ANALYSIS OF SEED FATS AND PREPARATION OF FATTY ACID DERIVATIVES

ABSTRACT THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

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IN

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BY

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Under the Supervision of

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DEPARTMENT OF CHEMISTRY
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ABSTRACT

Results of explanatory research are recorded in the thesis.

This thesis consists of two parts: The Part A has one chapter dealing with the positional distribution of fatty acids in triacylglycerols of four seed oils and Part B comprises of six chapters depicting the synthesis and characterization of selected fatty acids derivatives and their antimicrobial screening.

PART A ANALYSIS OF SEED FATS/ OILS

Chapter 1: Pancreatic Lipase Hydrolysis of Triacylglycerols

The fatty acids distribution pattern in triacylglycerols of four seed oils viz. *Mimusops elengi*, *Parkinsonia aculeate*, *Brassica rapa*, *Brassica oleracea* var. *botrytis* were determined using pancreatic lipase hydrolysis method. All the four seed oils contain high percentage of unsaturated acids. (*M. elengi* 64.8%, *P. aculeate* 82.7%, *B. rapa* 84.8%, *B. oleracea* var. *botrytis* 73.4%). *M. elengi* contains relatively greater amount of saturated acids 35.2% than the other seeds. The lipolytic data revealed that linoleic acid dominates at 2-position of triacylglycerols of all seed oils. Among four oils, three contain erucic acid *M. elengi* (0.3%), *B. rapa* (17.7%), *B. oleracea* var. *botrytis* (1.6%).

PART B SYNTHESIS OF FATTY ACID DERIVATIVES

Chapter 2: 3, 5-Disubstituted-1H-1,2,4-triazoles*

Rapid and efficient one-pot condensation reaction of long-chain alkyl and alkenyl acid hydrazides 1a-e and nitriles 2a-c was carried out to afford 3,5-disubstituted-1H-1,2,4-triazoles (Scheme 1). The compounds 3a-o were characterized on the basis of elemental analysis and spectral data.

The triazoles 3a-o were screened for in vitro antibacterial activity against the representative panel of two Gram-positive and two Gram-negative bacteria. All the synthesized compounds were also tested for their inhibitory action against five strains of fungus. The various compounds show potent inhibitory action against test organisms.

Chapter 3: 2,5-Disubstituted-1,3,4-oxadiazoles*

A series of novel 2,5-disubstituted-1,3,4-oxadiazoles 4a-f have been synthesized from long-chain alkanolic and alkenolic acids (Scheme 2). Furthermore, compounds were screened for in vitro antibacterial activity against the representative panel of two Gram-positive and two Gram negative bacteria. All the synthesized compounds were also tested for their inhibitory action against five strains of fungus. The various compounds show potent inhibitory action against test organisms.

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

A series of novel 3,5-disubstituted-1,2,4-oxadiazoles have been synthesized from alkenyl long-chain esters and acid chlorides. The derivatives of acids have been employed for the synthesis under conventional and solvent free microwave conditions. The synthesis under different reaction conditions has been discussed. The structures of the synthesized compounds were elucidated by IR, $^1$H NMR, $^{13}$C NMR, MS data and elemental analysis.

At first one-pot synthesis of 3,5-disubstituted-1,2,4-oxadiazoles 3a-t from easily synthesized fatty esters 1a-d and amidoximes 2a-e was carried out. The one-pot procedure provided 3a-t in 50-58% yield (Scheme 3).

In another method, 1'a-d was added dropwise to a stirred solution of the amidoxime 2a-e in pyridine (Scheme 4) and reaction was refluxed for 1-2.5 hours. In most of the cases the yield of product was 60-62%.
ABSTRACT

$$\text{Cl} \quad \text{R} \quad \text{NOH} \quad \text{NH}_2 \quad \text{Pyridine} \quad \text{O} \quad \text{N} \quad \text{R}$$

$1'a-d \quad 2a-e \quad 3a-t$

Scheme 4 Synthesis of 3,5-disubstituted-1,2,4-oxadizoles from long-chain acid chlorides and amidoximes

To get the pharmacophoric important moiety in higher yields and in shorter reaction time the synthesis of $3a-t$ was carried out under microwave irradiation (Scheme 5).

$$\text{R}_1 \quad \text{X} \quad \text{NOH} \quad \text{NH}_2 \quad \text{microwave} \quad \text{neutral alumina} \quad \text{O} \quad \text{N} \quad \text{R}$$

$1a-d (X=OMe) \quad 2a-e \quad 3a-t$

$1'a-d (X=Cl)$

Scheme 5 Synthesis of 3,5-disubstituted-1,2,4-oxadiazoles under microwave irradiation

Chapter 5: 2-Substituted-benzimidazoles, tetrahydrobenzimidazoles and imidazoles*

A series of novel 2-substituted benzimidazoles 4a-p and imidazoles 4q-t have been synthesized from long-chain alkenoic acids. The reactions occurred under relatively mild conditions and afforded the desired product in good yields (Scheme 6-8). The structures of these compounds have been elucidated by elemental and spectral (IR, $^1$H NMR, $^{13}$C NMR, Mass) analyses.

Furthermore, compounds were screened for *in vitro* antibacterial activity against the representative panel of two Gram-positive and two Gram-negative bacteria. All the synthesized compounds were also tested for their inhibitory action against five strains of fungus. The various compounds show potent inhibitory action against test organisms.

Scheme 6 Synthesis of 2-substituted-benzimidazoles

Scheme 7 Synthesis of 2-substituted-imidazoles

Scheme 8 Synthesis of 2-substituted-tetrahydobenzimidazoles
Chapter 6: 3,5,6-Trisubstituted-1,2,4-triazines*

The use of microwave irradiation has been an established tool in organic synthesis for achieving better selectivity, rate enhancement and reduction of thermally degradative by product. Rapid and efficient solvent-free synthesis of 3,5,6-trisubstituted-1,2,4-triazines 3a-l from fatty acid hydrazides 1a-f under microwave irradiation is described (Scheme 9). The one-pot synthesis on solid inorganic support provides the products in good yields. The newly synthesized compounds were screened for antimicrobial activity. The structural features of the synthesized 1,2,4-triazines were characterized by IR, $^1$H NMR, $^{13}$C NMR, mass and elemental analysis.

\[ \text{Scheme 9 Synthesis of 3,5,6-trisubstituted-1,2,4-triazines} \]

*Research paper entitled “Microwave assisted efficient one-pot synthesis of 3,5,6-trisubstituted-1,2,4-triazines from fatty acid hydrazides under solvent-free conditions and their antimicrobial activity” is published. (A. Rauf, S. Sharma, S. Gangal, ARKIVOC 2007, (xvi) 137-147).
Chapter 7: Isoxazoles and triazoles

The efficient cycloaddition reactions were carried out to afford the novel 3,5-disubstituted-dihydroisoxazoles, isoxazoles, triazole and triazolo-tetrazole from methyl undec-10-enoate 1 and methyl undec-10-ynoate 4. In this chapter, the straightforward synthesis of 3,5-disubstituted-4,5-dihydroisoxazoles 3a-c and bis-4,5-dihydroisoxazole 3d possessing ester moiety by one-pot process is reported. The reactions occurred under relatively mild conditions and afforded the desired product in good yields.

