\cdot\text{NH}_2 \]

2-propanol

\[ \text{Lii} \]

**Scheme 1.1.20**

The various aroylpropionic acid derivatives containing 1,3,4-oxadiazole nucleus (Liv) were synthesized by treating 3-aryloxypropionic acid (Liii) with various aryl acid hydrazides in presence of phosphorus oxy chloride\(^{43}\) (**Scheme 1.1.21**).
2,5-Disubstituted-1,3,4-oxadiazoles

Scheme 1.1.21

A facile, convenient and high yielding synthesis and in vitro anti-cancer activity of a series of 5-(3'-indolyl)-2-substituted-1,3,4-oxadiazoles (Lvi) has been described by Kumar et al. The key step of synthesis involved the cyclization of N-acylhydrazones (Lv) using [bis(trifluoro-acetoxy)iodo]benzene under solvent free condition (Scheme 1.1.22).

Scheme 1.1.22

Watanabe et al. synthesized the biologically important 2,5-diphenyl-1,3,4-oxadiazole (Lix) derivatives by reacting 4-iodo/bromo benzhydrazide (Lvii) with 4-dimethyl-aminobenzaldehyde (Lviii) in presence of ceric ammonium nitrate (CAN) (Scheme 1.1.23).
2,5-Disubstituted-1,3,4-oxadiazoles

The reaction of anthranilic acid derivatives (Lx) with (N-isocyanimino)triphenylphosphorane (Lxi) at room temperature afforded 2-substituted-1,3,4-oxadiazoles (Lxii) via an intramolecular aza-wittig reaction under neutral condition46 (Scheme 1.1.24).

The 3-substituted benzoic acid derivatives (Lxvi) and (N-isocyanimino)triphenylphosphorane (Lxvii) in dry solvent reacted together in a 1:1 ratio at room temperature to produce 1,3,4-oxadiazoles (Lxviii)47 (Scheme 1.1.25).
The cyclization of 2-toluic acid (Lxiii) and hydrazine hydrate (Lxiv) catalysed by polyphosphoric acid (PPA) yielded 2,5-di-p-tolyl-1,3,4-oxadiazole (Lxv) in good yield (Scheme 1.1.26).

The synthesis of imidazo[5,1-b][1,3,4]oxadiazole motif (Lxxi) by the double cyclodehydration of amino acid-derived acyl hydrazide amides (Lxix) has been described by Tran et al. (Scheme 1.1.27).
A primary aromatic amine and formaldehyde solution on reaction with 5-(2-thienyl)-1,3,4-oxadiazoline-2-thione (Lxxii) in ethanol gave 3-arylaminomethyl-5-(2-thienyl)-1,3,4-oxadiazoles (Lxxiii)\(^\text{50}\) (Scheme 1.1.28).

![Reaction scheme](image)

**Scheme 1.1.28**

The environmentally benign electroorganic synthesis of 5-substituted-2-amino-1,3,4-oxadiazoles (Lxxv) by electrocyclization of semicarbazone (Lxxiv) has been described by Sharma et al.\(^\text{51}\) (Scheme 1.1.29).

![Electrocyclization scheme](image)

**Scheme 1.1.29**

The synthesis of 2-styryl-5-(arylmethanesulfonylmethyl)-1,3,4-oxadiazoles (Lxxviii) was achieved by refluxing a mixture of arylmethanesulfonylacetic acid hydrazide (Lxxvi) with cinnamic acid (Lxxvii) in presence of phosphorous oxy chloride\(^\text{52}\) (Scheme 1.1.30).
The reaction of 4-aminoisothiazdcarboxylic-3-acid hydrazide (Lxxix) with isothiocyanates, Ar₁-NCS, followed by in situ cyclization of intermediate thiosemicarbazides with DCC afforded 2-(4-aminoisothiazolyl-3)-5-(arylamino)-1,3,4-oxadiazole (Lxxx) in good yields²³ (Scheme 1.1.31).

![Scheme 1.1.30](image)

Ar= 3,4-(OCH₂O)-C₆H₄, 3,4-(OCH₂CH₂O)-C₆H₄, 4-Cl-C₆H₄

The synthesis of 2-(4-isopropylthiazol-2-yl)-5-substituted-1,3,4-oxadiazoles (Lxxxii) was achieved by the reaction of 4-isopropylthiazole-2-carbahydrazide (Lxxxi) with appropriate aromatic acid in phosphorus oxy chloride under reflux condition²⁴ (Scheme 1.1.32).
2,5-Disubstituted-1,3,4-oxadiazoles

\[ \text{Lxxxii} \quad \text{RCOOH} \quad \text{POCl}_3, \text{reflux} \quad \text{Lxxx} \]

\( R = \text{CH}_3, \text{C}_6\text{H}_5, \text{C}_6\text{H}_4\text{Cl}, \text{C}_6\text{H}_4\text{C}_6\text{H}_4\text{NO}_2, \text{C}_6\text{H}_4\text{Br}, \text{C}_6\text{H}_4\text{Cl}, \text{C}_6\text{H}_4\text{OH} \)

Scheme 1.1.32
1.2. Synthesis, Antibacterial and Antifungal Activity of Some New 2,5-Disubstituted-1,3,4-oxadiazoles*

A wide variety of heterocyclic systems have been explored for developing pharmaceutically important molecules. Among them the derivatives of oxadiazoles have played an important role in the medicinal chemistry. 1,3,4-Oxadiazoles have demonstrated a broad spectrum of biological activities in both agrochemical and pharmaceutical fields. The substituted oxadiazoles serve both as biomimetic and reactive pharmacophores and are key elements with potential activities. The therapeutic effects of compounds containing 1,3,4-oxadiazole rings have been studied for a number of pathological conditions including inflammation\(^5\), pain\(^6\) and hypertension\(^7\). Furthermore, the synthesis of oxadiazoles has attracted wide attention due to the diversity of their applications as antibacterial\(^8\), antimycobacterial\(^9\), antifungal\(^10\) and antidepressant agents\(^11\). 2,5-Disubstituted-oxadiazoles have also attracted significant interest because of their application in material science for the development of electronic as well as optical devices\(^12\). In addition, the oxadiazoles have been used as models for studying intramolecular interactions and structural chemical design of new molecules with improved parameters\(^2\).

Various biological applications such as antimicrobial, antifungal, pesticidal and anticancer activities have also been reported for seed oils, long chain alkenoic acids and their derivatives. The symmetrically disubstituted 1,3,4-oxadiazoles have been prepared for the author’s laboratory.

The wide range of therapeutic values of alkenoic acids and oxadiazoles ring systems prompted us to synthesize the title compounds and screen them for various antimicrobial activities. The basic idea was to append the long chain alkenyl/hydroxyalkenyl moiety of the fatty acid to the oxadiazole nucleus so as to combine the beneficial effects in a single structure with expected biological activities.
1.3. Results and Discussion

In a typical reaction procedure the long chain alkenoic acid hydrazides (1a-d), used as the starting material were prepared from corresponding long chain alkenoic acids by esterification and further treatment with hydrazine hydrate. The reaction of 1a-d with phenyl isocyanate in dry benzene under reflux and removing excess of solvent under reduced pressure gave semicarbazides (2a-d). The treatment of 2a-d with phosphorus oxychloride yielded 2,5-disubstituted-1,3,4-oxadiazoles (3a-d) in good yields. The reaction sequence is outlined in Scheme 1.3.1.

\[
\begin{align*}
\text{1a-d} & \xrightarrow{\text{PhNCO}} \text{2a-d} \\
\text{2a-d} & \xrightarrow{\text{POCl}_3} \text{3a-d}
\end{align*}
\]

Scheme 1.3.1. Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles 3a-d

The characterization data of semicarbazides (2a-d) and 1,3,4-oxadiazoles (3a-d) is given in Table 1.3.1. As can be seen from Table 1.3.1, the scope of the reaction using olefinic (internal and terminal) and hydroxy fatty acids is found to be good. The yields of 1,3,4-oxadiazoles are excellent and independent of the substituent present in the precursor.
### Table 1.3.1. Characterization data of synthesized compounds 2a-d and 3a-d

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R</th>
<th>Mol. Formula</th>
<th>M. p. [°C]</th>
<th>Yield [%]</th>
<th>Analysis (%) found (calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>2a</td>
<td>C(_2)H(_8)N(_3)</td>
<td>C(<em>{18})H(</em>{27})O(_2)N(_3)</td>
<td>103-104</td>
<td>80</td>
<td>67.67(68.11) 8.44(8.56) 13.09(13.23)</td>
</tr>
<tr>
<td>2b</td>
<td>C(_2)H(_4)O(_2)N(_3)</td>
<td>C(<em>{25})H(</em>{41})O(_2)N(_3)</td>
<td>101-102</td>
<td>79</td>
<td>71.95(72.25) 9.86(9.93) 10.02(10.11)</td>
</tr>
<tr>
<td>2c</td>
<td>C(_2)H(_4)O(_3)N(_3)</td>
<td>C(<em>{25})H(</em>{41})O(_3)N(_3)</td>
<td>111-113</td>
<td>77</td>
<td>69.23(69.57) 9.50(9.56) 09.65(9.73)</td>
</tr>
<tr>
<td>2d</td>
<td>C(_2)H(_4)O(_3)N(_3)</td>
<td>C(<em>{25})H(</em>{41})O(_3)N(_3)</td>
<td>113-114</td>
<td>72</td>
<td>69.33(69.57) 9.20(9.56) 09.65(9.73)</td>
</tr>
<tr>
<td>3a</td>
<td>C(_2)H(_6)N(_3)</td>
<td>C(<em>{18})H(</em>{25})ON(_3)</td>
<td>133-134</td>
<td>92</td>
<td>72.06(72.21) 8.11(8.40) 13.90(14.03)</td>
</tr>
<tr>
<td>3b</td>
<td>C(_2)H(_6)N(_3)</td>
<td>C(<em>{25})H(</em>{39})ON(_3)</td>
<td>144-146</td>
<td>90</td>
<td>75.23(75.52) 9.74(9.87) 10.44(10.56)</td>
</tr>
<tr>
<td>3c</td>
<td>C(_2)H(_6)N(_3)</td>
<td>C(<em>{25})H(</em>{39})O(_2)N(_3)</td>
<td>133-136</td>
<td>87</td>
<td>72.35(72.60) 9.41(9.49) 10.00(10.15)</td>
</tr>
<tr>
<td>3d</td>
<td>C(_2)H(_6)N(_3)</td>
<td>C(<em>{25})H(</em>{39})O(_2)N(_3)</td>
<td>135-137</td>
<td>87</td>
<td>72.28(72.60) 9.36(9.49) 09.97(10.15)</td>
</tr>
</tbody>
</table>

The semicarbazide 2a showed IR bands at 3236 cm\(^{-1}\) (NH, NH-NH) and 1667 cm\(^{-1}\) (C=O). \(^1\)H NMR was more informative, characteristic peaks were observed at \(\delta\) 13.04 (1H, s, CO-NH-Ar), 9.16 (2H, br. s, CO-NHNH-CO), 8.17 (2H, d, \(J = 7.2\) Hz, Ar-H-2"/6"), 7.60 (1H, t, \(J = 7.4\) Hz, Ar-H-4") and 7.51 (2H, t, \(J = 7.4\) Hz, Ar-H-3"/5"). In \(^{13}\)C NMR peaks at \(\delta\) 168.2, and 165.4 were observed.

*The build up of 3a-d is evident from their spectral data. Compound 3a, 5-(Dec-9'-enyl)-2-phenylamine-1,3,4-oxadiazole, showed IR absorption bands at 3228, 1504, 1258*
2,5-Disubstituted-1,3,4-oxadiazoles

cm\(^{-1}\) due to stretching vibrations of NH, C=N and C-O-C functions. The \(^1\)H NMR was more informative in assigning the structure. Diagonastic peaks at \(\delta\) 9.07 (1H, s, NH), 7.27 (2H, d, \(J = 7.6\) Hz, Ar-H-2\(^\prime\)/6\(^\prime\)), 7.18 (1H, t, \(J = 7.5\) Hz, Ar-H-4\(^\prime\)) and 6.98 (2H, t, \(J = 7.3\) Hz, Ar-H-3\(^\prime\)/5\(^\prime\)) were observed. A triplet at \(\delta\) 2.24 was observed for methylene protons \(\alpha\) to oxadiazole ring. The methine proton of C-9 showed signal at 5.79. The C-10 methylene proton designated as \(H_E\) and \(H_Z\) displayed two distinct \(\delta\) values when coupled with adjacent C-9 methine protons. Thus, the \(^1\)H NMR showed two doublet of doublet at \(\delta\) 5.00 and 4.94 for \(H_Z\) and \(H_E\) protons, respectively. In \(^1^3\)C NMR peaks at \(\delta\) 172.9 and 155.6 were observed for ring carbon atoms. The mass spectra showed characteristic molecular ion peak which were in accordance with the molecular formula. Detailed spectral data of the titled compounds are given in experimental section.

**Antibacterial studies**

All the newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method\(^{69,70}\). The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 1.3.2.
2,5-Disubstituted-1,3,4-oxadiazoles

*Table 1.3.2. In vitro antibacterial activity of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles 3a-d*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. pyogenes</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>3a</td>
<td>18.0±0.5</td>
<td>17.7±0.6</td>
</tr>
<tr>
<td>3b</td>
<td>15.6±0.2</td>
<td>15.3±0.2</td>
</tr>
<tr>
<td>3c</td>
<td>21.2±0.3</td>
<td>20.2±0.4</td>
</tr>
<tr>
<td>3d</td>
<td>20.1±0.2</td>
<td>21.1±0.3</td>
</tr>
<tr>
<td>Standard</td>
<td>23.0±0.2</td>
<td>22.0±0.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (standard); Chloramphenicol and negative control (DMSO) measured by the Halo Zone Test (Unit, mm)

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The minimum inhibitory concentrations and minimum bactericidal concentrations (MBCs) are given in *Table 1.3.3.*
### Table 1.3.3. MIC and MBC results of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles 3a-d

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th></th>
<th></th>
<th>Gram negative bacteria</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td></td>
</tr>
<tr>
<td></td>
<td>S. pyogenes</td>
<td>S. aureus</td>
<td>P. aeruginosa</td>
<td>K. pneumoniae</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>3a</td>
<td>12.5</td>
<td>50.0</td>
<td>6.25</td>
<td>25.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td></td>
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<td>3c</td>
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<td>3d</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Positive control Chloramphenicol

MIC (µg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (µg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely.

The PBE (percentual bacteriostatic efficiency, %) was obtained as

\[
PBE = \frac{100}{MIC}
\]

The results have been depicted in Fig. 1.3.1.
2,5-Disubstituted-1,3,4-oxadiazoles

Fig. 1.3.1 Percentual bacteriostatic efficiency (PBE%) for compounds 3a-d compared to control drug Chloramphenicol (Ch)

The investigation of antibacterial screening data revealed that all the tested compounds have exerted significant inhibitory activity against the growth of tested bacterial strains. The data pertaining to Table 1.3.3 reveal that compounds 3a-d have significant influence on the antibacterial profile of subject bacterial strains at 6.25 μg/ml concentrations. The compounds 3c and 3d were found to be almost equally potent as the reference drug, Chloramphenicol, in case of *K. pneumoniae*. The MBC of few compounds was found to be the same as MIC but in most of the compounds it was two or three folds higher than the corresponding MIC results.

Among the synthesized oxadiazoles, the compounds with a hydroxyalkenyl chain substituent at 5th position of oxadiazoles were found to increase the antibacterial activity.
2,5-Disubstituted-1,3,4-oxadiazoles

in compounds 3c and 3d. However the position of the hydroxy group had no significant
effect on the magnitude of the antibacterial activity. Further, the compounds showed
parallel activity against Gram-positive and Gram-negative bacterial strains.

Antifungal studies

Antifungal activity was also done by disk diffusion method\textsuperscript{71,72}. For assaying
antifungal activity Candida albicans, Aspergillus fumigatus, Penicillium marneffei and
Trichophyton mentagrophytes (recultured) in DMSO strains were used. The fungal
activity of each compound was compared with Greseofulvin as standard drug. Inhibition
zones were measured and compared with the controls. The fungal zones of inhibition
values are given in Table 1.3.4.

\textit{Table 1.3.4. Antifungal activity of 5-Alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-
oxadiazoles 3a-d}

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA</td>
</tr>
<tr>
<td>3a</td>
<td>24.2±0.4</td>
</tr>
<tr>
<td>3b</td>
<td>26.1±0.3</td>
</tr>
<tr>
<td>3c</td>
<td>23.3±0.9</td>
</tr>
<tr>
<td>3d</td>
<td>23.2±0.2</td>
</tr>
<tr>
<td>Standard</td>
<td>30.0±0.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (Greseofulvin) and negative control (DMSO) measured by the Halo Zone
Test (Unit, mm).
\textbf{CA}= Candida albicans, \textbf{AF}= Aspergillus fumigatus, \textbf{TM}= Trichophyton
mentagrophytes, \textbf{PM}= Penicillium marneffei.
The results of minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) as determined by broth dilution technique are given in Table 1.3.5.

**Table 1.3.5. MIC and MFC of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles 3a-d**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>AF</th>
<th>TM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
</tr>
<tr>
<td>3a</td>
<td>25.0</td>
<td>50.0</td>
<td>25.0</td>
<td>100</td>
</tr>
<tr>
<td>3b</td>
<td>6.25</td>
<td>25.0</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>3c</td>
<td>25.0</td>
<td>100</td>
<td>12.5</td>
<td>50.0</td>
</tr>
<tr>
<td>3d</td>
<td>12.5</td>
<td>25.0</td>
<td>25.0</td>
<td>50.0</td>
</tr>
<tr>
<td>St.</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Positive control Greseofulvin
CA = Candida albicans, AF = Aspergillus fumigatus, TM = Trichophyton mentagrophytes, PM = Penicillium marneffei, St. = Standard. MIC (µg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (µg/ml) = minimum fungicidal

The ratio MFC/MIC was calculated in order to determine if the compound had a fungistatic (MFC/MIC ≥ 4) or fungicidal (MFC/MIC ≤ 4) activity and the results have been depicted in Fig. 1.3.2.
The antifungal screening data showed moderate to good activity. Among the screened compounds, 3b was found to be most active against all test fungal strains. The compound 3b was active against *P. marneffei*. The MFC of most of the compounds was two or three folds higher than the corresponding MIC results. Most of the synthesized compounds showed good fungistatic activity against the fungal strain *C. albicans*. The data revealed that compounds 3a-d have produced the marked enhancement in the potency of these analogues as antifungal agents. The compounds with an internal double bond in the long alkenyl substituent of synthesized oxadiazoles were found to be potent.
antifungal agents. Contrary to the antibacterial studies, the presence of the hydroxy on the alkenyl side chain turns out to be detrimental for the anti-fungal activity perhaps due to pharmacokinetic reasons.

In conclusion the present study showed that the synthesized compounds can be used as template for future development through modification and derivatization to design more potent and selective antimicrobial agents.
1.4. Experimental

Anhydrous conditions were achieved by oven drying flasks and equipments. The reaction progress and completion was monitored by thin layer chromatography on glass plates (20×5 cm) with a layer of silica gel G (Merck, Mumbai, India, 0.5mm thickness). Mixture of n-hexane-ethyl acetate-acetic acid (70:30:1, v/v) were used as developing solvents. Column chromatography was carried out on silica gel (Merck, Mumbai, India, 60-120 mesh). IR spectra were obtained on Shimadzu 8201 PC FT-IR using KBr pellet and nujol oil with absorption given in cm⁻¹. ¹H NMR and ¹³C NMR spectra were recorded in CDCl₃ on a Bruker DRX-400 instrument. The chemical shifts (δ) were measured relative to TMS as an internal standard. Coupling constants (J) are expressed in Hz. Mass spectra were obtained on a Jeol SX-102 (FAB) spectrometer. Melting points were taken in open capillary and are uncorrected.

All reagents and solvents were generally used as received from commercial suppliers and if required were dried and distilled before use. Undec-10-enoic (purity 98%) and (9Z)-octadec-9-enoic (97%) acids were purchased from Fluka Chemicals (Bucks, Switzerland). (9Z,12R)-12-Hydroxyoctadec-9-enoic (ricinoleic) and (9R,12Z)-9-hydroxyoctadec-12-enoic (isoricinoleic) acids were isolated from the natural sources i.e. from Ricinus communis and Wrightia tinctoria seed oils respectively.

Isolation and characterization of hydroxy fatty acids

The naturally occurring hydroxy fatty acids have the potentiality of utilization as an alternative source for the production of oleochemicals. In view to this, the isolation of hydroxy fatty in pure form and their characterization is of prime importance. The
hydroxy fatty acids viz. (9Z,12R)-12-hydroxyoctadec-9-enoic acid (ricinoleic acid) and (9R,12Z)-9-hydroxyoctadec-12-enoic acid (isoricinoleic acid) were isolated from *Ricinus communis* and *Wrightia tinctoria* seed oils respectively. The freshly extracted seed oils were saponified with alcoholic KOH. After the removal of unsaponifiable matter the soap solution was decomposed with 10% H₂SO₄. The mixed fatty acids so obtained were partitioned according to Gunstone’s partition procedure, between light petroleum and 80% aqueous methanol. The hydroxy acid present in each methanolic layer was collected and most of the methanol was removed under reduced pressure. The residue was taken up in ether and dried over sodium sulphate. Evaporation of the solvent gave the concentrate of hydroxy acids which on purification by column chromatography gave the 98% pure (9Z,12R)-12-hydroxyoctadec-9-enoic (ricinoleic) and (9R,12Z)-9-hydroxyoctadec-12-enoic (isoricinoleic) acids. Further their methyl esters were prepared by MeOH/H⁺ and were characterized on the basis of the spectral data.

**Spectroscopic data**

*(9Z,12R)-12-hydroxyoctadec-9-enoate*

IR (KBr): 3406, 1740 cm⁻¹

¹H NMR (400 MHz, CDCl₃): δ 6.31 (s, 3H, OCH₃), 5.42 (m, 2H, -CH=CH-), 3.84 (m, 1H, -CHCH), 2.30 (m, 1H, -CHOH-), 2.02 (m, 4H, -CH₂-CH=CH-CH₂-), 1.32 (br.s, 18H, chain CH₃), 0.84 (dist.t, 3H, CH₃).

*(9R,12Z)-9-hydroxyoctadec-12-enoate*

IR (KBr): 3406, 1738 cm⁻¹
\textbf{2,5-Disubstituted-1,3,4-oxadiazoles}

\textit{\textsuperscript{1}H NMR} (400 MHz, CDCl\textsubscript{3}): \delta 6.29 (s, 3H, OCH\textsubscript{3}), 5.37 (m, 2H, -CH=CH-), 3.82 (m, 1H, -CHOH), 2.27 (m, 1H, -CHOH-), 2.09 (m, 4H, -CH\textsubscript{2}-CH=CH-CH\textsubscript{2}-), 1.30 (br.s, 18H, chain CH\textsubscript{2}), 0.86 (dist.t, 3H, CH\textsubscript{3}).

\textit{General procedure for the synthesis of long chain alkenoic acid hydrazide} (1a-d)

The hydrazides of long chain alkenoic acids (1a-d) which are used as the starting material were prepared by the previously reported methods\textsuperscript{58}. The synthesized hydrazides 1a-d were characterized by melting points.

\textit{Synthesis of 1-(alkenoyl/hydroxyalkenoyl)-5-phenylsemicarbazide} (2a-d)

Hydrazide (1a-d) (1.0 mmole) was dissolved in abs. ethanol by heating to make a clear solution. An equal molar amount of phenyl isocyanate was added to it and the solution was refluxed for 5 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to precipitate. The precipitate was filtered, washed with abs. ethanol and dried to give analytically pure compounds 2a-d. The purity of the compounds was ascertained by TLC resolution studies using petroleum ether/ethyl acetate (4:1, v/v) and a few drops of acetic acid as mobile phase. The newly synthesized compounds were characterized from their spectral data.

\textbf{Spectroscopic Data}

\textit{1-(Undec-10-enoyl)-5-phenylsemicarbazides} (2a)

White powder; Yield 80%; Mp 103-104 \textdegree{}C.

\textit{IR} (KBr): 3236, 1667 cm\textsuperscript{-1}.

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2,5-Disubstituted-1,3,4-oxadiazoles

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 13.04 (s, 1H, CO-NH-Ar), 9.16 (br. s, 2H, CO-NHNH-CO), 8.17 (d, 2H, $J$= 7.2 Hz, Ar-H-2"/6"), 7.60 (t, 1H, $J$= 7.4 Hz, Ar-H-4"), 7.51 (t, 2H, $J$= 7.4 Hz, Ar-H-3"/5"), 5.82 (tdd, 1H, $J_{H-H_2}$ = 6.8 Hz, $J_{H-H_2}$ =10.0 Hz, $J_{H-H_2}$ =17.8 Hz, CH$_2$=CH-), 5.01 (dd, 1H, $J_{H-H_2}$=10.0 Hz, $J_{H-H_2}$=2.8 Hz, CH$_2$=CH), 4.94 (dd, 1H, $J_{H-H_2}$=17.8 Hz, $J_{H-H_2}$=2.8 Hz, CH$_2$=CH-), 2.54 (t, 2H, $J$= 7.8 Hz, CH$_2$-CO), 2.36 (m, 4H, CH$_2$=CH-CH$_2$), 1.82 (m, 2H, CH$_2$-CH$_2$-CO), 1.37-1.25 (br. s, 20H, (CH$_2$)$_{20}$), 0.89 (dist. t, 3H, terminus CH$_3$).

$^13$C NMR (100 MHz, CDCl$_3$): $\delta$ 168.2, 165.4, 139.2, 133.2, 131.5, 128.9, 128.6, 114.2, 114.2, 33.8, 29.9, 29.6, 29.3, 29.2, 29.1, 29.0, 28.9.

1-[(9Z)-Octadec-9-enoyl]-5-phenylsemicarbazides (2b)

White powder; Yield 79%; Mp 101-102 °C.

IR (KBr): 3222, 1665 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 13.18 (s, 1H, NH), 9.18 (br. s, 2H, NHNH), 8.08 (d, 2H, $J$= 7.2 Hz, Ar-H-2"/6"), 7.64 (t, 1H, $J$= 7.4 Hz, Ar-H-4"), 7.51 (t, 2H, $J$= 7.2 Hz, Ar-H-3"/5"), 5.31 (m, 2H, CH$_2$-CH=CH-CH$_2$), 2.54 (t, 2H, $J$= 7.8 Hz, CH$_2$-CO), 2.36 (m, 4H, CH$_2$-CH$_2$=CH-CH$_2$), 1.82 (m, 2H, CH$_2$-CH$_2$-CO), 1.37-1.25 (br. s, 20H, (CH$_2$)$_{20}$), 0.89 (dist. t, 3H, terminus CH$_3$).

$^13$C NMR (100 MHz CDCl$_3$): $\delta$ 167.4, 164.7, 139.2, 137.3, 133.2, 131.5, 128.8, 129.0, 31.9, 30.7, 30.4, 30.1, 29.9, 29.7, 29.6, 29.4, 29.2 "two signals are hidden", 29.1, 26.6, 22.7, 14.2.

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2,5-Disubstituted-1,3,4-oxadiazoles

1-[(9Z,12R)-12-hydroxy-octadec-9-enyl]-5-phenylsemicarbazides (2c)

Off-white powder; Yield 77%; Mp 111-113 °C.

IR (KBr): 3343, 3233, 1661 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 13.23 (s, 1H, NH), 9.17 (br. s, 2H, NH₂N), 8.12 (d, 2H, J= 7.4 Hz, Ar-H-2"/6"), 7.65 (t, 1H, J= 7.4 Hz, Ar-H-4"), 7.51 (t, 2H, J= 7.2 Hz, Ar-H-3"/5"), 5.37 (m, 2H, CH₂-CH=CH-CH₂), 4.87 (m, 1H, CH-OH), 2.55 (t, 2H, J= 7.6 Hz, CH₂-CO), 2.36 (m, 4H, CH₂-CH₂=CH-CH₂), 1.83 (m, 2H, CH₂-CH₂-CO), 1.76 (m, 1H, CH-OH), 1.44-1.28 (br. s, 18H, (CH₂)₉), 0.88 (dist. t, 3H, terminus CH₃).


1-[(9R,12Z)-9-hydroxy-octadec-12-enyl]-5-phenylsemicarbazide (2d)

Off-white powder; Yield 72%; Mp 113-114 °C.

IR (KBr): 3353, 3229, 1668 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 13.03 (s, 1H, NH), 9.09 (br. s, 2H, NH₂N), 8.12 (d, 2H, J= 7.2 Hz, Ar-H-2"/6"), 7.61 (t, 1H, J= 7.4 Hz, Ar-H-4"), 7.52 (t, 2H, J= 7.2 Hz, Ar-H-3"/5"), 5.38 (m, 2H, CH₂-CH=CH-CH₂), 4.89 (m, 1H, CH-OH), 2.55 (t, 2H, J= 7.6 Hz, CH₂-CO), 2.26 (m, 4H, CH₂-CH₂=CH-CH₂), 1.86 (m, 2H, CH₂-CH₂-CO), 1.68 (m, 1H, CH-OH), 1.40-1.25 (br. s, 18H, (CH₂)₉), 0.87 (dist. t, 3H, terminus CH₃).

¹³C NMR (100 MHz, CDCl₃): δ 167.6, 163.2, 138.4, 137.6, 131.2, 128.7, 128.3, 125.5, 70.56, 40.1, 39.9, 36.3, 24.8, 31.3, 29.1, 29.0, 28.9, 28.8, 28.5, 28.4, 25.1, 22.3, 14.01.
Synthesis of 5-(alkenyl/hydroxyalkenyl)-2-phenylamine 1,3,4-oxadiazoles (3a-d)

1-Alkenoyl/hydroxyalkenyl-5-phenyl carbamides (2a-d) (1.0 mmole) in phosphorus oxy chloride (6.0 ml) were refluxed for 4 h. The progress of the reaction was monitored by TLC. After the completion of the reaction the resulting mixture was then poured into NaOH/ice water solution, resulting in deposition that was filtered, washed, dried and recrystallized from aqueous ethanol and acetone (1:4, v/v) to give compounds 3a-d. All these novel compounds were characterized from their spectral data.

Spectroscopic Data

5-(Dec-9'-enoic)-2-phenylamine-1,3,4-oxadiazole (3a)

White powder; Yield 92%; Mp 133-134 °C.

IR (KBr): 3228, 1504, 1258 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 9.07 (s, 1H, NH), 7.27 (d, 2H, J= 7.6 Hz, Ar-H-2"/6"), 7.18 (t, 1H, J= 7.5 Hz, Ar-H-4"), 6.98 (t, 2H, J= 7.3 Hz, Ar-H-3"/5"), 5.79 (tdd, 1H, J₈₋₋ₓ= 6.6 Hz, J₈₋₋ₓ=10.1 Hz, J₈₋₋ₓ=16.9 Hz, CH₂=CH-), 5.00 (dd, 1H, J₈₋₋ₓ=10.1 Hz, J₈₋₋ₓ= 2.2 Hz, H₂C=CH), 4.94 (dd, 1H, J₈₋₋ₓ=16.9 Hz, J₈₋₋ₓ= 2.2 Hz, H₂C=CH), 2.24 (t, 2H, J = 7.5 Hz, CH₂ α to ring), 2.00 (m, 2H, CH₂=CH-CH₂), 1.58 (m, 2H, CH₂ β to ring), 1.21 (br, s, 10H, (CH₂)₅).

¹³C NMR (100 MHz, CDCl₃): δ 172.9, 155.6, 139.4, 139.0, 128.7, 122.2, 118.5, 114.2, 40.6, 40.2, 39.8, 39.4, 33.9, 29.2, 28.9, 25.4.

MS (ESI): m/z = 322.3 [M+Na]⁺, calculated = 322.4.
2,5-Disubstituted-1,3,4-oxadiazoles

5-[(8'Z)-Heptadecenoic]-2-phenylamine-1,3,4-oxadiazole (3b)

White crystals; Yield 90%; Mp 144-146 °C.

IR (KBr): 3218, 1505, 1242 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 9.39 (s, 1H, NH), 7.26 (d, 2H, J = 7.7 Hz, Ar-H-2''/6''), 7.17 (t, 1H, J = 7.5 Hz, Ar-H-4''), 6.98 (t, 2H, J = 7.3 Hz, Ar-H-3''/5''), 5.33 (m, 2H, CH₂-CH=CH-CH₂), 2.23 (t, 2H, J = 7.5 Hz, CH₂ α to ring), 2.01 (m, 4H, CH₂-CH=CH-CH₂), 1.56 (m, 2H, CH₂ β to ring), 1.29 (br, s, 20H, (CH₂)₁₀), 0.87 (dist. t, 3H, terminus CH₃).

¹³C NMR (100 MHz, CDCl₃): δ 172.9, 152.2, 133.5, 130.4, 129.3, 125.6, 123.2, 114.2, 40.6, 40.2, 39.8, 39.4, 38.6, 38.4, 33.9, 31.8, 31.4, 29.2, 28.4, 28.2, 27.9, 22.6, 14.0.

MS (ESI): m/z = 420.3 [M+Na]⁺, calculated = 420.5.

(8'Z,11'R)-5-(11'-Hydroxy-octadec-8'-enoic)-2-phenylamine-1,3,4-oxadiazole (3c)

Off-white powder; Yield 87%; Mp 133-136 °C.

IR (KBr): 3358, 3224, 1518, 1220 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 9.31 (s, 1H, NH), 7.62 (d, 2H, J = 7.2 Hz, Ar-H-2''/6''), 7.53 (t, 1H, J = 7.0 Hz, Ar-H-4''), 7.41 (t, 2H, J = 7.2 Hz, Ar-H-3''/5''), 5.37 (m, 2H, CH₂-CH=CH-CH₂), 4.69 (m, 1H, CH-OH), 2.38 (t, 2H, J = 7.5 Hz, CH₂ α to ring), 1.98 (m, 4H, CH₂-CH=CH-CH₂), 1.78 (m, 1H, CH-OH), 1.67 (m, 2H, CH₂ β to ring), 1.28 (br, s, 18H, (CH₂)₉), 0.88 (dist. t, 3H, terminus CH₃).

¹³C NMR (100 MHz, CDCl₃): δ 172.1, 163.2, 138.7 “one signal hidden”, 132.2, 128.1, 128.0, 127.0, 70.7, 40.1, 39.9, 39.7, 39.5, 39.3, 31.3, 30.4, 29.1, 29.0, 28.8, 28.6, 25.1,
2,5-Disubstituted-1,3,4-oxadiazoles

22.0, 13.6, 167.2, 139.8, 139.0, 133.5, 130.0, 129.8, 127.9, 77.1, 38.6, 38.4, 33.5, 31.7, 30.2, 29.9, 29.6, 29.3, 28.7, 28.5, 28.4, 28.3, 22.4, 14.4.

**MS (ESI):** \( m/z = 436.5 \ [M+Na]^+ \), calculated = 436.59.

*(8'R,11'Z)-5-(8'-Hydroxy-octadec-11'-enoic)-2-phenylamine-1,3,4-oxadiazole (3d)*

Off-white powder; Yield 87%; Mp 135-137 °C.

**IR** (KBr): 3353, 3217, 1494, 1225 cm\(^{-1}\).

**\(^1\)H NMR** (400 MHz, CDCl\(_3\)): \( \delta \) 9.88 (s, 1H, NH), 8.20 (d, 2H, \( J = 7.3 \) Hz, Ar-H-2"/6"), 7.63 (t, 1H, \( J = 7.4 \) Hz, Ar-H-4"), 7.54 (t, 2H, \( J = 7.9 \) Hz, Ar-H-3"/5"), 5.34 (m, 2H, CH\(_2\)-CH=CH-CH\(_2\)), 4.87 (m, 1H, CH-OH), 2.50 (t, 2H, \( J = 7.5 \) Hz, CH\(_2\) a to ring), 1.98 (m, 4H, CH\(_2\)-CH=CH-CH\(_2\)), 1.77 (m, 1H, CH-OH), 1.67 (m, 2H, CH\(_2\) b to ring), 1.28 (br, s, 18H, (CH\(_2\))\(_9\)), 0.89 (dist. t, 3H, terminus CH\(_3\)).

**\(^13\)C NMR** (100 MHz, CDCl\(_3\)): \( \delta \) 172.1, 165.7, 139.1, 139.0, 133.0, 130.7, 129.4, 127.6, 77.3, 38.6, 36.4, 30.4, 31.8, 29.8, 29.6, 29.4, 29.2, 29.0, 28.9, 28.6, 25.9, 22.2, 14.3.

**MS (ESI):** \( m/z = 436.4 \ [M+Na]^+ \), calculated = 436.59.

**Antibacterial studies**

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method\(^{69,70}\). A standard inoculums (1-2 \( \times 10^7 \) c.f.u./ml 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the
inoculums. The discs measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Chloramphenicol (30 µg) was used as positive control and the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37 °C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5 X10^5 c.f.u./ml of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC.

To obtain the minimum bacterial concentration (MBC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 18-24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The PBE (percentual bacteriostatic efficiency, %) was obtained as

\[ PBE = \frac{100}{MIC} \]
Antifungal studies

Antifungal activity was also done by disk diffusion method\textsuperscript{71,72}. For assaying antifungal activity *Candida albicans*, *Aspergillus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO strains were used. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Twenty millilitres of agar media was poured into each petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3-4 days. The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with the controls.

The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately $1.6 \times 10^4 - 6 \times 10^4$ c.f.u./ml. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC).

To obtain the minimum fungicidal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 48 h of incubation at 35 °C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed.
2,5-Disubstituted-1,3,4-oxadiazoles

The ratio MFC/MIC was calculated in order to determine if the compound had a fungistatic (MFC/MIC ≥ 4) or fungicidal (MFC/MIC ≤ 4) activity.
1.5. References


2,5-Disubstituted-1,3,4-oxadiazoles


46. A. Ramazani, A. Souldozi, Phosphorus, Sulfur and Silicon, 2009, 184, 2344.
47. A. Ramazani, A. Souldozi, Phosphorus, Sulfur and Silicon, 2009, 184, 3191.
2,5-Disubstituted-1,3,4-oxadiazoles


CHAPTER-2

Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles
2.1. Theoretical

Microwave assisted synthesis in organic chemistry is an important and a well established area of research due to a number of advantages over conventional heating methods\(^1\). The microwave assisted reactions occur more rapidly, safely and with higher chemical yields rendering this method superior to conventional methods which require longer reaction periods, tedious work up and use of large quantity of solvents and reagents causing environmental pollution. On the other hand, microwave induced organic reaction enhancement (MORE) technique provides a non-conventional technique for the rapid synthesis of organic compounds.

Nitrogen heterocycles of different ring sizes, with different substitution patterns and embedded in various molecular frameworks constitute extremely important structure classes in the search for bioactivity. Many compounds bearing five-membered heterocyclic rings in their structure have an extensive spectrum of pharmacological activities. Among them 1,3,4-oxadiazoles and their derivatives have attracted considerable interest in material and medicinal chemistry as surrogates of carboxylic acids, esters and carboxamides\(^2\). The 1,3,4-oxadiazole compounds have shown a wide array of biological activities in both agrochemical and pharmaceutical fields showing anti-convulsant\(^3\), anti-microbial\(^4\), insecticidal\(^5\), fungicidal\(^6\), anti-inflammatory\(^7\), anti-leishmanial\(^8\), hypotension\(^9\) and anti-tumor\(^10\) characteristics. Some of the members belonging to 1,3,4-oxadiazole class display 5-HT-receptor antagonists (i)\(^11\), muscarinic receptor agonists (ii)\(^12\), benzodiazepine receptor agonists (iii)\(^13\) and tyrosinase inhibitors\(^14\).
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

A fructose based 3-acetyl-2,3-dihydro-1,3,4-oxadiazole (GLB) (iv) and its 5-linear 5-alkyl derivatives (v) have shown some cytotoxic activities.