Scheme 10 Synthesis of 3,5-disubstituted-dihydroisoxazoles and bis-isoxazole

In similar reactions using methyl undec-10-ynoate (4) and Cerium (IV) ammonium nitrate in various acetones, the corresponding isoxazoles 5a-c and bis-isoxazoles 5d were obtained (Scheme 11).

Scheme 11 Synthesis of 3,5-disubstituted-isoxazoles and bis-isoxazole
The cycloaddition of azidoacetonitrile (6) with terminal alkyne gave corresponding triazole (7) which on further reaction with sodium azide and ammonium chloride in DMF gave tetrazole (8) (Scheme 12).

Scheme 12 Synthesis of 1-Cyanomethyl-4-(carbomethoxyoctyl)-1,2,3-triazole and [1-(1H-Tetrazol-5-yl-methyl)-4-carbomethoxyoctyl]-(1,2,3)-triazole

One more representative of 1,2,3-triazole (10) was synthesized in appreciable yield by utilizing dipolarophile (4), benzyl chloride (9), sodium azide in DMF (Scheme 13).

Scheme 13 Synthesis of 1-Benzyl-4-(carbomethoxyoctyl)-1,2,3-triazole
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CERTIFICATE

This is to certify that the work embodied in this thesis entitled "Analysis of Seed Fats and Preparation of Fatty Acid Derivatives" is the original work of Ms. Shweta Sharma carried out under my supervision. The thesis is suitable for submission for the award of the degree of Doctor of Philosophy in Chemistry.

Dr. Abdul Rauf
To My Mummy & Daddy
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Shweta Sharma
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Introduction
Oils and fats, main constituent of lipids, are significant physiologically as well as chemically. The calorie density for dietary fats is more than twice than that of proteins and carbohydrates. Fatty acids are essential dietary requirements for their link with the prostaglandins and their involvement in cell membrane formation. Recently the importance of oils and fats is continuously increasing in biochemical and biomedical sciences.

All multi-cellular organisms, use chemical messengers to send information between organelles and into other cells and as relatively small hydrophobic molecules, lipids are excellent candidates for signaling purposes. The fatty acid constituents have well-defined structural features, such as cis-double bonds in particular positions, which can carry information by binding selectively to specific receptors. In esterified form, they can infiltrate membranes or be translocated across them to carry signals to other cells. During transport, they are usually bound to proteins so their effective solution concentrations are very low, and they can be considered to be inactive until they reach the site of action and encounter the appropriate receptor.

India, predominantly an agricultural country flourishes in forest flora. There is a wide potential of agrichemicals derived from minor seed oils plentiful in specific kind of fatty acids. In recent years the deployment of fatty acids as agrichemicals finds their way into a variety of industrial uses and most of them primarily through derivatization.

The mounting costs of petrochemicals have attracted the attention of chemists to find new sources of oils containing unusual fatty acids and also to modify fatty acids for promising industrial uses. In nature large numbers of fatty acids are widely distributed in plants. The fatty acids with unusual structural features in seed oils have manifold industrial significance as they are utilized in dispersants, cosmetics, lubricants, protective coatings, plastics, urethane derivatives surfactants, varieties of synthetic intermediates, stabilizers in plastic formulations and in synthesis of other long-chain compounds. Such
fatty acids with unusual structures are vital for the collection of oleochemicals (fat derived chemicals). The ethoxylated hydroxyl fatty acids-containing seed oils are used as stabilizers of hydrophobic substances in industries such as perfumes and cosmetics. The polyethoxylated hydroxy fatty acids are non-ionic surfactants and are included in the formulations for cleaning clothes, dishes, hand surfaces and metals, textile processing. The hydroxyolefinic fatty acids are known to occur in a number of seed oils and are highly important to the chemical and pharmaceutical industry. The importance of organic acids such as sorbic, cinnamic, ricinoleic, myristic as antimicrobial drugs is well established in pharmaceutical industry.

Thus fatty acids have increasingly being found utilizable as explicit raw material for the upcoming chemical industry. They have also found applications in metal catalyzed metathesis and ring-opening reactions, polymerization, oxidation etc. Fatty esters are used as antifogging agents and found very useful for plasticizer in biodegradables 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 agents. Some fatty esters are found very effective for the treatment of dermatitis, cardiovascular, hepatic and renal diseases.

The heterocyclic derivatives of fatty acids account for utility in variety of applications. The fatty acid derivatives are used in various industrial applications such as coatings, cosmetics, perfumes, pharmaceuticals, pesticides, insecticides and polymers. The presences of heterocyclic rings in fatty acids are rare in nature.

For more than a century, heterocycles have constituted one of the largest areas of research in organic chemistry. They have contributed to the development of society from a biological and industrial point of view as well as to the understanding of life processes and to the efforts to improve the quality of life. Heterocycles play an important role in biochemical processes because the side groups of the most typical and essential constituents of living cells, DNA and RNA, are based on aromatic heterocycles. The
remarkable ability of heterocyclic to serve both as biomimetics and active pharmacophores has largely contributed to their unique value as traditional key elements of numerous drugs. Among the approximately 20 million chemical compounds identified by the end of the second millennium, more than two-thirds are fully or partially aromatic, and approximately half are heterocyclic. The heterocycles are present in all kinds of organic compounds such as in biology, pharmacology, optics, electronics, material sciences, and so on.

A number of cyclic derivatives of fatty acids containing nitrogen, sulfur, oxygen and phosphorus as heteroatoms have revealed fungi-static and bactericidal agents. Such oleochemicals are innate component in the manufacture of fungicides, insecticides, pharmaceuticals, lubricants, additives, greases, cosmetics etc. The fatty epoxy esters are utilized in the synthesis of PVC plasticizers and stabilizers.

Fatty acids are 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 classical reactions of organic chemistry, fatty acids also exhibited as potential substrate to undergo microwave assisted reactions. 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 in that it makes it possible to shorten the length of reactions significantly, to increase their selectivity, and to increase the product yields, which is particularly important in the case of high-temperature processes that take a long time.

One of the most fascinating aspects of olefinic long-chain carboxylic acids is conversion of double bond to triple bond which served as potential dipolarophile for the synthesis of diverse heterocycles.
The development of synthetic routes for the synthesis of long-alkyl and alkenyl chain heterocyclic moieties is highly desirable and advantageous as very few papers supports the synthesis of heterocyclic derived from fatty acids. These aspects created an active interest to undertake the present work which comprises of two parts:

(i) analysis of seed oils especially to investigate the positional distribution of fatty acids in neutral triacylglycerols.

(ii) to synthesize the fatty acid derivatives having biological importance.
Part A
Analysis of Seed Fats/Oils
Chapter 1
Pancreatic lipase hydrolysis of triacylglycerols
1.1 Theoretical

The seed oils consist predominantly of triacylglycerols (TAGs), which are esters of fatty acids with glycerol. Triacylglycerols are complex molecules present in all oils and fats. The wide variety of fatty acids that may attach to the glycerol backbone generates the large diversity of TAGs that share very similar physiochemical properties, thus making their separation and analysis difficult. Therefore one must employ a consecutive series of separation and characterization techniques to define TAGs composition of fat.

TAGs on hydrolysis produce fatty acids. The common fatty acids of TAGs contain even number of carbon atoms (4-24) in straight chain with a terminal carboxyl group; which may be fully saturated (SFA) or unsaturated (UFA). In the diene and triene derivatives, respectively, the double bonds are interrupted by a methylene group. Of the most commonly occurring acids are: lauric (C₁₂₆), myristic (C₁₄₆), palmitic (C₁₆₆), palmitoleic (C₁₆₆), stearic (C₁₈₆), oleic (C₁₈₆), linoleic (C₁₈₂), linolenic (C₁₈₃) and erucic (C₂₂₆) acid. Smith\(^1\) classified these ubiquitous compounds as usual fatty acids. Consequently, all fatty acids with different structure features are considered as unusual. Smith\(^1\) has classified fatty acids into three major groups:

(i) usual
(ii) unusual non-oxygenated
(iii) unusual oxygenated

In nature large number of other fatty acids has been discovered possessing unusual structural features. They include allenic, cyclopropane, cyclopropene, cyclopentene, furan, hydroxyl, epoxy, keto groups\(^3,9\).

For the chemist, the isolation and elucidation of rare fatty acid structures has always been a challenge, and nowadays the number of known fatty acids exceeds
Pancreatic lipase hydrolysis of triacylglycerols

thousands$^{10}$. The interest in fatty acids with unusual structure features and in oils, that can provide a high concentration of a single fatty acid, has increased as they can be of high value for the chemical and pharmaceutical industry$^{10-15}$. Global screening programs for the search of new plant oils as renewable sources have been initiated. This is also a preventive research as it is estimated that there is approximately only 50-100 years supply left in readily accessible fossil oil resources, which are actually the most important basis of the petrochemical industry. In contrast to fossil oil resources, the vegetable oils are renewable and have the ecological advantage to be CO$_2$-neutral$^{16}$. The complexity in the analysis of fatty acids has created many problems in their detection, isolation and structure determination. This picture was changed with the advances in the methodology of lipid analysis. The chromatographic techniques used in the analysis of oils are: thin layer chromatography (TLC), high performance liquid chromatography (HPLC), column chromatography, liquid column chromatography$^{17-19}$, partition adsorption, ion-exchange and complexation chromatography and gas liquid chromatography (GLC)$^{20}$. Gas chromatography has been employed successfully in the isolation of pure fractions from complex mixtures. Although gas chromatography is the dominant technique for the fatty acid analysis, high-performance liquid chromatography (HPLC)$^{21-30}$ has expanded dramatically into almost every area of chemical and biochemical research as well as food analysis$^{31}$. Likewise, various spectroscopic techniques, such as UV, IR, high resolution $^1$H NMR, $^{13}$C NMR$^{32}$, liquid chromatography mass spectrometry (LCMS)$^{33}$ gas chromatography mass spectrometry (GCMS)$^{34-36}$ and chemical ionization mass spectrometry (CIMS)$^{37}$ offer remarkable solutions and unexpected advantages for the analysis of unknown fatty compounds.

The wealthy vegetation growth of India is abundant in medicinally important seed oils. To study the biological or medicinal aspects of seed oils it is indispensable to study the chemistry of that particular oil as the two branches are interrelated. The fatty acids constitute the TAGs. The chemistry of oils and fats is basically the chemistry of
Pancreatic lipase hydrolysis of triacylglycerols

constituent fatty acids present in triacylglycerols (TAGs). The most important aspect of lipid analysis is to study the positional distribution of fatty acids in TAGs. It is of great importance to know the composition at all the three positions (i.e. sn-1, sn-2 and sn-3) of TAGs.

It is well established through literature that various methods like crystallization, Grignard procedure, enzymatic hydrolysis etc. are used for stereospecific TAGs analysis of fatty acids. Ester bonds to all three positions of the glycerol molecule are so similar in chemical reactivity that there is no simple chemical procedure for hydrolyzing one or other of them selectively. Most of the living organisms have developed lipolytic enzyme systems that are able to distinguish between various positions of glycerol or certain type of bonds in specific lipids and in many cases these enzymes can be isolated and used in simple in vitro incubations as an aid in structural analyses of lipids. Hence enzymatic hydrolysis is one of the most widely used methods. Enzymes used for this purpose are lipases. Lipases are enzymes that catalyze the hydrolysis of acylglycerols. In addition lipases are widely used in a variety of other reactions that include esterifications of acids with alcohols, oils and fats transesterifications as well as reactions leading to the synthesis of products such as sugars, esters, fatty amides etc. Lipases are ubiquitous in nature, being present in the animal, microbial and plant kingdoms. Enzymology of these lipases is well documented\(^{38,39}\). Several scientists and companies have studied enzymatic hydrolysis extensively\(^{40-47}\). Besides these, there are various other reports on enzymatic hydrolysis\(^{48-77}\).