The most general method involves the cyclization of diacylhydrazides with a variety of reagents such as thionyl chloride, phosphorus oxychloride and sulfuric acid, usually under harsh reaction conditions. Further, most of these protocols are multi-step in nature and involve long reaction times. Only a few reliable and operationally facile
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

examples have been reported for the one step synthesis of 1,3,4-oxadiazoles, especially from readily available carboxylic acids and acid hydrazides\textsuperscript{16,17}.

Thus, in an attempt to overcome these disadvantages of classical thermal reactions the microwave technique for the synthesis of 1,3,4-oxadiazoles has rapidly gained acceptance.

The organomercurials, 2-(aryl mercurithio)-5-[4'-methylquinolinyl-2-oxy methyl]-1,3,4-oxadiazoles (viii) were synthesized by reacting 2-mercapto-5-[4'-methylquinolinyl-2-oxy methyl]-1,3,4-oxadiazole (vi) in DMF, anhydrous K\textsubscript{2}CO\textsubscript{3} and aryl mercuric chloride (vii) under microwave irradiation\textsuperscript{18} (Scheme 2.1.1).

\begin{equation}
\begin{array}{c}
\text{vi} \\
\text{vii} \\
\text{viii}
\end{array}
\end{equation}

\[ R = \text{H, 4-CH}_3, \text{4-Cl, 4-Br, 4-OCH}_3 \]

\textbf{Scheme 2.1.1}

A novel procedure for the synthesis of 1,3,4-oxadiazoles (x) from 1,2-diacylhydrazines (ix) using polymer-supported burgess reagent under microwave conditions is described by Brain \textit{et al.}\textsuperscript{19} (Scheme 2.1.2).
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\[
\begin{align*}
\text{R} & = \text{Ph, 2-Methoxyphenyl, 2-Chlorophenyl, 2-Nitrophenyl, 2-Thiophenyl, 2-Furyl, 3-Pyridyl, PhSO}_2\text{CH}_2. \\
\text{R}_1 & = \text{Ph, Me, NHPh.}
\end{align*}
\]

Scheme 2.1.2

Wang et al.\textsuperscript{20} synthesized the 2-(4-chlorobenzoylamido)-5-aryloxymethyl-1,3,4-oxadiazoles (xii) by the cyclization of 1-aryloxyacetyl-4-(4-chlorobenzoyl)-thiosemicarbazides (xi) in the presence of mercuric acetate under the condition of microwave irradiation (Scheme 2.1.3).

\[
\begin{align*}
\text{Ar} & = \text{C}_6\text{H}_5, 2-\text{CH}_3\text{-C}_6\text{H}_4, 3-\text{CH}_3\text{-C}_6\text{H}_4, 4-\text{CH}_3\text{-C}_6\text{H}_4, 1-\text{Naphthyl, 4-Cl-C}_6\text{H}_4}
\end{align*}
\]

Scheme 2.1.3

A series of 2,5-diaryl-1,3,4-oxadiazoles (xvi) have been synthesized by reacting a mixture of corresponding aromatic acid (xiii), hydrazine dihydrochloride (xiv) and phosphorus pentoxide (xv) in orthophosphoric acid under microwave conditions\textsuperscript{21} (Scheme 2.1.4).
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

\[
\begin{align*}
\text{Ar} \cdot \text{Y} + \text{NH}_2\text{-NH}_2\cdot2\text{HCl} + \text{P}_2\text{O}_5 & \xrightarrow{\text{H}_3\text{PO}_4} \xrightarrow{\text{MW}} \text{Ar}^\circ\text{v}^\circ\text{Ar} \\
x_\text{iii} & x_\text{iv} & x_\text{v} & x_\text{vi}
\end{align*}
\]

\(\text{Ar}=\text{C}_6\text{H}_5, \text{3-OC}_6\text{H}_3\text{-C}_6\text{H}_4, \text{3-Pyridyl, 2-HO-C}_6\text{H}_4, \text{4-Cl-C}_6\text{H}_4.\)

Scheme 2.1.4

2-(4-Methoxylphenyloxyacetylamido)-5-aryloxymethyl-1,3,4-oxadiazoles (xix) were synthesized by the cyclization of 1-aryloxyaceteyl-4-(4-methoxylphenyloxyacetyl)-thiosemicarbazides (xviii) in presence of mercuric acetate under microwave irradiation\(^2^2\) (Scheme 2.1.5).

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Scheme 2.1.5
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

The microwave dielectric heating of potassium salt of 2-acyldithiocarbazinic acids (xx) gave the 5-substituted-2-mercapto-1,3,4-oxadiazoles (xxi) in good yields\(^{23}\) (Scheme 2.1.6).

\[
\begin{align*}
\text{xx} & \quad \text{MW} \quad \text{xxi} \\
R= \text{Ph, 4-Cl-C}_6\text{H}_4, 4-\text{CH}_3\text{C}_6\text{H}_4, 4-\text{Pyridyl, 4-OCH}_3\text{C}_6\text{H}_4, \text{C}_6\text{H}_5\text{CH}_2, 4-\text{OH-C}_6\text{H}_4
\end{align*}
\]

Scheme 2.1.6

The 2,5-disubstituted-1,3,4-oxadiazoles (xxiv) were obtained by Mashraqui \textit{et al.}\(^{24}\) by condensing monoaryl hydrazides (xxii) with acid chlorides (xxiii) in HMPA solvent under microwave heating (Scheme 2.1.7).

\[
\begin{align*}
\text{xxii} & \quad \text{HMPA, 1h, rt} \quad \text{xxiv} \\
\text{xxiii} & \quad \text{MW, 40 sec} \\
R= \text{Ph, 4-NO}_2\text{C}_6\text{H}_4, 4-\text{MeOC}_6\text{H}_4, 4-\text{Pyridyl.} \\
R_1= \text{Ph, 2-Furyl, CH}_2\text{Ph, CH}_3, 2-\text{Propyl, 2-Thienyl, 4-MeOPh.}
\end{align*}
\]

Scheme 2.1.7

2,5-Disubstituted-1,3,4-oxadiazoles (xxviii) were prepared by the oxidation of 1-aroyl-2-arylidine hydrazines (xxvii) with potassium permanganate on the surface of silica gel as well as in mixtures of acetone and water under microwave irradiation\(^{25}\) (Scheme 2.1.8).
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

\[
\begin{align*}
\text{R} & = \text{Ph, Me, 4-Cl-C}_6\text{H}_4 \\
\text{R}_1 & = \text{Ph, 4-NO}_2\text{C}_6\text{H}_4, 4-\text{Cl-C}_6\text{H}_4, 4-\text{Me-C}_6\text{H}_4, 4-\text{MeO-C}_6\text{H}_4, 4-\text{MeOOC-C}_6\text{H}_4, 4-(\text{Me})_2\text{N-C}_6\text{H}_4, \text{Me, CH}_3\text{CH}=\text{CH, Ph.}
\end{align*}
\]

Scheme 2.1.8

Khan et al. \textsuperscript{26} synthesized the 2,5-disubstituted-1,3,4-oxadiazoles \( \text{xxx i} \) from 3-pyridyl hydrazide \( \text{xxix} \) and benzoic acid \( \text{xxx} \) by microwave irradiation taking alumina as the solid support and phosphorus oxychloride as a dehydrating agent (Scheme 2.1.9).

\[
\begin{align*}
\text{xxix} & + \text{xxx} \xrightarrow{\text{POCl}_3, \text{MW}} \text{xxx i} \\
\text{R} & = 3-\text{Pyridyl} \\
\text{R}_1 & = \text{Phenyl}
\end{align*}
\]

Scheme 2.1.9

The reaction of isonicotinic acid hydrazide \( \text{xxx ii} \) and corresponding benzaldehyde \( \text{xxx iii} \) under microwave conditions gave the heterocyclyl acylhydrazones \( \text{xxx iv} \). The oxidation of \( \text{xxx iv} \) with iodobenzene diacetate (IBD) gave the heterocyclyl-1,3,4-oxadiazoles \( \text{xxx v} \) in a solid state\textsuperscript{27} (Scheme 2.1.10).
Microwave assisted 2,5-disubstituted 1,3,4-oxadiazoles

Scheme 2.1.10

Li et al.\textsuperscript{28} gave the solvent free synthesis of 2-aryl-5-(coumarin-3'-yl)-1,3,4-oxadiazoles (xxxviii) in high yields by reacting the coumarin-3-carboxylic acid (xxxvi) with unsubstituted benzoic acid hydrazides (xxxvii) in presence of PEG supported dichlorophosphate under microwave irradiation (Scheme 2.1.11).

Scheme 2.1.11

Natero et al.\textsuperscript{29} gave the one-step synthesis of 5-phenyl-2-chloromethyl-1,3,4-oxadiazoles (xLi) from commercially available acylhydrazides (xL) using 1-chloro-2,2,2-trimethoxyethane (xxxix) as a solvent under microwave conditions (Scheme 2.1.12).
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

Scheme 2.1.12

A library of 2,5-disubstituted-1,3,4-oxadiazoles (xLiv) have been synthesized under microwave irradiation and screened for their tyrosinase inhibition activities\(^{30}\) (Scheme 2.1.13).

Scheme 2.1.13

A single pot synthetic protocol for the synthesis of 2-sulfonamide-1,3,4-oxadiazoles (xLvii) from 1,2-diacylhydrazine (xLv) under microwave irradiation using PS-BEMP and corresponding sulfonyl chloride (xLvi) is reported by Baxendale et al.\(^ {31}\) (Scheme 2.1.14).
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

Scheme 2.1.14

Wang et al.\textsuperscript{32} gave the single step, rapid and efficient synthesis of 1,3,4-oxadiazoles (L) from carboxylic acids (xLviii) and acid hydrazides (xLix) by using commercially available PS-PPh\textsubscript{3} resin combined with microwave heating (Scheme 2.1.15).

\[
\begin{align*}
\text{R} & = \text{phenyl, tolyl, \text{NMe}_2, \text{NMe}_2, \text{MeO, F, Cl}} \nonumber \\
\text{R}_1 & = \text{phenyl, tolyl, \text{NMe}_2, \text{NMe}_2, \text{MeO, F, Cl}} \nonumber 
\end{align*}
\]

Scheme 2.1.15

The reaction of 9-ethylcarbazol-3-carbaldehyde (Li) with aroylhydrazines (Lii) under microwave condition gave the intermediate, 1-aryloxy-2-(9'-ethylcarbazol-3'-yl)methylidene) hydrazines (Liii). The further treatment of Liii with potassium
permanganate in DMF under microwave irradiation afforded the 2-aryl-5-(9'-ethylcarbazol-3'-yl)-1,3,4-oxadiazoles (Liv) in excellent yields\textsuperscript{33} (Scheme 2.1.16).

\[
\text{R= H, 4-Cl, 3-NO}_2, 3-\text{CH}_3, 2-\text{CH}_3, 4-\text{OH, 4-Br.}
\]

\textbf{Scheme 2.1.16}

5-Substituted-2-(2-methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazoles (Lvi) have been prepared under microwave irradiation using 2-methyl-4-nitro-1-imidazol-acethydrazide (Lvi), aromatic acid (Lv) and phosphorus oxy chloride as the dehydrating agent\textsuperscript{34} (Scheme 2.1.17).

\[
\text{R= C}_6\text{H}_5, 4-\text{CH}_3\text{C}_6\text{H}_4, 4-\text{OCH}_3\text{C}_6\text{H}_4, 4-\text{ClC}_6\text{H}_4, 4-\text{CH}_3\text{C}_6\text{H}_4, \text{C}_6\text{H}_4\text{N.}
\]

\textbf{Scheme 2.1.17}
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

The synthesis of 5-aryl-2-(2-hydroxyphenyl)-1,3,4-oxadiazoles (L₅x) by the reaction of salicylic hydrazide (Lviii) with carboxylic acids (Lix) in the presence of thionyl chloride under neat conditions is described\(^{35}\) (Scheme 2.1.18).

\[
\text{Lviii} + \text{Lix} \xrightarrow{\text{SOCl}_2, \text{MW}} \text{Lx}
\]

\(R = \text{H, 4-Me, 4-MeO, 3,4-(MeO)₂, 3,4,5-(MeO)₃, 3-Cl, 2-Br, 3-MeOC₆H₄CH₂.}\)

**Scheme 2.1.18**

Polshettiwar *et al.*\(^{36}\) gave a novel, one-pot, solvent free, green protocol for the synthesis of 1,3,4-oxadiazoles (Lxiii) by the condensation of acid hydrazide (Lxi) and triethyl orthoalkanates (Lxii) using solid supported Nafion\(^8\)NR50 and phosphorus pentasulfide in alumina as a catalyst (Scheme 2.1.19).

\[
\text{Lxi} + \text{Lxii} \xrightarrow{\text{Nafion}^8\text{NR50, MW}} \text{Lxiii}
\]

\(R = \text{H, F, OMe, 2-Furyl, 2-Thienyl, 4-Pyridyl.}\)

\(R_1 = \text{H, Et, Ph.}\)

**Scheme 2.1.19**

An efficient one pot synthesis of unsymmetric 2,5-disubstituted-1,3,4-oxadiazoles has been developed by Pore *et al.*\(^{37}\). The target oxadiazoles (Lxv) were formed by the
oxidation of acylhyrazones (Lxiv) using trichloroisocyanuric acid (TCCA) at an ambient temperature (Scheme 2.1.20).

\[
\text{R—N\textbf{—}NH} \xrightarrow{\text{TCCA, EtOH, rt}} \text{R—N\textbf{—}N}
\]

**Lxiv**

\[
\text{R= Ph, 4-ClC}_6\text{H}_4, 4-\text{OCH}_3\text{C}_6\text{H}_4. \]

\[
\text{R}_1= \text{Ph, 4-}\text{OCH}_3\text{C}_6\text{H}_4, 4-\text{CH}_3\text{C}_6\text{H}_4, 3,4-(\text{OCH}_3)\text{C}_6\text{H}_3.
\]

**Scheme 2.1.20**

The microwave assisted synthesis of new lanthanum (III) and praseodymium (III) complexes with oxadiazole functionalized dithiocarbazinates (Lxviii) is described (Scheme 2.1.21).

\[
\text{MCl}_3 + 3\text{LK} \xrightarrow{\text{Ethanol, MW}} [\text{M(L)}_3]
\]

**Lxvi**

**Lxvii**

**Lxviii**

\[
\text{M= La, Pr}
\]

\[
\text{L= N-(5-phenyl-1,3,4-oxadiazole-2-yl)dithiocarbinate (PODC), N-(5-o-chlorophenyl-1,3,4-oxadiazole-2-yl)dithiocarbinate (OCODC), N-(5-p-chlorophenyl-1,3,4-oxadiazole-2-yl)dithiocarbinate (PCODC), N-(5-o-methylphenyl-1,3,4-oxadiazole-2-yl)dithiocarbinate (MODC), N-(5-p-nitrophenyl-1,3,4-oxadiazole-2-yl)dithiocarbinate (NODC).}
\]

**Scheme 2.1.21**

Han *et al.* gave the microwave assisted synthesis of novel alkyl substituted fructose-based oxadiazoles and investigated them for their cytotoxic activity towards cancer cells. The reaction of \(E\)-3-alkylhydrazono-1,2:4,5-di-O-isopropylidene-\(\beta\)-D-erythro-2-hexulopyranose (Lxix) and \(Z\)-3-alkylhydrazono-1,2:4,5-di-O-isopropylidene-\(\beta\)-
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

D-erythro-2-hexulopyranose (Lxx) with acetic anhydride under microwave heating conditions gave (2R,3R,S,6'S,7'R)-3-alkyl-2',2',2"',2"'-tetermethyl-5-methyl-2,3-dihydro-1,3,4-oxadiazole-2-spiro-7'-(1',3'-dioxalano[4,5-c]pyrano)-6'-spiro-4"-(1",3"'-diaoxolane) (Lxxi) and (2S,3a'R,6'S,7a'R)-3-alkyl-2',2',2"',2"'-tetermethyl-5-methyl-2,3-dihydro-1,3,4-oxadiazole-2-spiro-7'-(1',3'-dioxalano[4,5-c]pyrano)-6'-spiro-4"-(1",3"'-diaoxolane) (Lxxii) in good yields (Scheme 2.1.22).

\[ \text{Lxxix} + \text{Lxx} \xrightarrow{30 \text{ min. Ac}_2\text{O}, \text{MW}} \text{Lxxi} + \text{Lxxii} \]

\( R = \text{CH}_3, \text{C}_2\text{H}_5, \text{n-C}_3\text{H}_7, \text{n-C}_4\text{H}_9, \text{n-C}_5\text{H}_{11}, \text{n-C}_6\text{H}_{13}, \text{n-C}_7\text{H}_{15}, \text{n-C}_8\text{H}_{17}, \text{n-C}_9\text{H}_{19}, \text{n-C}_{11}\text{H}_{19}, \text{n-C}_{13}\text{H}_{23}, \text{n-C}_{15}\text{H}_{31}, \text{n-C}_{17}\text{H}_{35} \)

**Scheme 2.1.22**

A novel approach to synthesize the glucose-based 3-acetyl-5-alkyl-2,3-dihydro-1,3,4-oxadiazoles with the assistance of microwave irradiation was developed by Wang et al. The reaction of a mixture of E/Z hydrazones (Lxxiii, Lxxiv) with acetic anhydride...
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

under microwave irradiation above 160°C, to produce the target 1,3,4-oxadiazoles (Lxxv, Lxxvi), which are a pair of isomers on the C-3 of furan ring (Scheme 2.1.23).

The reaction of 5-aryl (hetaryl)tetrazoles (Lxxvii) with phenyl isocyanate (Lxxviii) under the conditions of microwave activation formed the corresponding 2-anilino-5-aryl(hetaryl)-1,3,4-oxadiazoles (Lxxix) in high yields (Scheme 2.1.24).

\[ \text{R} = \text{CH}_3, \text{n-C}_3\text{H}_7, \text{n-C}_7\text{H}_{15}, \text{C}_6\text{H}_5, \text{C}_6\text{H}_4\text{Br}, \text{C}_6\text{H}_4\text{OMe}. \]

Scheme 2.1.23

The reaction of 5-aryl (hetaryl)tetrazoles (Lxxvii) with phenyl isocyanate (Lxxviii) under the conditions of microwave activation formed the corresponding 2-anilino-5-aryl(hetaryl)-1,3,4-oxadiazoles (Lxxix) in high yields (Scheme 2.1.24).
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

\[
\begin{align*}
\text{Lxxvii} & \quad \text{Lxxviii} & \quad \text{Lxxix} \\
\text{R} &= 4\text{-Me}_2\text{NC}_6\text{H}_4, 4\text{-MeOC}_6\text{H}_4, 4\text{-MeC}_6\text{H}_4, \text{Ph}, 4\text{-ClC}_6\text{H}_4, 4\text{-NO}_2\text{C}_6\text{H}_4, 2\text{-Pyridyl, 3-Pyridyl, 4-Pyridyl, 2-Furyl.}
\end{align*}
\]

**Scheme 2.1.24**

Xu *et al.* reported the microwave assisted synthesis and antifungal activity of 2,5-disubstituted-1,3,4-oxadiazoles containing azulene moiety. The 5-aryl-2-(3-methylazulen-1-yl)-1,3,4-oxadiazoles (Lxxxii) were obtained by the microwave irradiation of corresponding hydrazide (Lxxxi) with appropriate carboxylic acids in presence of phosphorus oxy chloride (**Scheme 2.1.25**).

\[
\begin{align*}
\text{Lxxx} & \quad \text{Lxxxi} & \quad \text{Lxxxii} \\
\text{Ar} &= \text{C}_6\text{H}_5, 4\text{-MeC}_6\text{H}_4, 4\text{-ClC}_6\text{H}_4, 4\text{-OHCC}_6\text{H}_4, 2\text{-BrC}_6\text{H}_4, 2\text{-OHCC}_6\text{H}_4, \text{C}_6\text{H}_5\text{CH}=&\text{CH}, 4\text{-MeC}_6\text{H}_5\text{CH}=&\text{CH}.
\end{align*}
\]

**Scheme 2.1.25**
2.2. A Facile One-pot Synthesis of Novel 2,5-Disubstituted-1,3,4-oxadiazoles under Conventional and Microwave Conditions and Evaluation of their \textit{in vitro} Antimicrobial Activities

1,3,4-Oxadiazole is apparently among the most significant heterocycle cores. The 1,3,4-oxadiazole derivatives may act as ester and amide bioisosteres and hence are of interest in pharmaceutical and agrochemical fields\textsuperscript{43}. The wide range of biological activities associated with 1,3,4-oxadiazoles include anti-viral\textsuperscript{44}, antimicrobial\textsuperscript{4}, antineoplastic\textsuperscript{45}, fungicidal\textsuperscript{6}, inhibition of tyrosinase\textsuperscript{30} and cathepsin K\textsuperscript{46}. Also, much attention has been focused on the oxadiazole core \Pi-systems as electron-transporting and hole-blocking materials in the area of organic light-emitting diodes (OLEDs)\textsuperscript{47}. Further 1,3,4-oxadiazole heterocycles can contribute substantially in increasing the pharmacological activity by participating in hydrogen bonding interactions with the receptors\textsuperscript{48}.

Several methods have been reported in literature for the synthesis of 1,3,4-oxadiazoles\textsuperscript{49,50}. Most of these protocols are multi-step in nature and generally involve the cyclization of acid hydrazides with a variety of reagents, such as, phosphorus oxychloride, sulfuric acid and thionyl chloride, usually under harsh conditions. Recently a few efficient examples have been reported for the synthesis of 1,3,4-oxadiazoles, especially from readily available carboxylic acids and acid hydrazides. However, these protocols use expensive catalysts and require longer times for the completion of the reaction\textsuperscript{51,52}. Consequently, the development of effective methods for the rapid and concise synthesis and modification of oxadiazole motif need to be developed.
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

The application of microwave irradiation in organic synthesis for conducting reactions at highly accelerated rates is an emerging technique\(^{53}\). In fact, in recent years, the use of microwaves have become popular among synthetic organic chemists both to improve classical organic reactions (shortening reaction times and/or improving yields) as well as to promote new reactions.

The substantial reduction in the reaction times under microwave irradiation and the pharmacological importance of 1,3,4-oxadiazole ring systems prompted us to synthesize the 2,5-disubstituted-1,3,4-oxadiazoles bearing an alkanyl/alkenyl/hydroxyalkenyl chain substituent at 5\(^{\text{th}}\) position and evaluate them for their antimicrobial activity. To the best of our awareness this contribution reports for the first time the simple and straightforward synthesis of 1,3,4-oxadiazoles under microwave condition having a long chain substituent at C-5.
2.3. Results and Discussion

Due to the beneficial pharmacological properties of certain molecules containing 1,3,4-oxadiazole moiety the synthesis of new 1,3,4-oxadiazole derivatives using procedures in which some aspect of green chemistry could be met is desirable. Our main strategy in this work was to synthesize new 1,3,4-oxadiazole derivatives with potential biological activities, using microwave-induced organic reaction enhancement methodology, which is extremely fast, cleaner than conventional reactions and lead to a higher atom economy (less chemical waste).

The conventional synthesis of the target 2,5-disubstituted-1,3,4-oxadiazoles (3a-l) was achieved by refluxing a mixture of fatty acid hydrazide (1a-f) and appropriate carboxylic acid (2a-b) in presence of phosphorus oxy chloride (Scheme 2.3.1). The product yields and the reaction times required for the completion of the reaction under reflux conditions are given in Table 2.3.1. As, can be seen from Table 2.3.1, the reaction time ranged within 8-14 h.

\[
\text{Scheme 2.3.1. Conventional synthesis of 2,5-disubstituted-1,3,4-oxadiazoles 3a-l.}
\]
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

Table 2.3.1. 2,5-Disubstituted-1,3,4-oxadiazoles 3a-l.

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>R₁</th>
<th>Product</th>
<th>Time (h.)</th>
<th>Yield (%)</th>
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<tr>
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<td>HE</td>
<td>bH</td>
<td>3a</td>
<td>10</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>5</td>
<td>3b</td>
<td>11</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>OH</td>
<td>3c</td>
<td>14</td>
<td>78</td>
</tr>
<tr>
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<td>6</td>
<td>OH</td>
<td>3d</td>
<td>14</td>
<td>79</td>
</tr>
<tr>
<td>5</td>
<td>H₂C</td>
<td>11</td>
<td>3e</td>
<td>8</td>
<td>86</td>
</tr>
<tr>
<td>6</td>
<td>H₂C</td>
<td>11</td>
<td>3f</td>
<td>8</td>
<td>85</td>
</tr>
<tr>
<td>7</td>
<td>H₂C</td>
<td>11</td>
<td>3g</td>
<td>11</td>
<td>80</td>
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<td>8</td>
<td>6</td>
<td>5</td>
<td>3h</td>
<td>12</td>
<td>82</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>OH</td>
<td>3i</td>
<td>13</td>
<td>75</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>CH₂</td>
<td>3j</td>
<td>14</td>
<td>78</td>
</tr>
<tr>
<td>11</td>
<td>H₂C</td>
<td>11</td>
<td>3k</td>
<td>9</td>
<td>85</td>
</tr>
<tr>
<td>12</td>
<td>H₂C</td>
<td>11</td>
<td>3l</td>
<td>9</td>
<td>83</td>
</tr>
</tbody>
</table>
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

Further to explore the probability of getting the pharmacophoric important moiety in higher yields and in shorter reaction times, our attention turned towards employing the microwave irradiation. The application of microwave energy to organic compounds for conducting synthetic reactions at highly accelerated rates is an emerging technique. In fact, in recent years, microwave has become popular among synthetic organic chemists both to improve classical organic reactions, shortening reaction times and/or improving yields, as well as to promote new reactions. Under microwave conditions, the reactions were carried out by irradiating a mixture of fatty acid hydrazide (1a-f) with the appropriate carboxylic acid (2a-b), supported on neutral alumina, using phosphorus oxychloride as the cyclizing agent (Scheme 2.3.2).

\[
\begin{align*}
\text{1a-f} + \text{2a-b} & \xrightarrow{\text{POCl}_3, \text{Al}_2\text{O}_3, \text{MW}} \text{3a-l}
\end{align*}
\]

Scheme 2.3.2. Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (3a-l) under microwave irradiation.

At first, the condensation reaction of hydrazide of undec-10-enoic acid (1a) with 2-furoic acid (2a) was chosen as a model to optimize the conditions for the preparation of compounds 3a-l (Table 2.3.2). In order to determine the optimum conditions for the synthesis of oxadiazoles, variations in molar ratios of reagents, the microwave irradiation time and the power level of microwave setup were investigated.
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

Table 2.3.2. Optimization of the microwave-assisted condensation of 1a and 2a on neutral alumina

<table>
<thead>
<tr>
<th>Entry</th>
<th>Support</th>
<th>Molar Ratio(1a/2a)</th>
<th>Time (Min.)</th>
<th>Power (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neutral alumina</td>
<td>0.2/0.5</td>
<td>5</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>Neutral alumina</td>
<td>0.5/0.8</td>
<td>7</td>
<td>40</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>Neutral alumina</td>
<td>0.8/1.0</td>
<td>12</td>
<td>50</td>
<td>71</td>
</tr>
<tr>
<td>4</td>
<td>Neutral alumina</td>
<td>1.0/1.0</td>
<td>16</td>
<td>60</td>
<td>90</td>
</tr>
</tbody>
</table>

After some experimentation, we found a set of conditions that generally provided the products in good yields. The optimum conditions were set up and the target 2,5-disubstituted-1,3,4-oxadiazoles (3a-l) were synthesized in appreciable yields (Table 2.3.3).

As per our requirement, the strategy was successfully worked out. All the reactions proceeded in shorter reaction time and an appreciable increment in the product yield was observed as compared to conventional reaction conditions. The generality and ability of this methodology in the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles was demonstrated by using a variety of structurally divergent fatty acid hydrazides and carboxylic acids. The scope of the reaction using saturated, olefin (internal and terminal) and hydroxy fatty acid hydrazides was found to be good. Moreover, the nature of hydrazide and the carboxylic acid did not show strongly obvious effects in terms of yields. The synthesized compounds were characterized by IR, $^1$H NMR, $^{13}$C NMR and mass spectral analysis.
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

Table 2.3.3. 2,5-Disubstituted-1,3,4-oxadiazoles (3a-I) under microwave conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>1</th>
<th>2</th>
<th>1/2</th>
<th>Power%(b)</th>
<th>Time(h.)</th>
<th>Product</th>
<th>Yield%(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>2a</td>
<td>1/1</td>
<td>60</td>
<td>16</td>
<td>3a</td>
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<tr>
<td>2</td>
<td>1b</td>
<td>2a</td>
<td>1/1</td>
<td>60</td>
<td>16</td>
<td>3b</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>2a</td>
<td>1/1</td>
<td>60</td>
<td>17</td>
<td>3c</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>2a</td>
<td>1/1</td>
<td>60</td>
<td>17</td>
<td>3d</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>2a</td>
<td>1/1</td>
<td>60</td>
<td>15</td>
<td>3e</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>1f</td>
<td>2a</td>
<td>1/1</td>
<td>60</td>
<td>15</td>
<td>3f</td>
<td>93</td>
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<td>7</td>
<td>1a</td>
<td>2b</td>
<td>1/1</td>
<td>60</td>
<td>19</td>
<td>3g</td>
<td>89</td>
</tr>
<tr>
<td>8</td>
<td>1b</td>
<td>2b</td>
<td>1/1</td>
<td>60</td>
<td>19</td>
<td>3h</td>
<td>90</td>
</tr>
<tr>
<td>9</td>
<td>1c</td>
<td>2b</td>
<td>1/1</td>
<td>60</td>
<td>20</td>
<td>3i</td>
<td>86</td>
</tr>
<tr>
<td>10</td>
<td>1d</td>
<td>2b</td>
<td>1/1</td>
<td>60</td>
<td>20</td>
<td>3j</td>
<td>88</td>
</tr>
<tr>
<td>11</td>
<td>1e</td>
<td>2b</td>
<td>1/1</td>
<td>60</td>
<td>18</td>
<td>3k</td>
<td>91</td>
</tr>
<tr>
<td>12</td>
<td>1f</td>
<td>2b</td>
<td>1/1</td>
<td>60</td>
<td>17</td>
<td>3l</td>
<td>91</td>
</tr>
</tbody>
</table>

(a) All reactions were carried out using fatty acid hydrazides (1eq) with respect to carboxylic acids and POCl₃ under microwave irradiation.
(b) Microwave equipment multimode was used.
(c) Monitored by TLC.
(d) All yields refer to isolated products and the products were characterized by IR, ¹H NMR, ¹³C NMR and mass spectral analysis.

The spectra of compound 3a showed the IR absorption bands at 1668, 1568, 1240 cm⁻¹ due to C=C, C=N and C-O-C functions. The ¹H NMR was more informative in assigning the structure. Diagnostic peaks at δ 7.64, 7.32 and 6.55 were observed for the furan ring system. A triplet at δ 2.36 was observed for the methylene protons α to the oxadiazole ring. The methine proton of C-9 showed a signal at 5.82. The C-10 methylene protons designated as Hₓ and Hᵧ displayed two distinct δ values when coupled with adjacent C-9 methine protons. Thus, the ¹H NMR showed two doublet of doublet at δ 5.01 and 4.94 for Hᵧ and Hₓ protons respectively. In the ¹³C NMR peaks at δ 172.4 and
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

168.2 were observed for the ring carbon atoms. The mass spectrum was also consistent with the assigned molecular formula.

Similarly, the spectra of compound 3g showed the IR absorption bands at 1665, 1563, 1242 cm$^{-1}$ due to C=C, C=N and C-O-C functions. A characteristic IR band at 748 cm$^{-1}$ was also observed for the aromatic ring system. The $^1$H NMR was more informative in assigning the structure. Diagnostic peaks at $\delta$ 7.98, 7.46 and 7.33 were observed for the aromatic ring system. A triplet at 2.35 was observed for the methylene protons $\alpha$ to the oxadiazole ring. The methine proton of C-9 showed a signal at 5.80. The C-10 methylene protons designated as $H_E$ and $H_Z$ displayed two distinct $\delta$ values when coupled with adjacent C-9 methine protons. Thus, the $^1$H NMR showed two doublet of doublet at $\delta$ 4.98 and 4.93 for $H_Z$ and $H_E$ protons respectively. The $^{13}$C NMR showed characteristic peaks at $\delta$ 172.9 and 168.4 for the oxadiazole ring carbon atoms. The mass spectrum was also consistent with the assigned molecular formula. Detailed spectral data of the titled compounds are given in experimental section.

**Antibacterial studies**

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), Methicillin resistant *Staphylococcus aureus* (MRSA +Ve), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method.$^{54,55}$ The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. Ciprofloxacin was used as the standard drug. The bacterial zones of inhibition values are given in Table 2.3.4.
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

Table 2.3.4. Antibacterial activity of 2,5-disubstituted-1,3,4-oxadiazoles 3a-l

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. pyogenes</td>
<td>MRSA*</td>
</tr>
<tr>
<td>3a</td>
<td>11.4±0.4</td>
<td>10.1±0.3</td>
</tr>
<tr>
<td>3b</td>
<td>21.3±0.4</td>
<td>20.8±0.3</td>
</tr>
<tr>
<td>3c</td>
<td>19.1±0.5</td>
<td>18.3±0.4</td>
</tr>
<tr>
<td>3d</td>
<td>13.4±0.4</td>
<td>13.2±0.4</td>
</tr>
<tr>
<td>3e</td>
<td>16.2±0.4</td>
<td>15.5±0.2</td>
</tr>
<tr>
<td>3f</td>
<td>22.5±0.2</td>
<td>21.6±0.4</td>
</tr>
<tr>
<td>3g</td>
<td>14.5±0.4</td>
<td>14.4±0.4</td>
</tr>
<tr>
<td>3h</td>
<td>17.1±0.2</td>
<td>16.6±0.3</td>
</tr>
<tr>
<td>3i</td>
<td>20.1±0.3</td>
<td>19.5±0.2</td>
</tr>
<tr>
<td>3j</td>
<td>20.2±0.4</td>
<td>19.7±0.3</td>
</tr>
<tr>
<td>3k</td>
<td>12.4±0.5</td>
<td>12.1±0.5</td>
</tr>
<tr>
<td>3l</td>
<td>21.4±0.3</td>
<td>20.5±0.4</td>
</tr>
<tr>
<td>Standard</td>
<td>23.0±0.2</td>
<td>22.0±0.2</td>
</tr>
</tbody>
</table>

Positive control (standard); Ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm). * Methicillin resistant Staphylococcus aureus (MRSA +Ve).

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The number of c.f.u. was counted after 18-24 h of incubation at 35 °C. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums was killed. The minimum inhibitory concentrations and minimum bactericidal concentrations are given in Table 2.3.5.
Table 2.3.5. MIC and MBC results of 2,5-disubstituted-1,3,4-oxadiazoles 3a-l.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th></th>
<th></th>
<th>Gram negative bacteria</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3a</td>
<td>3b</td>
<td>3c</td>
<td>3d</td>
<td>3e</td>
<td>3f</td>
</tr>
<tr>
<td></td>
<td>S. pyogenes</td>
<td>MRSA*</td>
<td>P. aeruginosa</td>
<td>K. pneumoniae</td>
<td>E.coli</td>
<td></td>
</tr>
<tr>
<td>MIC</td>
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<td>25</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
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<td>100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>MIC</td>
<td>50</td>
<td>50</td>
<td>12.5</td>
<td>50</td>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td>MBC</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>MBC</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>MBC</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>MIC</td>
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<td>50</td>
<td>50</td>
<td>100</td>
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<td>&gt;100</td>
</tr>
<tr>
<td>MBC</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Positive control Ciprofloxacin.

MIC (μg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely;
MBC (μg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely.

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Good inhibitory results were obtained against *S. pyogenes, S. aureus* and *E.coli* species. The structure activity data showed that variously substituted 1,3,4-oxadiazoles 3a-l have varying degree of microbial inhibition. The antibacterial activity seemed to be dependent on both the substituents. The presence of chlorobenzyl substituents at C-5 of the oxadiazole ring system in compounds 3a-l was found to enhance the bacterial inhibition effects. Further,
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

A marked enhancement in the antibacterial activity was observed for the compounds bearing a bromo or a hydroxy group in the long chain substituent at C-2 of the oxadiazole moiety. The compounds 3c, 3d, 3f, 3i, 3j and 3l showed good inhibition against *S. pyogenes*, *S. aureus* and *E. coli* species. The oxadiazoles 3f, 3j and 3l showed activity nearly equivalent to that of Ciprofloxacin. The MICs and MBCs also revealed good antibacterial activity.

**Antifungal studies**

Antifungal activity was also done by disk diffusion method. For assaying antifungal activity *Candida albicans*, *Aspergillus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by agar diffusion method\(^{56,57}\). The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 2.3.6.

The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) are given in Table 2.3.7.
### Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

#### Table 2.3.6. Antifungal activity of 2,5-disubstituted-1,3,4-oxadiazoles 3a-l.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>AF</th>
<th>TM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
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<td>3a</td>
<td>19.9±0.3</td>
<td>18.5±0.5</td>
<td>16.4±0.2</td>
<td>11.9±0.3</td>
</tr>
<tr>
<td>3b</td>
<td>26.1±0.3</td>
<td>21.1±0.2</td>
<td>18.3±0.3</td>
<td>13.7±0.2</td>
</tr>
<tr>
<td>3c</td>
<td>23.1±0.3</td>
<td>20.2±0.5</td>
<td>16.7±0.4</td>
<td>13.1±0.4</td>
</tr>
<tr>
<td>3d</td>
<td>21.1±0.3</td>
<td>18.5±0.4</td>
<td>14.7±0.5</td>
<td>13.2±0.4</td>
</tr>
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<td>3e</td>
<td>22.8±0.5</td>
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<td>17.1±0.3</td>
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<tr>
<td>3f</td>
<td>26.2±0.4</td>
<td>21.6±0.5</td>
<td>18.2±0.4</td>
<td>14.1±0.3</td>
</tr>
<tr>
<td>3g</td>
<td>22.2±0.4</td>
<td>19.7±0.3</td>
<td>15.8±0.9</td>
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</tr>
<tr>
<td>3h</td>
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<td>18.1±0.2</td>
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</tr>
<tr>
<td>3i</td>
<td>24.1±0.5</td>
<td>21.3±0.3</td>
<td>17.8±0.5</td>
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<tr>
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<tr>
<td>3k</td>
<td>21.3±0.3</td>
<td>19.6±0.4</td>
<td>16.4±0.2</td>
<td>12.9±0.3</td>
</tr>
<tr>
<td>3l</td>
<td>27.1±0.3</td>
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<td>Standard</td>
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<td>24.0±0.3</td>
<td>20.0±0.5</td>
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</table>

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>AF</th>
<th>TM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (Greseofulvin) and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

CA = *Candida albicans*, AF = *Aspergillus fumigatus*, TM = *Trichophyton mentagrophytes*, PM = *Penicillium marneffei.*
Table 2.3.7. MIC and MFC of 2,5-disubstituted-1,3,4-oxadiazoles 3a-l

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA MIC (ng/ml)</th>
<th>CA MFC (ng/ml)</th>
<th>AF MIC (ng/ml)</th>
<th>AF MFC (ng/ml)</th>
<th>TM MIC (ng/ml)</th>
<th>TM MFC (ng/ml)</th>
<th>PM MIC (ng/ml)</th>
<th>PM MFC (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3b</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>50</td>
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<td>25</td>
<td>100</td>
</tr>
<tr>
<td>3d</td>
<td>25</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>25</td>
<td>&gt;100</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>3e</td>
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<td>25</td>
<td>100</td>
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<td>100</td>
<td>25</td>
<td>100</td>
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<td>3f</td>
<td>6.25</td>
<td>12.5</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
<td>100</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>3g</td>
<td>25</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>25</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>3h</td>
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CA = Candida albicans, AF = Aspergillus fumigatus, TM = Trichophyton mentagrophytes, PM = Penicillium marneffei. MIC (ng/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (ng/ml) = minimum fungicidal concentration, i.e., the lowest concentration of the compound for killing the fungus completely.