One of the most important aspects of lipase mediated hydrolysis is that it requires a mild reaction condition which makes it a superior method over other classical processes which involves high temperature and pressure resulting in high energy consumption. Lipase catalyzed hydrolysis reactions require less energy causing essentially no thermal damage to reactants and products for example in color and formation of oxidation products. Also, certain important functionalities of fatty acids such as double bond
remains protected. Literature reports on the enzymatic fat-splitting show that rate of lipolysis reaction to be optimal in a pH range of 4.8-7.2 at 37°C. Therefore, despite relatively high price of the bio-catalysts, lipase catalyzed hydrolysis could be economically attractive for the preparation of thermally sensitive products of good commercial values, such as polyunsaturated fatty acid (PUFA), hydroxyl fatty acids etc.

The lipases are position specific and their specifications have classically been divided into five major types:

(i) lipid class
(ii) positional
(iii) fatty acids
(iv) stereochemical
(v) combinations

Lipases obtained from natural sources can be positionally non-specific or display one of the two kinds of positional specificity sn-1,3 specific or sn-2 specific. Non-specific lipases hydrolyze all three ester bonds of TAGs equally well.

None the less, from time to time various enzymes have been studied for determining the composition of position sn-1, sn-2 and sn-3 yet no lipolytic enzyme has been isolated that is capable of distinguishing position between 1 and 3 of triacyl-sn-glycerols.

A lipase that is completely specific for the primary ester bonds of TAGs is present in crude pig (and other animal) pancreas extract which is a sn-2 specific enzyme.
Pig pancreatin, a powder obtained by dehydrating pig pancreas with acetone and diethylether, is the most widely used source of the enzyme, it is stable for long period of time and is available commercially. A series of animals and vegetable fats has been subjected to hydrolysis with pancreatic lipase. A considerable amount of data has been obtained by means of pancreatic lipase hydrolysis on the composition of position \( sn-2 \) of the triacyl-\( sn \)-glycerols of seed oils, and this has been enhanced in recent years by stereospecific analysis. These investigations have shown that all straight-chain saturated fatty acids of whatever chain lengths are hydrolyzed from the primary positions at the same rate, but the ester bonds of long-chain PUFA are hydrolyzed more slowly, possibly as a result of steric hindrance caused by the proximity of functional groups to the ester bonds.

It is well established from studies involving with pancreatic lipase, position \( sn-2 \) of the TAGs of seed oils is abundant in the PUFA (specifically linoleic and linolenic acids), while saturated fatty acids are concentrated in the primary positions and monoenoic acids are relatively evenly distributed\(^7^9\).

Besides being used for analyzing the distribution pattern of TAGs in seed oils pancreatic lipase is also used for the detection of adulteration in fats, preparation of TAGs with desired composition (structured lipids), and preparation of interesterified blends and in lipid bioconversions.
1.2 Pancreatic Lipase Hydrolysis Four Selected Triacylglycerols

The genus *Mimusops* belongs to the family Sapotaceae and comprises 30 species including *M. elengi* L. It is cultivated in North and Peninsular India and Andaman Islands. It is grown as an avenue tree in many parts of India. *M. elengi* is an ornamental tree with sweet-scented flowers. Seeds are 1-2 per fruit, ovoid, compressed, greyish brown and shiny. The bark and fruit of this plant are used in the treatment of diarrhea and dysentery, and a decoction of the bark is used as a gargle. The pounded seeds pasted with oil are used for the treatment of obstinate constipation. Pillow stuffing made from the dried flowers induces nasal discharge and relieves headache. Tree is an evergreen tree with sweet scented flowers having ancient glamour. Garlands made of its flowers are ever in good demand due to its long lasting scent. Its bark is used as a gargle for odontopathy, ulitis and ulemorrhagia. Tender stems are used as tooth brushes. It is also useful in urethrorrhoea, cystorrhoea, diarrhoea and dysentery. Flowers are used for preparing a lotion for wounds and ulcers. Powder of dried flowers is a brain tonic and is useful as a snuff to relieve cephalgia. Unripe fruit is used as a masticatory and will help to fix loose teeth. Seeds are used for preparing suppositories in cases of constipation especially in children. The bark and seed coat are used for strengthening the gum and enter into the composition of various herbal tooth powders, under the name of "Vajradanti", where they may be used along with tannin-containing substances like catechu (*Acacia catechu*), pomegranate (*Punica granatum*) bark, etc. The bark is used as snuff for high fever accompanied by pains in various parts of the body. The flowers are considered expectorant and smoked in asthma. A lotion prepared from unripe fruits and flowers is used for smearing on sores and wounds. In Ayurveda, the important preparation of *Mimusops* is "Bakuladya Taila", applied on gum and teeth for strengthening them, whereas in Unani system, the bark is used for the diseases of genitourinary system of males. *M. elengi* has recorded highly significant antifungal activity against *Fusarium proliferatum*.
In order to search for antimicrobial phytochemicals, two antibacterial compounds were detected from the seeds of *M. elengi* Linn. After characterization, the compounds were identified as 2,3-dihyro-3,3',4',5,7-pentahydroxyflavone and 3,3',4',5,7-pentahydroxyflavone. The compounds showed strong inhibitory activity against Gram positive and Gram negative bacteria. *Parkinsonia aculeata* belongs to Leguminosae. It is reported to be highly medicinal, whole plant is used as antipyretic, and leaves are considered to be diaphoretic and abortifacient. The leaves, stems and flowers contain alkaloids and steroids. The seeds are edible and contain albumin and glutelin as principal proteins. Seeds are mucilaginous and reported to contain golden colored fatty oil and neutral sugars. Leite *et al.* investigated the antidiabetic effect of water soluble fraction made of aerial parts (leaves and flowers) of the *P. aculeata* in alloxan diabetic rats.

*Brassica rapa* belongs to family *Brassicaceae*. Leaves are used raw or cooked. The cooked leaves make an acceptable vegetable, though they are coarser than the related cabbage. They are more often used as a spring greens, sowing the plants in the autumn and allowing them to overwinter. Young leaves can also be added in small quantities to salads, they have a slightly hot cabbage-like flavor and some people find them indigestible. A nutritional analysis is available. Often used as a cooked vegetable, the young roots can also be grated and eaten in salads, they have a slightly hot flavor like a mild radish. A nutritional analysis is available. A decoction of the leaves or stems is used in the treatment of cancer. The powdered seed is said to be a folk remedy for cancer. The root when boiled with lard is used for breast tumours. A salve derived from the flowers is said to help skin cancer. The crushed ripe seeds are used as a poultice on burns. *B. rapa* root peelings contain a natural insecticide. The chopped roots can be brewed into a tea with flaked soap, this is then strained before use. It is effective against aphids, red spider mites and flies.
Thiols, polysulfides, isothiocyanates, nitriles, carbonyl compounds and terpenes were isolated from the head space of *Brassica oleracea* var. *botrytis*. Dimethyltrisulfide, prop-2-enylisothiocyanate, pentanal, hexanal, heptanal, pentanol and octanol were identified. The oil is suitable for domestic consumption\(^4\).

### 1.3 Results and discussion

The oils from all the seed samples were isolated using Soxhlet apparatus and their analytical values are given in Table 1.1. Neutral TAGs were then isolated from the seed oils using column chromatographic technique. The isolated TAGs was transesterified for fatty acid methyl esters (FAMEs) analysis. The TAGs composition based on lipolytic data, which includes the molar percent of different acids at 2-position along with enrichment factor is given in Table 1.2. The total TAGs composition is given in Table 1.3.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Source</th>
<th>Oil content (%)</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IV(^a)</td>
</tr>
<tr>
<td>1</td>
<td><em>Mimusops elengi</em></td>
<td>32.34</td>
<td>87.8</td>
</tr>
<tr>
<td></td>
<td>(Sapotaceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Parkinsonia aculeata</em></td>
<td>29.13</td>
<td>120.8</td>
</tr>
<tr>
<td></td>
<td>(Leguminosae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Brassica rapa</em></td>
<td>38.57</td>
<td>107.9</td>
</tr>
<tr>
<td></td>
<td>(Brassicaceae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Brassica oleracea</em></td>
<td>41.23</td>
<td>102.8</td>
</tr>
<tr>
<td></td>
<td>var. <em>botrytis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Brassicaceae)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\)\(^a\)= Iodine value  \(\)\(^b\)= Saponification value  \(\)\(^c\)= Refractive index

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8
Pancreatic lipase hydrolysis of triacylglycerols

The neutral TAGs of *M. elengi* composed of oleic (27.9%), linoleic (35.7%), linolenic (0.9%) and erucic (0.3%) acids as unsaturated components. The myristic (8.1%), palmitic (10.2%) and stearic (16.9%) acids are also present in appreciable amount. The combined percentage of unsaturated acids (64.8%) shows that degree of unsaturation is quite higher in *M. elengi* neutral TAGs with linoleic acid as major component. The fatty acids present at 2-position are myristic (4.2%), palmitic (7.3%), stearic (11.1%), oleic (33.7%), linoleic (40.9%), linolenic (2.2%) and erucic (0.6%). The composition at 1,3-position is depicted in Table 1.2. It was linoleic (33.1%) acid, which dominates at 1,3-position followed by oleic acid (25%). Another major component present at 1,3-position was stearic acid (19.8%). Compositions of other fatty acids present in TAGs, 2-position and 1,3-position are listed in Table 1.2. Molar present (M) of linolenic acid at 2-position in *M. elengi* is listed in Table 1.2 was greater as compared to other acids i.e. myristic, palmitic, stearic, oleic, linoleic, erucic acids. The enrichment factor (E, Table 1.2) shows that it was maximum for linolenic acid (E value 2.44). Table 1.3 is further strengthening the calculated composition of saturated and unsaturated acylglycerols of *M. elengi*. It was triunsaturated acylglycerols that were found in majority 40.44%.

The fatty acid profile of *P. aculeata* (Table 1.2) revealed by GLC indicated that the oil has requisite amount of unsaturated fatty acids (74.1%) with linoleic acid (40.5%) as major component. The other unsaturated acid present in major quantity was oleic acid (33.6%). Among saturated acids, stearic acid dominates (11.2%) over myristic (0.8%) and palmitic acid (5.3%). The lipolytic data of *P. aculeata* (Table 1.2) clearly indicates that within the limits of experimental error oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) were preferentially acylated at 2-position of glycerol. Linoleic and linolenic acids were also present at 1,3-position in major amount. Major components at 2-position (43.7%) as well as 1,3-position (38.9%) was linoleic. The molar percentage at 2-position was maximum for oleic acid (34.22). The enrichment factor E was within the reported
range and Table 1.2 shows that it was maximum for erucic acid (E value 1.25). Table 1.3 indicates that monosaturated acylglycerols (GSU₂) were found in majority, 66.34 %.
Table 1.2 *Fatty acid composition based on lipolytic data*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Source</th>
<th>Methyl Ester Composition by GLC as mol %</th>
<th>14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>22:1</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td><em>M. elengi</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TAG(^a)</td>
<td>8.1</td>
<td>10.2</td>
<td>16.9</td>
<td>27.9</td>
<td>35.7</td>
<td>0.9</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-MAG(^b)</td>
<td>4.2</td>
<td>7.3</td>
<td>11.1</td>
<td>33.7</td>
<td>40.9</td>
<td>2.2</td>
<td>0.6</td>
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<tr>
<td></td>
<td>1,3-position</td>
<td>10.1</td>
<td>11.6</td>
<td>19.8</td>
<td>25</td>
<td>33.1</td>
<td>0.25</td>
<td>0.15</td>
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<tr>
<td></td>
<td>E</td>
<td>0.51</td>
<td>0.71</td>
<td>0.65</td>
<td>1.21</td>
<td>1.14</td>
<td>2.44</td>
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<td></td>
<td>M(^d)</td>
<td>17.28</td>
<td>23.85</td>
<td>20.19</td>
<td>40.26</td>
<td>38.18</td>
<td>81.48</td>
<td>66.66</td>
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<td><em>P. aculeata</em></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>TAG(^a)</td>
<td>0.8</td>
<td>5.3</td>
<td>11.2</td>
<td>33.6</td>
<td>40.5</td>
<td>8.6</td>
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<td></td>
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<tr>
<td></td>
<td>2-MAG(^b)</td>
<td>0.6</td>
<td>2.3</td>
<td>8.1</td>
<td>34.5</td>
<td>43.7</td>
<td>10.8</td>
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<td></td>
<td>1,3-position</td>
<td>0.9</td>
<td>6.8</td>
<td>12.8</td>
<td>33.2</td>
<td>38.9</td>
<td>7.5</td>
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<tr>
<td></td>
<td>E</td>
<td>0.75</td>
<td>0.43</td>
<td>0.72</td>
<td>1.03</td>
<td>1.07</td>
<td>1.25</td>
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<tr>
<td></td>
<td>M(^d)</td>
<td>25</td>
<td>14.46</td>
<td>24.11</td>
<td>34.22</td>
<td>30.89</td>
<td>26.54</td>
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<tr>
<td>3.</td>
<td><em>B. rapa</em></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>TAG(^a)</td>
<td>0.6</td>
<td>7.9</td>
<td>6.7</td>
<td>26.9</td>
<td>39.7</td>
<td>0.5</td>
<td>17.7</td>
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<tr>
<td></td>
<td>2-MAG(^b)</td>
<td>0.3</td>
<td>4.6</td>
<td>3.8</td>
<td>30.1</td>
<td>41.1</td>
<td>1.3</td>
<td>18.8</td>
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<tr>
<td></td>
<td>1,3-position</td>
<td>0.75</td>
<td>9.6</td>
<td>8.2</td>
<td>25.3</td>
<td>39</td>
<td>0.1</td>
<td>17.2</td>
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<tr>
<td></td>
<td>E</td>
<td>0.5</td>
<td>0.58</td>
<td>0.57</td>
<td>1.11</td>
<td>1.04</td>
<td>2.6</td>
<td>1.06</td>
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<tr>
<td></td>
<td>M(^d)</td>
<td>16.66</td>
<td>19.41</td>
<td>18.90</td>
<td>37.29</td>
<td>34.51</td>
<td>86.66</td>
<td>35.40</td>
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<tr>
<td>4.</td>
<td><em>B. oleracea var. botrytis</em></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>TAG(^a)</td>
<td>0.5</td>
<td>10.9</td>
<td>15.2</td>
<td>33.8</td>
<td>37.7</td>
<td>0.3</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-MAG(^b)</td>
<td>0.3</td>
<td>6.3</td>
<td>9.6</td>
<td>38.4</td>
<td>42.5</td>
<td>0.6</td>
<td>2.3</td>
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<tr>
<td></td>
<td>1,3-position</td>
<td>0.6</td>
<td>13.2</td>
<td>18</td>
<td>31.5</td>
<td>35.3</td>
<td>0.2</td>
<td>1.3</td>
<td></td>
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<tr>
<td></td>
<td>E</td>
<td>0.6</td>
<td>0.58</td>
<td>0.63</td>
<td>1.13</td>
<td>1.12</td>
<td>2</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M(^d)</td>
<td>20</td>
<td>19.26</td>
<td>21.05</td>
<td>37.86</td>
<td>37.57</td>
<td>66.66</td>
<td>47.91</td>
<td></td>
</tr>
</tbody>
</table>