The antifungal screening data showed moderate to good activity. The 2,5-disubstituted-1,3,4-oxadiazoles 3a-l showed same antifungal activity trends as for the bacterial strains. The good inhibition results were obtained against C. albicans, A. fumigatus and P. marneffei fungal strains. Moderate activity was obtained in case of T. mentagrophytes fungal strains. The compounds 3f, 3j and 3l were found to be the most potent antifungal agents. The MBC of few compounds was found to be the same as MIC.
but in most of the compounds it was two or three or four folds higher than the corresponding MIC results.

Thus, the nature of substituents has a strong influence on the extent of antibacterial and antifungal activities. The data analysis revealed that all the compounds have produced a marked enhancement in the potency of these analogues as antibacterial and antifungal agents.
2.4. Experimental

The sources of all fatty acids and instrumentation details are same as given in Chapter 1 (pg. 31). The microwave irradiations were carried out using a modified domestic microwave oven.

**General procedure for the synthesis of fatty acid hydrazides (1a-d)**

The hydrazides of long chain alkenoic acids (1a-d) used as the starting material were prepared by previously reported method. The synthesized hydrazides were characterized by their melting points.

**General procedure for the conventional synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (3a-l)**

Phosphorus oxy chloride (5 ml) was added to a mixture of substituted carboxylic acid (0.01 mol) and fatty acid hydrazide (0.01 mol) in abs. ethanol. The reaction mixture was refluxed for 8-14 h on a water bath. After the completion of the reaction, the contents were cooled at room temperature and poured into crushed ice. The precipitated crude product was washed with 10% solution of NaHCO$_3$ and further recrystallized from 95% ethanol (Table 2.3.1)

**General procedure for the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (3a-l) under solvent-free microwave conditions.**

A mixture of fatty acid hydrazide (0.01 mol), carboxylic acid (0.01 mol) and alumina (1.50g) were mixed and ground in a mortar and pestle until a fine homogenous powder was obtained (5 min). Phosphorus oxy chloride (5 ml) was added. The mixture was irradiated under microwave irradiation for the required time (Table 2.3.3). After the completion of the reaction (TLC analysis), ice cold water (10 ml) was added to the
reaction mixture and the precipitated crude product was filtered. The crude 1,3,4-
oxadiazole was washed with 10% solution of NaHCO₃ and further purified by column
chromatography over silica gel using petroleum ether-diethyl ether mixture as eluent.

The synthesized 2,5-disubstituted-1,3,4-oxadiazoles (3a-l) were characterized
from their spectral data (IR, ¹H NMR, ¹³C NMR and mass spectra).

Spectroscopic data

5-(Dec-9'-enoic)-2-furyl-1,3,4-oxadiazole (3a)

IR (KBr): 1668, 1568, 1240 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 7.64 (m, 1H, furan 5⁻-H), 7.32 (m, 1H, furan 2⁻-H), 6.55
(m, 1H, furan 4⁻-H), 5.82 (td, 1H, J₅⁻⁻⁻⁻= 6.6 Hz, J₅⁻⁻⁻⁻=10.2 Hz, J₅⁻⁻⁻⁻=17.0 Hz,
CH₂=CH⁻), 5.01 (dd, 1H, Jₜ⁻⁻⁻⁻⁻⁻=11.8 Hz, Jₜ⁻⁻⁻⁻⁻⁻= 1.9 Hz, CH₂C=CH), 4.94 (dd, 1H,
Jₜ⁻⁻⁻⁻⁻⁻=16.7 Hz, Jₜ⁻⁻⁻⁻⁻⁻=2.2 Hz, CH₂C=CH), 2.36 (t, 2H, J = 7.4 Hz, CH₂ α to ring), 2.02
(m, 2H, CH₂=CH-CH₂), 1.64 (m, 2H, CH₂ β to ring), 1.40-1.25 (br, s, 10H, (CH₂)₅).

¹³C NMR (100 MHz, CDCl₃): δ 172.4, 168.2, 142.7, 139.4, 139.0, 114.7, 111.6, 40.2,

MS (ESI): m/z = 297.08 [M+Na]⁺, calculated = 297.12.

(8'Z,11'R)-5-(11'-Hydroxy-octadec-8'–enoic)-2-furyl-1,3,4-oxadiazole (3c)

IR (KBr): 3358, 1668, 1427, 1394, 1390, 114.7, 111.6, 40.2,

MS (ESI): m/z = 297.08 [M+Na]⁺, calculated = 297.12.
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

2H, J = 7.5 Hz, CH₂ α to ring), 2.10 (m, 1H, CH-OH), 1.96 (m, 4H, CH₂-CH=CH-CH₂), 1.58 (m, 2H, CH₂ β to ring), 1.28 (br, s, 18H, (CH₂)₉), 0.86 (dist. t, 3H, terminus CH₃).

¹³C NMR (100 MHz, CDCl₃): δ 172.3, 168.6, 142.8, 131.1, 124.9, 111.8, 70.5, 40.3, 38.9, 38.6, 31.5, 31.3, 29.0, 28.9, 28.3, 27.9, 27.3, 26.4, 26.1, 22.6, 14.2.

MS (ESI): m/z = 411.18 [M+Na]⁺, calculated = 411.18.

(8'R,11'Z)-5-(8'-Hydroxy-octadec-11'-enoic)-2-furyl-1,3,4-oxadiazole (3d)

IR (KBr): 3353, 1669, 1557, 1251 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 7.63 (m, 1H, furan 5"-H), 7.30 (m, 1H, furan 2"-H), 6.55 (m, 1H, furan 4"-H), 5.36 (m, 2H, CH₂-CH=CH-CH₂), 3.65 (m, 1H, CH-OH), 2.36 (t, 2H, J = 7.5 Hz, CH₂ α to ring), 2.08 (m, 1H, CH-OH), 1.98 (m, 4H, CH₂-CH=CH-CH₂), 1.54 (m, 2H, CH₂ β to ring), 1.30 (br, s, 18H, (CH₂)₉), 0.88 (dist. t, 3H, terminus CH₃).

¹³C NMR (100 MHz, CDCl₃): δ 172.5, 168.1, 142.7, 131.9, 125.1, 111.1, 70.6, 40.3, 38.7, 38.3, 31.7, 31.6, 29.5, 28.7, 28.4, 27.6, 27.4, 26.8, 26.3, 22.5, 14.1.


5-Pentadec-2-furyl-1,3,4-oxadiazole (3e).

IR (KBr): 1667, 1571, 1249 cm⁻¹.

¹H NMR (CDCl₃): δ 7.63 (m, 1H, furan 5"-H), 7.32 (m, 1H, furan 2"-H), 6.54 (m, 1H, furan 4"-H), 2.36 (t, 2H, J = 7.4 Hz, CH₂ α to ring), 1.69 (m, 2H, CH₂ β to ring), 1.25 (br, s, 24H, (CH₂)₁₂), 0.87 (dist. t, 3H, terminus CH₃).
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.1, 168.2, 142.2, 111.7, 40.6, 40.4, 40.2, 40.0, 39.8, 39.6, 33.9, 33.7, 29.2, 28.9, 28.7, 26.4, 25.4, 25.1, 14.0.


5-[I-Bromopentadec$\beta$-furyl]-1,3,4-oxadiazole (3f).

IR (KBr): 728, 1665, 1569, 1248 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): $\delta$ 7.65 (m, 1H, furan 5$^\alpha$-H), 7.33 (m, 1H, furan 2$^\alpha$-H), 6.56 (m, 1H, furan 4$^\alpha$-H), 2.38 (t, 2H, $J=7.4$ Hz, CH$_2$ $\alpha$ to ring), 1.65 (m, 2H, CH$_2$ $\beta$ to ring), 1.29 (br, s, 24H, (CH$_2$)$_{12}$), 0.87 (dist. t, 3H, terminus CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.5, 168.5, 143.0, 111.6, 52.1, 40.7, 40.5, 40.1, 39.2, 38.9, 34.0, 33.3, 29.0, 28.7, 28.1, 26.3, 24.9, 25.5, 14.1.

MS (ESI): $m/z =$ 448.04 [M+Na]$^+$, calculated = 448.07.

5-(Dec-9$'$-enoic)-2-chlorobenzyl-1,3,4-oxadiazole (3g)

IR (KBr): 1665, 1563, 1242, 748 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.99 (m, 1H, Ar-H-3$''$), 7.47 (m, 2H, Ar-H-4$''$/5$''$), 7.34 (m, 1H, Ar-H-6$''$), 5.80 (tdd, 1H, $J_{\beta-\gamma}$ = 6.6 Hz, $J_{\alpha-\gamma}$ = 10.2 Hz, $J_{\alpha-\beta}$ = 16.9 Hz, CH$_2$=CH$^-$), 4.98 (dd, 1H, $J_{\alpha-\beta}$ = 11.8 Hz, $J_{\beta-\gamma}$ = 1.9 Hz, H$_2$C=CH), 4.93 (dd, 1H, $J_{\alpha-\beta}$ = 16.7 Hz, $J_{\gamma-\alpha}$ = 2.2 Hz, H$_2$C=CH$^-$), 2.35 (t, 2H, $J = 7.4$ Hz, CH$_2$ $\alpha$ to ring), 2.02 (m, 2H, CH$_2$=CH-CH$_2$), 1.63 (m, 2H, CH$_2$ $\beta$ to ring), 1.29-1.40 (br, s, 10H, (CH$_2$)$_5$).
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.9, 168.4, 139.6, 131.4, 128.7, 128.3, 125.8, 122.6, 114.3, 40.1, 39.7, 38.6, 37.4, 32.0, 29.5, 28.6, 26.6.

MS (ESI): $m/z = 341.5$ [M+Na]$^+$, calculated = 341.6.

5-[(8'Z)-Heptadecenoic]-2-chlorobenzyl-1,3,4-oxadiazole (3h)

IR (KBr): 1667, 1562, 1248, 743 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.98 (m, 1H, Ar-H-3"), 7.46 (m, 2H, Ar-H-4"/5"), 7.33 (m, 1H, Ar-H-6"), 5.34 (m, 2H, CH$_2$-CH=CH-CH$_2$), 2.33 (t, 2H, $J = 7.4$ Hz, CH$_2$ α to ring), 2.03 (m, 4H, CH$_2$-CH=CH-CH$_2$), 1.62 (m, 2H, CH$_2$ β to ring), 1.26 (br, s, 20H, (CH$_2$)$_{10}$), 0.87 (dist. t, 3H, terminus CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.4, 168.3, 131.6, 131.4, 128.9, 128.6, 125.7, 125.3, 122.2, 40.9, 40.5, 38.6, 38.3, 33.6, 32.7, 31.8, 30.5, 29.8, 28.7, 21.9, 20.6, 20.3, 14.0.


(8'Z,11'R)-5-(11'-Hydroxy-octadec-8'-enoic)-2-chlorobenzyl-1,3,4-oxadiazole (3i)

IR (KBr): 3352, 1665, 1569, 1243, 745 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.90 (m, 1H, Ar-H-3"), 7.42 (m, 2H, Ar-H-4"/5"), 7.29 (m, 1H, Ar-H-6"), 5.27 (m, 2H, CH$_2$-CH=CH-CH$_2$), 3.58 (m, 1H, CH=OH), 2.34 (t, 2H, $J = 7.4$ Hz, CH$_2$ α to ring), 2.09 (m, 1H, CH=OH), 1.98 (m, 4H, CH$_2$-CH=CH-CH$_2$), 1.56 (m, 2H, CH$_2$ β to ring), 1.25 (br, s, 18H, (CH$_2$)$_9$), 0.81 (dist. t, 3H, terminus CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.4, 168.3, 131.6, 131.1, 128.6, 128.5, 125.9, 125.2, 122.6, 70.1, 40.2, 38.5, 37.9, 31.8, 31.1, 29.3, 28.7, 27.4, 27.0, 26.3, 26.0, 22.5, 14.1.
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

**MS (ESI):** $m/z = 455.62$ [M+Na]$^+$, calculated = 455.66.

*(8'R,11'Z)-5-(8'-Hydroxy-octadec-11'-enoic)-2-chlorobenzyl-1,3,4-oxadiazole (3j)*

**IR (KBr):** 3353, 1667, 1565, 1244, 741 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.91 (m, 1H, Ar-H-3"), 7.40 (m, 2H, Ar-H-4"/5"), 7.27 (m, 1H, Ar-H-6"), 5.29 (m, 2H, CH$_2$-CH=CH-CH$_2$), 3.56 (m, 1H, CH-OH), 2.30 (t, 2H, $J = 7.4$ Hz, CH$_2$ α to ring), 2.11 (m, 1H, CH-OH), 1.95 (m, 4H, CH$_2$-CH=CH-CH$_2$), 1.57 (m, 2H, CH$_2$ β to ring), 1.22 (br, s, 18H, (CH$_2$)$_9$), 0.81 (dist. t, 3H, terminus CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.6, 168.7, 131.9, 131.1, 128.6, 128.3, 125.5, 125.2, 122.1, 70.1, 40.6, 38.5, 37.5, 31.8, 31.0, 29.3, 28.1, 27.4, 27.0, 26.9, 26.5, 22.5, 14.1.

**MS (ESI):** $m/z = 455.62$ [M+Na]$^+$, calculated = 455.66.

*S-Pentadec-2-chlorobenzyl-1,3,4-oxadiazole (3k).*

**IR (KBr):** 1663, 1565, 1244, 743 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): $\delta$ 8.00 (m, 1H, Ar-H-3"), 7.47 (m, 2H, Ar-H-4"/5"), 7.34 (m, 1H, Ar-H-6"), 2.35 (t, 2H, $J = 7.4$ Hz, CH$_2$ α to ring), 1.64 (m, 2H, CH$_2$ β to ring), 1.27 (br, s, 24H, (CH$_2$)$_{12}$), 0.87 (dist. t, 3H, terminus CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 172.2, 168.1, 131.3, 128.7, 128.5, 125.9, 122.3, 40.3, 40.1, 40.0, 39.6, 39.3, 33.5, 33.0, 29.1, 28.3, 26.9, 25.1, 25.0, 14.0.

**MS (ESI):** $m/z = 413.64$ [M+Na]$^+$, calculated = 413.65.
Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

5-[1-Bromopentadec-2-chlorobenzyl-1,3,4-oxadiazole (3I).

IR (KBr): 1668, 1569, 1249, 748, 726 cm⁻¹.

¹H NMR (CDCl₃): 8 8.00 (m, 1H, Ar-H-3'''), 7.47 (m, 2H, Ar-H-4''''/5''), 7.35 (m, 1H, Ar-H-6''), 2.37 (t, 2H, J=7.4 Hz, CH₂α to ring), 1.66 (m, 2H, CH₂β to ring), 1.26 (br, s, 24H, (CH₂)₁₂), 0.88 (dist. t, 3H, terminus CH₃).

¹³C NMR (100 MHz, CDCl₃): δ 172.5, 168.3, 131.5, 128.6, 128.3, 125.6, 122.7, 52.4, 40.6, 40.4, 39.9, 39.2, 37.9, 34.6, 33.1, 29.6, 28.7, 28.0, 26.4, 24.6, 25.1, 14.0


Anal. Found: C, 55.42; H, 08.76; N, 10.76. C₁₈H₃₅N₃OBr requires C, 55.66; H, 08.81; N, 10.81%.

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against Escherichia coli (ATCC-25922), Methicillin resistant Staphylococcus aureus (MRSA +Ve), Pseudomonas aeruginosa (ATCC-27853), Streptococcus pyogenes and Klebsiella pneumoniae (Clinical isolate) bacterial strains by disc diffusion method. Ciprofloxacin (30 μg) was used as positive control. While the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 2.3.4.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). MBC was defined as
the lowest drug concentration at which 99.9% of the inoculums was killed. The minimum inhibitory concentrations and minimum bactericidal concentrations are given in Table 2.3.5.

**Antifungal studies**

Antifungal activity was also done by disk diffusion method. For assaying antifungal activity *Candida albicans, Aspergillus fumigatus, Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by agar diffusion method. The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 2.3.6. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). MFC was defined as the lowest drug concentration at which 99.9% of the inoculums was killed. The minimum inhibitory concentrations and minimum fungicidal concentrations are given in Table 2.3.7.
2.5. References

Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles


Microwave assisted 2,5-disubstituted-1,3,4-oxadiazoles

CHAPTER-3

2,5-Di substituted-1,3,4-thiadiazoles
3.1. Theoretical

Understanding five membered heterocycles is a long standing objective because these heterocycles constitute the primary skeletons of more than half of the compounds produced by nature and they play a vital role in biological activities\(^1\). During recent years there has been much investigation of the five membered heterocycles. 1,3,4-Thiadiazoles, having one sulfur and two nitrogen atoms, many of which have been found to be pharmacologically active\(^2\). The advent of sulfur drugs and the discovery of mesoionic compounds further accelerated the rate of progress in the field of sulfur containing heterocycles.

A large number of compounds containing 1,3,4-thiadiazole moiety have been investigated as precursors to therapeutically interesting drug candidates because of their diverse properties such as antimicrobial\(^3\), antitubercular\(^4\), anticancer\(^5\), anti-inflammatory\(^6\), anti-convulsant\(^7\), analgesic\(^8\), anti-secretory\(^9\), antidepressant and anxiolytic agents\(^10\). Moreover much interest has also been focused on the cardiotonic\(^11\), diuretic\(^12\) and herbicidal\(^13\) activities displayed by the compounds incorporating this heterocyclic system. The heterocyclic mercaptans incorporating 1,3,4-thiadiazoles have been found to possess anti-proliferative activities against human cancer cell lines\(^14\) and tumor associated carbonic anhydrase isoenzymes I, II and IX\(^15\). Nitroheteroaryl-1,3,4-thiadiazole derivatives based on megazole have shown impressive antimicrobial and antiparasitic activity particularly against trypanosomatic protozoa\(^16\). The Nitroimidazolyl-1,3,4-thiadiazole based compounds have been reported as anti-leshmanial agents. The compounds of 1,3,4-thiadiazole series have been found to exhibit antiphlogistic (i) and 5-LO and CO inhibitory (ii) properties\(^17\).
The 1,3,4-thiadiazoles and its derivatives have become very useful compounds in medicine. The acetazol (iii), a carbonic anhydrase inhibitor is used systematically (i.e. an oral medication) for the treatment of glaucoma. It is believed to reduce the intraocular pressure by lowering the fluid formation in the eye.

The substituted 1,3,4-thiadiazoles have been very useful in agriculture and many fields of technology such as dyes, lubricating compositions, analytical reagents, optically active liquid crystals, photographic materials and many other uses. The 2-amino-5-phenyl-1,3,4-thiadiazole (APT) (iv) and its derivatives are found to inhibit corrosion of steel and copper.
The macrocyclic polyether compounds containing a 1,3,4-thiadiazole moiety (n-MCTH) (v) has been used in the corrosion inhibition of C38 carbon steel in acidic media \(^2^6\).

![Image](image.png)

The mercapto-5-\(R\)-amino-1,3,4-thiadiazole derivatives have been studied for their electrochemical behaviors at carbon paste electrode \(^2^7\). The poly(2-amino-5-mercapto-1,3,4-thiadiazole) (PMAT) (vi) film has been used for selective determination of L-cysteine \(^2^8\) and folic acid \(^2^9\).

![Image](image.png)

The 2,5-dimercapto-1,3,4-thiadiazole and its analogs have been found to possess a broad range of potential applications such as solid state organic crystals, bioorganic catalysis etc \(^3^0\). The thiadiazoles containing the imine (-C=N-) group have long been of interest as luminophores for optical applications owing to their electron accepting nature \(^3^1\).

The immobilization of 5-amino-1,3,4-thiadiazole-thiol (ATT) onto analogue of heulandite (vii) for divalent toxic metals removal has also been reported \(^3^2\).
The discovery of the diverse pharmacological and material properties of 1,3,4-thiadiazoles and its derivatives has stimulated a substantial interest in the chemistry and synthesis of these important heterocycles and their analogs.

Xinping et al.$^{33}$ synthesized 6-aryl-3-(5-methylisoxazole-3-yl)-s-triazolo[3,4-b]-1,3,4-thiadiazole (ix) from 4-amino-5-mercapto-3-(5-methylisoxazole-3-yl)-1,2,4-triazole (viii) by treating with various aromatic acids in presence of phosphorus oxy chloride (Scheme 3.1.1).

Scheme 3.1.1

The synthesis of 6-aryl-3-cinchophenyl-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles (xi) from 3-cinchophenyl-4-amino-5-thio-1,2,4-triazole (x) by treating with aromatic acid in phosphorus oxy chloride was reported by Pengfei et al.$^{34}$ (Scheme 3.1.2).
2,5-Disubstituted-1,3,4-thiadiazoles

\[ \text{ArCOOH} \]

\[ \text{POCl}_3 \]

\[ 2,5\text{-Disubstituted-1,3,4-thiadiazoles} \]

\[ \text{Ar} = 4\text{-Br-C}_6\text{H}_4, 4\text{-Cl-C}_6\text{H}_4. \]

**Scheme 3.1.2**

The reaction of 4-amino-5-(3-chlorobenzo[6]thien-2-yl)-3-mercapto-1,2,4-triazole (xii) with acetic anhydride under reflux condition for 18 h yielded the cyclized product 3-(3-chlorobenzo[6]thien-2-yl)-6-methyl-1,2,4-triazolo[3,4-b][1,3,4]-thiadiazole (xiii) (Scheme 3.1.3).

\[ \text{Ac}_2\text{O} \]

**Scheme 3.1.3**

Zheng *et al.*\(^{36}\) reported the synthesis of 2-amino-5-aryloxymethyl-1,3,4-thiadiazoles (xvi) under microwave conditions from thiosemicarbazide (xv) and aryloxyacetic acid (xiv) (Scheme 3.1.4).
2,5-Disubstituted 1,3,4-thiadiazoles

\[
\text{ArO—OH} + \text{H}_2\text{NN—S—NH—NH}_2 \xrightarrow{\text{MW, 8 min}} \text{ArO—S—NH—NH}_2
\]

\text{xiv} \quad \text{xv} \quad \text{xvi}

\text{Ar}= \text{C}_6\text{H}_4, 2-\text{CH}_3\text{C}_6\text{H}_4, 4-\text{Cl}\text{C}_6\text{H}_4, 1\text{-Naphthyl}.

\text{Scheme 3.1.4}

The condensation of triazoles (xvii) with heteroaromatic acids (xviii) in the presence of phosphorus oxy chloride produced various triazolo thiadiazoles (xix) (Scheme 3.1.5) while its condensation with heteroaromatic aldehydes (xx) afforded a 5,6-dihydrotriazolo thiadiazole derivative (xxi)\(^{37}\) (Scheme 3.1.6).

\[
\text{Ar} = \text{Phenyl, Methyl, H.}
\]

\text{Ar}= 2\text{-Chloro-5-methoxy phenyl, 3,4-Dimethoxy benzyl, 2-Methyl-3-furanyl}.

\text{Scheme 3.1.5}
2,5-Disubstituted-1,3,4-thiadiazoles

\[
\text{Ar'} \quad \text{N—N} \quad } \text{H} \\
\text{H} \quad \text{SH} + \text{Ar'} \\
\text{DMF} \\
\text{Ar} \\
\text{N—N} \quad } \text{H} \\
\text{NH}_2 \\
xvii \quad \text{xx} \quad \text{xxi}
\]

\(\text{Ar'} = 3\text{-Phenyl-4-quinolinyl, 4-Quinolinyl, 2,6-Dihydroxy-4-pyridinyl, 5-Methoxy-3-indolyl methyl.}\)

\(\text{Ar} = 2\text{-Chloro-5-methoxy phenyl, 3,4-Dimethoxy benzyl, 2-Methyl-3-furanyl.}\)

**Scheme 3.1.6**

A fast and efficient microwave assisted synthesis of 2-arylamino-5-(1-adamantyl)-1,2,4-triazolo[3,4-b][1,3,4]-thiadiazoles (xxiii) is achieved by the desulfurization of corresponding \(N,N'\)-disubstituted thioureas (xxii)\(^{38}\) (Scheme 3.1.7).

\[
\text{N—N} \quad } \text{H} \\
\text{HN} \quad \text{NH—Ar} \\
\text{xxii} \quad \text{xxiii}
\]

\(\text{Ar} = \text{C}_6\text{H}_5, \text{4-Br-C}_6\text{H}_4, \text{4-Cl-C}_6\text{H}_4, \text{3-F-C}_6\text{H}_4, \text{4-F-C}_6\text{H}_4.\)

**Scheme 3.1.7**

Aamir et al.\(^{39}\) reported the synthesis of 5-[2-(4-i-butylphenyl)ethyl]-2-alkyl/arylamino-1,3,4-thiadiazoles (xxv) from thiosemicarbazide (xxiv) by gradually adding to it a cold solution of concentrated sulphuric acid (Scheme 3.1.8).
Scheme 3.1.8

Aryl-N-\{[(4-[2-(2-chlorophenyl)-4-oxo-(3-hydroquinazolin-3-yl)]-phenyl)-
carbonylamino)-amino]-thioxomethyl\}-amides (xxvi) when stirred in concentrated
sulphuric acid gave aryl-N-5-{4-[2-(2-chlorophenyl)-4-oxo-(3-hydroquinazolin-3-yl)]-
phenyl}-1,3,4-thiadiazole-2-yl-amides (xxvii) in excellent yields\(^4\) (Scheme 3.1.9).

Scheme 3.1.9

Pintilie et al.\(^4\) synthesized the 2-[1-(3-3-nitrobenzoylamino)-3-(methylthio)]-
propyl-5-(alkyl/phenyl) amino-1,3,4-thiadiazoles (xxix) from 1-[\(N-(3-nitrobenzoyl)\)-D,L-
methionyl]-4-alkyl/phenyl-thiosemicarbazides (xxviii) by stirring at room temperature
with concentrated sulphuric acid (Scheme 3.1.10).
2,5-Disubstituted-1,3,4-thiadiazoles

The synthesis of 5-[(biphenyl-4-yloxy)methyl]-2-aryl amino-1,3,4-thiadiazoles (xxxi) from $\text{N}^1[2-(\text{biphenyl-4-yloxy})\text{ethanoyl}]-\text{N}^2-$aryl-thiosemicarbazides (xxx) was reported by Kumar et al.\(^{42}\) (Scheme 3.1.11).

$\text{Ar}=\text{C}_6\text{H}_5, 4-\text{F-C}_6\text{H}_4, 2-\text{Cl-C}_6\text{H}_4, 4-\text{Cl-C}_6\text{H}_4.$

$\text{Ar}=\text{CH}_3, \text{C}_6\text{H}_5, 4-\text{CH}_3\text{C}_6\text{H}_4, 4-\text{Br-C}_6\text{H}_4, \text{CH}_2-\text{CH}=\text{CH}_2.$

**Scheme 3.1.10**

The synthesis of 5-[(biphenyl-4-yloxy)methyl]-2-aryl amino-1,3,4-thiadiazoles (xxxi) from $\text{N}^1[2-(\text{biphenyl-4-yloxy})\text{ethanoyl}]-\text{N}^2-$aryl-thiosemicarbazides (xxx) was reported by Kumar et al.\(^{42}\) (Scheme 3.1.11).

$\text{Ar}=\text{C}_6\text{H}_5, 4-\text{F-C}_6\text{H}_4, 2-\text{Cl-C}_6\text{H}_4, 4-\text{Cl-C}_6\text{H}_4.$

**Scheme 3.1.11**

The $N$-ethyl-5-substituted-1,3,4-thiadiazol-2-amines (xxxiii), with high cytotoxicity were synthesized from $N$-ethyl hydrazinecarbothioamides (xxxii) in sulfuric acid medium\(^{43}\) (Scheme 3.1.12).
The thiosemicarbazide of 6-chloro-2-aminobenzothiazole (xxxiv) on cyclization with different carboxylic acids in phosphorus oxy chloride provided the corresponding 2-aryl-5-(6'-chloro-1,3'-benzothiazole-2-yl-amino)-1,3,4-thiadiazole (xxxv) of pharmaceutical importance\(^4^4\) (Scheme 3.1.13).

Padmavathi \(\text{et al.}^4\) converted 2-arylsulfonylmethyl/arylmethanesulfonylmethyl-5-aryl-1,3,4-oxadiazoles (xxxvi) to 2-arylsulfonylmethyl/arylmethanesulfonylmethyl-5-aryl-1,3,4-thiadiazoles (xxxvii) by treatment with a two fold excess of thiourea in tetrahydrofuran (Scheme 3.1.14).
The reaction of ester (xxxviii) with hydrazine in ethanol gave the aroyl hydrazides (xxxix) which were converted to 5-substituted-2-mercapto-1,3,4-thiadiazoles (xli) via potassium aroyl dithiocarbazates (xl) (Scheme 3.1.14).

Augustine et al.\textsuperscript{47} have given the propelphosphonic anhydride (T3P) mediated synthesis of 1,3,4-thiadiazoles (xliv) by the reaction of carboxylic acid (xlili), hydrazide (xliii) and $P_2S_5$ in triethylamine (TEA) (Scheme 3.1.15).
Various 1,4-bis(6-substituted)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (xLvi) were synthesized from terphthalic dihydrazide (xLv) via the formation of bis-potassium dithiocarbazinate (xLvi) and 1,4-phenylene-bis(4-amino-4H-1,2,4-triazole-3-thiol) (xLvii) (Scheme 3.1.17).

Abdelhamid et al.\textsuperscript{49} reported the synthesis of 2,3-dihydro-1,3,4-thiadiazoles (L) by stirring a mixture of appropriate hydrazonoyl halide (xLix) with 2-(benzofuran-2-ylcarbonyl)-3-mercaptop-3-methylsulfanylacelonitrile (L) in ethanol (Scheme 3.1.18).
A novel approach to 3,6-disubstituted[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (Liv) using silica-supported dichlorophosphate as a recoverable cyclodehydrant, carboxylic acids (Lii) and thiocarbohydrazide (Liii) as starting material is described by Li and Zhao\(^5\) (Scheme 3.1.19).

\[ R= C_6H_5, \, 4-CH_3OC_6H_4, \, 2-CH_3-C_6H_4, \, 2-Cl-C_6H_4, \, \text{Fur-2-yl}. \]

The biologically active 3,6-disubstituted-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazoles (Lvi) were obtained by refluxing a mixture of 4-amino-5-benzyl-
2,5-Disubstituted-1,3,4-thiadiazoles

4H-1,2,4-triazole-3-thiol (Lv), aryl/aroyl acid and phosphorus oxy chloride for 8-12 h \(^{51}\) (Scheme 3.1.20).

\[
\text{Lv} \quad \begin{array}{c}
\text{R} = \text{C}_6\text{H}_5-\text{CH}_2, \text{C}_6\text{H}_5-\text{OCH}_2, 2-\text{OHC}_6\text{H}_4. \\
\text{R}_1 = \text{C}_6\text{H}_5\text{CONHCH}_2, 2-\text{Br-}C_6\text{H}_4, 2-\text{C}_6\text{H}_5\text{CO-}C_6\text{H}_4, \text{C}_{10}\text{H}_7\text{CH}_2, \text{C}_8\text{H}_6\text{NCH}_2, \text{C}_6\text{H}_5\text{COCH}_2
\end{array}
\]

\[
\text{Lvi} \quad \begin{array}{c}
\text{R}_1\text{COOH} \\
\text{POCl}_3
\end{array}
\]

Scheme 3.1.20

The interaction of alkyl dithioester (Lvii) with hydrazonyl halide (Lviii) in ethanol containing triethylamine at reflux temperatures gave 1,5-dihydro-1,3,4-thiadiazoles (Lix) \(^{52}\) (Scheme 3.1.21).

\[
\text{Lvii} \quad \begin{array}{c}
\text{R} = \text{CH}_2, \text{CH}_2\text{Ph.} \\
\text{R}_2 = \text{COCH}_3, \text{COOEt}, \text{C}_6\text{H}_5\text{CO.}
\end{array}
\]

\[
\text{Lviii} \quad \begin{array}{c}
\text{R}_1 = \text{C}_6\text{H}_5, \text{o-CH}_3\text{C}_6\text{H}_4, \text{p-ClC}_6\text{H}_4. \\
\text{X} = \text{Cl}, \text{Br.}
\end{array}
\]

Scheme 3.1.21
Efimova et al.\textsuperscript{53} examined the microwave-assisted reaction of 5-aryl(hetaryl)-tetrazoles (Lx) with phenyl isothiocyanate (Lxi) and obtained the corresponding 2-aryl(hetaryl)-5-phenylamino-1,3,4-thiadiazoles (Lxii) (Scheme 3.1.22).

\begin{equation}
\text{Lx} + \text{Lxi} \xrightarrow{\text{MW}} \text{Lxii}
\end{equation}

\text{R}= 4-\text{Me}_2\text{NC}_6\text{H}_4, 4-\text{Me}_2\text{OC}_6\text{H}_4, 4-\text{MeC}_6\text{H}_4, \text{Ph}, 4-\text{ClC}_6\text{H}_4, 4-\text{O}_2\text{NC}_6\text{H}_4, \text{Pyridine-2-yl}.

\textbf{Scheme 3.1.22}

The condensation of bis-aldehyde (Lxiii) with 2-amino-5-mercapto-1,3,4-thiadiazole (Lxiv) and further cyclization in presence of dibromoalkanes afforded the azathia crown macrocycles containing two 1,3,4-thiadiazoles rings as subunits (Lxv)\textsuperscript{54} (Scheme 3.1.23).

\begin{equation}
\text{Lxiii} + \text{Lxiv} \xrightarrow{\text{Br-Br}} \text{Lxv}
\end{equation}

\text{X}= (\text{CH}_2)_2, (\text{CH}_2)_3, (\text{CH}_2)_4.

\text{Y}= (\text{CH}_2)_2, (\text{CH}_2)_4.

\textbf{Scheme 3.1.23}
Lamani et al.\textsuperscript{55} reported the synthesis of 2-amino-5-benzof[\(d\)]isoxazol-3-ylmethyl-[1,3,4]thiadiazole (Lxviii) by refluxing 1,2-benzisoxazole-3-acetic acid (Lxvi) with thiosemicarbazide (Lxvii) in presence of phosphorus oxy chloride (Scheme 3.1.24).

\[
\text{Lxvi} + \text{Lxvii} \xrightarrow{POCl_3, \text{Reflux}} \text{Lxviii}
\]

Scheme 3.1.24

The pharmacologically important 3-[(3-substituted[1,2,4]triazolo[3,4-\(b\)])[1,3,4]thiadiazol-6-yl)methyl]-1H-pyrazolo[3,4-\(d\)]pyrimidine-4,6-dithione (Lxxi) were obtained by stirring a mixture of [1H-pyrazolo[3,4-\(d\)]pyrimidine-2,4-dithione-5-yl]acetonitrile (Lxx), 4-amino-5-substituted[1,2,4]triazole-3-thiol (Lxix) and phosphoric acid at 100\(^\circ\)C for 2 h\textsuperscript{56} (Scheme 3.1.25).

\[
\text{Lxix} + \text{Lxx} \xrightarrow{PPA, 100 ^\circ C} \text{Lxxi}
\]

\(R = \text{CH}_3, \text{CH}_2\text{Ph}, \text{CH}_2-\text{O-Ph(p-Cl)}.\)

Scheme 3.1.25
The refluxing reaction of 1,2-bis(4-chloro-5H-1,2,3-dithiazol-5-ylidene)hydrazine (Lxxii) in PhCl and benzyltriethylammonium iodide (Lxxiii) under an argon atmosphere afforded the 1,3,4-thiadiazole-2,5-dicarbonitrile (Lxxiv) (Scheme 3.1.26).

Umamatheswari et al.\textsuperscript{58} reported the synthesis of 5-spiro-(3-methyl-2,6-diphenyl tetrahydropyran-4-yl)-4,5-dihydro-[1,3,4]-thiadiazole (Lxxvii) by the treatment of respective thiosemicarbazone (Lxxv) with freshly distilled acetic anhydride (Lxxvi) (Scheme 3.1.27).
The 2,5-diphenyl-1,3,4-thiadiazole containing vinyl monomers [PTBEMA (Lxxviii), PVPT (Lxxix), PTPEMA (Lxxx)] and their polymers with considerable fluorescent properties have been synthesized.

![Chemical Structure of PTBEMA](Lxxviii)

2-[4-(5-Phenyl-1,3,4-thiadiazole-2-yl)benzyl]oxyethyl methacrylate (PTBEMA)

![Chemical Structure of PVPT](Lxxix)

2-Phenyl-5-(4-vinylphenyl)-1,3,4-thiadiazole (PVPT)

![Chemical Structure of PTPEMA](Lxxx)

2-[4-(5-Phenyl-1,3,4-thiadiazole-2-yl)phen oxy]ethyl methacrylate (PTPEMA)
Li et al.\textsuperscript{60} synthesized the 3,6-disubstituted-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles (Lxxxii) and screened them for their inhibitory activity to \textit{E. coli} methionine aminopeptidase (EcMetAPI). The synthesis was achieved by refluxing 4-amino-5-[2-(4-chlorophenoxy)methylbenzimidazole]-1-methylene]-3-mercapto-1,2,4-triazole (Lxxxi) with various aromatic acids in presence of phosphorus oxy chloride (Scheme 3.1.28).

\begin{align*}
\text{Aromatic acid} & \xrightarrow{\text{POCl}_3} \text{Lxxxii} \\
\text{Lxxxi} & \xrightarrow{\text{Y= C}_6\text{H}_5, \text{CH}_3\text{C}_6\text{H}_4, \text{CH}_3\text{OC}_6\text{H}_4, \text{ClC}_6\text{H}_4, \text{FC}_6\text{H}_4, \text{O}_2\text{NC}_6\text{H}_4,} \text{Lxxxii}
\end{align*}

\textbf{Scheme 3.1.28}

The reaction of 4-alkyl/aryl-1-((6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl)acetyl)-3-thiosemicarbazide (Lxxxiii) with concentrated sulfuric acid at room temperature resulted in the formation of corresponding 2-alkyl/arylamino-5-((6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl)thiazol-3-yl)methyl)-1,3,4-thiadiazoles (Lxxxiv)\textsuperscript{61} (Scheme 3.1.29).
A facile protocol for the synthesis of 5-substituted-2-amino-1,3,4-thiadiazoles (Lxxxvii) in water has been described by Aryanasab et al.\textsuperscript{62}. The synthesis involved the reaction of acid hydrazide (Lxxxvi) with dithiocarbamate (Lxxxv) (Scheme 3.1.30).

![Scheme 3.1.29](image)

\[ \text{Lxxxiii} \quad \xrightarrow{H_2SO_4, \text{reflux}} \quad \text{Lxxxiv} \]

\( R = \text{CH}_3, \text{C}_2\text{H}_5, \text{C}_3\text{H}_7, \text{C}_4\text{H}_9, \text{CH}_2\text{CH} = \text{CH}_2, \text{C}_6\text{H}_5, \text{C}_6\text{H}_4\text{-Br}, \text{C}_6\text{H}_4\text{-Cl}, \text{C}_6\text{H}_4\text{-CH}_3. \)

**Scheme 3.1.29**

A facile protocol for the synthesis of 5-substituted-2-amino-1,3,4-thiadiazoles (Lxxxvii) in water has been described by Aryanasab et al.\textsuperscript{62}. The synthesis involved the reaction of acid hydrazide (Lxxxvi) with dithiocarbamate (Lxxxv) (Scheme 3.1.30).