\[1,3\text{-position} = \frac{(3 \times \text{TAG}) - (2\text{-MAG})}{2}\]

\[E = \text{mole percent acid at 2-position of TAG}\]

\[M = \text{mole percent of acid in 2-MAG} \times 100\]

\[3 \times \text{mole percent of same in TAG}\]

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The fatty acid composition of neutral TAGs of *B. rapa* as by GLC analysis indicated that the oil was composed of myristic (0.6%), palmitic (7.9%), stearic (6.7%), oleic (26.9%), linoleic (39.7%), linolenic (0.5%), erucic acid (17.7%). The GLC analysis of lipolytic product of *B. rapa* (Table 1.2) shows that linoleic acid dominates at 2-position (41.1%) and 1,3-position (39%). Among saturated acids the major component at 2-position were myristic (0.3%), palmitic (4.6%) and stearic (3.8%) acids. The percent composition of fatty acids at 1,3-position were myristic (0.75%), palmitic (9.6%), stearic (8.2%), oleic (25.3%), linoleic (39%), linolenic (0.1%), erucic acid (17.2%). E values of *B. rapa* for different acids (Table 1.2) show that linolenic acid has the highest enrichment factor value (E value 2.6). M value was found maximum for linolenic acid (86.66). TAGs composition of *B. rapa* (Table 1.3) indicated that triunsaturated acylglycerols (GU₃) were the most abundant (72.34%) in the seed oil.

**Table 1.3 Triacylglycerol composition* of seed oils**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Source</th>
<th>Trisaturated acylglycerols (GS₃)</th>
<th>Disaturated acylglycerols (GS₂U)</th>
<th>Monosaturated acylglycerols (GSU₂)</th>
<th>Triunsaturated acylglycerols (GU₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>M. elengi</em></td>
<td>6.18</td>
<td>19.25</td>
<td>34.13</td>
<td>40.44</td>
</tr>
<tr>
<td>2.</td>
<td><em>P. aculeate</em></td>
<td>0.03</td>
<td>1.40</td>
<td>66.34</td>
<td>33.23</td>
</tr>
<tr>
<td>3.</td>
<td><em>B. rapa</em></td>
<td>0.51</td>
<td>1.61</td>
<td>25.54</td>
<td>72.34</td>
</tr>
<tr>
<td>4.</td>
<td><em>B. oleracea</em></td>
<td>1.3</td>
<td>19.25</td>
<td>33.64</td>
<td>56.46</td>
</tr>
</tbody>
</table>

* Composition of minor TAGs are not included
GLC analysis of methyl esters of neutral TAG of *B. oleracea* var. *botrytis* showed that linoleic acid (37.7%) was present in maximum amount. Other than linoleic acid, the oil composed of myristic (0.5%), palmitic (10.9%), stearic (15.2%), oleic (33.8%), linolenic (0.3%), erucic acid (1.6%). The GLC analysis of 2-MG of *B. oleracea* var. *botrytis* showed that at 2-position was predominantly occupied by linoleic acid (42.5%). Composition of other components present at 2-MG are myristic (0.3%), palmitic (6.3%), stearic (9.6%), oleic (38.4%), linoleic (42.5%), linolenic (0.6%) and erucic acid (2.3%). The fatty acid present in maximum amount at 1,3-position is linoleic (35.3%). The molar percent of linolenic acid (66.66) was found to be maximum at 2-position. E factor was found to be maximum for erucic acid (E value 1.44). According to data given in Table 1.3, the molar percent of triunsaturated acylglycerols (G13) was quite high 56.46 % with a very little amount of trisaturated acylglycerols (G13 1.3 %).