![Scheme 3.1.30](image)

\[ \text{Lxxxv} + \text{Lxxxvi} \xrightarrow{\text{Et}_3\text{N}, \text{H}_2\text{O, reflux}} \text{Lxxxvii} \]

\( R_1 = \text{Ph, 3,4-Cl}_2\text{C}_6\text{H}_3, \text{n-Bu, PhCH}_2, (R)-1\text{-Phenylethyl, Adamantyl.} \)

\( R_2 = \text{CH}_2\text{CH}_2\text{CN, Et.} \)

\( R_3 = \text{Ph, PhCH}_2, 4\text{-MeC}_6\text{H}_4, 4\text{-Pyridine, 2-HOC}_6\text{H}_4. \)

**Scheme 3.1.30**
3.2. Synthesis, Antibacterial and Antifungal Activity of Some New 2,5-Disubstituted-1,3,4-thiadiazoles*

The ever increasing demand for novel medicinally active compounds and the laborious process of lead discovery and optimization have resulted in the continuous search for simple and efficient compounds containing such useful scaffolds. Among the well known heterocyclic systems the derivatives of thiadiazoles possess broad-spectrum biological activities and many other uses. The therapeutic effects of compounds containing 1,3,4-thiadiazole rings have been studied for a number of pathological conditions including inflammation, pain and depression. This scaffold represents a pharmacophore itself as it has been observed in several substances showing activity against a broad range of therapeutic targets. Furthermore, the synthesis of thiadiazoles has attracted wide attention due to the diversity of their applications as antimicrobial, anti-tubercular, anticancer and anticonvulsant agents.

The thiosemicarbazides have been effectively used as synthons for the synthesis of various nitrogen heterocyclic. The semicarbazide moiety gives a platform for various cyclocondensation as well as addition cyclization reactions to take place. Bearing in mind the aforementioned pharmacological applications associated with 1,3,4-thiadiazoles extensive research activity is being pursued towards the synthesis of thiadiazoles decorated with different functional groups. The present stratagem is aimed in the

direction of developing new 1,3,4-thiadiazoles having alkenyl/hydroxyalkenyl and phenylamine moieties as substituents. The structures of the synthesized compounds have been characterized by IR, $^1$H NMR, $^{13}$C NMR, and Mass spectroscopy.

Further the increasing number of multidrug resistant pathogens led us to screen the newly synthesized derivatives against the representative panel of Gram-positive, Gram-negative bacteria and fungi.
3.3. Results and Discussion

The typical reaction procedure for the synthesis of 3a-d involved the reaction of long chain alkenoic acid hydrazide (1a-d) with phenyl isothiocyanate to furnish the corresponding thiosemicarbazides (2a-d) which on dehydrative cyclization by Ac₂O produced 2,5-disubstituted-1,3,4-thiadiazoles (3a-d) in excellent yields. To the prior, the long chain acid hydrazides (1a-d) were synthesized from corresponding long chain alkenoic acids by esterification and further treatment with hydrazine hydrate. The reaction sequence is outlined in Scheme 3.3.1.

![Scheme 3.3.1. Synthesis of 2,5-disubstituted-1,3,4-thiadiazoles 3a-d](image)

The characterization data of 2,5-disubstituted-1,3,4-thiadiazoles (3a-d) is given in Table 3.3.1.
### Table 3.3.1. Characterization data of synthesized 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-thiadiazoles 3a-d.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R</th>
<th>Mol. Formula</th>
<th>M.p. [°C]</th>
<th>Yield [%]</th>
<th>Analysis (%) found (calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>H</td>
<td>C_{18}H_{25}SN_{3}</td>
<td>134-135</td>
<td>95</td>
<td>68.30 (68.53) 7.78 (7.98) 13.07 (13.31)</td>
</tr>
<tr>
<td>3b</td>
<td>(6)</td>
<td>C_{25}H_{39}SN_{3}</td>
<td>138-140</td>
<td>91</td>
<td>72.23 (72.59) 9.44 (9.49) 10.10 (10.15)</td>
</tr>
<tr>
<td>3c</td>
<td>OH</td>
<td>C_{25}H_{39}OSN_{3}</td>
<td>136-138</td>
<td>85</td>
<td>69.61 (69.89) 9.04 (9.14) 09.78 (9.78)</td>
</tr>
<tr>
<td>3d</td>
<td>(5)</td>
<td>C_{25}H_{39}OSN_{3}</td>
<td>139-141</td>
<td>83</td>
<td>69.54 (69.89) 9.02 (9.14) 09.72 (9.78)</td>
</tr>
</tbody>
</table>

The data in Table 3.3.1 reveals that the yields of the 1,3,4-thiadiazoles 3a-d are found to be excellent and are independent of the substituents present in the precursor. The scope of the reaction using olefinic (internal and terminal) and hydroxy acids was found to be good. The newly synthesized compounds were analyzed for C, H and N content and the structures were confirmed on the basis of IR, $^1$H NMR, $^{13}$C NMR and mass spectral data.

5-(Dec-9'-enyl)-2-phenylamine-1,3,4-thiadiazole 3a gave significant IR bands at 3221 cm$^{-1}$ (NH), 1488 cm$^{-1}$ (C=N) and 707 cm$^{-1}$ (C-S-C). $^1$H NMR peak at $\delta$ 12.20 (1H, s, NH), 8.16 (2H, d, $J = 8.5$ Hz, Ar-H-2'/6''), 7.55 (1H, t, $J = 7.3$ Hz, Ar-H-4'') and 7.44 (2H, t, $J = 7.8$ Hz, Ar-H-3''/5'') were observed. In addition to normal fatty acid chain peaks a signal of methine proton of C-9 at $\delta$ 5.73 was observed. The C-10 methylene designated as H$_{E}$ and H$_{Z}$ displayed two distinct $\delta$ values when coupled with C-9 methine
protons. The spectrum showed two doublets of doublet at $\delta$ 4.98 and 4.85 for $H_Z$ and $H_F$ protons respectively. In $^{13}$C NMR peaks at $\delta$ 165.2 and 153.0 were observed for ring carbon atoms. The mass spectra showed characteristic molecular ion peak which were in accordance with the molecular formula. The newly synthesized compounds have been confirmed by their spectral data.

*Antibacterial studies*

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 3.3.2.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) are given in Table 3.3.3.
Table 3.3.2. Antibacterial activity of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-thiadiazoles 3a-d.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. pyogenes</td>
<td>S. aureus</td>
</tr>
<tr>
<td>3a</td>
<td>13.5±0.3</td>
<td>13.1±0.5</td>
</tr>
<tr>
<td>3b</td>
<td>13.1±0.7</td>
<td>12.9±0.3</td>
</tr>
<tr>
<td>3c</td>
<td>22.9±0.8</td>
<td>21.6±0.3</td>
</tr>
<tr>
<td>3d</td>
<td>21.1±0.2</td>
<td>20.8±0.4</td>
</tr>
<tr>
<td>Standard</td>
<td>23.0±0.2</td>
<td>22.0±0.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (standard); Chloramphenicol and negative control (DMSO) measured by the Halo Zone Test (Unit, mm)

Table 3.3.3. MIC and MBC results of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-thiadiazoles 3a-d

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. pyogenes</td>
<td>S. aureus</td>
</tr>
<tr>
<td>3a</td>
<td>25.0</td>
<td>50.0</td>
</tr>
<tr>
<td>3b</td>
<td>12.5</td>
<td>100</td>
</tr>
<tr>
<td>3c</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>3d</td>
<td>6.25</td>
<td>25.0</td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Positive control Chloramphenicol. MIC (µg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (µg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely.
The PBE (percentual bacteriostatic efficiency, %) was obtained as

\[
PBE = \frac{100}{MIC}
\]

The results have been reported in Fig. 3.3.1.

![Bar chart showing PBE% for compounds 3a-d compared to control drug Chloramphenicol (Ch)].

**SP** = *S. pyogenes*, **SA** = *S. aureus*, **PA** = *P. aeruginosa*, **KP** = *K. pneumonia*, **EC** = *E. Coli*

*Fig. 3.3.1 Percentual bacteriostatic efficiency (PBE%) for compounds 3a-d compared to control drug Chloramphenicol (Ch)*

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The structural activity study showed that the synthesized 1,3,4-thiadiazoles have varying degree of microbial inhibition. Compounds 3c and 3d showed good inhibition against all Gram-positive and Gram-negative bacterial strains at 6.25 μg/ml concentrations. The compounds 3c and 3d were found to be almost equally
potent as the reference drug, Chloramphenicol, in case of *K. pneumoniae*. The MBC of few compounds was found to be the same as MIC but in most of the compounds it was two or three folds higher than the corresponding MIC results. Among the synthesized thiadiazoles, the compounds with a hydroxyalkenyl chain substituent at 5\textsuperscript{th} position of thiadiazoles were found to increase the antibacterial activity in compounds 3c and 3d. However the position of the hydroxy group had no significant effect on the magnitude of the antibacterial activity.

Further, the compounds showed parallel activity against gram-positive and gram-negative bacterial strains.

*Antifungal studies*

In another set of experiments, the synthesized 1,3,4-thiadiazoles were also screened for their antifungal activity. The antifungal activity was assessed against *Candida albicans, Aspergillus fumigatus, Penicillium marneffei and Trichophyton mentagrophytes* (recultured) in DMSO by agar diffusion method\textsuperscript{68,69}. The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 3.3.4.

The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) were determined by broth dilution technique and the results are given in Table 3.3.5.
Table 3.3.4. **Antifungal activity of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-thiadiazoles 3a-d**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of zone of inhibition (mm)</th>
<th>CA</th>
<th>AF</th>
<th>TM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td></td>
<td>19.5±0.5</td>
<td>16.5±0.4</td>
<td>14.1±0.3</td>
<td>10.2±0.5</td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td>27.2±0.2</td>
<td>23.8±1.2</td>
<td>20.3±0.7</td>
<td>16.1±0.2</td>
</tr>
<tr>
<td>3c</td>
<td></td>
<td>25.1±0.7</td>
<td>22.4±0.3</td>
<td>18.9±0.5</td>
<td>15.1±0.9</td>
</tr>
<tr>
<td>3d</td>
<td></td>
<td>24.9±1.4</td>
<td>21.8±0.2</td>
<td>19.0±0.2</td>
<td>15.2±1.2</td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td>30.0±0.2</td>
<td>27.0±0.2</td>
<td>24.0±0.3</td>
<td>20.0±0.5</td>
</tr>
<tr>
<td>DMSO</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CA= Candida albicans, AF= Aspergillus fumigatus, TM= Trichophyton mentagrophytes, PM= Penicillium marneffei.
Positive control (Greseofulvin) and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

Table 3.3.5. **MIC and MFC of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-thiadiazoles 3a-d**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>MIC</th>
<th>MFC</th>
<th>AF</th>
<th>MIC</th>
<th>MFC</th>
<th>TM</th>
<th>MIC</th>
<th>MFC</th>
<th>PM</th>
<th>MIC</th>
<th>MFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td></td>
<td>25.5</td>
<td>50.0</td>
<td></td>
<td>25.0</td>
<td>100</td>
<td></td>
<td>25.0</td>
<td>50.0</td>
<td></td>
<td>25.0</td>
<td>100</td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td>6.25</td>
<td>12.5</td>
<td></td>
<td>6.25</td>
<td>25.0</td>
<td></td>
<td>6.25</td>
<td>12.5</td>
<td></td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>3c</td>
<td></td>
<td>12.5</td>
<td>25.0</td>
<td></td>
<td>12.5</td>
<td>50.0</td>
<td></td>
<td>12.5</td>
<td>25.0</td>
<td></td>
<td>25.0</td>
<td>50.0</td>
</tr>
<tr>
<td>3d</td>
<td></td>
<td>25.0</td>
<td>50.0</td>
<td></td>
<td>25.0</td>
<td>50.0</td>
<td></td>
<td>25.0</td>
<td>100</td>
<td></td>
<td>25.0</td>
<td>100</td>
</tr>
<tr>
<td>St.</td>
<td></td>
<td>6.25</td>
<td>12.5</td>
<td></td>
<td>6.25</td>
<td>12.5</td>
<td></td>
<td>6.25</td>
<td>12.5</td>
<td></td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

CA= Candida albicans, AF= Aspergillus fumigatus, TM= Trichophyton mentagrophytes, PM= Penicillium marneffei, St.= Standard. MIC (µg/ml) = minimum inhibitory concentration, i.e the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (µg/ml) = minimum fungicidal.
Positive control Greseofulvin
The ratio MFC/MIC was calculated in order to determine if the compound had a fungistatic (MFC/MIC ≥ 4) or fungicidal (MFC/MIC ≤ 4) activity and the results have been summarized in Fig. 3.3.2

\[ \text{CA= C. albicans, AF= A. fumigatus, TM= T. mentagrophytes and PM= P. marneffei.} \]

\textit{Fig. 3.3.2 MFC/MIC of compound 3a-d compared to control drug Greseofulvin (Gr)}

The antifungal screening data showed moderate to good activity. The excellent inhibition results were obtained against all the test strains by compound 3b. Moderate activity was shown by compounds 3c and 3d against the tested fungal strains. The compound 3a showed significantly lower inhibitory activity. The compound 3b showed maximum activity
against *C. albicans*, *A. fumigates* and *T. metagrophyte* strains. The MFC of most of the compounds was two or three folds higher than the corresponding MIC results. Most of the synthesized 1,3,4-thiadiazoles showed good fungistatic activity against the fungal strain *C. albicans*. Hence it could be concluded that the higher anti-fungal potency of the compound 3b may be attributed to the presence of an internal double bond in the long chain alkenyl substituent of the synthesized 1,3,4-thiadiazoles. Contrary to the antibacterial studies, the presence of the hydroxy on the alkenyl side chain turns out to be detrimental for the anti-fungal activity perhaps due to pharmacokinetic reasons.

Thus, the synthesized compounds can be used as template for future development through investigation regarding the structure-activity relationship, toxicity and their biological effects to design more potent and selective antimicrobial agents for therapeutic use.
3.4. Experimental

The sources of all the fatty acids and instrumentation details are the same as given in Chapter 1 (pg. 31).

General procedure for the synthesis of long-chain alkenoic acid hydrazide (1a-d)

The hydrazides of long chain alkenoic acids (1a-d) which are used as the starting material were prepared by the previously reported methods^5. 

Synthesis of 1-(alkenoyl/hydroxyalkenoyl)-5-phenylthiosemicarbazide (2a-d)

1-Alkenoyl-5-phenylthiosemicarbazides (2a-d) were prepared by the reported literature method^70. The synthesized compounds, 2a-d, were characterized by their melting points and spectral data. The spectral data of 2a and 2e is given below.

1-(Undec-10-enoyl)-5-phenylsemicarbazides (2a)

White powder; Yield 80%; Mp 104-107 °C.

IR (KBr): 3236, 1667, 1236 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 11.04 (s, 1H, CS-NH-Ar), 9.35 (br. s, 2H, CO-NHNH-CS), 8.14 (d, 2H, J= 7.2 Hz, Ar-H-2"/6"), 7.56 (t, 1H, J= 7.4 Hz, Ar-H-4"), 7.48 (t, 2H, J= 7.4 Hz, Ar-H-3"/5"), 5.85 (tdd, 1H, J_H-H₂ = 6.8 Hz, J_H-N =10.0 Hz, J_H-H₅ =17.8 Hz, CH₂=CH-), 5.04 (dd, 1H, J_H₂-H₅ =10.0 Hz, J_H₂-H₅ =2.8 Hz, H₂C=CH), 4.91 (dd, 1H,
2,5-Disubstituted-1,3,4-thiadiazoles

\[ J_{\text{H-H}} = 17.8 \text{ Hz}, \quad J_{\text{H-C}} = 2.8 \text{ Hz}, \quad H_2C=\text{CH-}, \quad 2.42 (t, 2H, J = 7.4 \text{ Hz}, \text{CH}_2\text{-CO}), \quad 2.03 (m, 2H, \text{CH}_2=\text{CH-CH}_2), \quad 1.85 (m, 2H, \text{CH}_2\text{CH}_2\text{-CO}), \quad 1.45-1.25 (\text{br. s, 10H, (CH}_2)_5). \]

\[^{13}C\text{NMR}\] (100 MHz, CDCl\textsubscript{3}): \( \delta \) 168.2, 165.4, 139.2, 133.2, 131.3, 128.9, 128.6, 114.2, 33.8, 29.9, 29.6, 29.3, 29.2, 29.1, 29.0, 28.9.

\( l^{-}(9Z,12R)-12\text{-hydroxy-octadec-9-enyl}-5\text{-phenylsemicarbazides (2c)} \)

Off-white powder; Yield 77%; Mp 148-150 \(^\circ\)C.

\[ ^1H\text{NMR}\] (400 MHz, CDCl\textsubscript{3}): \( \delta \) 11.07 (s, 1H, CS-NH-Ar), 9.32 (br. s, 2H, CO-NH\textsubscript{2}-CS), 8.14 (d, 2H, J= 7.4 Hz, Ar-H-2"/6"), 7.59 (t, 1H, J= 7.4 Hz, Ar-H-4"), 7.50 (t, 2H, J= 7.2 Hz, Ar-H-3"/5"), 5.32 (m, 2H, CH\textsubscript{2}-CH=CH-CH\textsubscript{2}), 4.83 (m, 1H, CH-OH), 2.51 (t, 2H, J = 7.6 Hz, CH\textsubscript{2}-CO), 2.36 (m, 4H, CH\textsubscript{2}-CH\textsubscript{2}=CH-CH\textsubscript{2}), 1.87 (m, 2H, CH\textsubscript{2}-CH\textsubscript{2}-CO), 1.71 (m, 1H, CH-OH), 1.44-1.28 (br. s, 18H, (CH\textsubscript{2})\textsubscript{9}), 0.86 (dist. t, 3H, terminus CH\textsubscript{3}).

\[^{13}C\text{NMR}\] (100 MHz, CDCl\textsubscript{3}): \( \delta \) 166.4, 163.7, 138.7, 136.3, 132.2, 128.1, 128.0, 127.8, 70.7, 40.1, 39.9, 39.7, 39.4, 39.2, 31.3, 30.4, 29.4, 29.2, 29.0, 28.8, 26.1, 22.0, 14.1.

\textit{Synthesis of 2-Phenylamine-5-(alkenyl/hydroxyalkenyl)-1,3,4-thiadiazoles (3a-d)}

One mmole of compounds (2a-d) in acetic anhydride (Ac\textsubscript{2}O) (6.0 ml) was refluxed for 5 h. The resulting mixture was poured into crushed ice (100gm) with stirring.
The product thus obtained was filtered, washed with cold water, dried and recrystallized from aqueous ethanol and acetone (1:4 ml v/v) to give analytically pure compounds 3a-d. These novel compounds 3a-d, prepared according to the above procedure were characterized from their spectral data.

**Spectroscopic Data**

*2-Phenylamine-5-(dec-9'-enoic)-1,3,4-thiadiazole (3a)*

White powder; Yield 95%; Mp 134-135 °C.

**IR** (KBr): 3221, 1488, 707 cm⁻¹.

**¹H NMR** (CDCl₃): 8 12.20 (s, 1H, NH), 8.16 (d, 2H, J = 8.5 Hz, Ar-H-2''/6''), 7.55 (t, 1H, J = 7.3 Hz, Ar-H-4''), 7.44 (t, 2H, J = 7.8 Hz, Ar-H-3''/5''), 5.73 (tdd, 1H, J_H-H₂ = 6.7 Hz, J_H-H₂ = 10.1 Hz, J_H-H₂ = 16.9 Hz, CH₂=CH-), 4.98 (dd, 1H, J_H-H₂ = 10.1 Hz, J_H-H₂ = 2.1 Hz, H₂C=CH), 4.85 (dd, 1H, J_H-H₂ = 16.9 Hz, J_H-H₂ = 2.1 Hz, H₂C=CH), 2.98 (t, 2H, J = 7.5 Hz, CH₂ α to ring), 1.96 (m, 2H, CH₂=CH-CH₂), 1.76 (m, 2H, CH₂ β to ring), 1.25 (br. s, 10H, (CH₂)₅).

**¹³C NMR** (CDCl₃): δ 165.2, 153.0, 139.1, 133.4, 131.3, 129.8, 127.9, 114.2, 33.7, 29.1, 28.9, 28.8, 28.7, 28.4, 26.0, 25.8.

**ESI-MS** found [M+Na]⁺ 338.2; C₁₈H₂₅SN₃ [M+Na]⁺ requires 338.47.
2,5-Disubstituted-1,3,4-thiadiazoles

2-Phenylamine-5-(Z)(heptadec-8'-enoic)-1,3,4-thiadiazole (3b)

White powder; Yield 91%; Mp 138-140 °C.

**IR (KBr):** 3219, 1468, 701 cm⁻¹.

**¹H NMR** (CDCl₃): δ 12.10 (s, 1H, NH), 8.14 (d, 2H, J= 8.5 Hz, Ar-H-2"/6"), 7.55 (t, 1H, J= 7.4 Hz, Ar-H-4"), 7.45 (t, 2H, J= 7.8 Hz, Ar-H-3"/5"), 5.30 (m, 2H, CH₂-CH=CH-CH₂), 2.98 (t, 2H, J = 7.7 Hz, CH₂ α to ring), 2.36 (m, 4H, CH₂-CH₂=CH-CH₂), 1.76 (m, 2H, CH₂ β to ring), 1.28 (br. s, 20H, (CH₂)₁₀), 0.80 (dist. t, 3H, terminus CH₃).

**¹³C NMR** (CDCl₃): δ 165.4, 158.0 “one signal hidden”, 139.1, 137.2, 133.2, 131.3, 128.8, 128.6, 31.9, 29.9, 29.7, 29.5 “two signals are hidden”, 29.4 “two signals are hidden”, 29.3, 29.1, 28.7, 26.4, 22.7, 14.2.

**ESI-MS** found [M+Na]⁺ 436.3; C₂₅H₃₉SN₃ [M+Na]⁺ requires 436.6.

2-Phenylamine-5-{(8'Z,11'R)-11'-hydroxy-heptadec-8'-enyl]-1,3,4-thiadiazole (3c)

White powder; Yield 85%; Mp 136-138 °C

**IR (KBr):** 3310, 3219, 1467, 695 cm⁻¹.

**¹H NMR** (CDCl₃): δ 12.37 (s, 1H, NH), 8.23 (d, 2H, J= 8.5 Hz, Ar-H-2"/6"), 7.62 (t, 1H, J= 7.4 Hz, Ar-H-4"), 7.52 (t, 2H, J= 7.8 Hz, Ar-H-3"/5"), 5.37 (m, 2H, CH₂-CH=CH-CH₂), 3.59 (s, 1H, CH-OH), 3.00 (t, 2H, J = 7.5 Hz, CH₂ α to ring), 2.38 (m, 4H, CH₂-
2,5-Disubstituted-1,3,4-thiadiazoles

CH₂=CH-CH₂), 1.83 (m, 2H, CH₂ β to ring), 1.71 (m, 1H, CH-OH), 1.26 (br. s, 18H, (CH₂)₉), 0.87 (dist. t, 3H, terminus CH₃).

¹³C NMR (CDCl₃): δ 170.9, 165.0, 133.3, 131.1, 130.7, 130.2, 128.8, 128.5, 73.9, 34.1, 31.5, 29.8, 29.7, 29.5, 29.3, 29.1, 28.9, 27.1, 25.2, 23.1, 22.5, 21.3, 14.6.

ESI-MS found [M+Na]⁺ 452.4; C₁₈H₃₉OSN₃ [M+Na]⁺ requires 452.6

2-Phenylamine-5-(8'R,11'Z)-8'-hydroxy-heptadec-11'-enyl]-1,3,4-thiadiazole (3d)

Off-white powder; Yield 83%; Mp 139-141 °C

IR (KBr): 3222, 3236, 1461, 699 cm⁻¹.

¹H NMR (CDCl₃): δ 12.17 (s, 1H, NH), 8.31 (d, 2H, J= 8.4 Hz, Ar-H-2")/6"), 7.43 (t, 1H, J= 7.4 Hz, Ar-H-4"), 7.25 (t, 2H, J= 7.4 Hz, Ar-H-3"/5"), 5.36 (m, 2H, CH₂-CH≡CH-CH₂), 3.57 (s, 1H, CH-OH), 2.81 (t, 2H, J= 7.5 Hz, CH₂ α to ring), 2.23 (m, 4H, CH₂-CH₂=CH-CH₂), 2.04 (m, 2H, CH₂ β to ring), 1.71 (m, 1H, CH-OH), 1.33 (br. s, 18H, (CH₂)₉), 0.90 (dist. t, 3H, terminus CH₃).

¹³C NMR (CDCl₃): δ 168.9, 163.1, 137.6, 133.2, 131.2, 128.7, 128.3, 125.5, 70.56, 40.1, 39.9, 36.3, 24.8, 31.3, 29.1, 29.0, 28.9, 28.8, 28.5, 28.4, 25.1, 22.3, 14.01.

ESI-MS found [M+Na]⁺ 452.5; C₁₈H₃₉OSN₃ [M+Na]⁺ requires 452.6.

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against Escherichia coli (ATCC-25922), Staphylococcus aureus (ATCC-25923),
*Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method\(^{66,67}\). Chloramphenicol (30 µg) was used as positive control and the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 3.3.2.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The minimum inhibitory concentrations and minimum bactericidal concentrations (MBCs) are given in Table 3.3.3. The PBE (percentual bacteriostatic efficiency, \%) was obtained as

\[
PBE = \frac{100}{MIC}
\]

The results have been reported in Fig. 3.3.1.

**Antifungal studies**

Antifungal activity was also done by disk diffusion method. For assaying antifungal activity *Candida albicans, Aspergillus fumigatus, Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by agar diffusion method\(^{68,69}\). The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 3.3.4.
The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) as determined by broth dilution technique are given in Table 3.3.5.

The ratio MFC/MIC was calculated in order to determine if the compound had a fungistatic (MFC/MIC ≥ 4) or fungicidal (MFC/MIC ≤ 4) activity and the results have been summarized in Fig. 3.3.2.
3.5. References


2,5-Disubstituted-1,3,4-thiadiazoles


2,5-Disubstituted-1,3,4-thiadiazoles


CHAPTER 4

3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones.
4.1 Theoretical

Triazinone is a heterocyclic compound which is structurally similar to fragment of some natural macromolecules. As a widely used biologically active compound the derivatives of triazinone exhibit anticancer\(^1\), antidiuretic and neurodepressant\(^2\), antimicrobial\(^3\), cardiotonic as well as blood platelet aggregation-inhibition effects\(^4\). The biological effects of 1,2,4-triazinone derivatives are largely determined by their ability to take up lose proton\(^5\).

The 1,2,4-triazinone derivative (i) is a useful antifungal agent. It can be administered parenterally, orally or locally to warm blooded animals in form of conventional solid pharmaceutical preparations\(^6\).

![Image of triazinone derivative]

The 2-aryl-1,2,4-benzotriazin-3-ones class of compounds have established themselves as attractive antimicrobial and antiviral drugs\(^7\). The azoloannealed triazinones (ii) has been proved to be neuraminidase inhibitors\(^8\). They have also displayed high activity against different kind of viruses including influenza and bird flu (culture H5N1)\(^9,10\). The 1,2,4-triazinone has recently much attention as a core structure of vardenafil (levitra) (iii), a potent and effective PDE5 inhibitor for the treatment of erectile disfunction\(^11\).
In the field of agriculture, the 1,2,4-triazinone class of compounds are used as insecticides, herbicides, plant growth regulators and increasing crop yields\textsuperscript{12,13}. Pymetrozine (iv), represents a typical triazinone compound, which has high efficiency, low toxicity and is environment friendly\textsuperscript{14}. Another herbicide metribuzin (v) is widely used for the control of grasses and broad-leaved weeds in soyabean, sugarcane and numerous other crops\textsuperscript{15}.

The various methods for the synthesis of 1,2,4-triazinone derivatives are reported by numerous workers. A common method for the synthesis of 1,2,4-triazinones is the cyclization of \( \alpha \)-(acylamino) carboxyhydrazides\textsuperscript{16}. The reaction of nitrilimines with \( \alpha \)-aminoacetetonitrile affords 1,2,4-triazinone in excellent yield\textsuperscript{17}. Further, cyclocondensation reactions of nitrilimines with 2-hydrazinoacetate or with \( \alpha \)-amino esters represent an important synthetic route for the preparation of 1,2,4-triazinone \textsuperscript{18,19}.
The synthesis of various 1,2,4-triazinone derivatives employing amidrazones and α-halo acid esters has been reported.

6-Aryl-3-pyridyl-1,2,4-triazinone (ix) have been prepared by the reaction of a keto-carboxylic acid or its ester (vi) with 2-pyridyl-hydrazine (vii), via the formation of an intermediate (viii) in presence of an inert solvent. The synthesized 1,2,4-triazinone exhibit a strong antifungal activity against the pathogenic fungi *Trichophyton rubrum* and *Candida albicans* (Scheme 4.1.1).

![Scheme 4.1.1](image)

vi vii viii ix

R= H, C₁-C₄ Alkyl  \hspace{1cm} \text{Ar} = \text{C}_6\text{H}_4\text{X, C}_1\text{C}_8 \text{ Alkyl, C}_1\text{C}_8 \text{ Alkylthio, Hydroxyl, Amino.}

Scheme 4.1.1

Various synthetic routes to access a variety of novel 1-aryl-2H,4H-tetrahydro-1,2,4-triazin-3-one analogs from various starting materials have been developed by Bhatia *et al.*. The synthesis of parent structure 1-phenylperhydro-1,2,4-triazin-3-one (xii), a selective inhibitor of 5-LO in broken cell, intact cell and human blood assay, was achieved by the reaction of 2-chloroethyl isocyanate (x) with (3-fluorophenyl)hydrazine (Scheme 4.1.2).
3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones

\[ \text{Et}_3\text{N, H}_2\text{NNHR}_1 \quad \xrightarrow{\text{CH}_2\text{Cl}_2} \quad \text{Nal, DMF} \quad 75^\circ \text{C} \]

\[ \begin{align*}
R_1 &= 3\text{-Fluorophenyl} \\
R_2 &= \text{H}
\end{align*} \]

Scheme 4.1.2

Zou et al.\textsuperscript{22} gave the synthesis of 1,10-phenanthroline[5,6-\text{e}]-1,2,4-triazin-3-one (xiv) by heating a mixture of phenanthroline-5,6-dione semicarbazone (xiii) with acetic acid and ammonium acetate (Scheme 4.1.3).

\[ \text{acetic acid} \quad \xrightarrow{\text{NH}_4\text{Ac}} \quad \text{xiv} \]

Scheme 4.1.3

The refluxing of a mixture of 9-amino-7-(4'-chlorophenyl)-8,9-dihydro-8-imino-6\text{H},7\text{H}-[1]benzopyran[3',4',5,6]-pyrano[2,3-d]-pyrimidine-6-one (xv), ethyl chloroacetate, methanol and sodium metal for 6 h gave 15-(4'-chlorophenyl)-3,4-dihydro-2\text{H},14\text{H},15\text{H}-[1]benzopyran[3',4',5,6]-pyrano[2,3-d]-pyrimido-[1,6-b][1,2,4]-triazine-3,14-dione (xvi)\textsuperscript{23} (Scheme 4.1.4).
Heim-Riether et al. synthesized the imidazo[5,1-\textit{f}][1,2,4]-triazinones (xix) which are of pharmaceutical importance as isosteres of purine. The key step involved the formation of N-aminoimidazoles (xviii) from 3\textit{H}-imidazoles (xvii) by treatment with lithium hexamethyldisilazane and o-(diphenylphosphinyl)hydroxylamine, and further cyclization with formamide to yield corresponding imidazotriazinones (xix) (Scheme 4.1.5).

A facile method for the synthesis of substituted bis(indole)-1,2,4-triazinones (xxii) is given by Garg et al. The access to the target triazinones involved the cyclocondensation reaction between amidrazone (xx) and ketoester (xxi) functionalities in presence of MgSO\textsubscript{4} in methanol and refluxing in DMF (Scheme 4.1.6).
3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones

The conversion of nitroguanidine imidacloprid (xxiii) to its methyltriazinone derivative (xxv) via the formation of its aminoguanidine (xxiv) derivative is described by Kanne et al.\textsuperscript{26} (Scheme 4.1.7).
The 1,2,4-triazolo[3,4-f][1,2,4]triazinone (xxvii) was synthesized from the reaction of the corresponding hydrazine derivative (xxvi) with carbon disulfide (Scheme 4.1.8).

\[
\text{xxvi} \quad \text{CS}_2 \quad \text{xxvii}
\]

Scheme 4.1.8

The diazotization of o-(alkylazo)anilines (xxviii) gave the o-(alkylazo)aryldiazonium tetrafluoroborates (xxix) which on further reaction with an excess of sodium cyanate in MeCN yielded the 2-alkyl-1,2,4-benzotriazin-3(2H)-ones (xxx) (Scheme 4.1.9).

\[
\text{xxviii} \quad \text{NOBF}_4 \quad \overrightarrow{\text{NaNCO MeCN}} \quad \text{xxx}
\]

Scheme 4.1.9

A simple and high yielding method for the integration of a 1,2,4-triazole ring with 1,2,4-triazine-5-one is developed by Kamble et al. The bromination and 1,3-dipolar cycloaddition reaction of 3-arylsyndone (xxxi) formed 3-aryl-5-methyl-2-oxo-Δ^4-1,3,4-
oxadiazoles (xxxii). The reaction of (xxxii) with semicarbohydrazide formed an intermediate (xxxiii) which underwent cyclization with pyruvic acid in presence of acetic acid fused with sodium acetate to yield 6-methyl-4-(3'-methyl-5'-oxo-1'-aryl-1,5-dihydro-1,2,4-triazol-4'-yl)-3-oxo/thioxo-3,4-dihydro-2H-1,2,4-triazin-5-one (xxxiv) (Scheme 4.1.10).

![Chemical Reaction Diagram](image)

\[ R = \text{C}_6\text{H}_5, \text{p-CH}_3\text{C}_6\text{H}_4, \text{p-OCH}_3\text{C}_6\text{H}_4, \text{p-ClC}_6\text{H}_4, \text{p-BrC}_6\text{H}_4 \quad X = \text{O, S} \]

**Scheme 4.1.10**

The reaction of diethyl aminomalonate (xxxv) with dimethylformamide dimethyl acetal gave the amidine (xxxvi) which on condensation with hydrazine afforded the dihydrotriazinone (xxxvii). The (xxxvii) could be oxidized to corresponding triazinone (xxxviii) by treatment with iodobenzene diacetate\(^{30}\) (Scheme 4.1.11).
Sanudo et al.\textsuperscript{31} gave the synthesis of a new class of dipeptidyl ureas, 3-hydroxy-6-oxo[1,2,4]triazin-1-yl alaninamides (\textit{xLiii}). The Ugi reaction of cyclohexyl/benzyl isocyanide (\textit{xLi}), benzoyl/-4-methoxybenzoyl formic acid (\textit{xL}) and semicarbazones (\textit{xxxix}) gave the Ugi adduct (\textit{xLii}), which on stirring with sodium ethoxide in ethanol gave the desired triazinone (\textit{Scheme 4.1.12}).
6-Amino-3-benzylmercapto-1,2,4-triazolo[3,4-$f$][1,2,4]-triazin-8(7$H$)-one (xLvi) were synthesized by stirring a solution of 6-amino-3-thio(2$H$)-1,2,4-triazolo-[3,4-$f$][1,2,4]-triazin-8(7$H$)-one (xLiv) and benzyl bromide (xLv) in methanolic ammonia$^{32}$ (Scheme 4.1.13).

![Scheme 4.1.13](image)

The synthesis of 6-methyl-2,5,5-trihydro-3-oxo-4-amino-1,2,4-triazine (xLix) from 2-oxo-5-methyl-1,3,4-oxadiazol-3-acetone (xLvii) via the formation of 6-methyl-2,5,5-trihydro-3-oxo-4-acetamide-1,2,4-triazine (xLviii) is given by Zhang et al.$^{33}$ (Scheme 4.1.14).

![Scheme 4.1.14](image)

The exposure of amidrazone (L) to ketoester (Li) in presence of MgSO$_4$ in DMF afforded the anti- (Li$ii$) and syn-triazinone (Li$iii$) as the major and minor products respectively$^{34}$ (Scheme 4.1.15).
Dalloul et al.\textsuperscript{18} gave the synthesis of 4,5-dihydro-1,2,4-triazinones (Lvii) by the reaction of an $\alpha$-amino ester (Lv) with nitrilimines (Liv). The synthesis proceeded via the formation of an open-chain amidrazone ester intermediate (Lvi) which underwent cyclization to yield the title 1,2,4-triazinones (Scheme 4.1.16).

The substituted 4,5-dihydro-1,2,4-triazin-6-ones (Lx) were prepared by the direct interaction of hydrazonyl halides (Lviii), nitrilimines precursors with $\alpha$-amino esters in presence of triethylamine as a base or by treatment of amidrazones (Lix) with $\alpha$-haloesters\textsuperscript{35} (Scheme 4.1.17).
Thaher et al.\textsuperscript{36} reported the synthesis of dihydro-1,2,4-triazin-6-one containing nitroarginine moiety. The reaction of nitrilimines (Lxi) with nitroarginine methyl ester (Lxii) at room temperature, through a cyclocondensation reaction yielded 1-aryl-3,5-disubstituted-1,2,4-triazin-6-ones (Lxiii) (Scheme 4.1.18).
An efficient method for the incorporation of $^{15}$N-isotope into 1,2,4-triazolo[5,1-c][1,2,4]triazines have been developed by Shestakova et al. The diazoles (Lxv) were obtained by the treatment of 2-\(R\)-amino-1,2,4-triazole (Lxiv) with H$^{15}$NO$_2$ (86% label) under acidic conditions. The reaction of (Lxv) with ethyl nitroacetate in presence of sodium carbonate gave the salt (Lxvi) which was acidified to give the [5-$^{15}$N]-6-nitro-1,2,4-triazolo[5,1-c][1,2,4]-triazin-7-ones (Lxvii) (Scheme 4.1.19).
The reaction of amidrazone (Lxviii) with formaldehyde in presence of p-toluenesulfonic acid yielded 4-(2-chloropenyl)-2-phenyl-6-(piperidin-1-yl)-3,4-dihydro-1,2,4-triazin-5(2H)-one (Lxix)\(^{38}\) (Scheme 4.1.20).

Scheme 4.1.20
Mullick et al.\textsuperscript{39} gave the synthesis of 5,6-diphenyl-1,2,4-triazine-3(2\textsubscript{H})-thioxo/thione (Lxxi) by the reaction of benzyl (Lxx) with an equimolar amount of semicarbazide or thiosemicarbazide in presence of acetic acid (Scheme 4.1.21).

\begin{center}
\begin{tikzpicture}
  \node at (0,0) {\textbf{Lxx}};
  \node at (2,0) {\textbf{Lxxi}};
  \draw[->] (0.5,0) -- (2,0);
  \draw (0.5,0) -- (1,0.5);
  \draw (0.5,0) -- (1,-0.5);
  \draw (1,0.5) -- (1.5,1);
  \draw (1,0.5) -- (1.5,-1);
  \draw (1,-0.5) -- (1.5,-1);
  \draw (1.5,1) -- (2,0.5);
  \draw (1.5,-1) -- (2,-0.5);
  \node at (1,0.8) {\textbf{Semicarbazide/Thiosemicarbazide}};
  \node at (1,-0.8) {\textbf{acetic acid}};
\end{tikzpicture}
\end{center}

$X = S, O$

\textbf{Scheme 4.1.21}

The synthesis of 8-(4-chlorophenyl)-2,6-dioxo-1,3,4,6-tetrahydro-2\textit{H}-pyrido[1,2-b][1,2,4]-triazine-7,9-dicarbonitrile (Lxxiii) was achieved by refluxing a mixture of 4-aryl-1,6-diamino-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (Lxxii) and monochloroacetic acid in DMF\textsuperscript{40} (Scheme 4.1.22).

\begin{center}
\begin{tikzpicture}
  \node at (0,0) {\textbf{Lxxii}};
  \node at (3,0) {\textbf{Lxxiii}};
  \draw[->] (0.5,0) -- (3,0);
  \draw (0.5,0) -- (1,0.5);
  \draw (0.5,0) -- (1,-0.5);
  \draw (1,0.5) -- (1.5,1);
  \draw (1,-0.5) -- (1.5,-1);
  \draw (1.5,1) -- (2,0.5);
  \draw (1.5,-1) -- (2,-0.5);
  \node at (1,0.8) {\textbf{ClCH\textsubscript{2}COOH}};
  \node at (1,-0.8) {\textbf{DMF}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 4.1.22}
4.2. Facile One-pot Synthesis and in vitro Antimicrobial Activity of Novel 3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones.