According to Gunstone *et al.*95, among the unsaturated C18 acids, oleic and linolenic acids show a slight preference for the 1-position while linoleic acid shows preference for the 2-position. The results in this chapter though not entirely but closely consent with this theory. Under the mentioned conditions of lipase digestion, because of incredibly fast hydrolysis and a prompt handling of the products no substantial acyl shifting took place in either mono- or diacylglycerols. From the present work it is concluded that the order of major unsaturated acids for 2-position in *M. elengi* is C18:2 > C18:1 > C18:0 and in *P. aculeata* the order is C18:2 > C18:1 > C18:3. In *B. rapa* it was linolenic acid which was acylated at 2-position preferentially and the order of preference in this oil is C18:3 > C18:2 > C22:1 whereas in *B. oleracea* var. *botrytis* the order of preference is C18:2 > C18:1 > C18:0.
1.4 Experimental

Dried samples of seeds were purchased from Pratap Nursery, Dehradun (INDIA). Analytical grades reagents and adsorbent (silica gel G) used for thin layer chromatography (TLC) and column chromatography (silica gel, 60-120 mesh) were Merck Ltd., Mumbai (INDIA). Distilled solvents were used throughout. Porcine pancreatic lipase was purchased from Sigma Chemicals Co. Centrifugation was carried out at 2000rpm on R-23, Remi centrifuge.

Lipid Extraction

Cleaned and dried samples of seeds were ground in a disintegrator. The powered seeds were extracted repeatedly with light petroleum ether (40-60 °C) in a Soxhlet apparatus. The extracted oils were dried over anhydrous Na₂SO₄. The solvent was removed in vacuum. The analytical values of the oils and seeds were determined according to the AOCS methods.

Purification of Triacylglycerols (TAGs) by Column Chromatography

The TAGs were purified from the total lipid by column chromatography using silica gel (mesh 60-120). Activated silica gel (30g per 1g of the sample) was packed in dry petroleum ether (40-60 °C) and total lipid was added to it. The column was washed with 200 ml of petroleum-ether/ Et₂O (95:5, v/v) to afford TAGs fractions. These fractions were further concentrated in vacuum and the purity was checked by TLC. The purified TAGs showed only one spot indicating that these preparations contained no detectable lipid contaminants.

Thin Layer Chromatography (TLC)

Analytical TLC was performed on glass plates coated with 0.25 mm or 1 mm thick layer of silica gel G with 20% diethyl ether in n-hexane as developing solvent. The plates were
Pancreatic lipase hydrolysis of triacylglycerols

rendered visual by spraying with 20% aqueous solution of perchloric acid and heating in an oven (110 °C) for 10 min. DNPH (2,4-nitrophenyl hydrazine)\(^9\), picric acid\(^9\) and Halphen's tests\(^9\) were then performed to check for any unusual moiety viz. keto, epoxy or cyclopropene moiety respectively.

**Deacylation of TAGs by pancreatic Lipase**

Lipase hydrolysis of the neutral TAGs was almost same as that described by Luddy *et al*.\(^{100}\).

To the lipid sample (100 mg in a screw-capped tube) were added tris-hydroxymethylaminomethane buffer (3 ml, 1 mol/ L) at pH 8, pig pancreatin lipase (20 mg) was added and shaken vigorously on a magnetic stirrer. Bile salt solution (0.5 ml, 1 gm/L) and CaCl\(_2\) solution (0.2 ml, 2.2%, w/v) were added. The reaction mixture was kept at 40±0.5 °C for 3 minute while shaking. The content was cooled under running tap water and hydrochloric acid (1 ml, 6 mol/L) was then added to terminate the reaction. Reaction mixture was immediately extracted with diethyl-ether (3x10 ml). Combined ethereal solution was washed with water and dried over anhydrous Na\(_2\)SO\(_4\). The dried samples were immediately fractionated by boric acid TLC.

**Isolation of 2-MAGs by boric acid TLC**

The boric acid TLC plates were prepared by mixing silica gel in saturated solution of boric acid and then spreading the slurry of silica gel on glass plates.

The lipolytic products (dissolved in 1 ml of chloroform) were applied on preparative boric acid plates in streaks. The chromatoplates were developed with a mixture of petroleum ether-diethyl ether-acetic acid (70: 30: 2, v/v). bands were detected under UV light after spraying the chromatoplates with 2', 7'- dichlorofluorescein. The compounds were separated out into four defined zones in the following order of increasing height travelled: monoacylglycerols, diacylglycerols, free fatty acids and...
unreacted triacylglycerols. The band corresponding to 2-monoacylglycerols (identified using standard sample run in the side of the plates) was scrapped off and extracted with CH₂Cl₂. The CH₂Cl₂ extract was washed with water and dried over anhydrous Na₂SO₄.

*Esterification of TAGs and 2-MAGs*

TAGs and MAGs were refluxed for 1 hour in a large excess of absolute MeOH containing 1% H₂SO₄ (v/v). Resulting mixtures were diluted to the cloud point with water, chilled in an ice bath and then extracted repeatedly with Et₂O. Combined extracts were dried over anhydrous Na₂SO₄ and evaporated in vacuum.

*Quantitative analysis of methyl esters*

The quantitative examination of methyl esters was done by comparing the gas chromatographs (GLCs) with those of the standard samples. GLC was carried out by using Varian Vista 600 instrument equipped with FID (290°C) detector using stainless steel column (2M x 2mm i.d.) packed with 15% of OV 275 on chromosorb-W (80-100 mesh). Separations were carried out at a programmed temperature of 140°C-200°C (10°C min⁻¹). The peak areas were determined by Hewlett Packard HP 3396 series-II integrator, which was used to determine the composition of esters. The fatty acid esters were identified by comparing with those of standard mixed fatty acid methyl esters. TAGs structures were also determined using pancreatic hydrolysis data applying Gunstone’s¹⁰¹ method of calculation.
1.5 References

Pancreatic lipase hydrolysis of triacylglycerols


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Pancreatic lipase hydrolysis of triacylglycerols


21


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Part B

Synthesis

of

Fatty Acid Derivatives
CHAPTER-2

3,5-Disubstituted-
1H-1,2,4-triazoles
2.1 Theoretical

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. Despite the large availability of methods to construct nitrogen heterocycles, there is still a strong need to further explore synthetic methods to efficiently synthesize novel heterocyclic structures. This proposal aims at the development of novel methodology for the synthesis of nitrogen heterocyclic structural motifs, containing varied groups.