In the recent years the chemistry of triazinone derivatives has been a subject of great interest due to the use of such ring systems as the core structure in many heterocyclic compounds covering a wide range of pharmacological applications. Several reports have revealed that 1,2,4-triazinone derivatives possess significant biological activities, such as antimicrobial, antibacterial, fungicide, pesticide, herbicide and crop protection, as well as blood platelet aggregation-inhibition. Researchers have also reported that certain 1,2,4-triazin-6-one possess antitumor activity against ovarian cancer, leukemia, breast cancer and small and large lung cancer cells. Apart from the pharmacological applicability, the 1,2,4-triazinones have also received significant attention as ligands to transition metals. The reaction of 1,3,5-trisubstituted 4,5-dihydro-1,2,4-triazine oximes with metal acetates afforded various complexes depending on the reagents and conditions used.

In continuation to the research work done in our laboratory, involving the synthesis of various derivatives of fatty acids, of synthetic and biological importance, we here report a facile one-pot synthesis of various 1,6-dihydro-1,2,4-triazin-5(2H)-ones, bearing a long alkanyl/alkenyl/hydroxy alkenyl chain at C-3. In this chapter, we for the first time report a simple and effective synthesis of various 1,6-dihydro-1,2,4-triazinone derivatives using various fatty acid hydrazides and chloroacetamide as the starting
material. The reaction described herein has a remarkable synthetic utility and is a valuable addition to the synthesis and manipulation of fatty acid derivatives because it makes the target compounds available in one step starting from fatty acid hydrazides. The newly synthesized compounds were also screened for their antibacterial and antifungal activities against a panel of Gram-positive and Gram-negative strains of bacteria and selected fungal strains.
4.3. Results and Discussion

To the prior the fatty acid hydrazides, 1a-f used as the starting materials were prepared from the fatty acids following the previously reported method\textsuperscript{51}. The 3-substituted 1,6-dihydro-1,2,4-triazin-5-(2H)-ones 3a-f were synthesized by the cyclocondensation of long chain saturated and olefinic carboxylic acid hydrazides (1a-f) with chloroacetamide (2) in $N,N$-dimethylformamide (DMF) under reflux conditions for a duration of 26-30 h (Scheme 4.3.1).

\begin{center}
\textbf{Scheme 4.3.1. Synthesis of 3-substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones 3a-f}
\end{center}
Table 4.3.1. 3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones 3a-f

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting from</th>
<th>R</th>
<th>Product</th>
<th>Yield(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a, 2</td>
<td></td>
<td>3a</td>
<td>78</td>
</tr>
<tr>
<td>2</td>
<td>1b, 2</td>
<td></td>
<td>3b</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>1c, 2</td>
<td></td>
<td>3c</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>1d, 2</td>
<td></td>
<td>3d</td>
<td>78</td>
</tr>
<tr>
<td>5</td>
<td>1e, 2</td>
<td></td>
<td>3e</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>1f, 2</td>
<td></td>
<td>3f</td>
<td>85</td>
</tr>
</tbody>
</table>

As can be seen from Table 4.3.1, the scope of the reaction using saturated, olefinic (internal and terminal) and hydroxy acid hydrazides was found to be good. The yields of synthesized 3-substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones (3a-f) did not depend on the length of chain of acid hydrazide and were found to be appreciable in all cases. The synthesized compounds were identified on the basis of elemental analysis, IR, $^1$H NMR and mass spectral data.

The structure of compound 3a was confirmed by the appearance of absorption band at 3224 cm$^{-1}$ due to N-H stretch. Another absorption band at 2921 cm$^{-1}$ was due to aliphatic stretch. The absorption band for C=O group was observed at 1675 cm$^{-1}$. The other prominent absorption band observed in the IR spectrum was at 1599 cm$^{-1}$ corresponding to C=N group. The $^1$H NMR spectrum of 3a was more informative and showed a singlet at $\delta$ 2.52 ppm attributable to CH$_2$ protons of the triazinone ring. The NH protons resonated as two broad singlets at $\delta$ 9.45 ppm and $\delta$ 8.60 ppm. The methine
proton of C-9 showed a signal at \( \delta 5.71 \). The C-10 methylene protons designated as \( H_E \) and \( H_Z \) displayed two distinct \( \delta \) values when coupled with adjacent C-9 methine proton. Thus, the \(^1H\) NMR showed two doublet of doublet at \( \delta 4.91 \) and 4.84 for \( H_Z \) and \( H_E \) protons respectively. Further evidence for the formation of 1,6-dihydro-1,2,4-triazin-5-(2H)-ones (3a-f) were obtained by recording the mass spectrum. The mass spectrum of compound 3a showed the molecular ion peak at \( m/z 237.32 \) corresponding to the molecular formula \( C_{13}H_{23}N_3O \). Similarly, the structures of compounds 3b-f were confirmed from their spectral data and elemental analysis in a straightforward manner. Detailed spectra of title compounds are given in experimental section.

Antibacterial studies

All the synthesized compounds were further tested for their antibacterial activity by disk diffusion assay\(^{52,53}\) with slight modifications against an assortment of two Gram-positive (\( \text{S. pyogenes} \) and \( \text{S. aureus} \)) and three Gram-negative (\( \text{P. aeruginosa}, \text{K. pneumoniae} \) and \( \text{E. coli} \)) strains of bacteria. Ciprofloxacin was used as the standard drug whereas DMSO was used as the negative control. The screening data was obtained as diameter of zone of inhibition and the results are summarized in Table 4.3.2. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The data pertaining to Table 4.3.2 showed that the compounds 3c, 3d and 3f have a significant influence on the anti-bacterial profile of \( \text{S. pyogenes}, \text{S. aureus} \) and \( \text{E. coli} \) species at 6.25 \( \mu g/ml \) concentrations.
### Table 4.3.2 Antibacterial activity of newly synthesized compounds (3a-f)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. pyogenes</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td><strong>3a</strong></td>
<td>11.3</td>
<td>11.2</td>
</tr>
<tr>
<td><strong>3b</strong></td>
<td>14.1</td>
<td>13.3</td>
</tr>
<tr>
<td><strong>3c</strong></td>
<td>17.1</td>
<td>16.3</td>
</tr>
<tr>
<td><strong>3d</strong></td>
<td>17.1</td>
<td>16.5</td>
</tr>
<tr>
<td><strong>3e</strong></td>
<td>9.2</td>
<td>8.9</td>
</tr>
<tr>
<td><strong>3f</strong></td>
<td>18.6</td>
<td>17.3</td>
</tr>
<tr>
<td><strong>Standard</strong></td>
<td>23.0</td>
<td>22.0</td>
</tr>
<tr>
<td><strong>DMSO</strong></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (standard); Ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm)

The Fig. 4.3.1 indicates that the *in-vitro* screening of synthesized compounds gives promising results as compared to the reference drug Ciprofloxacin.
3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones

![Bar graph showing percent inhibition for compounds 3a-f over reference drug against bacterial strains.]

**Fig. 4.3.1. Percent inhibition for compounds 3a-f over reference drug against bacterial strains.**

SP = *S. pyogenes*, SA = *S. aureus*, PA = *P. aeruginosa*, KP = *K. pneumonia*, EC = *E. Coli*

The MICs and MBCs of the synthesized compounds were also determined by broth dilution technique and the results are given in Table 4.3.3. The MBC of few compounds was found to be the same as MIC but in most of the compounds it was two or three folds higher than the corresponding MIC results.
Table 4.3.3. MIC and MBC results of synthesized compounds (3a-f)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. pyogenes</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>3a</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3b</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>3c</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>3d</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>3e</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3f</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Standard</td>
<td>12.5</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Positive control Ciprofloxacin; MIC (µg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (µg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely.

Antifungal studies

In another set of experiments, the synthesized compounds 3a-f were also examined for their antifungal activity against Candida albicans, Aspergillus fumigatus, Trichophyton mentagrophytes and Penicillium marneffei fungal strains and the results in the form of diameter of zone of inhibition are tabulated in Table 4.3.4. The antifungal screening data revealed that the compounds showed moderate activity. Among
the screened compounds 3d and 3f showed good fungicidal activity against C. albicans, 
*A. fumigatus* and *P. marneffei* fungal strains.

Table 4.3.4. Antifungal activity of synthesized compounds (3a-f).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>AF</th>
<th>TM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>15.8</td>
<td>12.9</td>
<td>8.7</td>
<td>7.1</td>
</tr>
<tr>
<td>3b</td>
<td>16.1</td>
<td>13.1</td>
<td>9.9</td>
<td>6.9</td>
</tr>
<tr>
<td>3c</td>
<td>16.3</td>
<td>13.2</td>
<td>10.9</td>
<td>7.1</td>
</tr>
<tr>
<td>3d</td>
<td>22.9</td>
<td>22.8</td>
<td>17.1</td>
<td>12.6</td>
</tr>
<tr>
<td>3e</td>
<td>15.1</td>
<td>11.8</td>
<td>8.1</td>
<td>7.2</td>
</tr>
<tr>
<td>3f</td>
<td>23.9</td>
<td>20.6</td>
<td>17.1</td>
<td>12.9</td>
</tr>
<tr>
<td>Standard</td>
<td>30</td>
<td>27</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (Greseofulvin) and negative control (DMSO) measured by the Halo Zone Test (Unit, mm). CA= *Candida albicans*, AF= *Aspergillus fumigatus*, TM= *Trichophyton mentagrophytes*, PM= *Penicillium marneffei.*

The Fig. 4.3.2 depicts the antifungal potency of synthesized compounds over reference drug Greseofulvin in terms of % inhibition.
3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones

Fig 4.3.2. Percent inhibition of compounds 3a-f over reference drug against fungal strains.

CA = Candida albicans, AF = Aspergillus fumigatus, TM = Trichophyton mentagrophytes, PM = Penicillium marneffei.

The MICs and MFCs of compounds 3a-f were determined and the results are given in Table 4.3.5. The data in Table 4.3.5 reveals that MFC of most of the compounds was two or three folds higher than the corresponding MIC results.
### Table 4.3.5. MIC and MFC of synthesized compounds (3a-f).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>AF</th>
<th>TM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
</tr>
<tr>
<td>3a</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3b</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>3c</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>3d</td>
<td>12.5</td>
<td>25</td>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td>3e</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3f</td>
<td>12.5</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
</tr>
</tbody>
</table>

CA = Candida albicans, AF = Aspergillus fumigatus, TM = Trichophyton mentagrophytes, PM = Penicillium marneffei. MIC (µg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (µg/ml) = minimum fungicidal concentration, i.e. the lowest concentration of the compound for killing the fungus completely.

The antimicrobial screening data also revealed that the compounds 3a-f have produced a marked enhancement in the potency of the 1,2,4-triazinone analogs as antibacterial and antifungal agents.
4.4 Experimental

The sources of all the fatty acids and instrumentation details are same as given in Chapter 1 (pg. 31).

General procedure for the synthesis of 3-alkanyl/alkenyl/hydroxyl alkenyl-1,6-dihydro-1,2,4-triazin-5-(2H)-one (3a-f)

The fatty acid hydrazides, 1a-f (0.01 mol) and chloroacetamide, 2 (0.01 mol) were refluxed in DMF (180 ml) for 26-30 h. After the completion of the reaction (monitored by TLC), the reaction mixture was concentrated, cooled and poured in ice cold water (100 gm). The desired triazinone which separated out was filtered and dried. Further purification by column chromatography over silica gel using petroleum ether-diethyl ether mixture as eluent afforded the compounds 3a-f (Scheme 4.3.1). The novel compounds 3a-f were characterized from their spectral data.

Spectroscopic Data

3-(Dec-9'-enyl)-1,6-dihydro-1,2,4-triazin-5-(2H)-one (3a)

Brown powder; Yield 78%; Mp 151-153 °C.

IR (KBr): 3224, 2921, 1675, 1599 cm⁻¹.

$^1$H NMR (400 MHz, CDCl₃): $\delta$ 9.27 (s, 1H, NH), 7.42 (s, 1H, NH), 5.71 (tdd, 1H, $^\gamma$-H, $^\alpha$-H, $^\beta$-H =0.2 Hz, $^\gamma$-$^\beta$ =17.0 Hz, CH₂=CH), 4.91 (dd, 1H, $^\alpha$-H, $^\gamma$-H =10.1 Hz, $^\gamma$-$^\beta$ =2.2 Hz, H₂C=CH), 4.84 (dd, 1H, $^\alpha$-H, $^\gamma$-H =16.7 Hz, $^\gamma$-$^\beta$ =2.2 Hz, H₂C=CH), 2.52
3-(Z)-(Heptadec-8'-enyl)-1,6-dihydro-1,2,4-triazin-5-(2H)-one (3b).

Brown solid; yield 85%; mp 150-153 °C.

IR (KBr): 3219, 2920, 1680, 1597 cm^{-1}.

^{1}H NMR (CDCl₃): δ 9.55 (s, 1H, NH), 8.02 (s, 1H, NH), 5.31 (m, 2H, CH₂-CH=CH(CH₂), 2.58 (s, 2H, CH₂ of triazinone ring), 2.24 (t, 2H, J=7.4 Hz, CH₂ α to ring), 1.99 (m, 4H, CH₂-CH=CH(CH₂), 1.59 (m, 2H, CH₂ β to ring), 1.25 (br. s, 20H, (CH₂)₁₀), 0.87 (dist. t, 3H, terminus CH₃).

MS (ESI): m/z = 358 [M+Na]⁺, calculated = 358.17.

Anal. Found: C, 71.39; H, 10.74; N, 12.43. C₂₀H₃₇N₃O requires C, 71.60; H, 11.10; N, 12.52%.

3-[(8'Z, 11'R)-11'-Hydroxyoctadec-8'-enyl]-1,6-dihydro-1,2,4-triazin-5-(2H)-one (3c).

Sticky solid; yield 67%; mp 153-154 °C.

IR (KBr): 3224, 2925, 1732, 1597 cm⁻¹.
3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones

$^1$H NMR (CDCl$_3$): $\delta$ 9.45 (s, 1H, NH), 8.04 (s, 1H, NH), 6.38 (m, 2H, CH$_2$-CH=CH-CH$_2$), 3.57 (m, 1H, CH-OH), 2.57 (s, 2H, CH$_2$ of triazinone ring), 2.24 (t, 2H, $J=7.5$ Hz, CH$_2$ α to ring), 2.15 (m, 1H, CH-OH), 2.01 (m, 4H, CH$_2$-CH=CH-CH$_2$), 1.55 (m, 2H, CH$_2$ β to ring), 1.25 (br, s, 18H, (CH$_2$)$_9$), 0.87 (dist. t, 3H, terminus CH$_3$).

MS (ESI): $m/z = 374$ [M+Na]$^+$, calculated = 374.16.

Anal. Found: C, 68.05; H, 10.47; N, 11.77. C$_{20}$H$_{37}$N$_3$O$_2$ requires C, 68.34; H, 10.60; N, 11.95%.

3-[(8'R, 11'Z)-8'-Hydroxyoctadec-11'-enyl]-1,6-dihydro-1,2,4-triazin-5-(2H)-one (3d).

Brown powder; Yield 78%; Mp 152-154 °C.

IR (KBr): 3228, 2920, 1683, 1606 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): $\delta$ 9.59 (s, 1H, NH), 8.00 (s, 1H, NH), 5.34 (m, 2H, CH$_2$-CH=CH-CH$_2$), 3.58 (m, 1H, CH-OH), 2.57 (s, 2H, CH$_2$ of triazinone ring), 2.25 (t, 2H, $J=7.4$ Hz, CH$_2$ α to ring), 2.01 (m, 4H, CH$_2$-CH=CH-CH$_2$), 2.12 (m, 1H, CH-OH), 1.58 (m, 2H, CH$_2$ β to ring), 1.29 (br, s, 18H, (CH$_2$)$_9$), 0.87 (dist. t, 3H, terminus CH$_3$).

MS (ESI): $m/z = 374$ [M+Na]$^+$, calculated = 374.16.

Anal. Found: C, 67.98; H, 10.40; N, 11.71. C$_{20}$H$_{37}$N$_3$O$_2$ requires C, 68.34; H, 10.60; N, 11.95%.
3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones

3-Pentadec-1,6-dihydro-1,2,4-triazin-5-(2H)-one (3e).

Brown powder; Yield 82%; Mp 148-150 °C.

IR (KBr): 3207, 2920, 1678, 1596 cm⁻¹.

¹HNMR (CDCl₃): δ 9.38 (s, 1H, NH), 8.00 (s, 1H, NH), 2.56 (s, 2H, CH₂ of triazinone ring), 2.20 (t, 2H, J=7.4 Hz, CH₂ α to ring), 1.61 (m, 2H, CH₂ β to ring), 1.25 (br, s, 24H, (CH₂)₁₂), 0.87 (dist. t, 3H, terminus CH₃).

MS (ESI): m/z = 232 [M+Na]^⁺, calculated = 332.15.


3-[1-Bromopentadec]-1,6-dihydro-1,2,4-triazin-5-(2H)-one (3f).

Brown powder; Yield 85%; Mp 150-152 °C.

IR (KBr): 3228, 2920, 1678, 1596, 724 cm⁻¹.

¹HNMR (CDCl₃): δ 9.32 (s, 1H, NH), 8.02 (s, 1H, NH), 2.59 (s, 2H, CH₂ of triazinone ring), 2.24 (t, 2H, J=7.4 Hz, CH₂ α to ring), 1.60 (m, 2H, CH₂ β to ring), 1.25 (br, s, 24H, (CH₂)₁₂), 0.87 (dist. t, 3H, terminus CH₃).

MS (ESI): m/z = 411 [M+Na]^⁺, calculated = 411.05.

Anal. Found: C, 55.42; H, 08.76; N, 10.76. C₁₈H₃₅N₃OBr requires C, 55.66; H, 08.81; N, 10.81%.
Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method. Ciprofloxacin was used as positive control. While the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 4.3.2.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) are given in Table 4.3.3.

Antifungal studies

Antifungal activity was also done by disk diffusion method. The antifungal activity was assayed against *Candida albicans*, *Aspergillus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) by agar diffusion method. The fungal activity of each compound was compared with Greseofulvin which was used as a standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 4.3.4.
The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) as determined by broth dilution technique are given in Table 4.3.5.

The percent inhibition (\% inhibition) values of antibacterial (Fig. 4.3.1) and antifungal (Fig. 4.3.2) activity for different synthesized compounds over reference drug, were calculated using the following formula

\[
\% \text{ inhibition} = \frac{\alpha - \beta}{\alpha} \times 100
\]

where \( \alpha \) stands for the zone of inhibition of positive control (reference drug).

and \( \beta \) stands for the zone of inhibition of synthesized compounds.
4.5. References


3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H)-ones


CHAPTER-5

Fatty acid alkenoates
5.1 Theoretical

The esterification of carboxylic acids with alcohol is one of the most fundamental and important reactions in organic synthesis. The reaction gains its popularity due to the wide utility of esters in the field of organic, bio-organic and related fine chemical synthesis. The esters owing to their stability and easy interconvertibility serve to be the common intermediate in a large number of chemical reactions.

The fatty ester analogs have received little attention despite of the fact that such molecules have shown diverse biological activities such as antioxidant\(^1\), antifeedent\(^2\), bacterial\(^3\), herbicidal\(^4\), anti-inflammatory\(^5\) and neuroprotective activities\(^6\). Some fatty acids have also been effectively used in the treatment of cardiovascular, dermatitis\(^7\), hepatic and renal disorders\(^8\). Thus fatty acid esters, due to their great biological importance can be explored as new pharmacophores for the development of potential pharmaceutical molecules.

A large number of methods have been reported for the esterification of fatty acids\(^9\). The esterification reaction is usually performed by reacting fatty acids with a suitable alcohol in presence of different catalysts. A large number of catalysts such as alkyl chloroformate and Et\(_3\)N\(^10\), phenyl dichlorophosphate\(^11\), SiO\(_2\)/NaHSO\(_4\)\(^12\), amberlyst 15\(^13\), USY zeolite\(^14\), MoO\(_2\)/ZrO\(_2\)\(^15\), MgSO\(_4\)/H\(_2\)SO\(_4\)\(^16\), salycilic resin/FeCl\(_3\)\(^17\), SiO\(_2\)\(^18\), celite/CsF\(^19\), dowex 50WXZ\(^20\), beta zeolite\(^21\), H\(^+\)/PhH\(^22\), CH\(_2\)N\(_2\) in ether\(^23\), CH\(_3\)SO\(_3\)H\(^24\), SOCl\(_2\)\(^25\), ZrCl\(_4\)\(^26\), PPh\(_3\)/CH\(_3\)CN/NC\(_3\)H\(_4\)-N=N-C\(_3\)H\(_4\)N\(^27\), anhydride/iPr\(_2\)NEt/(+)\()-\)BTM/CH\(_2\)Cl\(_2\)\(^28\) etc. have been involved in this transformation. Inspite of the fact that a variety of catalyst are available the \(N,N\)-dicyclohexyl-carbodiimide (DCC) remains the most widely used because most of the reported methods suffer from some drawback or
the other which include, the use of transition metal, long reaction time, high temperature etc.

Garazd et al.\textsuperscript{29} reported the synthesis of 7-Boc-aminoacyloxy-3-phenoxy-2-trifluoromethylchromone (ii) from 7-hydroxy-3-phenoxy-2-trifluoromethylchromone (i) and Boc-promoted amino acid using DCC and a catalytic amount of 4-dimethylaminopyridine (DMAP) (Scheme 5.1.1).

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme511}
\end{center}

\textbf{Scheme 5.1.1}

New organosilane compounds (v) containing cholesteryl group have been synthesized by esterification reaction between 3,3,5,5-tetramethyl-3,5-disila-4-oxa-cyclohexane-1-carboxylic acid (iii) and \(\omega\)-hydroxyalkyl cholesteryl carbonate (iv) in presence of DCC and DMAP. Their phase behaviours have also been investigated\textsuperscript{30} (Scheme 5.1.2).
Sonpatki et al. developed an improved method by synthesizing new keto-ester in presence of DCC and DMAP. 2-Bromothiophene (vi), succinic anhydride and anhydrous aluminium chloride in nitrobenzene were stirred at -5 to -10 °C to yield the γ-keto acid (vii). The γ-keto acid reacted with dodecan-1-ol in presence of DCC and DMAP in dichloromethane to give the γ-keto ester (viii) (Scheme 5.1.3).

Scheme 5.1.2

Scheme 5.1.3
The synthesis of liquid crystalline promoter (x) was achieved by Dardel et al.\textsuperscript{32} by the reaction of (ix) with 4-hydroxycyanobiphenyl in presence of DCC and DMAP at room temperature (Scheme 5.1.4).

![Scheme 5.1.4](image)

A new approach to the design of systems for specific and nonspecific transport of nucleoside analogs using spacers with moderately labile ester bonds has been developed\textsuperscript{33}. Azidothymidine (xii) and geraniol monosuccinate (xi) in anhydrous ethyl acetate; DCC and DMAP to yield geraniol-azidothymidine succinate (xiii) as the major product, which possessed considerable antiviral potential (Scheme 5.1.5).

![Scheme 5.1.5](image)
Fatty acid alkenoates

Greetings et al. synthesized natural compounds like hydroxytyrosol (xiv) and reacted them with three equivalents of steric acid (xv) and DCC in presence of DMAP to obtain a triester (xvi) (Scheme 5.1.6).

\[
\text{xiv} + 3 \text{v} \xrightarrow{\text{DCC/DMAP, rt}} \text{xvi}
\]

Scheme 5.1.6

The synthesis of 4-benzyloxybenzyl crotonate (xix) was achieved by condensing 4-benzyloxybenzyl alcohol (xvii) and crotonic acid (xviii) in presence of DCC and DMAP in dichloromethane under argon at room temperature (Scheme 5.1.7).

\[
\text{xvii} + \text{xviii} \xrightarrow{\text{DCC, DMAP}} \text{xix}
\]

Scheme 5.1.7

The synthesis and liquid crystal properties of aryl esters of laterally substituted 5-pyrimidinecarboxylic acids has been reported by Mikhaleva. The addition of DMAP
and DCC to a suspension of acid (xx) and the appropriate phenol yielded the aryl ester (xxi) (Scheme 5.1.8).

\[
\text{BuO-} + \text{R} = \text{CH}_3, \text{OCH}_3 \quad \text{Ar} = 4-\text{Bu-C}_6\text{H}_4, 4-\text{CN-C}_6\text{H}_4
\]

\[
\text{xx} \quad \text{OH-Ar} \quad \text{DCC, DMAP} \quad \text{xxi}
\]

Abd-El-Aziz and coworkers\(^{36}\) reported the functionalization of azo dye moieties with organoiron complexes. The organoiron benzothiazole complex (xxiv) was formed by a condensation reaction of cyclopentadienyliron complex (xxii) containing a carbonyl group with the terminal hydroxy group of the benzothiazole azo dye (xxiii) using DCC and DMAP (Scheme 5.1.9).
Kavganko et al.\textsuperscript{37} synthesized the esters (xxvi) in excellent yields by reacting 1-(4-hydroxyphenyl)-2-octen-1-one (xxv) with aromatic acid in presence of DCC and DMAP (Scheme 5.1.10).

\textbf{Scheme 5.1.10}

The synthesis and anti-cancer activity of fatty acid esters of podophyllotoxin against human cancer cell lines has been described\textsuperscript{38}. The coupling of a \(\omega\)-hydroxy fatty acid (xxviii) with \(\text{C}_4\)-\(\alpha\)-hydroxy function of podophyllotoxin (xxvii) in dry
dichloromethane, DMAP and DCC gave the ester (xxix) in quantitative yields (Scheme 5.1.11).

\[ \text{Scheme 5.1.11} \]

4-Substituted-aryl-16-bromohexadecanoate (xxxii) were prepared from readily available starting material by Angelova and coworkers. 16-Bromohexadecanoic acid (xxx) acid, benzyl alcohol (xxxi) and DMAP were stirred in dry ether under argon atmosphere. DCC was added in a few portions in solid and the mixture was stirred at room temperature to obtain the desired ester (xxxii) (Scheme 5.1.12).

\[ \text{Scheme 5.1.12} \]
Tuchnaya et al.\textsuperscript{40} reported the synthesis of mixed disuccinates of 1,12-dodecanediol, 3'-azido/2',3'-didehydro-3-deoxythymidine (XXXV) using DMAP and DCC (Scheme 5.1.13).

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\includegraphics[width=\textwidth]{scheme5113.png}};
\end{tikzpicture}
\end{center}

Yuan et al.\textsuperscript{41} synthesized 12α-deoxoartemisinyl ester 4'-O-demethyl-4β-(4''-nitroanilino)-4-deoxypodophyllotoxin (XXXVIII). A mixture of 4'-O-dimethyl-4β-(4''-nitroanilino)-4-deoxypodophyllotoxin (XXXVI), artesunate (XXXVII), DMAP and DCC in dichloromethane was stirred for 6 h at 0°C to obtain the title ester, which acted as a topoisomerase inhibitor possessing multi target specificity (Scheme 5.1.14).
The pyridine acid ester derivatives of podophyllotoxin and 4'-demethylepipodophyllotoxin have been prepared and assessed for their antitumor activities (Scheme 5.1.15).

Ponedel’kina et al. obtained the azidothymidine hemisuccinate (xLiii) by the reaction of azidothymidine (xii) with succinic anhydride (xLii) in presence of DMAP.
The carboxy group was further activated by the transformation of hemisuccinate (xLiii) to \(N\)-hydroxysuccinimide ester (xLv) via reaction with an equimolar amount of \(N\)-hydroxysuccinimide (xLiv) and DCC at 0-20 °C for 10-20 min (Scheme 5.1.16).

Scheme 5.1.16

Koskinen et al.\(^{44}\) treated the alcohols (xLvi) with monoester of malonic acid (xLvii) in presence of DCC and DMAP to provide the corresponding malonates (xLviii) (Scheme 5.1.17).
Fatty acid alkenoates

Scheme 5.1.17
5.2.1. Synthesis, Characterization and Preliminary Antimicrobial Activity of 7-Hydroxy-coumarin Derivatives*

Morbidity and mortality because of enteric bacterial infections are the major health problems in some areas like Indian subcontinent, portions of South America and tropical fractions of Africa. Every year millions of people are killed due to food poisoning, rheumatic, salmonellosis and diarrhea caused by some Gram-positive and Gram-negative bacterial strains. The pharmacological drugs available for the treatment of above diseases are either too expensive or have undesirable side effects or contraindications. Furthermore, some microbial strains resistant to the available drugs have also begun to appear. Therefore, it is desirable to search for new lead molecules which can be effectively used against microbial infections.

Natural plant drugs are frequently considered to be less toxic and freer from side effects than synthetic ones. Among the most significant classes of natural compounds, an important position is occupied by oxygen-containing heterocyclic compounds, coumarins. Coumarin is a parent organic compound of a class of naturally occurring phytochemicals found in many plant species. They form an important group of organic compounds that are used as additives to food and cosmetics, optical brightening agents and dispersed fluorescent and laser dyes. The derivatives of coumarin usually occur as secondary metabolites in various plant species and are found to act as plant growth regulators.

7-Hydroxy coumarin (7-HC); a benzopyrone in nature, is an elite class of compounds. It is a major human metabolite, which plays a role as dietary antioxidant. Coumarin and its derivatives are biologically active compounds and have found application in several therapeutic areas. Medically, coumarin glycosides (e.g., Apterin, xLix) have been shown to dilate the coronary artery and also found to possess anti-tumor and antimetastatic activities. Skimmin (7-O-β-glucopyranosylumbeliferone, L), an O-glycosylated derivative of coumarin is found to show promising neuroprotective effects. Dicumarol, another coumarin glycoside better known as warfarin (Li), has found commercial application as an anticoagulant.

\[
\begin{align*}
\text{Apterin (xLix)} & \quad \text{Skimmin (L)} \\
\text{Coumarin Glycosides.} & \\
\text{Warfarin (Li)} & \quad \text{Lipid Lowering Agent (Lii)} \\
\text{Biologically important coumarin derivatives}
\end{align*}
\]
Fatty acid alkenoates

The coumarin heterocyclic ring is a common feature of various bioactive compounds such as calanolides A & B (Liii, Liv), which act as HIV protease inhibitors and AChE inhibitors\(^{55}\). Recent studies have revealed that coumarin and its derivatives exhibit several other medicinal applications such as anti-HIV and anti-tumor\(^{56}\), antiamoebic\(^{57}\), tuberculostatic\(^{58}\), antimicrobial\(^{59}\), insecticidal\(^{60}\), anthelmintic\(^{61}\), hypnotics\(^{62}\), blood glucose reducing agents\(^{63}\) and lipid-lowering agents\(^{64}\) (Lii). Optimization of coumarin based structures has resulted in marketed medicines such as novobiocin and chlorobiocin, commonly used as antibiotics. Further, it has also been found that other coumarin compounds, after suitable structural modification can be used as drugs and incorporation of the coumarin nuclei may be an important synthetic strategy in drug discovery\(^{(65,66)}\).

![Calanolide A (Liii) and Calanolide B (Liv)](image)

**Coumarin derivatives as anti-HIV agents.**

Bearing in mind the wide range of bioactivities associated with coumarins and fatty-ester analogs it was thought worthwhile to synthesize new coumarin esters. Thus the purpose of the study was to synthesize new coumarin esters with long chain (C-11 or C-18) acid incorporated at C-7 hydroxy group of the coumarin and to find their novel
bioactivity by an *in vitro* evaluation against a panel of Gram-positive and Gram-negative strains of bacteria and some fungal strains.
5.2.2. Results and Discussion

Fatty acids and their derivatives have been reported as antimicrobial agents\textsuperscript{67}. It is expected that the incorporation of the hydroxy and non-hydroxy FA chain may increase the antimicrobial activity of certain organic moieties\textsuperscript{68}. The present study is based on the synthesis, characterization and evaluation of antimicrobial activities of 7-HC derivatives derived from different fatty acids. A typical procedure in a single step involves the reaction of 7-hydroxy coumarin with fatty acid in the presence of DCC and 4-dimethyl aminopyridine (DMAP) in dichloromethane by stirring at room temperature to furnish corresponding 7-O-coumarinyl alkenoates (Scheme 5.2.2.1). The completion of the reaction was checked by thin layer chromatography (TLC). The reaction time varied from 2–3 h. The purity of the compounds was checked by TLC and the compounds under the study were characterized by the spectral data.

\[ \begin{align*}
R - \text{O} & \quad + \quad \text{HO} - \text{O} - \text{CO} \\
1-4 & \quad \text{DCC/DMAP} & \quad 25^\circ\text{C} & \quad \text{R} - \text{O} - \text{O} - \text{CO} \\
5 & \quad 6-9
\end{align*} \]

\textit{Scheme 5.2.2.1. Synthesis of 7-O-coumarinyl alkenoates (6-9).}
As evident from the Table 5.2.2.1, the scope of the reaction using olefinic (internal and terminal) and hydroxy fatty acids was found to be good and the products 6-9 (7-O-coumarinyl alkenoates) were obtained in excellent yields. It may also be observed that the yields are independent of the substituent present in the precursor.

In general, the IR spectra showed ester peak at 1729 cm\(^{-1}\) and CH\(_2\) (methylene) peak at 2921 and 2849 cm\(^{-1}\). In the \(^1\)H NMR spectra, the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of all the compounds showed peaks at d 6.39-7.69 ppm corresponding to the protons of 7-hydroxy coumarin. The \(^{13}\)C NMR spectra of all the compounds were also in good agreement. Characteristic molecular ion peaks [M+Na]\(^{+}\) was observed for all the compounds under study. A detailed spectral description for compound 6 is discussed below.
Fatty acid alkenoates

IR spectrum of compound 6 revealed characteristic band at 1729 cm\(^{-1}\) (ester C–O stretching). In the \(^1\)H-NMR the olefinic protons, \(\text{C}_{11}\text{H}_2=\text{C}_{10}\text{H}\) were observed at \(\delta H 5.81\) (tdd, 1H, \(J_{10-9} = 6.8, J_{10-H_2} = 16.8, J_{10-H_3} = 10.4, \text{CH}_2=\text{CH}\)), \(4.99\) (dd, 1H, \(J_{H_2-H_3} = 16.8, J_{H_2-H_4} = 3.2, \text{CH}_2=\text{CH}\)), \(4.93\) (dd, 1H, \(J_{H_3-H_4} = 10.4, J_{H_3-H_4} = 3.2, \text{H}_2\text{C}=\text{CH}\)) and were correlated with observations in the \(^{13}\)C NMR which gave signals at \(\delta C 139.18\) and \(114.24\) respectively. Besides these a characteristic carbon signal for the fatty acid chain at \(\delta C 171.68\) (d–, ester C=O) was recorded. The structure was further supported by its mass spectral data, which showed a molecular ion peak at \([\text{M}+\text{Na}]^+ 351.2\) consistent with the molecular formula \(\text{C}_{20}\text{H}_{24}\text{O}_4\). The COSY data also gave good support to the assigned structure. Similarly other compounds were characterized from their spectral data. Spectral studies have illustrated that the change in the nature of FA at \(C_7\) has not significantly influenced the pattern of proton and carbon signals of the 7-HC moiety.

A variety of acylated 7-hydroxycoumarins were investigated in the early 1970s for antibiotic and antifungal activity\(^6^9\). Acyl groups in the study included acetyl, propionyl and butanoyl. These compounds were found to possess activity against a number of fungal strains (MIC approximately 125 \(\mu g/ml\)) and Gram negative bacteria (MIC sub 500 \(\mu g/ml\)). It was observed that the ester derivatives of 7-hydroxycoumarins increased the antimicrobial activity of 7-hydroxycoumarin. Thus it is expected that the use of long chain hydroxy and non-hydroxy acid groups can further increase the antimicrobial potency of the coumarin ester derivatives.
Taking in account the observations stated above, all the newly synthesized compounds were screened for their *in vitro* antimicrobial potency against an assortment of three Gram-positive bacteria *Bacillus subtilis* (ATCC 6501), *Staphylococcus aureus* (ATCC-25923), *Streptococcus pyogenes* (recultured), three Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC-27853), *Salmonella typhimurium* (recultured), *Escherichia coli* (ATCC-25922) and four strains of fungus *Candida albicans* (ATCC-24433), *Candida krusei* (ATCC-6528), *Candida parapsilosis* (ATCC-22019) and *Cryptococcus neoformans* (recultured), by the disk diffusion method and measured by Halo Zone Test\textsuperscript{70-72}. The MIC of synthesized compounds against bacterial and fungal strains was performed by macro dilution test and the results were recorded visually and spectrophotometrically. The screening results for the antibacterial and antifungal activity have been summarized in Tables 5.2.2.2 and 5.2.2.3.
### Fatty acid alkenoates

**Table 5.2.2. Antibacterial and Antifungal Activity of newly synthesized compounds (6-9).**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Compounds</th>
<th></th>
<th></th>
<th></th>
<th>Chloram.</th>
<th>Fluconazol</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Zone of inhibition (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NT</td>
<td>NT</td>
<td>-</td>
</tr>
</tbody>
</table>

**Gram positive**

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td>21.9±0.5</td>
<td>18.9±0.4</td>
<td>22.8±0.5</td>
<td>23.1±0.2</td>
<td>26.8±0.5</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>19.5±0.3</td>
<td>16.2±0.8</td>
<td>21.6±0.5</td>
<td>22.3±0.7</td>
<td>23.4±0.4</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>18.9±0.5</td>
<td>16.8±0.8</td>
<td>20.1±0.3</td>
<td>20.5±0.4</td>
<td>22.±0.5</td>
<td>NT</td>
<td>-</td>
</tr>
</tbody>
</table>

**Gram negative**

<p>| | | | | | | | |</p>
<table>
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<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>16.5±0.4</td>
<td>14.3±0.2</td>
<td>16.5±0.4</td>
<td>17.1±0.2</td>
<td>19.1±0.3</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>17.2±0.5</td>
<td>15.1±0.5</td>
<td>21.1±0.4</td>
<td>21.2±0.8</td>
<td>25.2±0.2</td>
<td>NT</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>16.1±0.2</td>
<td>14.3±0.7</td>
<td>18.5±0.4</td>
<td>19.3±0.2</td>
<td>20.5±0.5</td>
<td>NT</td>
<td>-</td>
</tr>
</tbody>
</table>

**Fungi**

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
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<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>18.6±1.4</td>
<td>17.8±0.2</td>
<td>20.3±1.2</td>
<td>22.5±0.5</td>
<td>NT</td>
<td>29.3±0.5</td>
<td>-</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>16.8±0.2</td>
<td>14.5±0.3</td>
<td>18.7±0.4</td>
<td>20.7±0.4</td>
<td>NT</td>
<td>27.4±0.5</td>
<td>-</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>15.9±0.3</td>
<td>14.4±0.4</td>
<td>17.8±0.4</td>
<td>19.8±0.4</td>
<td>NT</td>
<td>24.5±0.5</td>
<td>-</td>
</tr>
<tr>
<td><em>C. neoformans</em></td>
<td>15.8±1.2</td>
<td>13.8±1.2</td>
<td>16.9±0.2</td>
<td>17.1±0.5</td>
<td>NT</td>
<td>19.5±0.5</td>
<td>-</td>
</tr>
</tbody>
</table>

NT = Not Tested, Chloram. = Chloramphenicol
### Fatty acid alkenoates

**Table 5.2.2.3. Minimum inhibition concentration (MIC) fatty acid analogs of 7-hydroxy coumarin (6-9).**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Compounds</th>
<th>MIC (µg/ml)</th>
<th>Chloram.</th>
<th>Fluconazol</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
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<tr>
<td>Gram positive</td>
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<tr>
<td><em>B. subtilis</em></td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>32</td>
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<tr>
<td><em>S. pyogenes</em></td>
<td>64</td>
<td>128</td>
<td>64</td>
<td>32</td>
<td>32</td>
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<tr>
<td><em>S. aureus</em></td>
<td>128</td>
<td>128</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Gram negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>128</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td>64</td>
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<tr>
<td><em>S. typhimurium</em></td>
<td>64</td>
<td>128</td>
<td>64</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>128</td>
<td>128</td>
<td>64</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
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<td></td>
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<tr>
<td><em>C. albicans</em></td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>NT</td>
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<tr>
<td><em>C. krusei</em></td>
<td>256</td>
<td>512</td>
<td>256</td>
<td>256</td>
<td>NT</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>32</td>
<td>64</td>
<td>16</td>
<td>16</td>
<td>NT</td>
</tr>
<tr>
<td><em>C. neoformans</em></td>
<td>16</td>
<td>32</td>
<td>16</td>
<td>8</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT= Not Tested, Chloram. = Chloramphenicol
Fatty acid alkenoates

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The data pertaining to Tables 5.2.2.2 and 5.2.2.3 reveals that compounds 6-9 have significant effect on the antibacterial profile of Gram-positive bacteria. This higher susceptibility of Gram-positive bacteria may be attributed to the absence of the unique outer peptidoglycon membrane, which leaves the bacterial wall permeable to these derivatives. Compounds 9 showed good inhibition against *S. pyogens*, *S. aureus* and *E. coli* species at 32µg/ml whereas compound 8 showed good inhibition against *S. aureus* at 32 µg/ml concentrations. Compounds 8 and 9 showed promising antibacterial activity nearly equivalent to that of standard drug (Chloramphenicol) against most of the bacterial strains.

The investigation of antifungal screening data revealed that all the tested compounds showed moderate to good fungal inhibition. Compound 8 showed good antifungal activity against all strains of fungi. Compound 9 exerted significant inhibitory activity nearly equivalent to that of standard drug (Fluconazole) against *C. parapsilosis* at 8 µg/ml, against *C. neoformans* at 16 µg/ml and against *C. albicans* at 2 µg/ml.
5.2.3. Experimental

The sources of all the fatty acids and instrumentation details are the same as chapter 1 (pg. 31).

**General procedure for the synthesis of fatty acid derivatives of 7-hydroxy-coumarin**

(7-O-coumarinyl alkenoates, 6-9)

A solution of FA (5mmol), DCC (5.5mmol), and 7-hydroxy coumarin (5mmol) in dichloromethane (50mL) with catalytic amount of DMAP were stirred mechanically at room temperature until esterification was complete. The \( N,N \)-dicyclohexylurea was filtered off and the filtrate was washed with water (3×50 mL), 5% acetic acid (3×50 mL), again with water (3×50 mL) and then dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to give the esters 6-9 (Scheme 5.2.2.1) which were chromatographed over a column of silica gel using n-hexane-ethyl acetate (94:6, v/v) as eluent. All these novel compounds were characterized from their spectral data.

**Spectroscopic data**

**7-O-Coumarinyl undec-10-enoate (6)**

White powder; Yield: 91%; Mp 168 °C.

\( IR \) (KBr): 3080, 2921, 2849, 1729, 1624, 1400, 1269, 1122 cm\(^{-1}\).

**ESI-MS** found \([M+Na]^+\) 351.2; \( C_{20}H_{24}O_4 \) [M+Na]\(^+\) requires 351.17.
The $^1$H NMR, $^{13}$C NMR and COSY data is given in Table 5.2.3.1.

**7-O-Coumarinyl (9Z)-octadec-9-enoate (7)**

Viscous solid; Yield: 89%.

**IR (KBr):** 3120, 2921, 2849, 1708, 1651, 1405, 1270, 1122 cm$^{-1}$.

**ESI-MS** found [M+Na]$^+$ 449.3; C$_{27}$H$_{38}$O$_4$ [M+Na]$^+$ requires 449.28.

The $^1$H NMR, $^{13}$C NMR and COSY data is given in Table 5.2.3.2.

**7-O-Coumarinyl (9Z,12R)-12-hydroxyoctadec-9-enoate (8)**

Viscous solid; Yield: 85%.

**IR (KBr):** 3384, 3080, 2920, 2858, 1732, 1618, 1401, 1267, 1122 cm$^{-1}$.

**ESI-MS** found [M+Na]$^+$ 465.3; C$_{27}$H$_{38}$O$_5$ [M+Na]$^+$ requires 465.27.

The $^1$H NMR, $^{13}$C NMR and COSY data is given in Table 5.2.3.3.

**7-O-Coumarinyl (12Z,9R)-9-hydroxyoctadec-12-enoate (9)**

Viscous solid; Yield: 87%.

**IR (KBr):** 3421, 3002, 2920, 2852, 1736, 1639, 1396, 1270, 1122 cm$^{-1}$.

**ESI-MS** found [M+Na]$^+$ 465.3; C$_{27}$H$_{38}$O$_5$ [M+Na]$^+$ requires 465.27.

The $^1$H NMR, $^{13}$C NMR and COSY data is given in Table 5.2.3.4.
### Table 5.2.3.1. $^1$H NMR, $^{13}$C NMR and COSY data of (6) in CDCl$_3$

<table>
<thead>
<tr>
<th>H number</th>
<th>$\delta$ (ppm)</th>
<th>Integration</th>
<th>Multiplicity</th>
<th>J (Hz)</th>
<th>COSY</th>
<th>C number</th>
<th>$\delta$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7.69</td>
<td>1H</td>
<td>d</td>
<td>$J = 9.6$</td>
<td>H-3</td>
<td>4</td>
<td>142.96</td>
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<tr>
<td>5</td>
<td>7.49</td>
<td>1H</td>
<td>d</td>
<td>$J = 8.4$</td>
<td>H-6</td>
<td>5</td>
<td>118.50$^a$</td>
</tr>
<tr>
<td>8</td>
<td>7.10</td>
<td>1H</td>
<td>s</td>
<td>$J = 9.2$</td>
<td>H-4</td>
<td>8</td>
<td>110.46</td>
</tr>
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<td>6</td>
<td>7.04</td>
<td>1H</td>
<td>d</td>
<td>$J = 8.4$</td>
<td>H-5</td>
<td>6</td>
<td>116.61$^a$</td>
</tr>
<tr>
<td>3</td>
<td>6.39</td>
<td>1H</td>
<td>d</td>
<td>$J = 9.2$</td>
<td>H-4</td>
<td>3</td>
<td>116.03$^a$</td>
</tr>
<tr>
<td>10'</td>
<td>5.81</td>
<td>2H</td>
<td>tdd</td>
<td>$J_{10'-9'} = 6.8$; $J_{10'-H_2} = 16.8$; $J_{10'-H_2} = 10.4$</td>
<td>H-11'</td>
<td>10'</td>
<td>139.18</td>
</tr>
<tr>
<td>11'(H$_Z$)</td>
<td>4.99</td>
<td>1H</td>
<td>ddd</td>
<td>$J_{H_2-10'} = 16.8$; $J_{H_2-10'} = 3.2$</td>
<td>H-10'</td>
<td>11'</td>
<td>114.24</td>
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<tr>
<td>11'(H$_E$)</td>
<td>4.93</td>
<td>1H</td>
<td>ddd</td>
<td>$J_{H_2-10'} = 10.4$; $J_{H_2-10'} = 3.2$</td>
<td>H-10'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2'</td>
<td>2.59</td>
<td>2H</td>
<td>t</td>
<td>$J = 7.6$</td>
<td>H-3'</td>
<td>2'</td>
<td>33.02$^b$</td>
</tr>
<tr>
<td>9'</td>
<td>2.03</td>
<td>2H</td>
<td>qd</td>
<td>$J = 7.2$</td>
<td>H-10'</td>
<td>9'</td>
<td>34.35$^b$</td>
</tr>
<tr>
<td>3'</td>
<td>1.75</td>
<td>2H</td>
<td>q</td>
<td>$J = 12.4$</td>
<td>H-2'</td>
<td>3'-8'</td>
<td>29.30-</td>
</tr>
<tr>
<td>24.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4'-8'</td>
<td>1.45-1.32</td>
<td>5×2H</td>
<td>br. s</td>
<td></td>
<td></td>
<td>10</td>
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<td></td>
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<td></td>
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<td>9</td>
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<td></td>
<td>7</td>
<td>154.70$^c$</td>
</tr>
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<td></td>
<td>2</td>
<td>160.44</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1'</td>
<td>171.68</td>
</tr>
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</table>

$^{a,b,c}$ Assignments may be reversed.
# Table 5.2.3.2. $^1$H NMR, $^{13}$C NMR and COSY data of (7) in CDCl$_3$

<table>
<thead>
<tr>
<th>H number</th>
<th>$\delta$ (ppm)</th>
<th>Integration</th>
<th>Multiplicity</th>
<th>$J$ (Hz)</th>
<th>COSY</th>
<th>C number</th>
<th>$\delta$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7.70</td>
<td>1H</td>
<td>d</td>
<td>$J = 9.6$</td>
<td>H-3</td>
<td>4</td>
<td>142.91</td>
</tr>
<tr>
<td>5</td>
<td>7.49</td>
<td>1H</td>
<td>d</td>
<td>$J = 8.4$</td>
<td>H-6</td>
<td>5</td>
<td>127.76$^a$</td>
</tr>
<tr>
<td>8</td>
<td>7.09</td>
<td>1H</td>
<td>s</td>
<td></td>
<td></td>
<td>8</td>
<td>115.76</td>
</tr>
<tr>
<td>6</td>
<td>7.03</td>
<td>1H</td>
<td>d</td>
<td>$J = 8.4$</td>
<td>H-5</td>
<td>6</td>
<td>118.34$^b$</td>
</tr>
<tr>
<td>3</td>
<td>6.37</td>
<td>1H</td>
<td>d</td>
<td>$J = 9.6$</td>
<td>H-4</td>
<td>3</td>
<td>116.44$^b$</td>
</tr>
<tr>
<td>9'-10'</td>
<td>5.35</td>
<td>2H</td>
<td>m</td>
<td></td>
<td></td>
<td>10'</td>
<td>130.07$^c$</td>
</tr>
</tbody>
</table>

| 2'       | 2.59           | 2H          | t            | $J = 7.6$| H-3'  | 9'       | 129.84$^c$    |
| 8', 11'  | 2.03           | 4H          | m            |          | H-9'-10'| 2'       | 34.17$^d$     |
| 11'      |                |             |              |          |       | 3'       | 31.79$^d$     |
| 3'       | 1.75           | 2H          | q            | $J = 11.6$| H-2'  | 3'-7', 12'-17'| 29.64-22.57 |
| 4'-7', 12'-17' | 1.41-1.26 | 10×2H | br. s          |          |       | 18'      | 14.03          |
| 18'      | 0.87           | 3H          | dist. t      |          |       | 10       | 127.75$^a$    |
|          |                |             |              |          |       | 9        | 153.15$^e$    |
|          |                |             |              |          |       | 7        | 154.48$^e$    |
|          |                |             |              |          |       | 2        | 160.31        |
|          |                |             |              |          |       | 1'       | 171.47        |

$^a,b,c,d,e$ Assignments may be reversed.
### Table 5.2.3.3. $^1H$ NMR, $^{13}C$ NMR and COSY data of (8) in CDCl₃

<table>
<thead>
<tr>
<th>H number</th>
<th>$\delta$ (ppm)</th>
<th>Integration</th>
<th>Multiplicity</th>
<th>$J$ (Hz)</th>
<th>COSY</th>
<th>C number</th>
<th>$\delta$ (ppm)</th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>7.61</td>
<td>1H</td>
<td>d</td>
<td>$J = 9.6$</td>
<td>H-3</td>
<td>4</td>
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<td>7.03</td>
<td>1H</td>
<td>s</td>
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<td>8</td>
<td>115.99</td>
</tr>
<tr>
<td>6</td>
<td>6.97</td>
<td>1H</td>
<td>d</td>
<td>$J = 8.4$</td>
<td>H-5</td>
<td>6</td>
<td>118.49</td>
</tr>
<tr>
<td>3</td>
<td>6.31</td>
<td>1H</td>
<td>d</td>
<td>$J = 9.6$</td>
<td>H-4</td>
<td>3</td>
<td>116.60</td>
</tr>
<tr>
<td>9'</td>
<td>5.33</td>
<td>1H</td>
<td>m</td>
<td></td>
<td>H-10', H-8'</td>
<td>9'</td>
<td>128.57</td>
</tr>
<tr>
<td>10'</td>
<td>5.48</td>
<td>1H</td>
<td>m</td>
<td></td>
<td>H-11', H-9'</td>
<td>10'</td>
<td>133.31</td>
</tr>
<tr>
<td>12'</td>
<td>3.55</td>
<td>1H</td>
<td>q</td>
<td>$J = 8.4$</td>
<td>H-11'</td>
<td>12'</td>
<td>71.57</td>
</tr>
<tr>
<td>2'</td>
<td>2.51</td>
<td>2H</td>
<td>t</td>
<td>$J = 7.6$</td>
<td>H-3'</td>
<td>2'</td>
<td>35.32</td>
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<td>11'</td>
<td>2.14</td>
<td>2H</td>
<td>t</td>
<td>$J = 7.2$</td>
<td>H-12', H-10'</td>
<td>11'</td>
<td>36.81</td>
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<tr>
<td>8'</td>
<td>1.97</td>
<td>2H</td>
<td>qd</td>
<td>$J = 7.6$</td>
<td>H-9'</td>
<td>8'</td>
<td>34.31</td>
</tr>
<tr>
<td>3'</td>
<td>1.68</td>
<td>2H</td>
<td>q</td>
<td>$J = 11.2$</td>
<td>H-2'</td>
<td>3'-7', 13'-17'</td>
<td>31.84-</td>
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<td>1H</td>
<td>br. m</td>
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<td></td>
</tr>
<tr>
<td>4'-7', 13'-17'</td>
<td>1.43-1.21</td>
<td>9×2H</td>
<td>br. s</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>18'</td>
<td>0.80</td>
<td>3H</td>
<td>dist. t</td>
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</table>

Assignments may be reversed.
### Table 5.2.3.4. $^1$H NMR, $^{13}$C NMR and COSY data of (9) in CDCl$_3$

<table>
<thead>
<tr>
<th>H number</th>
<th>$\delta$ (ppm)</th>
<th>Integration</th>
<th>Multiplicity</th>
<th>$J$ (Hz)</th>
<th>COSY</th>
<th>C number</th>
<th>$\delta$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7.73</td>
<td>1H</td>
<td>d</td>
<td>$J = 9.6$</td>
<td>H-3</td>
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<td>143.37</td>
</tr>
<tr>
<td>5</td>
<td>7.55</td>
<td>1H</td>
<td>d</td>
<td>$J = 8.8$</td>
<td>H-6</td>
<td>5</td>
<td>128.90$^a$</td>
</tr>
<tr>
<td>8</td>
<td>7.02</td>
<td>1H</td>
<td>s</td>
<td></td>
<td></td>
<td>8</td>
<td>110.19$^b$</td>
</tr>
<tr>
<td>6</td>
<td>6.98</td>
<td>1H</td>
<td>d</td>
<td>$J = 8.4$</td>
<td>H-5</td>
<td>6</td>
<td>116.61$^b$</td>
</tr>
<tr>
<td>3</td>
<td>6.30</td>
<td>1H</td>
<td>d</td>
<td>$J = 9.6$</td>
<td>H-4</td>
<td>3</td>
<td>115.75$^b$</td>
</tr>
<tr>
<td>12'-13'</td>
<td>5.27</td>
<td>1H</td>
<td>m</td>
<td></td>
<td>H-11', H-14'</td>
<td>12'</td>
<td>129.99$^a$</td>
</tr>
<tr>
<td>9'</td>
<td>3.44</td>
<td>1H</td>
<td>q</td>
<td>$J = 8.4$</td>
<td></td>
<td>9'</td>
<td>70.53</td>
</tr>
<tr>
<td>2'</td>
<td>2.52</td>
<td>2H</td>
<td>t</td>
<td>$J = 7.6$</td>
<td>H-3'</td>
<td>2'</td>
<td>33.88$^c$</td>
</tr>
<tr>
<td>11', 14'</td>
<td>2.02</td>
<td>2×2H</td>
<td>m</td>
<td></td>
<td>H-12'-13'</td>
<td>14'</td>
<td>34.18$^c$</td>
</tr>
<tr>
<td>3'</td>
<td>1.67</td>
<td>2H</td>
<td>q</td>
<td>$J = 12.0$</td>
<td>H-2</td>
<td>3'-7', 11', 15'-17'</td>
<td>30.95-</td>
</tr>
<tr>
<td>22.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11'</td>
<td>22.50</td>
</tr>
<tr>
<td>OH</td>
<td>1.55</td>
<td>1H</td>
<td>br. m</td>
<td></td>
<td></td>
<td>10'</td>
<td>37.39$^d$</td>
</tr>
<tr>
<td>4'-8', 10', 15'-17'</td>
<td>1.40-1.02</td>
<td>9×2H</td>
<td>br. s</td>
<td></td>
<td>18'</td>
<td>14.11</td>
<td>39.83$^d$</td>
</tr>
<tr>
<td>18'</td>
<td>0.80</td>
<td>3H</td>
<td>dist. t</td>
<td></td>
<td>8'</td>
<td>39.83$^d$</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>154.51$^e$</td>
<td>2</td>
</tr>
<tr>
<td>1'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1'</td>
<td>171.52</td>
<td></td>
</tr>
</tbody>
</table>

$^a$, $^b$, $^c$, $^d$, $^e$ Assignments may be reversed.
Antibacterial studies

The in-vitro antibacterial activities of 6 to 9 were tested using the bacterial cultures of Bacillus subtilis (ATCC 6501), Staphylococcus aureus (ATCC-25923), Streptococcus pyogenes (recultured), Pseudomonas aeruginosa (ATCC-27853), Salmonella typhimurium (recultured), Escherichia coli (ATCC-25922) and fungal cultures of Candida albicans (ATCC-24433), Candida krusei (ATCC-6528), Candida parapsilosis (ATCC-22019) and Cryptococcus neoformans (recultured), by the disk diffusion method\textsuperscript{70,71}. Chloramphenicol (30 μg) was used as positive control. While the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 5.2.2.2.

The minimum inhibitory concentration (MIC) was assessed by the macro dilution test using standard inoculums of 5 X 10\textsuperscript{5} c.f.u. /ml. Initially the compounds were dissolved in DMSO after that serial dilution of the test compounds were set to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 μg /ml. To each tube was added 100 μl of 24 h old inoculums. The growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations are given in Table 5.2.2.3.
Antifungal studies

Antifungal activity was also done by disk diffusion method\(^2\). For assaying antifungal activity *Candida albicans*, *Candida krusei*, *Candida parapsilosis*, *Cryptococcus neoformans* were used as the test strains. Fluconazole (30 µg) was used as positive control. While the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against albicans and non-albicans strains of fungi. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 5.2.2.2.

The minimum inhibitory concentration (MIC) was assessed by the macro dilution test using standard inoculums of $1.6 \times 10^4$-6 $\times 10^4$ c.f.u. /ml. Initially the compounds were dissolved in DMSO after that serial dilution of the test compounds were set to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg /ml. To each tube was added 100 µl of 48-72 h old inoculums. The growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of fungi was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations are given in Table 5.2.2.3.
5.3.1 Synthesis and in vitro Antimicrobial Activity of β-Sitosteryl Esters of Hydroxy and Non-hydroxy Olefinic Fatty Acids

Phytosterols are bioactive compounds found in almost all plants as structural component of plant membranes. Their function appears to be to control membrane fluidity, permeability and signal transduction. They are structurally similar and functionally analogous to the cholesterol in vertebrate animals. Plant contains more than 200 different types of phytosterols, the most abundant being β-sitosterol (90%). There has been an increasing interest in the use of phytosterols. They reduce the serum low-density lipoprotein (LDL) cholesterol levels by means of reduction in absorption of cholesterol and thus protect against cardiovascular diseases through their hypocholesterolemic action. The β-sitosterol and lactose β-sitosterol are found to have an inhibitory effect on the pathophysiological process in asthma. The β-sitosterol-3-O-glucopyranoside (Lv), isolated from natural source was found to be a promising candidate for bacterial sortase inhibitor. The non-glucosidic β-sitosterols are found effective against benign prostatic hyperplasia (BPH) and help in increasing the urine flow thereby improving the urologic symptoms. The in vitro studies have suggested that it may show anti-cancer activity against colon cancer (HT-29) cells, prostrate cancer (LNCaP) cells and breast cancer (MDA-MB-231) cells by inhibition of tumor growth and induction of apoptosis in cancerous cells.

Fatty acid alkanoates

P-Sitosterol-3-O-glucopyranoside (Lv)
(bacterial sortase inhibitor)

β-Sitosterol has also been found to exert estrogenic effects in mammals\(^1\) and clastogenic effect on mitotically dividing chromosomes of bone marrow erythroblasts. The genotoxic effects of thermally oxidized derivatives of β-sitosterol have also been studied\(^2\). It has been suggested to prevent temporary immune weakness that typically occurs during recovery from endurance exercise\(^3\). In addition, the purified β-sitosterol has applications in biological studies and they have been reported to exert anti-atherosclerotic\(^4\), anti-inflammatory\(^5\) and anti-oxidative\(^6\) activities in animals and have been found to play an important role in multi stage treatment of HIV\(^7\).

Furthermore, the fatty acid esters of phytosterols have water holding property and are widely used as ingredients of cosmetics and bath additives\(^8\). Due to their physiological activity of lowering plasma cholesterol concentrations, the esters of phytosterols are being incorporated into a growing spectrum of functional foods, including margarine blended with fatty acid sterol esters (FASE) and salad oil dressing with added sterol\(^9\).
In view to the above mentioned pharmacological applications of phytosterols and in continuation of our search of biologically active molecules, the design, synthesis and evaluation of antimicrobial potency of hitherto unknown β-sterryl esters fatty acids has been carried out.
5.3.2. Results and Discussion

The application of catalysts in organic chemistry for conducting synthetic reactions at highly accelerated rates has been an emerging technique. In fact, use of catalysts has become popular among synthetic organic chemists for improving classical organic reactions, shortening reaction time and/or improving yields, as well as for promoting new reactions. To explore the probability of getting the pharmacophoric important moieties from natural products in higher yields and in shorter reaction time, our attention has turned to employ the use of catalyst.

3β-Sitosteryl alkenoates 11-14 were synthesized in quantitative yields by condensing long chain fatty acids with β-sitosteryl in dichloromethane using DCC and a catalytic amount of DMAP. The reaction was carried out at room temperature (Scheme 5.3.2.1).
The generality and scope of the synthetic procedure was demonstrated by subjecting β-sitosterol with olefinic (terminal and internal) and hydroxy olefinic carboxylic acid derivatives. The use of catalyst for the synthesis of fatty alkenoates provided higher yield of products. In most of the cases the yield of product was 74-88%.

The newly compounds were characterized by IR, $^1$H NMR, $^{13}$C NMR, Mass spectra. IR absorptions characteristic of ester carbonyl was observed in all the newly synthesized compounds.

IR spectrum of compound 11 revealed characteristic band at 1732 cm$^{-1}$ (ester C–O stretching). The two olefinic protons, C$^{11}$H$_2$=C$^{10}$H and C$_6$-H were observed at δH 5.81 (tdd, 1H, $J_{H^{-}\text{CH}_2}$ = 6.7 Hz, $J_{H^{-}\text{CH}_2}$ =10.1 Hz, $J_{H^{-}\text{H}_2}$ =16.9 Hz, CH$_2$=CH$^-$), 5.36 (d,
1H, J = 4.8 Hz, C6-H), 4.98 (dd, 1H, J_{H_2-H} = 10.1 Hz, J_{H_2-H_6} = 2.8 Hz, H_2C=CH), 4.93 (dd, 1H, J_{H_3-H} = 16.9 Hz, J_{H_3-H_2} = 2.8 Hz, H_8C=CH-) and correlated with observations at δC 114.23, 122.67, 139.77, 139.27 respectively. A multiplet at δH 4.61 was observed for C3β-H and correlated with observation at δC 73.75. Besides few other significant carbon signals for the fatty acid chain characteristic peak at δC 174.07 (C1, ester C=O) was recorded. Spectral studies have illustrated that the change in the nature of fatty aids at β-C3 has not significantly influenced the pattern of proton and carbon signals of the β-sitosterol moiety. Similarly other compounds were characterized from their spectral data detailed in experimental section.

**Antibacterial studies**

The newly synthesized compounds were further screened for their in vitro antibacterial activity against *Escherichia coli* (K-12), *Staphylococcus aureus* (ATCC-25923), *Salmonella typhimurium* (MTCC-98), *Bacillus subtilis* (ATCC 6051) bacterial strains by disc diffusion method\(^{70,71,93}\). Chloramphenicol (30 μg) was used as positive control. While the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against various Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 5.3.2.1.
Table 5.3.2.1. Antibacterial activity of the newly synthesized fatty alkenoates (11-14).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
<td>S. aureus</td>
</tr>
<tr>
<td>11</td>
<td>17.8±0.4</td>
<td>17.3±0.5</td>
</tr>
<tr>
<td>12</td>
<td>15.6±0.3</td>
<td>15.2±0.4</td>
</tr>
<tr>
<td>13</td>
<td>21.9±0.8</td>
<td>20.6±0.3</td>
</tr>
<tr>
<td>14</td>
<td>20.1±0.2</td>
<td>20.2±0.3</td>
</tr>
<tr>
<td>Standard</td>
<td>23.0±0.2</td>
<td>22.0±0.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (standard); Chloramphenicol and negative control (DMSO) measured by the Halo Zone Test (Unit, mm)

Minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBCs) were also determined for the title compounds by broth dilution technique. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC. MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The MIC and MBC for the newly synthesized compounds are given in Table 5.3.2.2.
**Table 5.3.2.2. MIC and MBC results of newly synthesized fatty alkenoates (11-14).**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>B. subtilis</strong></td>
<td><strong>S. aureus</strong></td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>11</td>
<td>12.5</td>
<td>25</td>
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<td>13</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>14</td>
<td>6.25</td>
<td>25</td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

MIC (µg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely; MBC (µg/ml) = minimum bacterial concentration, i.e., the lowest concentration of the compound for killing the bacteria completely.

Positive control: Chloramphenicol

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Compounds 11 showed good inhibition against *B. subtilis, S. aureus, S. typhimurium* and *E. coli* while as compound 12 was moderately active against *S. typhimurium*. In most of the cases compounds 13 and 14 showed good antibacterial activity nearly equivalent to that of Chloramphenicol. The MBC of few compounds was found to be the same as MIC but in most of the compounds it was two or three or four folds higher than the corresponding MIC results.
Antifungal studies

In other set of experiments, the compounds 11-14 were screened for their antifungal activity. Antifungal activity was also done by disk diffusion method. For assaying antifungal activity Candida albicans, Aspergillus niger, Penicillium marneffei and Helminthosporum oryzae fungal strains were used. The fungal activity of each compound was compared with Greseofulvin which was used as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 5.3.2.3.

Table 5.3.2.3. Antifungal activity of newly synthesized fatty alkenoates (11-14).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>AN</th>
<th>HO</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>26.1±0.3</td>
<td>23.1±0.2</td>
<td>18.6±0.4</td>
<td>17.3±0.5</td>
</tr>
<tr>
<td>12</td>
<td>22.2±0.4</td>
<td>20.6±0.4</td>
<td>21.2±0.2</td>
<td>16.2±0.3</td>
</tr>
<tr>
<td>13</td>
<td>23.1±0.7</td>
<td>22.3±0.7</td>
<td>19.9±0.6</td>
<td>15.1±0.9</td>
</tr>
<tr>
<td>14</td>
<td>23.6±0.4</td>
<td>20.8±0.2</td>
<td>19.0±0.3</td>
<td>15.2±0.2</td>
</tr>
<tr>
<td>Standard</td>
<td>30.0±0.2</td>
<td>27.0±0.2</td>
<td>24.0±0.3</td>
<td>20.0±0.5</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (Greseofulvin) and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

Minimum inhibitory concentrations, MICs (the lowest concentration (highest dilution) required to arrest the growth of fungus) and minimum fungicidal concentration,
MFCs (the lowest drug concentration at which 99.9% of the inoculums was killed) were also determined and the results have been tabulated in Table 5.3.2.4.

Table 5.3.2.4. MIC and MFC results of newly synthesized fatty alkenoates (11-14).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>AN</th>
<th>HO</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
</tr>
<tr>
<td>11</td>
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<td>25</td>
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<td>50</td>
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<td>6.25</td>
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<tr>
<td>14</td>
<td>12.5</td>
<td>50</td>
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<td>50</td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

CA = Candida albicans, AN = Aspergillus niger, HO = Helminthosporum oryzae, PM = Penicillium marneffei. MIC (µg/ml) = minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of fungus completely; MFC (µg/ml) = minimum fungicidal concentration, i.e., the lowest concentration of the compound for killing the fungus completely.

Positive control Greseofulvin.

The antifungal screening data showed only moderate activity. Among the screened compounds, 11 showed good inhibition against Candida albicans. Compounds 13 and 14 revealed good results against Candida albicans and Helminthosporum oryzae and moderate activity against Aspergillus niger and Penicillium marneffei. The MFC of all the compounds was two or three or four folds higher than the corresponding MIC results.

The varied divergence in the antimicrobial activity of these compounds validates the reason of this study. The importance of such kind of work lies in the possibility that the new compounds might be more efficient against bacteria and a thorough study...
regarding the structure–activity relationship, toxicity and their biological effects would be helpful in designing more effective antimicrobial agents for therapeutic use.
5.3.3. Experimental

The sources of all the fatty acids and the instrumentation details are the same as Chapter 1 (pg. 31). β-sitosterol were isolated from *Ficus krishnae* following the literature procedure. 

*General procedure for the synthesis of fatty acid analogs of β-sitosterol (11-14)*

Fatty acid (5mmol), DCC (5.5mmol), and (5mmol) β-sitosterol in dichloromethane (20mL) with catalytic amount of 4-dimethylaminopyridine (DMAP) were stirred mechanically at room temperature until esterification was complete. *N,N*-dicyclohexylurea formed was filtered off. The filtrate was washed with water (3×20 mL), 5% acetic acid (3×20 mL) again with water (3×20 mL) and dried over anhydrous sodium sulphate. Solvent was removed under the reduced pressure to give the ester 11-14 (Scheme 53.2.1) which were chromatographed over a column of silica gel using n-hexane-ethyl acetate (96:4 v/v) as eluent. All these newly synthesized compounds were characterized from IR, 'H NMR, 13C NMR and mass spectral data.

**Spectroscopic Data**

*3β-β-Sitosterylundec-10′-enoate (11)*

White solid; *Rf* = 0.76 (*n*-hexane/ethyl acetate, 3:2 v/v, as developer), Isolated yield: 88%; Mp 93°C.

*IR* (KBr): 2931, 2860, 1736 cm⁻¹.
Fatty acid alkenoates

$^1$H NMR (CDCl₃): δ 5.80 (tdd, 1H, $J_{H^5-H^6} = 6.6$ Hz, $J_{H^5-H^6} = 10.2$ Hz, $J_{H^5-H^6} = 16.4$ Hz, CH₂=CHH), 5.38 (d, 1H, $J = 4.4$ Hz, C₆-H), 4.95 (dd, 1H, $J_{H^5-H^6} = 10.2$ Hz, $J_{H^5-H^6} = 2.6$ Hz, H₂C=CH), 4.91 (dd, 1H, $J_{H^5-H^6} = 16.4$ Hz, $J_{H^5-H^6} = 2.8$ Hz, H₂C=CH), 4.62 (m, 1H, C₃β-H), 2.31–1.07 (br m, 46H), 1.01 (s, 3H, C₁₀-CH₃), 0.92 (d, 3H, $J = 6.7$ Hz, C₂₀-CH₃), 0.86 (dd, 6H, $J = 6.4$ Hz C₂₅-(CH₃)₂), 0.84 (d, 3H, $J = 6.4$ Hz, C₂₉-CH₃), 0.68 (s, 3H, C₁₁-CH₃).

$^{13}$C NMR (CDCl₃): δ 173.41, 139.77, 139.27, 122.67, 114.23, 73.75, 56.74, 56.17, 50.06, 42.37, 39.77, 39.59, 38.22, 37.78, 37.06, 36.66, 36.25, 35.88, 34.78, 33.89, 31.97, 31.91, 29.38, 29.30, 29.17, 29.15, 28.97, 28.32, 28.10, 27.88, 25.13, 24.36, 23.90, 22.92, 22.66, 21.10, 19.41, 18.79, 14.9, 11.93.

ESI-MS found [M+Na]$^+$ 603.83; C₄₀H₆₈O₂ [M+Na]$^+$ requires 603.77.

3β-β-Sitosteryloctadec-9'-(Z)-enoate (12)

Viscous compound; $R_f$ = 0.56 (n-hexane/ethyl acetate, 3:2 v/v, as developer); Isolated yield 85%.

IR (KBr): 2932, 2854, 1732 cm⁻¹

$^1$H NMR (CDCl₃): δ 5.37 (d, 1H, $J = 4.6$ Hz, C₆-H), 5.35 (m, 2H, C₉=H=C₁₀-H) 4.61 (m, 1H, C₃β-H), 2.31–1.05 (br m, 56H), 1.01 (s, 3H, C₁₀-CH₃), 0.91 (d, 3H, $J = 6.7$ Hz, C₂₀-
**Fatty acid alkenoates**

.CH3), 0.89 (dist. t, 3H, C18H3), 0.86 (dd, 6H, J = 6.6 Hz C25-(CH3)2), 0.84 (d, 3H, J = 6.4 Hz C29-CH3), 0.67 (s, 3H, C13-CH3).


**ESI-MS** found [M+Na]+ 702.10; C47H82O2 [M+Na]+ requires 702.06.

**3β-β-Sitosteryll-12'-[(R)-hydroxyoctadec-9'-(Z)-enoate (13)**

Oily compound; Rf = 0.52 (n-hexane/ethyl acetate, 3:2 v/v, as developer); isolated yield: 78%.

**IR** (nujol): 3310, 2926, 2855, 1737 cm⁻¹.

**1H NMR** (CDCl3): δ 5.40 (m, 2H, C₉H=C₁₀H), 5.34 (d, 1H, J = 4.6 Hz, C₆-H), 4.85 (m, 1H, OH, D₂O exchangeable), 4.61 (m, 1H, C₃β-H), 3.60 (m, 1H, C₁₂-H-OH), 2.30–1.03 (br m, 54H), 1.01 (s, 3H, C₁₀-CH₃), 0.92 (d, 3H, J = 6.4 Hz, C₂₀-CH₃), 0.89 (dist. t, 3H, C₁₈H₂), 0.85 (dd, 6H, J = 6.6 Hz C₂₅-(CH₃)₂), 0.82 (d, 3H, J = 6.4 Hz, C₂₅-CH₃), 0.67 (s, 3H, C₁₃-CH₃).

**13C NMR** (CDCl₃): δ 170.42, 139.77, 139.27, 127.67, 124.23, 77.10, 68.5, 47.46, 44.39, 42.20, 42.10, 42.07, 39.75, 39.59, 37.21, 37.06, 36.43, 36.25, 36.19, 36.11, 35.83, 35.72,
Fatty acid alkenoates

35.14, 34.75, 33.39, 31.91, 29.38, 29.33, 29.17, 29.15, 28.37, 28.22, 28.10, 27.33, 25.13,

ESI-MS found [M+Na]^+ 718.09; C_{47}H_{82}O_{3} [M+Na]^+ requires 718.06.

3β,β-Sitosteryl-9'(R)-hydroxyoctadec-12'(Z)-enoate (14)

Oily compound; Rf = 0.54 (n-hexane/ethyl acetate, 3:2 v/v, as developer); Isolated yield:
74%.

IR (nujol): 3342, 2922, 2859, 1737 cm\(^{-1}\).

\(^1H\)NMR (CDCl\(_3\)): δ 5.40 (m, 2H, C\(_{11}\)H=C\(_{12}\)H), 5.33 (d, 1H, J = 4.6 Hz, C\(_6\)-H), 4.64 (m, 1H, OH, D\(_2\)O exchangeable), 4.57 (m, 1H, C\(_{3β}\)-H), 3.60 (m, 1H, C\(_9\)-H-OG), 2.31–1.07 (br m, 54H), 1.01 (s, 3H, C\(_{10}\)-CH\(_3\)), 0.92 (d, 3H, J = 6.5 Hz, C\(_{20}\)-CH\(_3\)), 0.88 (dist. t, 3H, C\(_{18}\)-H\(_3\)), 0.85 (dd, 6H, J = 6.6 Hz C\(_{25}\)-(CH\(_3\))\(_2\)), 0.81 (d, 3H, J = 6.4 Hz, C\(_{29}\)-CH\(_3\)), 0.67 (s, 3H, C\(_{13}\)-CH\(_3\)).

\(^13C\)NMR (CDCl\(_3\)): δ 170.42, 139.72, 139.19, 127.64, 124.23, 78.33, 67.53, 46.23, 44.77,
42.71, 42.39, 42.37, 39.76, 39.44, 37.22, 37.16, 36.20, 36.15, 36.00, 35.88, 35.71, 34.71,
34.34, 33.99, 31.65, 31.11, 29.38, 29.34, 29.30, 29.06, 29.02, 28.81, 28.32, 28.10, 27.32,

ESI-MS found [M+Na]^+ 718.11; C\(_{47}\)H\(_{82}\)O\(_3\) [M+Na]^+ requires 718.06.
**Antibacterial studies**

The newly synthesized compounds were screened for their in vitro antibacterial activity against *Escherichia coli* (K-12), *Staphylococcus aureus* (ATCC-25923), *Salmonella typhimurium* (MTCC-98), *Bacillus subtilis* (ATCC 6051) bacterial strains by disc diffusion method\(^{70,71,93}\). Chloramphenicol (30 µg) was used as positive control. While the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 5.3.2.1.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). MBC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum bactericidal concentration are given in Table 5.3.2.2.

**Antifungal studies**

Antifungal activity was also done by disk diffusion method. For assaying antifungal activity *Candida albicans*, *Aspergillus Niger*, *Penicillium marneffei* and *Heliminthosporum oryzae* in DMSO by agar diffusion method\(^{72,94}\). The fungal activity of each compound was compared with Greseofulvin as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are
given in Table 5.3.2.3. The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) as determined by broth dilution technique are given in Table 5.3.2.4.
5.4 References


Fatty acid alkenoates


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Fatty acid alkenoates


91. Y. Hirota, T. Nagao, Y. Watanabe, M. Suenaga, S. Nakai, M. Kitano, A.
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94. R. S. Varma, Antifungal Agents: Past, Present and Future prospects, National
Publications & Presentations
Papers Published/Communicated

1. Synthesis, Characterization and in vitro Antimicrobial Activities of 5-Alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles and thiadiazole


3. DCC/DMAP Mediated Esterification of Hydroxy and Non-hydroxy Olefinic Fatty Acids with $\beta$-Sitosterol: in vitro Antimicrobial Activity.

5. Fatty Acid Composition of Leaf Oil and Analgesic Activity of Alcoholic Extract of *Ficus bengalensis* leaves.


6. Facile One-pot Synthesis of Novel 3-Substituted-1,6-dihydro-1,2,4-triazin-5-(2H) ones from Fatty Acid Hydrazides and their *in vitro* Antimicrobial Activity.


**Papers Presented in Conferences.**

1. A. Rauf and *Nida N. Farshori*

   *Synthesis of 5-Alkenyl/Hydroxyalkenyl-2-phenyl-1,3,4-oxadiazoles and thia diazoles from Fatty Acid Hydrazides via Dehydrative Cyclization of Semicarbazide and Thiosemicarbazide*, National Symposium on Recent Trends in Chemical Sciences, February 24-25, 2010, Department of Chemistry, Aligarh Muslim University, Aligarh.
2. A. Rauf and Nida N. Farshori


**Conferences Attended**


Synthesis, characterization, and in vitro antimicrobial activities of 5-alkenyl/hydroxyalkenyl-2-phenylamine-1,3,4-oxadiazoles and thiadiazoles

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Spectral data
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A wide variety of heterocyclic systems have been explored for developing pharmaceutically important molecules. Among them the derivatives of oxadiazoles and thiadiazoles have played an important role in the medicinal chemistry. These heterocycles have been found to possess broad-spectrum antimicrobial activity and many other uses.1,2 The therapeutic effects of compounds containing 1,3,4-thiadiazole rings have been studied for a number of pathological conditions including inflammation3, pain,4,5 and hypertension.6 Furthermore, the synthesis of thiadiazole and oxadiazole has attracted wide attention due to the diversity of their applications as antibacterial7, antimycobacterial8,9, anti-fungal10-12 and antidepressant agents.13 Various biological applications such as antimicrobial16,17, antifungal18, pesticidal,19 and anticancer activities20 have also been reported for seed oils, long-chain alkenoic acids and their derivatives.

The wide range of therapeutic values of alkenoic acids and oxadiazoles/thiadiazole ring systems prompted us to synthesize the title compounds and screen them for various antimicrobial activities. The basic idea was to append the long-chain alkenyl/hydroxyalkenyl moiety of the long-chain alkenoic acid to the oxadiazole/thiadiazole nucleus so as to combine the beneficial effects in a single structure with expected biological activities.

In this communication we are reporting the synthesis of 1,2,5-oxadiazoles/thiadiazole ring systems prompted us to synthesize the title compounds and screen them for various antimicrobial activities.

The long-chain alkenoic acid hydrazides (1a–d) on reaction with phenylisocyanate and phenylthiocyanate gave their corresponding semicarbazides (2a–d) and thiosemicarbazides (4a–d), which on further refluxing with POCl3 and Ac2O yielded corresponding 1,3,4-oxadiazoles (3a–d) and thiadiazoles (5a–d), respectively. The structure elucidation of synthesized compounds is based on the elemental analysis and spectral data (IR, 1H NMR, 13C NMR and MS). The synthesized oxadiazoles and thiadiazoles have been screened for antibacterial and antifungal activities. The investigation of antimicrobial screening revealed that compounds 3c, 3d, 5c, 5d and compounds 3b, 5b showed good antibacterial and antifungal activities, respectively.

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A B S T R A C T

The long-chain alkenoic acid hydrazides (1a–d) on reaction with phenylisocyanate and phenylthiocyanate gave their corresponding semicarbazides (2a–d) and thiosemicarbazides (4a–d), which on further refluxing with POCl3 and Ac2O yielded corresponding 1,3,4-oxadiazoles (3a–d) and thiadiazoles (5a–d), respectively.

The structure elucidation of synthesized compounds is based on the elemental analysis and spectral data (IR, 1H NMR, 13C NMR and MS). The synthesized oxadiazoles and thiadiazoles have been screened for antibacterial and antifungal activities. The investigation of antimicrobial screening revealed that compounds 3c, 3d, 5c, 5d and compounds 3b, 5b showed good antibacterial and antifungal activities, respectively.

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The long-chain alkenoic acid hydrazides (1a–d), used as the starting material were prepared from corresponding long-chain alkenoic acids by esterification and further treatment with hydrazine hydrate.21 Reacting 1a–d with phenyl isocyanate in dry benzene under reflux and removing excess of solvent under reduced pressure gave semicarbazides (2a–d). The treatment of 2a–d with POCl3 yielded 2,5-disubstituted-1,3,4-oxadiazoles (3a–d) in good yields. The reaction of 1a–d with phenyl isothiocyanate gave corresponding thiosemicarbazides (4a–d) which on dehydration cyclization by Ac2O produced 2,5-disubstituted-1,3,4-thiadiazoles (5a–d) in excellent yields. The reaction sequences are outlined in Schemes 1 and 2.

The semicarbazide 2a showed IR bands at 3236 cm⁻¹ (NH, NH-CONH) and 1667 cm⁻¹ (C=O). 1H NMR was more informative, characteristic peaks were observed at δ 13.04 (1H, s, CO-NH-Ar), 9.16 (2H, br s, CO-NHNH-CO), 8.17 (2H, J = 7.2 Hz, Ar-H-2'/5'), 7.60 (1H, t, J = 7.4 Hz, Ar-H-4') and 7.51 (2H, t, J = 7.4 Hz, Ar-H-3'/5'). In 13C NMR peaks at δ 168.2 and 165.4 were observed. The build up of 3a–d is evident from their spectral data. Compound 3a, 5-(Dec-9'-eny)-2-phenylamine-1,3,4-oxadiazole, showed IR absorption bands at 3228 cm⁻¹, 1504 cm⁻¹, 1258 cm⁻¹ due to stretching vibrations of NH, C=N and C-O-C functions. The 1H NMR was more informative in assigning the structure. Diagnostic peaks at δ 9.07 (1H, s, NH), 7.27 (2H, d, J = 7.6 Hz, Ar-H-2'/5'), 7.18 (1H, t, J = 7.5 Hz, Ar-H-4') and 6.98 (2H, t, J = 7.3 Hz, Ar-H-3'/5')

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E-mail address: abdulaochef@gmail.com (A. Rauf).
were observed. A triplet at δ 2.24 was observed for CH₂ protons α to oxadazole ring. In 13C NMR peaks at δ 172.9 and 155.6 were observed for ring carbon atoms. The mass spectra showed characteristic molecular ion peak which were in accordance with the molecular formula.

5-(Dec-9-enyl)-2-phenylamino-1,3,4-thiadiazole 5a gave significant IR bands at 3221 cm⁻¹ (NH), 1488 cm⁻¹ (C=N), and 707 cm⁻¹ (C=S-C). 1H NMR peak at δ 2.20 (1H, s, NH), 8.16 (2H, d, J = 8.5 Hz, Ar-H-2/6'), 7.55 (1H, t, J = 7.3 Hz, Ar-H-4'), and 7.44 (2H, t, J = 7.8 Hz, Ar-H-3/5') were observed in addition to peaks of normal fatty acid chain. In 13C NMR peaks at δ 165.2 and 153.0 were observed for ring carbon atoms. The mass spectra showed characteristic molecular ion peak which were in accordance with the molecular formula. The newly synthesized compounds have been confirmed by their spectral data.

The characterization data of semicarbazides (2a-d), oxadiazoles (3a-d), and thiadiazoles (5a-d) is given in Table 1.

Undec-10-enic (purity, 98%) and (Z)-octadec-9-enic (97%) acids were purchased from Fluka chemicals (Buck Switzerland) (9Z,12Z)-12-hydroxyoctadec-9-enic acid (racemic acid, 98%) and (9Z,12Z)-9-hydroxyoctadec-12-enic acid (nonracemic acid, 98%) were isolated from Ruminis communis and Wightta tincton seed oils, respectively, following Gunstone's partition procedure. Phenyl thiocyanate, phenyl isocyanate, phosphorous trichloride, and acetic anhydride were purchased from Merck, Mumbai, India. Thin layer chromatography (TLC) was done on glass plates (20 x 5 cm) with a layer of silica gel G (Merck, Mumbai, India, 60–120 mesh). IR spectra were recorded on Shima­dzu 8201 PC spectrophotometer. 1H NMR spectra were recorded with Bruker DRX 400 spectrometer (400 MHz) in CDCl₃, using TMS as internal standard. Chemical shifts (δ) are quoted in ppm and coupling constants (J) are given in Hz. 13C NMR spectra were recorded at Bruker DRX 400 spectrometer in CDC1₃ with CDC1₃ (δ = 77.00).

Antibacterial studies. The newly prepared compounds were screened for their antibacterial activity against Escherichia coli (ATCC-25922), Staphylococcus aureus (ATCC-25923), Pseudomonas aeruginosa (ATCC-27853), Streptococcus pyogenes, and Klebsiella pneumoniae (Clinical isolate) bacterial strains by disc diffusion method. A standard inoculum (1 x 10⁶ cfu/ml) was used. The bacterial plates were dried by placing in an incubator at 37 °C for 24 h. The number of cfu was counted after 18–24 h of incubation. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC.

The results have been reported in Table 6. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Compounds 3a, 5b, 5c, 5d were found to be almost equally potent as the reference drug, chloramphenicol, in case of K pneumoniae. The MIC of few compounds was found to be the same as MIC but in most of the compounds it was two or three folds higher than the corresponding MIC results.

Antifungal studies. Antifungal activity was also done by disk diffusion method. For assaying antifungal activity Candida albicans, Aspergillus fumigatus, Penicillium marneffei, and Trichophyton mentagrophytes (recultured) in DMSO by agar diffusion method were used as negative control. The plates were inverted and incubated for 24 h at 37 °C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 2.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contained log–analytic seriesly two fold diluted amount of test compound and controls were inoculated with approximately 5 x 10⁶ cfu/ml of actively dividing bacterial cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC.

The MBC of few compounds was determined by broth dilution technique. The nutrient broth, which contained log–analytic seriesly two fold diluted amount of test compound and controls were inoculated with approximately 5 x 10⁶ cfu/ml of actively dividing bacterial cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as MIC.

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Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R</th>
<th>Mol formula</th>
<th>Mp (°C)</th>
<th>Yield (%)</th>
<th>Analysis (%) found (calculated)</th>
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<tr>
<td>2a</td>
<td></td>
<td>C_{8}H_{14}O_{2}N_{2}</td>
<td>103-104</td>
<td>80</td>
<td>6.76 (54.11) 8.44 (8.56) 13.06 (13.23)</td>
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<td>2b</td>
<td></td>
<td>C_{8}H_{14}O_{2}N_{2}</td>
<td>101-102</td>
<td>79</td>
<td>7.19 (72.25) 9.86 (9.93) 10.02 (10.11)</td>
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<tr>
<td>2c</td>
<td></td>
<td>C_{8}H_{14}O_{2}N_{2}</td>
<td>111-113</td>
<td>77</td>
<td>6.92 (69.57) 9.50 (9.56) 09.65 (9.73)</td>
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<tr>
<td>2d</td>
<td></td>
<td>C_{8}H_{14}O_{2}N_{2}</td>
<td>113-114</td>
<td>72</td>
<td>6.93 (69.57) 9.20 (9.26) 09.65 (9.73)</td>
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<tr>
<td>3a</td>
<td></td>
<td>C_{8}H_{14}O_{2}N_{2}</td>
<td>133-134</td>
<td>92</td>
<td>7.20 (72.21) 8.11 (8.40) 13.90 (14.03)</td>
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<td>C_{8}H_{14}O_{2}N_{2}</td>
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<td>90</td>
<td>7.52 (75.52) 9.74 (9.87) 10.44 (10.56)</td>
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<td>7.25 (72.60) 9.41 (9.49) 10.00 (10.15)</td>
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<td>3d</td>
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<td>C_{8}H_{14}O_{2}N_{2}</td>
<td>135-137</td>
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<td>7.28 (72.60) 9.36 (9.49) 09.97 (10.15)</td>
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<td>4a</td>
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<td>C_{8}H_{14}O_{2}N_{2}</td>
<td>134-135</td>
<td>95</td>
<td>6.80 (68.53) 7.78 (7.98) 13.07 (13.31)</td>
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<td>4b</td>
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<td>C_{8}H_{14}O_{2}N_{2}</td>
<td>138-140</td>
<td>91</td>
<td>7.23 (72.59) 9.44 (9.49) 10.10 (10.15)</td>
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<td>85</td>
<td>6.91 (69.89) 9.04 (9.14) 09.78 (9.78)</td>
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<td>139-141</td>
<td>83</td>
<td>6.94 (69.89) 9.02 (9.14) 09.72 (9.78)</td>
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Table 2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of zone of inhibition (mm)</th>
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<tr>
<td>3a</td>
<td>18.0±0.5 17.7±0.6 17.3±0.8 16.8±0.8 19.8±0.6</td>
</tr>
<tr>
<td>3b</td>
<td>15.0±0.2 15.3±0.2 19.3±0.5 14.9±0.3 17.5±0.3</td>
</tr>
<tr>
<td>3c</td>
<td>21.2±0.3 20.2±0.4 25.6±0.2 19.7±0.2 22.8±0.3</td>
</tr>
<tr>
<td>3d</td>
<td>20.1±0.2 21.1±0.3 19.4±0.2 20.2±0.1 21.6±0.2</td>
</tr>
<tr>
<td>4a</td>
<td>13.5±0.3 13.1±0.5 17.2±0.4 12.8±0.2 15.2±0.4</td>
</tr>
<tr>
<td>4b</td>
<td>13.1±0.7 12.9±0.3 16.1±0.6 12.1±0.5 14.8±0.4</td>
</tr>
<tr>
<td>4c</td>
<td>22.9±0.8 21.6±0.3 25.2±0.2 22.7±0.6 25.5±0.5</td>
</tr>
<tr>
<td>4d</td>
<td>21.1±0.2 20.8±0.4 25.9±0.6 19.9±0.9 22.8±0.2</td>
</tr>
<tr>
<td>Standard</td>
<td>23.0±0.2 22.0±0.2 32.0±0.3 19.0±0.2 27.0±0.2</td>
</tr>
</tbody>
</table>

The fungal activity of each compound was compared with gresero-fulvin as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 4. The nutrient broth, which obtained logarithmic senally two fold diluted amount of test compound and controls was inoculated with approximately 1.6 x 10^6 - 6 x 10^6 c.f.u./ml. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC).

To obtain the minimum fungicidal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of c.f.u. was counted after 48 h of incubation at 35 °C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum fungicidal concentration are given in Table 5. The ratio MFC/MIC was calculated in order to determine if the compound had a fungistatic (MFC/MIC >4) or fungicidal (MFC/MIC <4) activity and the results have been summarized in Table 6.
The antifungal screening data showed moderate to good activity. Among the screened compounds 3b and 5d were found to be most active against all test fungal strains. The compound 5b showed maximum activity against C. albicans, A. fumigatus, and T. mentagrophytes. The compound 3b was active against P. marneffei. The MIC of most of the compounds was two or three folds higher than the corresponding MIC results. Most of the synthesized compounds showed good fungistatic activity against the fungal strain C. albicans.

The present study describes the synthesis of new 4,5-disubstituted-1,3,4-oxadiazoles and thiaxidazoles using long-chain alkenolic acids as the starting material. The described compounds showed good antifungal activity against S. pyogenes, C. albicans, A. fumigatus, T. mentagrophytes, and P. marneffei fungal strains. Among the synthesized oxadiazoles/thiaxidazoles, the compounds with a hydroxalkenyl chain substituent at fifth position of oxa/thiadiaxal were found to be potent antifungal agents. Contrary to the antibacterial studies, the presence of the hydroxyl on the alkenyl side chain turns out to be detrimental for the antifungal activity perhaps due to pharmaco-kinetic reasons.
References and notes

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CH₃-CH₂-CH₂), 1.76 (2H, m, CH₂ to ring), 1.25 (10H, br s, (CH₂)₁₀, ¹³C NMR (100 MHz, CDCl₃, δ ppm) 165.2, 153.0, 139.1, 133.4, 131.3, 129.8, 127.9, 114.2, 33.7, 29.1, 28.9, 28.8, 28.7, 28.4, 26.0, 25.8 MS (ESI) m/z = 338.2 [M+Na]+, calcd = 338.47

S-(R)-1-Heptadec-8'-enyl)-2-Phenylamine-1,3,4-thiadiazole (5b) white powder. Yield 91%, mp 138-140 °C IR (νmax, cm⁻¹, KBr) 3219 (NH), 1468 (C=N), 701 (C-S-C) ¹H NMR (400 MHz, CDCl₃, δ ppm) 12.10 (1H, s, NH), 8.14 (2H, d, J = 8.5 Hz, Ar-H-2''), 7.55 (1H, t, J = 7.4 Hz, Ar-H-4''), 7.45 (2H, t, J = 7.8 Hz, Ar-H-3''), 5.30 (2H, m, CH₂-CH=CH-CH₂), 2.86 (2H, t, J = 7.7 Hz, CH₂ to ring), 2.36 (4H, m, CH₂-CH=CH-CH₂), 1.76 (2H, m, CH₂ to ring), 1.28 (20H, br s, (CH₂)₁₀), 0.80 (3H, d, J = 6.8 Hz, terminus CH₃) ¹³C NMR (100 MHz, CDCl₃, δ ppm) 165.4, 158.0 (one signal hidden), 139.1, 137.2, 133.2, 131.3, 128.8, 128.6, 31.9, 29.9, 29.7, 29.5 (two signals are hidden), 29.3, 29.1, 28.7, 28.4, 27.7, 14.2 MS (ESI) m/z = 436.3 [M+Na]+, calcd = 436.6

5-[(R)-11'-Hydroxy-heptadec-8'-enyl]-2-Phenylamine-1,3,4-thiadiazole (5c) white powder. Yield 83%, mp 139-141 °C IR (νmax, cm⁻¹, KBr) 3332 (OH), 3236 (NH), 1461 (C=N), 608 (C-S-C) ¹H NMR (400 MHz, CDCl₃, δ ppm) 12.17 (1H, s, NH), 8.31 (2H, d, J = 8.4 Hz, Ar-H-2''), 7.43 (1H, t, J = 7.4 Hz, Ar-H-4''), 7.25 (2H, t, J = 7.4 Hz, Ar-H-3''), 5.36 (2H, m, CH₂-CH=CH-CH₂), 3.57 (1H, s, CH-OH), 2.81 (2H, t, J = 7.5 Hz, CH₂ to ring), 2.23 (4H, m, CH₂-CH=CH-CH₂), 1.04 (2H, m, CH₂ to ring), 1.71 (1H, m, CH-OH), 1.33 (18H br s, (CH₂)₁₀), 0.90 (3H, d, J = 6.8 Hz, terminus CH₃) ¹³C NMR (100 MHz, CDCl₃, δ ppm) 168.9, 163.1, 137.8, 133.2, 131.2, 128.7, 128.4, 125.5, 70.56, 60.1, 39.9, 36.3, 24.8, 31.3, 29.1, 29.0, 28.9, 28.5, 28.4, 25.1, 22.3, 14.01 MS (ESI) m/z = 452.5 [M+Na]+, calcd = 452.6
Original article

Synthesis and characterization of novel fatty acid analogs of cholesterol: In vitro antimicrobial activity

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ABSTRACT

In the present study we synthesized, characterized and checked the antimicrobial activity of fatty acid analogs of cholesterol. The synthesized compounds were characterized using IR, $^1$H NMR, $^{13}$C NMR and mass spectral data and tested for their antimicrobial activity by disk diffusion assay with slight modifications against Gram-positive, Gram-negative strains of bacteria as well as fungal strains. Minimum inhibitory concentration (MIC) of all the synthesized compounds was also determined. Compounds 7-14 showed inhibitory action against both the groups of bacteria and four strains of fungus. In vitro antimicrobial activity of the test compounds show that the compounds 10 and 13 are excellent antibacterial agents, whereas compounds 13 and 14 are the excellent antifungal agents among the eight synthesized compounds.

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1. Introduction

Morbidity and mortality because of enteric bacterial infection are the major health problems. Every year millions of people are being killed by some Gram-positive and Gram-negative strains of bacteria. These bacteria mostly lead to food poisoning, rheumatic, salmonellosis and diarrhea [1]. In addition drug resistance in food poisoning, rheumatic, salmonellosis and diarrhea is being developed against the commonly used antimicrobial agents which are being extensively used for the treatment of above diseases. These diverse parasitic bacteria have significant effect on the human mucosa. These micro organisms may cause great damage to host tissue and severe infections [2].

More than 90% of the cases of vaginitis are of candidiasis, trichomoniasis and bacterial vaginosis [3]. Almost 75% women encompass fungal vulvovaginitis at least on one occasion or more in their lives and nearly 40–50% women encompass second incident of vulvovaginal candidiasis in their life. In view of the above, the design and synthesis of newer antimicrobials will always remain an area of immense significance.

Many seed oils, fatty acids (FA) and their derivatives are known for their antimicrobial [4,5], antifungal [6], and pesticidal [7] activities. A number of investigations have demonstrated that a variety of modified FA are promising molecules in cancer prevention and have potential in the treatment of cancers [8–10]. FA-ester analogs have received very little attention despite the fact that such molecules have been found to be associated with diverse biological activities such as antioxidant [11], antifeedant [12], anti-inflammatory [13], antiparasitic [14], antimicrobial [15] and neuroprotective [16]. Some fatty esters have been also found very effective for treatment of dermatitis [17], cardiovascular, hepatic and renal disorders [18]. Thus FA-esters may lead to a new route to potential pharmaceutical molecules.

In the earlier studies it has been reported that direct use of N,N-Dicyclohexylcarbodiimide (DCC) promotes esterification of primary and secondary alcohols under very mild conditions while tertiary alcohols result in poor yields [19]. A number of methods [20] reported for esterification of fatty acids either requires acidic or basic conditions or application of the heat. Literature reveals that some fatty acids have been esterified with cholesterol [21–23].

The purpose of this study was to find the novel bioactivity of various choleseryl esters. It was discussed that cholesterol derivatives could have antimicrobial activity [24]. In view of the significance of long-chain FA as potential pharmacophores; we report here the synthesis and spectral studies of new cholesterol analogs containing...
C11 and C18 FA along with their in vitro evaluation against a panel of Gram-positive, Gram-negative strains of bacteria and some strains of fungus FA incorporated at the C3 α-hydroxy group of cholesterol are undec-10-enoic, 10,11-dibromo-undecanoic, 10,11-epoxy-undecanoic, (9Z)-octadec-9-enoic (9Z,12R)-12-hydroxyoctadec-9-enoic (ricinolic) and (9R,12Z)-9-hydroxyoctadec-12-enoic (isoricinolic) acids (Scheme 1).

2. Chemistry

Undec-10-enoic (purity 98%) and (9Z)-octadec-9-enoic (97%) acids were purchased from Fluka Chemicals (Bucks, Switzerland), (9Z,12R)-12-Hydroxyoctadec-9-enoic (ricinolic, 98%) acid and (9R,12Z)-9-hydroxyoctadec-12-enoic (isoricinolic, 98%) acid were isolated from Ricinus communis and Wrightia tinctoria seed oils.

![Scheme 1 Synthesis of fatty acid analogs of cholesterol](image-url)
respectively following Gunstone’s partition procedure [25]. Cholesterol was purchased from S-d fine-chem (Mumbai, India) 
3-β-Hydroxy-5α,6β-dibromocholesterol [26], 10,11-dibromo-unde
ecanoic acid [27], and 10,11-epoxy-undecanoic acid [28] were 
synthesized following the literature methods.

A solution of FA, DCC and cholesterol/dibromocholesterol in 
dichloromethane with catalytic amount of 4-dimethylaminoipy
didine (DMAP) were stirred mechanically at room temperature until
esterification was complete. Products were purified by column 
chromatography and identified using different spectral techniques.

3. Pharmacology

3.1 Antibacterial studies

The in vitro antibacterial activities of 7-14 were tested using the 
bacterial cultures of Streptococcus mutans UA159 (ATCC#497), 
*Pseudomonas aeruginosa* (ATCC-27853), *Staphylococcus aureus* (ATCC- 
29253), *S. pyogenes* (recultured), *Salmonella typhimurium* (recultured), 
*Escherichia coli* (ATCC-25922) and fungal cultures of *Candida albicans* 
(ATCC-24433), *Candida krusei* (ATCC-6578), *Candida parapsilosis* 
(42219), *Cryptococcus neoformans* (recultured), by the disk 
diffusion method [29,30]. A standard inoculums (1-2 x 10⁷ c f u / 
ml. 0.5 McFarland standards) was introduced on to the surface 
of sterile agar plates, using a sterile glass spreader for even distribution of the 
microorganisms. Disks measuring 6 mm in diameter, sterilized by dry heat 
at 140 °C for 1 h, were prepared from Whatman No 1 filter paper. The 
sterile discs previously soaked in a known concentration of the test 
compounds were placed in nutrient agar medium. Solvent and growth controls were 
kept. Chloramphenicol (30 µg/mL) was used as positive control. While the disk 
poured in DMSO was used as negative control. The plates were inverted and incubated for 48–72 h at 
35 °C. The susceptibility was assessed on the basis of diameter of zone of 
inhibition against albicans and non-albicans strains of fungi. 
Table 1 shows the inhibition zones against Gram-positive and Gram-negative strains of bacteria. 
Table 2 shows the inhibition zones against fungi.

The minimum inhibitory concentration (MIC) was assessed by 
the macro dilution test using standard inoculums of 1 x 10⁹- 
6 x 10⁸ c f u /mL. Initially the compounds were dissolved in DMSO 
after that serial dilution of the test compounds were set to final 
concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/mL. To each tube 
was added 100 µl of 48–72 h old inoculums. The growth was monitored visually and spectrophotometrically. The lowest 
concentration (highest dilution) required to arrest the growth of bacteria was 
regarded as minimum inhibitory concentration (MIC). The 
minimum inhibitory concentrations are given in Table 2.

Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive bacteria</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S. mutans</td>
</tr>
<tr>
<td>7</td>
<td>205 ± 0.2</td>
</tr>
<tr>
<td>8</td>
<td>203 ± 0.2</td>
</tr>
<tr>
<td>9</td>
<td>215 ± 0.5</td>
</tr>
<tr>
<td>10</td>
<td>233 ± 0.1</td>
</tr>
<tr>
<td>11</td>
<td>219 ± 0.5</td>
</tr>
<tr>
<td>12</td>
<td>185 ± 0.4</td>
</tr>
<tr>
<td>13</td>
<td>228 ± 0.5</td>
</tr>
<tr>
<td>14</td>
<td>173 ± 0.5</td>
</tr>
<tr>
<td>Chloramp</td>
<td>268 ± 0.05</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control Chloramphenicol and negative control (DMSO) measured by the Halo Zone Test (Unit mm)

The investigation of antibacterial screening data revealed that 
all the tested compounds showed moderate to good bacterial 
inhibition. Compounds 13, 11, 10 and 7 showed good inhibition 
against *S. mutans*, *S. pyogenes*, *S. aureus* and *E. coli* cells at 32 µg/mL concentrations. Compounds 13, 11, 10 and 7 showed good 
antibacterial activity nearly equivalent to that of Chloramphenicol.

3.2 Antifungal studies

Antifungal activity was also done by disk diffusion method. For 
assaying antifungal activity *C. albicans*, *C. krusei*, *C. parapsilosis*, *C. neoformans* were inoculated in Sabouraud Dextrose broth medium 
(Hi-Media Mumbai) and incubated for 48–72 h at 35 °C, and 
successively a suspension of about 16 x 10⁶-6 x 10⁵ c f u /mL was 
introduced on to the surface of sterile agar plates, and a sterile glass 
spreader was used for even distribution of the inoculums. The discs 
measuring 6 mm in diameter were prepared from Whatman No 1 
filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile 
discs previously soaked in a known concentration of the test 
compounds were placed in Sabouraud Dextrose Agar (SDA) plates. 
Solvent and growth controls were kept. Fluconazole (30 µg/mL) was 
used as positive control. While the disk poured in DMSO was used as 
negative control. The plates were inverted and incubated for 48–72 h at 
35 °C. The susceptibility was assessed on the basis of diameter of zone 
of inhibition against albicans and non-albicans strains of fungi. 
Table 3 shows the inhibition zones against fungi.

The minimum inhibitory concentration (MIC) was assessed by 
the macro dilution method using standard inoculums of 1 x 10⁹– 
6 x 10⁸ c f u /mL. Initially the compounds were dissolved in DMSO 
after that serial dilution of the test compounds were set to final 
concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 µg/mL. To each tube 
was added 100 µl of 48–72 h old inoculums. The growth was monitored visually and spectrophotometrically. The lowest 
concentration (highest dilution) required to arrest the growth of fungi was 
regarded as minimum inhibitory concentration (MIC). The 
minimum inhibitory concentrations are given in Table 4.

The investigation of antifungal screening data revealed that 
all the tested compounds showed moderate to good fungal inhibition 
Compounds 14 and 13 showed good antifungal activity (almost 
equivalent to Fluconazole) against all strains of fungi. Compounds 11 
and 8 exhibited antifungal activity nearly equivalent to that of 
Fluconazole against *C. parapsilosis* at 8 µg/mL and compounds 11 and 8 
showed good antifungal activity against C. albicans at 1 µg/mL.

4. Results and discussion

FA and their derivatives are reported as antimicrobial agents 
[5,6]. It has been suggested that FA may increase antimicrobial 
activity of certain organic moieties [17]. The present study is based
on the synthesis and antimicrobial activities of cholesterol derivat­ives derived from different FA. Six FA—undec-10-enoic (1), 10,11-dibromo-undecanoic (2), 10,11-epoxy-undecanoic (3), (9Z)-octadec-9-enoic (4), (9Z,12R)-12-hydroxyoctadec-9-enoic (5) and (9R,12Z)-9-hydroxyoctadec-12-enoic (6) acids were esterified with the C3β-hydroxy group of cholesterol with the help of DCC in the presence of a catalytic amount of DMAP to produce 7, 8, 11–14 in quantitative yields (Scheme 1). Similarly compounds 1 and 2 on reacting with 3-β-Hydroxy-5a,6P-dibromocholesterol afforded ester 9 and 10 respectively. The products 7–14 were purified on a silica gel column with n-hexane/ethyl acetate as eluent. The purity of the compounds was checked by TLC. The signals of the FA and cholesterol moieties in the 1H and 13C NMR spectra of compounds 7–14 were successfully assigned. The high-resolution mass (electronization) spectral studies have further confirmed their structures. A detailed spectral description for compound 7 is discussed below.

IR spectrum of compound 7 revealed characteristic band at 1732 cm–1 (ester C=O stretching). The two olefinic protons, C17H32C=OCH2 and C17H32C=O were observed at δH 5.81 (tdd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.85 (tdd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 5.36 (d, 1H, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–) and correlated with observation at δC 173.2 cm–1 (ester C=O stretching). The two olefinic protons, C17H32C=OCH2 and C17H32C=O were observed at δH 5.81 (tdd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.85 (tdd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 5.36 (d, 1H, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–) and correlated with observation at δC 173.2 cm–1 (ester C=O stretching). The two olefinic protons, C17H32C=OCH2 and C17H32C=O were observed at δH 5.81 (tdd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.85 (tdd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 5.36 (d, 1H, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–) and correlated with observation at δC 173.2 cm–1 (ester C=O stretching).

<table>
<thead>
<tr>
<th>Com磅nses</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA</td>
</tr>
<tr>
<td>7</td>
<td>18.3 ± 0.2</td>
</tr>
<tr>
<td>8</td>
<td>19.5 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>15.5 ± 0.5</td>
</tr>
<tr>
<td>11</td>
<td>17.8 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td>19.6 ± 1.4</td>
</tr>
<tr>
<td>13</td>
<td>18.7 ± 0.5</td>
</tr>
<tr>
<td>14</td>
<td>20.3 ± 1.2</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>22.5 ± 0.5</td>
</tr>
</tbody>
</table>

CA: Candida albicans, CK: Candida krusei, CP: Candida parapsilosis, CN: Cryptococcus neoformans

5. Conclusion

Here we have reported the application of DCC coupling chem­istry procedure to synthesize the fatty acid analogs of cholesterol. The structures of compounds were extensively characterized. The preliminary biological testing revealed that compounds 14 and 13 showed good antifungal activity (almost equivalent to Fluconazole) against all strains of fungi. Compounds 13, 11, 10 and 7 showed good antibacterial activity nearly equivalent to that of Chloramphenicol. The varied divergence in the antimicrobial activity of these compounds validates the reason of this study. The importance of such kind of work lies in the possibility that the new compounds might be efficient antimicrobials. A detailed study regarding the structure–activity relationship, toxicity and other biological effects of such analogs would be helpful in designing more effective antimicrobial agents for therapeutic use.

6. Experimental protocol

6.1. Physical and spectroscopic measurements

Thin layer chromatography was done on glass plates (20 × 5 cm) with a layer of silica gel G (Merck, Mumbai, India, 0.5 mm thickness). Mixture of petroleum ether–ethyl acetate–acetic acid (50:50:1 v/v) were used as developing solvents. Column chromatography was carried out on silica gel (Merck, Mumbai, India, 60–120 mesh). 1H NMR was recorded with Bruker DRX 400 spectrometer at 400 MHz and 13C NMR was recorded at 100 MHz in CDC13. Chemical shifts (δ) are quoted in ppm. Melting points were taken in open capillary and are uncorrected.

6.2. Synthesis of fatty acid analogs of cholesterol

A solution of FA (5 mmol), DCC (5.5 mmol) and cholesterol/dibromocholesterol (5 mmol) in dichloromethane (50 mL) with catalytic amount of 4-dimethylaminopyridine (DMAP) were stirred mechanically at room temperature until esterification was complete. The N,N-dicyclohexylurea was filtered off and the filtrate was washed with water (3 × 50 mL), 5% acetic acid (3 × 50 mL) again with water (3 × 50 mL) and then dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure to give the ester 7–14 (Scheme 1) which were chromatographed over a column of silica gel using n-hexane–ethyl acetate (94:6 v/v) as eluent. All these novel compounds were characterized from their spectral data.

6.2.1. 3β-Cholesteryl-undec-10-enoate (7)

White amorphous compound. Rf = 0.83 (n-hexane/ethyl acetate, 1:1 v/v, as developer), isolated yield, 92%. m.p. 86 °C. IR (KBr, cm–1): 2927, 2851, 1732, 1466; 1H NMR (CDCl3, δH): 5.81 (tdd, 1H, JH,–H = 6.7 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.85 (tdd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 5.36 (d, 1H, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–), 4.98 (dd, 1H, JH,–H = 10.1 Hz, JH,–H = 6.7 Hz, CH2=CH–).
M R. Banday et al / EuropeanJoumol 0/Medicinal Chemistry 45 (20W) 1459-1464
yHz-H, = 2 8 H z , H z C = C H - ) , 4 9 3 ( d d . I H , J H , - H = 1 6 9 H Z ,
JH,-HI = 2 8 HZ. H £ C = C H - ) , 4 6 1 ( m , I H , CjP-H). 2 3 5 1 - 1 0 7 (far m,
44H). 101 (s, 3H, C,o-CH3), 0 9 0 (d, 3H,J = 6 5 Hz. C20-CH3), 0 8 6 (dd.
6H.7 = 6 6 Hz C25-(CH3)2), 0 6 7 (s, 3H, C n - C H j ) " C NMR (CDCI3. k)
173 41.139 77.139 27.122 6 7 114 2 3 , 7 3 7 5 . 5 6 7 4 . 5 6 1 7 . 50 0 6 . 4 2 37.
3 7 7 8 . 3 9 59. 3 8 22. 3 7 0 6 , 3 6 66, 3 6 2 5 3 5 8 8 . 3 4 7 8 . 33 8 9 . 3 1 9 7 .
3 1 9 1 . 2 9 3 8 . 2 9 3 0 . 2 9 1 7 . 2 9 1 5 , 2 8 97, 2 8 3 2 , 2 8 1 0 , 2 7 8 8 , 2 5 1 3
2 4 3 6 , 2 3 90, 2 2 92, 2 2 66, 2 1 1 0 , 1 9 4 1 , 18 7 9 , 1 1 9 3 , ESl-MS found
[M + Na]+ 5 7 4 4 1 , C38H6402 [M + N a ] * requires 575 3 5

622 3P-Cholesteryl-W.n'-dibromo-undecanoate
(8)
Off-white solid. Rf = 0 77 (n-hexane/ethyl acetate, 1 1 v/v, as
developer), isolated yield, 94X m p, 84 °C IR (KBr cm ') 2940.
2853.1732.1464. ' H NMR (CDCI3. «H) 5 36(d. 1 H , / = 4 8 Hz, Ce-H),
461 (m, IH, C3P-H), 416 (m, IH, Cio-H), 3 85 (dd, 2H,i = 10 0 Hz,
j = 2 4 Hz. C„ -H2). 2 31 - 1 0 7 (6r m. 44H), 104 (s, 3H, C10-CH3). 0 94
(d. 3H.y = 6 6 Hz. C20-CH3). 0 85 (dd, 6H,7 = 6 6 Hz C25-(CH3)2), 0 67
(s, 3H, C,3-CH3) " C NMR (CDCI3, <5c) 173 37,139 75,122 68 73 77,
56 73, 5616, 53 20, 50 05, 42 36, 39 77, 39 58, 38 22, 37 05, 36 66,
3643, 3624, 3605, 35 87, 34 76, 3197, 3105, 29 25, 29 21, 2911,
28 80, 28 31. 28 09, 2788, 26 78. 25 09, 24 36, 23 89 22 92, 22 65,
2109,1941,18 78,1193 , ESl-MS found [M + Na]* 733 78/735 76/
73769, C38H6402Br2 [M + Nal+ requires 733 35/735 35/73735
623 5a.60-Dibromo-3p-cholesteryl-undec-lO'-enoate (9)
White amorphous compound, Rf-0 78 (n-hexane/ethyl acetate,
1 1 v/v, as developer), isolated yield. 90* m p, 82 °C IR (KBr, cm"')
2932, 2850, 1738, 1466. ' H NMR (CDCI3, <5H) 5 73 (tdd, IH
JH-9<H, = 6 7 H Z , JH-HZ = 10 1 Hz, ; „ _ „ , = 16 9Hz, CH2=CH-),
4 9 5 (dd, 1H,;H,_H = 10 1 HZ,JHZ-HE = 2 8 HZ, H Z C = C H ) , 4 8 8 (dd,
1H,;H,-H = 1 6 9 H Z , ; H J », = 2 8Hz.H£C=CH-),475(m,lH,C3P-

H), 3 63 (t, I H , ; = 4 8 Hz, Cg-H), 2 23-1 07 (br m, 44H,), 1 01 (s 3H,
C10-CH3), 082 (t, 3H, J = 6 6 Hz, C20-CH3), 0 79 (dd, 6H,i = 6 4 Hz
C25-(CH3)2). 0 6 3 (s. 3H. C,3-CH3) " C NMR (CDCI3. 5c) 173 27,
139 25, 11423, 8819, 7179, 56 20, 5610, 5517, 4721 42 72, 4198,
4190, 3961. 39 54. 3724, 36 54. 3616. 3581, 3461, 33 86, 3103
3084, 2935, 2926, 2912, 28 94, 28 22, 28 07, 26 27, 25 04, 24 09,
23 84. 22 89. 22 63. 2132, 20 21, 18 71, 12 25 ESl-MS found
[M + NaJ+ 733 75/735 79/73776, C38H6302Br2 [M + N a r requires
733J5/735J5/73735
624 Sa.ep.lO'.U'-Tetrabromo-SP-cholesteryl-undecanoate (10)
Off-white solid, Rf = 0 74 (n-hexane/ethyl acetate 1 1 v/v as
developer), isolated yield. 74* m p , 88 °C IR (KBr cm ' ) 2945,
2886.1732.1465, ' H NMR(CDCI3. (5H) 4 82 (m. 1H, C3P-H), 416 (m,
IH,Cw-H), 3 84(dd, 2H,; = 1 0 0 Hz,/ = 2 4 Hz, Ci, -H2), 3 63 (t. 1H,
; = 4 8 Hz, Cs-H), 2 58-1 07 (br m. 44H,), 1 00 (s, 3H, Co-CHs), 0 90
(d, 3H,J = 6 6 Hz, C20-CH3). 0 85 (dd. 6H, J = 6 6 Hz C25-(CH3)2).
0 6 4 (s, 3H, C13-CH3) " C NMR (CDCI3, 5c) 17318, 7179, 5619
5614, 56 09, 47 26, 44 89, 42 70, 42 50, 39 67, 39 60 39 53, 39 48
37 22, 36 52, 36 40, 3615, 36 09, 36 01, 35 79, 35 71, 34 71, 34 56
3164, 3101, 30 83, 29 23, 2917, 28 77, 28 05, 26 95, 26 75, 24 99,
23 83, 22 63. 2141,18 70,11 91 , ESl-MS found [M + Na]+ 89131/
893 67/895 81/89773/899 91, C38H6302Br4 (M + Nal+ requires
891 35/893 35/895 35/89735/899 35
6,25 S^-Cholesteryl-lff.W-epoxyundecnoate
(11)
White solid R/= 0 74 (n-hexane/ethyl acetate, 1 1 v/v, as developer), isolated yield, 72% m p , 94 "C IR (KBr c m " ' ) 2932, 2854
1732,1464,1174, ' H NMR (CDC3I, <5H) 5 36 (d, IH.i = 4 4 Hz, Ce-H)
461 (m, IH, C3P-H). 2 90 (m. IH. Cw-H). 2 75 (dd 2H,7 = 101 Hz
y = 2 6 Hz, Cu -H2), 2 31 -1 05 (br m, 44H), 1 01 (s 3H, C10-CH3), 0 95
(d. 3H,; = 6 4 Hz, C20-CH3). 0 85 (dd. 6H.7 = 6 6 Hz C25-(CH3)2) 0 67
(s. 3H, C13-CH3) " C NMR (CDCI3. 6c) 173 18,139 75,122 68, 73 77,
56 73, 5616, 53 20, 50 05, 42 36, 39 77, 39 58, 38 22, 37 05, 36 66

1463

36 43, 36 24, 3605, 35 87, 34 76, 3197, 3105, 29 25, 29 21, 2911,
28 80, 28 31, 28 09, 27 88, 26 78, 25 09, 24 36, 23 89, 22 92. 22 65,
2109,19 41,18 78,11 9 1 , ESI-MS found JM + Na]+ 571 74, C38H64O3
IM + Nal+ requires 591 57
6 2 6 3|3-Cholestery(octadec-9'-fZ>enoQte (12)
Viscous pale-yellow oil, R/= 0 70 (n-hexane/ethyl acetate, 1 1 v/v,
as developer), isolated yield 80% IR(KBr, cm"') 2930,2852,1737,
1463, ' H NMR(CDCl3. 6H) 5 36(d.IH.J = 4 8 Hz.Ce-H), 533 (m,2H.
C9.H=Cio'H). 4 62 (m. 1 H. C3P-H). 2 31 -107 (br m, 53H.). 101 (s. 3H,
C10-CH3). 0 90 (d. 3H. j = 6 5 Hz. C20-CH3). 0 88(dist t, 3H. Cis H3),
0 8 5 (dd. 6H.J = 6 6 Hz C25-(CH3)2), 0 6 7 (s, 3H, C13-CH3) " C NMR
(CDCI3 &c) 170 42,139 77,139 27,127 67,124 23, 8810,47 26.44 89.
42 70. 42 50, 42 37, 39 78, 39 59, 3722, 3706, 3640, 3615, 3609,
3601, 35 88, 35 71, 3478, 33 89, 3197, 3191, 29 38, 2930, 2917,
2915, 2897, 2832. 2810, 2788, 2513, 24 36, 2408, 23 83, 2289,
22 69 22 63 2131,2018,1941,18 66,1421 ESl-MS found IM + Na)*
674 03, C45H78O2 (M + Na]-^ requires 673 42
6 2 7 3(J-Cho(estery/-I2'-(Rj-hydroxyoctadec-9'-(Z;-enoate (13;
Viscous colorless oil, R; = 0 67 (n-hexane/ethyl acetate. 1 1 v/v,
as developer), isolated yield, 75% IR (nujol, cm ' ) 3452, 2935,
2863,1737,1463, ' H NMR(CDCl3, «H) 5 41 (m,2H.C9H=Ci(yH).5 36
(d, l H , i = 4 6 Hz, Cs-H), 4 87 (m, IH, OH, D2O exchangeable), 461
(m, 1H, C3P-H), 3 61 (m, 1H, C,2 H-OH), 2 31 - 1 0 4 (br m, 51H,), 101
(s, 3H, C,o-CH3). 0 94 (d, 3H, J = 6 5 Hz, C20-CH3), 0 89 (dist t, 3H,
C,8 H3) 0 85 (dd, 6H, j = 6 6 Hz C25-(CH3)2), 0 67 (s, 3H, C13-CH3)
"C NMR (CDCI3, 6c) 17042, 139 77, 139 27, 12767, 12423, 7710,
68 5, 4746, 44 39, 42 20, 4210, 42 07, 39 75, 39 59, 3721, 3706,
36 43, 36 25, 3619, 3611, 35 83, 35 72, 34 75, 33 39, 3191, 29 38,
29 33, 2917, 2915, 28 37, 28 22, 2810, 2733, 2513, 2446, 2418,
23 83, 22 89, 22 69, 22 63, 21 31, 2018,19 41,18 66,13 6 7 , ESl-MS
found [M + Nal+ 690 07, C45H78O3 (M + Nal+ requires 689 42
628 3(3-C/iolesteryl-9'-(R>hydroxyoctadec-12 -(Z;-enoate (14)
Viscous colorless oil, R/= 0 8 (n-hexane/ethyl acetate. 1 1 v/v. as
developer), isolated yield. 98% lR(nujol,cm"') 3449.2922.2859,
1737, 1466, ' H NMR (CDCI3, 6») 5 40 (m, 2H, Ci, H=Ci2H), 5 33
(d, 1H,J = 4 8 Hz, Ce-H), 4 6 4 (m, IH, OH, D2O exchangeable), 4 57
(m, IH C3P-H), 3 60 (m, IH, C9 H-OH,), 2 31-107 (br m, 51H,), 101
(s, 3H, C10-CH3), 0 92 (d, 3H, J = 6 5 Hz, C20-CH3), 0 88 (dist t. 3H.
C,8 H3). 0 85 (dd. 6H. J = 6 6 Hz C25-(CH3)2). 0 67 (s. 3H. C^-CHs)
"C NMR (CDCI3. 6c) 170 42. 139 72,13919,127 64,124 23, 78 33,
67 53, 46 23, 44 77, 42 71, 42 39, 42 37, 39 76. 39 44. 37 22. 3716,
36 20, 3615 36 00, 35 88, 35 71, 34 34, 33 99, 3165, 3111, 29 38,
29 34, 29 30 29 06, 29 02, 28 81, 28 32, 2810, 27 32, 25 33, 24 36,
24 76, 2313, 22 89, 22 21, 2213, 21 31, 2018, 19 41,1419 , ESl-MS
found [M + Nal+ 69009, C,5H7803 (M + N a r requires 689 42
6 3 Pharmacology
Antibaaerial activity and antifungal activity of the synthesized
compounds was completed by disk diffusion method and measured
by Halo Zone Test [29 30) The MIC of synthesized compounds
against bacterial and fungal strains was performed by macro dilution
test and results were observed visually and spectrophotometncally
Acknowledgements
The authors thank the chairman. Department of Chemistry,
AMU, Aligarh for providing necessary facilities and SAIF Panjab
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2007 (SR)1


References

DCC/DMAP mediated esterification of hydroxy and non-hydroxy olefinic fatty acids with β-sitosterol: In vitro antimicrobial activity

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b Department of Biochemistry, Aligarh Muslim University Aligarh 202002, India

Received 6 November 2009

Abstract

A new series of fatty alkenoates were synthesized using an appropriate synthetic route involving DCC and DMAP as catalysts. Compounds were characterized by their spectral data. All the synthesized compounds were evaluated for their in vitro antimicrobial activity. The minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC) and minimum fungicidal concentration (MFC) were determined for test compounds as well as for reference standards. Among the compounds tested, compounds having hydroxy group at the fatty acid chain showed the most potent antibacterial as well as antifungal activities.

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Keywords Fatty acids, β-Sitosterol, DCC, DMAP, IR, NMR, Mass, Antimicrobial activity

The negative health trends call for a new interest in infectious disease in the medical and public health communities and renewed strategies on treatment and prevention. Solutions outlined by the Centre for Disease Control and Prevention (CDC) include: prevention, (such as vaccination); improved monitoring; and the development of new treatments. Last solution would encompass the development of new antimicrobials by which these infectious diseases can be defeated [1]. Many seed oils, fatty acids and their derivatives are known for their antimicrobial [2], antifungal [3] and pesticidal [4] activities. A number of investigations have demonstrated that a variety of modified fatty acids are promising molecules in cancer prevention and have potential in the treatment of cancers [5,6]. Fatty alkenoates have received very little attention despite the fact that such molecules have been found to be associated with diverse biological activities such as antioxidant [7], antifeedant [8], anti-inflammatory [9], antiparasitic [10], antimicrobial [11] and neuroprotective [12]. Some fatty esters have been also found very effective for treatment of dermatitis [13], cardiovascular, hepatic and renal disorders [14]. Thus fatty alkenoates may lead to a new route to potential pharmaceutical molecules.

A number of methods [15] reported for esterification of fatty acids require either acidic or basic conditions or application of the heat. N,N-Dicyclohexylcarbodiimide (DCC) is used as a dehydrating/activating agent in organic synthesis [16] and is extensively used for building the peptide and amide linkages [17]. Literature reveals that long-chain fatty acids have not been esterified with β-sitosterol in the presence of DCC.

* Corresponding author
E-mail address abduJoafchem@gmail.com (A. Rauf)
The purpose of this study was to find the novel bioactivity of \( \beta \)-sitosteryl esters. It was discussed that \( \beta \)-sitosterol derivatives could have antimicrobial activity \[18,19\]. In view of the significance of long-chain fatty acids as potential pharmacophores, we report here the synthesis and spectral studies of new \( \beta \)-sitosterol analogs containing C11 and C18 fatty acids along with their \textit{in vitro} evaluation against a panel of Gram-positive, Gram-negative strains of bacteria and some strains of fungus.

1. Experimental

Undec-10-enioic (purity 98\%) and (9Z)-octadec-9-enoic (97\%) acids were purchased from Fluka Chemicals (Buchs, Switzerland). (9Z,12R)-12-Hydroxyoctadec-9-enoic (ricinoleic) and (9R,12Z)-9-hydroxyoctadec-12-enoic (isorinicoleic) acids were isolated from the natural sources, i.e. from \textit{Ricinus communis} and \textit{Wrightia tinctoria} seed oils respectively. The concentrate of pure hydroxy acids was obtained by Gunstone's partitioning \[20\] of freshly prepared acids and further purified by column chromatography. \( \beta \)-Sitosterol was isolated from \textit{Ficus krishnae} following the literature procedure \[21\].

Fatty acid (5 mmol), DCC (5.5 mmol), and \( \beta \)-sitosterol (5 mmol) in dichloromethane (20 mL) with catalytic amount of 4-dimethylaminopyridine (DMAP) were stirred mechanically at room temperature until esterification was complete. \( N,N \)-dicyclohexylurea formed was filtered off. The filtrate was washed with water (3 \( \times \) 20 mL), 5\% acetic acid (3 \( \times \) 20 mL) again with water (3 \( \times \) 20 mL) and dried over anhydrous sodium sulphate. Solvent was removed under the reduced pressure to give the ester 6-9 (Scheme 1) which were chromatographed over a column of silica gel using \( n \)-hexane-ethyl acetate (96:4, v/v) as an eluent. All these newly synthesized compounds were characterized by IR, \(^1\)H NMR, \(^{13}\)C NMR and mass spectral data \[22\].

The newly synthesized compounds were screened for their \textit{in vitro} antibacterial activity against selected bacterial strains by disk diffusion method \[23\]. Chloramphenicol (30 \( \mu \)g) was used as positive control. While the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 1. MICs and MBCs were determined by broth dilution technique and the results are given in Table 2.

Antifungal activity was also done by disk diffusion method. For assaying antifungal activity was measured by agar diffusion method \[24\]. The fungal activity of each compound was compared with griseofulvin as a standard drug. The fungal zones of inhibition values are given in Table 3. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) are given in Table 4.

2. Results and discussion

The use of catalysts has become popular among synthetic organic chemists for improving classical organic reactions, shortening reaction time and/or improving yields, as well as promoting new reactions. To explore the probability of getting the pharmacophoric important moiety from natural products in higher yields and in shorter
### Table 1
Antibacterial activity of the newly synthesized fatty alkenoates

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter of zone of inhibition (mm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B subtilis</td>
<td>S aureus</td>
</tr>
<tr>
<td>6</td>
<td>17.8 ± 0.4</td>
<td>17.3 ± 0.5</td>
</tr>
<tr>
<td>7</td>
<td>15.6 ± 0.3</td>
<td>15.2 ± 0.4</td>
</tr>
<tr>
<td>8</td>
<td>21.9 ± 0.8</td>
<td>20.6 ± 0.3</td>
</tr>
<tr>
<td>9</td>
<td>20.1 ± 0.2</td>
<td>20.2 ± 0.3</td>
</tr>
<tr>
<td>Standard</td>
<td>23.0 ± 0.2</td>
<td>22.0 ± 0.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive control (standard), chloramphenicol and negative control (DMSO) measured by the Halo Zone Test (unit, mm)

### Table 2
MIC and MBC results of newly synthesized fatty alkenoates positive control chloramphenicol

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter of zone of inhibition (mm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B subtilis</td>
<td>S aureus</td>
</tr>
<tr>
<td>6</td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>7</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>9</td>
<td>6.25</td>
<td>25</td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

MIC (μg/mL), minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of bacteria completely. MBC (μg/mL), minimum bactericidal concentration, i.e. the lowest concentration of the compound for killing the bacterium completely.

### Table 3
Antifungal activity of newly synthesized fatty alkenoates

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA</td>
</tr>
<tr>
<td>6</td>
<td>26.1 ± 0.3</td>
</tr>
<tr>
<td>7</td>
<td>22.2 ± 0.4</td>
</tr>
<tr>
<td>8</td>
<td>23.1 ± 0.7</td>
</tr>
<tr>
<td>9</td>
<td>23.6 ± 0.4</td>
</tr>
<tr>
<td>Standard</td>
<td>30.0 ± 0.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
</tr>
</tbody>
</table>

CA, Candida albicans, AN, Aspergillus niger, HO, H. oryzae, PM, P. mameffei. MIC (μg/mL), minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of fungus completely. MFC (μg/mL), minimum fungicidal concentration, i.e. the lowest concentration of the compound for killing the fungus completely.

### Table 4
MIC and MFC results of newly synthesized fatty alkenoates positive control greseofulvin

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CA</th>
<th>AN</th>
<th>HO</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diameter of zone of inhibition (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIC</td>
<td>MFC</td>
<td>MIC</td>
<td>MFC</td>
</tr>
<tr>
<td>6</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>12.5</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>6.25</td>
<td>25</td>
<td>6.25</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>12.5</td>
<td>50</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Standard</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

CA, Candida albicans, AN, Aspergillus niger, HO, H. oryzae, PM, P. mameffei. MIC (μg/mL), minimum inhibitory concentration, i.e. the lowest concentration of the compound to inhibit the growth of fungus completely. MFC (μg/mL), minimum fungicidal concentration, i.e. the lowest concentration of the compound for killing the fungus completely.
reaction time, our attention has turned to employ the use of catalysts. The use of catalysts for the synthesis of fatty alkenoates provided higher yield of products. In most of the cases the yield of product was 74–88%. At first the esterification of β-sitosterol with undec-10-enoc acid was chosen as a model to optimize the conditions for the preparation of compounds 6–9. In order to determine the optimum conditions for the synthesis fatty alkenoates, variations in molar ratios of reagents and the catalysts were investigated. After some experimentation, we found a set of conditions that generally provide products in good yield and thus optimum conditions for molar ratio of fatty acids, β-sitosterol, DCC and DMAP were set up. The generality and scope of the synthetic procedures were demonstrated by subjecting β-sitosterol with olefinic (terminal and internal) and hydroxy olefinic carboxylic acids. IR absorptions characteristic of ester (1732–1737 cm\(^{-1}\)) were observed in all the newly synthesized compounds.

In the \(^1\)H NMR spectra of compound 6, the olefinic protons were observed at \(\delta_H 5.80\) (tdd, 1H, \(J_{H-CH_2} = 6.6\) Hz, \(J_{H-He} = 10.2\) Hz, \(J_{H-Hg} = 16.4\) Hz, \(CH_2=CH-\)), \(5.38\) (d, 1H, \(J = 4.4\) Hz, \(C_9-H\)), \(4.95\) (dd, 1H, \(J_{Hg-H_{10}} = 10.2\) Hz, \(J_{Hg-Hz} = 2.6\) Hz, \(H_2C=CH-\)), \(4.91\) (dd, 1H, \(J_{Hz-Hg} = 16.4\) Hz, \(J_{Hz-Hz} = 2.8\) Hz, \(H_{10}C=CH-\)) and correlated with observations at \(\delta_C 114.23, 122.67, 139.77, 139.27\) respectively. A multiplet at \(\delta_H 4.62\) was observed for \(C_7\beta-H\) and correlated with observation at \(\delta_C 73.75\). Besides few other significant carbon signals for the fatty acid chain characteristic peak at \(\delta_C 174.41\) (C=O, ester C=O) were recorded. Spectral studies have illustrated that the change in the nature of fatty acids at \(p-C\) has not significantly influenced the pattern of proton and carbon signals of the β-sitosterol moiety.

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. In most of the cases compounds 8 and 9 showed good antibacterial activity nearly equivalent to that of chloramphenicol. The MBC of few compounds was found to be the same as MIC but in most of the compounds it was two or three folds higher than the corresponding MIC results. The antifungal screening data showed only moderate activity. Among the screened compounds, 6 showed good inhibition against Candida albicans. Compounds 8 and 9 revealed good results against C. albicans and Helminthisporum oryzae and moderate activity against Aspergillus niger and Penicillium marneffei. The MFC of all the compounds was two or three folds higher than the corresponding MIC results.

The varied divergence in the antimicrobial activity of these compounds validates the reason of this study. The importance of such kind of work lies in the possibility that the new compounds might be more efficient against bacteria and a thorough study regarding the structure–activity relationship, toxicity and their biological effects would be helpful in designing more effective antimicrobial agents for therapeutic use.

Acknowledgments

The authors thank the Chairman, Department of Chemistry, AMU, Aligarh, for providing necessary facilities and SAIF Panjab University, Chandigarh for recording the spectra.

References

Selected spectroscopic data: 3β-sitosterolundec-10'-enoate 6. White solid, mp 93 °C. Rf = 0.76 (n-hexane/ethyl acetate, 3 : 2, v/v, as developer), isolated yield, 88% IR (KBr, cm⁻¹) 2931, 2860, 1736. ¹H NMR (CDCl₃) δH 5.80 (tdd, JH-H² = 6.6 Hz, JH-H¹ = 10.2 Hz, JH-H⁰ = 16.4 Hz, CH₂=C═CH), 5.38 (d, J = 4.4 Hz, C⁷-H), 4.95 (dd, JH-H⁰ = 10.2 Hz, JH-H¹ = 2.6 Hz, H₆=C═CH), 4.91 (dd, JH-H⁰ = 16.4 Hz, JH-H¹ = 2.8 Hz, H₅=C═CH⁻), 4.62 (m, JH-H¹ = 1.9 Hz, C¹⁴-β-H), 2.31-1.07 (br m, 46H), 1.01 (s, 3H, C₁₀-CH₃), 0.92 (s, 3H, C₁₀-CH₃), 0.86 (dd, JH-H³ = 4.4 Hz, C₂⁶-(CH₃)₃), 0.84 (d, JH-H³ = 6.4 Hz, C₂⁶-(CH₃)_, 0.68 (s, 3H, C₁₂-CH₃) ¹³C NMR (CDCl₃) δC 173.41, 139.77, 139.27, 122.67, 114.23, 73.75, 56.74, 56.17, 50.06, 42.37, 39.77, 39.59, 38.22, 37.78, 37.06, 36.66, 36.25, 35.88, 34.78, 33.89, 31.97, 31.91, 29.38, 29.30, 29.17, 29.15, 28.97, 28.32, 28.10, 27.88, 25.13, 24.36, 23.90, 22.92, 22.66, 21.10, 19.41, 18.79, 14.9, 11.93, ESI-MS found [M+Na]+ 603.90, C₄₀H₆₈O₂ [M+Na]+ requires 603.83


7-Hydroxy-coumarin derivatives: synthesis, characterization and preliminary antimicrobial activities

Nida N. Farshori · Mudasir R. Banday · Anis Ahmad · Asad U. Khan · Abdul Rauf

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Abstract A new series of 7-O-coumarinyl alkenoates were synthesized from 7-hydroxy-coumarin and fatty acids using DCC and DMAP as catalyst. The synthesized compounds were characterized on the basis of their spectral data. All the target compounds were evaluated for their in vitro antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogene, Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli and fungal cultures of Candida albicans, Candida krusei, Candida parapsilosis and Cryptococcus neoformans. The minimum inhibitory concentration (MIC) was determined for the test compounds as well as for reference standards. Among the tested compounds, 7-O-coumarinyl (9Z, 12R)-12-hydroxyoctadec-9-enoate and 7-O-coumarinyl (12Z, 9R)-9-hydroxyoctadec-12-enoate showed the most potent antifungal as well as antibacterial activities.

Keywords 7-Hydroxy-coumarin · Fatty acids · DCC · DMAP · Antimicrobial activity

Abbreviations
FA Fatty acid
7-HC 7-Hydroxy-coumarin
DCC N,N'-Dicyclohexylcarbodiimide
DMAP 4-Dimethylaminopyridine

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Introduction

Morbidity and mortality because of enteric bacterial infection are the major health problems in some areas like Indian subcontinent, portions of South America and tropical fraction of Africa (Qadri et al., 2005; Devasia et al., 2006). Every year millions of people are being killed by some or the other Gram-positive and Gram-negative strains of bacteria. These bacteria mostly lead to food poisoning, rheumatic, salmonellosis and diarrhoea (Khan et al., 2008). In addition, drug resistance is being developed by these bacteria against the commonly used antimicrobial agents which are being extensively used for the treatment of above diseases. Furthermore, the pharmacological drugs available are either too expensive or have undesirable side effects or contraindications (Berger, 1985). Many traditional plant treatments for the antimicrobial infections exist, and there lies a hidden wealth of potentially useful natural products for the control of microbial diseases (Gray and Flatt, 1997). Natural plant drugs are frequently considered to be less toxic and free from side effects than synthetic ones (Morin, 1987). Among the most significant classes of natural compounds, an important position is occupied by oxygen-containing heterocyclic compounds. 7-Hydroxy coumarin (7-HC) is a benzopyrone in nature, which is a major human metabolite and plays a role as dietary antioxidant in the human diet (fruits and vegetables). 7-HC has been reported to have antitumor (Kofinas et al., 1998), aldose reductase inhibitor (Okada et al., 1995) and xanthine oxidase inhibitor (Mills and Bone 2000) activities. The parent compound coumarin has been reported to reduce blood glucose levels (Marles and Farnsworth, 1996). A number of natural and synthetic coumarin (2-oxo-2H-chromene) derivatives have been reported to exert antimicrobial (Czerpack and Skolska, 1982; Jund et al., 1971), antifungal (El-Ansary et al., 1992; Reddy and Somayojulu, 1981), antiamoebic (Iqbal et al., 2009) and tuberculostatic (Abd Allah, 2000) activities. Moreover, the antibiotic novobiocin belongs to the hydroxy coumarin series. Coumarin may be a prodrug and 7-HC is the pharmacologically active agent (Ritschel et al., 1981). Many seed oils, fatty acids (FA) and their derivatives are known for their antimicrobial (Rauf and Parveen, 2005; Kabara et al., 1972), antifungal (Ahmed et al., 1985) and pesticidal (Khan et al., 1983) activities. A number of investigations have demonstrated that a variety of modified FA are promising molecules in cancer prevention and have potential in the treatment of cancers (Mujeebur-Rahman et al., 2005; Nagao et al., 1991; Lie Ken Jie et al., 1990). FA-ester analogs have received very little attention despite the fact that such molecules have been found to be associated with diverse biological activities such as antioxidant (Viklund et al., 2003), antifeedant (Mallavadhani et al., 2003), anti-inflammatory (Feng et al., 2009), antiparasitic (Grunberg et al., 1973), antimicrobial (Mod et al., 1975) and neuroprotective (Takahashi et al., 2003). Some fatty esters have been found to be very effective for the treatment of dermatitis (Csőka et al., 2007) cardiovascular, hepatic and renal disorders (Greeellings et al., 2003). Thus, FA-esters may lead to a new route to potential pharmaceutical molecules. Literature reveals that long-chain fatty acids have not been esterified with 7-HC in presence of DCC and DMAP.
The purpose of this study is to find the novel bioactivity of 7-HC esters. As discussed, the coumarin derivatives possess antimicrobial activity and in view of the significance of long-chain FA as potential pharmacophores; we herein report the synthesis and spectral studies of new coumarin derivatives containing C11 and C18 FA chain. These derivatives were further tested for their in vitro antimicrobial activity against a panel of Gram-positive, Gram-negative strains of bacteria and selected strains of fungus. FA incorporated at the C7 hydroxy group of coumarin are undec-10-enioic, (9Z)-octade-9-enioic, (9Z, 12R)-12-hydroxyoctadec-9-enioic (ricinoleic) and (9R, 12Z)-9-hydroxyoctadec-12-enioic (isoricinoleic) acids (Scheme 1).

Results and discussion

Chemistry

FA and their derivatives have been reported as antimicrobial agents. It is expected that the incorporation of the hydroxyl and non-hydroxyl FA chain may increase the antimicrobial activity of certain organic moieties. This study is based on the synthesis, characterization and evaluation of antimicrobial activities of 7-HC derivatives derived from different FA. 7-Hydroxy coumarin reacts with fatty acid in the presence of DCC and 4-dimethyl aminopyridine (DMAP) in dichloromethane by stirring at room temperature. The completion of the reaction was checked by thin layer chromatography (TLC). The reaction time varied from 2 to 3 h. The purity of the compounds was checked by TLC and the compounds under the study were characterized by the spectral data. In general, the IR spectra showed ester peak at 1729 cm⁻¹ and CH₃ (methylene) peak at 2921 and 2849 cm⁻¹. In the ¹H-NMR spectra, the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts, multiplicities and coupling constants.
The spectra of all the compounds showed peaks at δ 6.39–7.69 ppm corresponding to the protons of 7-hydroxy coumarin. The $^{13}\text{C}$-NMR spectra of all the compounds were also in good agreement. Characteristic molecular ion peaks [M + Na] was observed for all the compounds under study. A detailed spectral description for compound 6 is discussed below.

IR spectrum of compound 6 revealed characteristic band at 1729 cm$^{-1}$ (ester C=O stretching). In the $^1\text{H}$-NMR the olefinic protons, C$_{11}$H$_2$=C$_{10}$H were observed at δH 5.81 (ddd, 1H, $J_{10'}-\gamma = 6.8$, $J_{10'}-\zeta = 16.8$, $J_{10}-\zeta = 10.4$, CH$_2$=CH$-$), 4.99 (dd, 1H, $J_{12}-\zeta = 16.8$, $J_{12}-\eta = 3.2$, H$_2$C=CH), 4.93 (dd, 1H, $J_{10'-1}\zeta = 10.4$, $J_{10'}-\zeta = 3.2$, H$_2$C=CH$-$) and were correlated with observations in the $^{13}\text{C}$-NMR which gave signals at δC 139.18 and 114.24, respectively. Besides these a characteristic carbon signal for the fatty acid chain at δC 171.68 (C$_{15'}$, ester C=O) was recorded. Similarly other compounds were characterized from their spectral data. Spectral studies have illustrated that the change in the nature of FA at C$_7$ has not significantly influenced the pattern of proton and carbon signals of the 7-HC moiety.

Pharmacology

A variety of acylated 7-hydroxycoumarins were investigated in the early 1970s for antibiotic and antifungal activities (Jund et al., 1971). Acyl groups in the study included acetyl, propanoyl and butanoyl. These compounds were found to possess activity against a number of fungal strains (MIC approximately 125 μg/ml) and Gram-negative bacteria (MIC sub 500 μg/ml). It was observed that the ester derivatives of 7-hydroxycoumarins increased the antimicrobial activity of 7-hydroxycoumarin. Thus, it is expected that the use of long-chain hydroxyl and non-hydroxyl fatty acid groups can further increase the antimicrobial potency of the coumarin ester derivatives.

The determination of MIC of synthesized compounds against bacterial and fungal strains was performed by macro dilution test and the results were recorded visually and spectrophotometrically. The investigation of antibacterial screening data (Table 5) revealed that all the tested compounds showed moderate to good bacterial inhibition. Compound 9 showed good inhibition against S. pyogens, S. aureus and E. coli species at 32 μg/ml where as compound 8 showed good inhibition against S. aureus at 32 μg/ml concentrations. Compounds 8 and 9 showed good antibacterial activity nearly equivalent to that of standard drug (Chloramphenicol) against most of the bacterial strains.

The investigation of antifungal screening data revealed that all the tested compounds showed moderate to good fungal inhibition. Compound 8 showed good antifungal activity against all strains of fungi. Compound 9 exhibited antifungal activities nearly equivalent to that of standard drug (Fluconazole) against C. parapsilosis at 8 μg/ml, against C. neoformans at 16 μg/ml and C. albicans at 2 μg/ml.

One of the reasons for activity difference may be based on the several unique characteristics of Gram-negative bacteria such as the structure of the outer membrane. The outer leaflet of the membrane comprises a complex lipopolysaccharide whose lipid portion acts as an endotoxin. This outer membrane protects the
bacteria from several antibiotics, dyes and detergents which would normally
damage the inner membrane or cell wall (peptidoglycan). The outer membrane
provides these bacteria with resistance to lysozyme and penicillin. That is why most
of the times Gram-negative bacteria have higher MIC values as compared to Gram-
positive bacteria. In case of antifungal activity, Candida albicans are generally
susceptible for most of the antifungals while non-albicans like Candida krusei, Candida parapsilosis, Cryptococcus neoformans are resistant to most of the
antifungal drugs. Furthermore, the compounds having a hydroxyl group in the
alkenyl side chain showed greater activity.

Experimental

Chemicals and instruments

Undec-10-enoic (purity 98%) and (9Z)-octadec-9-enoic (97%) acids were purchased
from Fluka Chemicals (Bucks, Switzerland). (9Z, 12R)-12-Hydroxyoctadec-9-enoic
(ricinoleic, 98%) acid and (9R, 12Z)-9-hydroxyoctadec-12-enoic (isoricinoleic, 98%) acid were isolated from Ricinus communis and Wrightia tinctoria seed oils,
respectively, following Gunstone’s (1954) partition procedure. 7-Hydroxy-coumarin
was purchased from S-d fine-chem. (Mumbai, India). Thin layer chromatography
was done on glass plates (20 × 5 cm) with a layer of silica gel G (Merck, Mumbai,
India, 0.5-mm thickness). Mixture of petroleum ether–ethyl acetate–acetic acid
(50:50:1, v/v) were used as developing solvents. Column chromatography
was carried out on silica gel (Merck, Mumbai, India, 60–120 mesh). 1H NMR
was recorded with Bruker DRX 400 spectrometer at 400 MHz and 13C NMR was
recorded at 100 MHz in CDCl3. Chemical shifts (δ) are quoted in ppm. Melting
points were taken in open capillary and are uncorrected.

Chemistry: synthesis of fatty acid derivatives of 7-hydroxy-coumarin

A solution of FA (5 mmol), DCC (5.5 mmol) and 7-hydroxy-coumarin (5 mmol) in
dichloromethane (50 ml) with catalytic amount of DMAP were stirred mechanically
at room temperature until esterification was complete. The N,N-dicyclohexylurea
was filtered off and the filtrate was washed with water (3 × 50 ml), 5% acetic acid
(3 × 50 ml) again with water (3 × 50 ml) and then dried over anhydrous sodium
sulphate. The solvent was removed under reduced pressure to give the esters 6–9
(Scheme 1) which were chromatographed over a column of silica gel using
n-hexane–ethyl acetate (94:6, v/v) as eluent. All these novel compounds were
characterized from their spectral data. 1H, 13C NMR and COSY spectra of
synthesized compounds shown in Tables 1, 2, 3, 4.

7-O-Coumarinyl undec-10-enoate (6)

White powder; Yield: 91%; mp: 168°C; Rf: 0.59; IR (KBr): 3080 (C=C aromatic
ring), 2921 (C–H asymm.), 2849 (C–H symm.), 1729 (C=O ester), 1624 (C=O
Table 1 $^1$H-NMR, $^13$C-NMR and COSY data of (6) in CDCl$_3$

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* Assignments may be reversed
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* Assignments may be reversed

coumarin carbonyl. 1400 (C-O), 1269 (C=C), 1122 (C-H aromatic ring) cm$^{-1}$. ESI-MS found [M + Na]$^+$ 351.2; C$_{20}$H$_{24}$O$_4$ [M + Na]$^+$ requires 351.17.

7-O-Coumarinyl (9Z)-octadec-9-enoate (7)

Viscous solid; Yield: 89%; Rf: 0.57; IR (KBr): 3120 (C=C aromatic ring), 2921 (C-H asymm.), 2849 (C-H symm.), 1728 (C=O ester), 1651 (C=O coumarin carbonyl), 1405 (C-O), 1270 (C=C), 1122 (C-H aromatic ring) cm$^{-1}$. ESI-MS found [M + Na]$^+$ 449.3; C$_{27}$H$_{38}$O$_4$ [M + Na]$^+$ requires 449.28.

7-O-Coumarinyl (9Z, 12R)-12-hydroxyoctadec-9-enoate (8)

Viscous solid; Yield: 85%; Rf: 0.52; IR (KBr): 3384 (O-H), 3080 (C=C aromatic ring), 2920 (C-H asymm.), 2858 (C-H symm.), 1732 (C=O ester), 1618 (C=O coumarin carbonyl), 1401 (C-O), 1267 (C=C), 1122 (C-H aromatic ring) cm$^{-1}$. ESI-MS found [M + Na]$^+$ 465.3; C$_{27}$H$_{38}$O$_5$ [M + Na]$^+$ requires 465.27.

7-O-Coumarinyl (12Z, 9R)-9-hydroxyoctadec-12-enoate (9)

Viscous solid; Yield: 87%; Rf: 0.53; IR (KBr): 3421 (O-H), 3002 (C=C aromatic ring), 2920 (C-H asymm.), 2852 (C-H symm.), 1736 (C=O ester), 1639 (C=O
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Table 3 $^1$H-NMR, $^{13}$C-NMR and COSY data of (8) in CDCl$_3$

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* Assignments may be reversed

Coumarin carbonyl), 1396 (C-O), 1270 (C=C), 1122 (C–H aromatic ring) cm$^{-1}$. ESI–MS found [M + Na]$^+$ 465.3; C$_{27}$H$_{38}$O$_5$ [M + Na]$^+$ requires 465.27.

Pharmacology

Antibacterial studies

The minimum inhibitory concentration (MIC) was assessed by the macro dilution test using standard inoculums of 5 × 10$^5$ c.f.u./ml. Initially, the compounds were dissolved in DMSO after that serial dilution of the test compounds were set to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 μg/ml. To each tube was added 100 μl of 24-h old inoculums. The growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations are given in Table 5.
### Table 4: $^1$H-NMR, $^{13}$C-NMR and COSY data of (9) in CDCl$_3$

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<th>COSY</th>
<th>C number</th>
<th>$\delta$ (ppm)</th>
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<td>d</td>
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<td>4</td>
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<td>7.55</td>
<td>1H</td>
<td>d</td>
<td>8.8</td>
<td>H-6</td>
<td>5</td>
<td>128.90*</td>
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<td>8</td>
<td>7.02</td>
<td>1H</td>
<td>s</td>
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<td>8</td>
<td>110.19*</td>
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<tr>
<td>6</td>
<td>6.98</td>
<td>1H</td>
<td>d</td>
<td>8.4</td>
<td>H-5</td>
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<td>d</td>
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<td>12'-13'</td>
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<td>H-11', H-14'</td>
<td>12'</td>
<td>129.99*</td>
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<tr>
<td>9'</td>
<td>3.44</td>
<td>1H</td>
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<td>2.52</td>
<td>2H</td>
<td>t</td>
<td>7.6</td>
<td>H-3'</td>
<td>2'</td>
<td>33.88*</td>
</tr>
<tr>
<td>11', 14'</td>
<td>2.02</td>
<td>2H - 2H</td>
<td>m</td>
<td></td>
<td>H-12'-13'</td>
<td>14'</td>
<td>34.18*</td>
</tr>
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<td>1.67</td>
<td>2H</td>
<td>q</td>
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<td>H-2</td>
<td>3' - 7', 11', 15' - 17'</td>
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<tr>
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<td>1H</td>
<td>br m</td>
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<td>4', 8', 10', 15'-17'</td>
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<td>2H - 2H</td>
<td>br s</td>
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<tr>
<td>18'</td>
<td>0.80</td>
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<td>dst t</td>
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<td>18'</td>
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</tbody>
</table>

* Assignments may be reversed

### Antifungal studies

The minimum inhibitory concentration (MIC) was assessed by the macro dilution test using standard inoculums of $1.6 \times 10^6$ - $6 \times 10^6$ c.f.u./ml. Initially, the compounds were dissolved in DMSO after that serial dilution of the test compounds were set to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 ng/ml. To each tube was added 100 µl of 48-72-h-old inoculums. The growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of fungi was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations are given in Table 5.

### Conclusion

It is conceivable that these derivatives showing antimicrobial activity can be further modified to exhibit better potency than the standard drugs. Compound 9 showed good antibacterial activity nearly equivalent to that of chloramphenicol. The varied divergence in the antimicrobial activity of these compounds validates the reason of...
Table 5  Minimum inhibition concentration (MIC) fatty acid analogs of 7-hydroxy coumarin

<table>
<thead>
<tr>
<th>Strains</th>
<th>Compounds</th>
<th>Chloram</th>
<th>Fluconazol</th>
<th>DMSO</th>
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<tbody>
<tr>
<td></td>
<td>MIC (µg/ml)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gram positive</td>
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<td></td>
<td></td>
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<tr>
<td>B subtilis</td>
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<td>64</td>
<td>64</td>
<td>32</td>
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<tr>
<td>S Pyogenes</td>
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<td>128</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>S aureus</td>
<td>128</td>
<td>128</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Gram negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P aeruginosa</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td>64</td>
</tr>
<tr>
<td>S typhimurium</td>
<td>64</td>
<td>128</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>E coli</td>
<td>128</td>
<td>128</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>Fungi</td>
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<tr>
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<td>C krusei</td>
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<td>512</td>
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<tr>
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<td>64</td>
<td>16</td>
<td>16</td>
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<tr>
<td>C neoformans</td>
<td>16</td>
<td>32</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>

NT not tested, Chloram chloramphenicol

this study. Compounds 8 and 9 showed good antifungal activity against all strains of fungi. The importance of such kind of work lies in the possibility that the new compounds might be more efficient against bacteria for which a thorough study regarding the structure–activity relationship, toxicity and in their biological effects would be helpful in designing more effective antimicrobial agents.

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References