Many compounds bearing five-membered heterocyclic rings in their structure have an extensive spectrum of pharmacological activities. Among them, 1,2,4-triazole derivatives have attracted considerable interest and can be used as antifungal, antibacterial, anti-inflammatory, antiasthmatic, antidepressant, tuberculotherapeutic, hypoglycemic and diuretic activities.

The 1H-1,2,4-triazole compounds are considered interesting heterocycles since they possess important pharmacological activities such as antifungal and antiviral activities. Examples of antifungal drugs are fluconazole (i), ICI 153066 (ii), itraconazole (iii), ravuconazole (iv), voriconazole (v), and posaconazole (vi).

![Chemical structures](image)
Some 3-amino-1H-1,2,4-triazoles have been used as herbicides and defoliants; meanwhile they were described as catalase inhibitors\textsuperscript{20} and blockers for certain ethanol-induced behavior effects\textsuperscript{21}. It has been reported that only certain enantiomers of triazoles containing oxazolidine rings (e.g., (vii), 4(R), 5(R)) are active against \textit{Candida albicans} infections in mice\textsuperscript{22}.

Ribose N-glycoside (viii)\textsuperscript{23-27} is a broad spectrum antiviral agent containing the 3-aminocarbonyltriazole moiety. It is active against both RNA and DNA virus and is used in an aerosol for lower respiratory tract viral disease as well as in the treatment of influenza, Lassa fever and Hantaan virus\textsuperscript{28, 29}. Amidine and guanidine derivatives (ix) (R = H-HCl, Me, CN) exhibiting a broad spectrum of antiviral activity\textsuperscript{30} have been prepared.
Some triazole derivatives are considered as angiotensin II receptor antagonists. These compounds, such as (x) and (xi), are used to increase the blood pressure.

In addition, it was reported that compounds having triazole moieties, such as vorozole (xii), letrozole (xiii), and anastrozole (xiv), appeared to be very effective aromatase inhibitors, which in turn prevented breast cancer.

It is known that 1,2,4-triazole moieties interact strongly with heme iron, and aromatic substituents on the triazoles are very effective for interacting with the active site of aromatase.
Morpholine (xv) and N-methyl piperazine (xvi) derivative of 1-aminomethyl-3-substituted-4-[5-(4-methoxy-2-nitrophenyl)-2-furfurylidene]-amino-1,2,4-triazole-5-thiones show potent anticancer activity.\(^{40}\)

\[
\text{Ar} - \text{O} - \text{N} - \text{N} - \text{SH} \quad \text{Ar} - \text{O} - \text{N} - \text{N} - \text{NH}_{2}
\]

The 3-[(4-methylphenoxy)-methyl]-4-(N-pyrazin-2'-yl-carboxamido)-5-mercapto-1,2,4-triazoles (xvii) and 3-[(4-methylphenoxy)-methyl]-4-(N-pyrazin-2'-yl-carboxamido)-5-hydrazino-1,2,4-triazoles (xviii) are shown to possess anti-inflammatory and analgesic activity.\(^{41}\)

The various methods for the synthesis of 1,2,4-triazole derivatives are reported by numerous workers. Some of them are discussed here briefly. The reaction of hydrazine or substituted hydrazines with suitable electrophiles is the most common method for the preparation of the triazoles. Examples where hydrazines provide the triazole ring\(^{42-44}\) are described in Scheme 2.1-2.3.
The conversion of a non-triazole ring system into a triazole usually included the substitution of nitrogen for another heteroatom in a five-membered ring, only a few typical examples\textsuperscript{45-47} are illustrated in Scheme 2.4-2.5.

Scheme 2.4
Microwave irradiation has become a widely used method to synthesize many useful organic chemicals rapidly, with good yields and high selectivity\textsuperscript{48-52}. By applying the microwave irradiation method, several 1,2,4-triazole derivatives were recently reported. 3,5-Disubstituted 4-amino-1,2,4-triazoles (xxx) from the reaction of aromatic nitriles (xxx) with NH\textsubscript{2}NH\textsubscript{2}-2HCl in the presence of NH\textsubscript{2}NH\textsubscript{2}-2H\textsubscript{2}O excess in ethylene glycol under microwave irradiation\textsuperscript{53} (Scheme 2.6).

An efficient microwave-assisted one-pot and three-component synthesis of substituted 1,2,4-triazoles (xxxv) has been achieved utilizing substituted primary amines\textsuperscript{54} (Scheme 2.7).

The 1-[(1,5-dialkyl-1H-1,2,4-triazol-3-yl] methylthymines (xxxviii)\textsuperscript{55} have been prepared from cycloaddition of the 1-cyanothymine (xxxvi)\textsuperscript{56} with the reactive cumulenes (xxxvii), as potential anti-HIV agents since some acyclic 1,2,4-triazole C-
nucleosides\textsuperscript{57} showed remarkable antiviral properties against HSV-1 and -2 along with other virus (Scheme 2.8).

![Chemical structure of 3,5-disubstituted 1H-1,2,4-triazoles](xxxvi) + (xxxvii) → (xxxviii)

\textbf{Scheme 2.8}

A series of new 1,2,4-triazoloalkylphthalimides (xl) has been synthesized from cycloaddition of the cyanoalkylphthalimides (alkylmethyl, ethyl, propyl) (xxxix) respectively, with the reactive cumulenes (xxxvii), as promising inhibitors of TNF-\(\alpha\) production\textsuperscript{58} (Scheme 2.9).

![Chemical structure of 3,5-disubstituted 1H-1,2,4-triazoles](xxxxvii) + (xxxix) → (xl)

\textbf{Scheme 2.9}

1,2,4-triazole derivatives carrying piperazine residue, (xl\textsubscript{ii}) and (xl\textsubscript{iv})\textsuperscript{59}, have been synthesized from cycloaddition of (xxxvii) with the 1,4-bis(cyanomethyl) piperazine (xli)\textsuperscript{60} and 1-cyanomethyl-4-methylpiperazine (xli\textsubscript{ii})\textsuperscript{61}, respectively (Scheme 2.10-2.11).
The short chain, alicyclic and variously substituted aromatic carboxylic acids have been utilized for the synthesis of 1,2,4-triazole derivatives. But 1,2,4-triazoles from long-chain carboxylic acid hydrazides have not been reported earlier. These considerations led to the series of present investigations to synthesize 3,5-disubstituted-1H-1,2,4-triazoles from fatty acid hydrazides.
2.2 Synthesis, Antibacterial and Antifungal Activity of Some Novel 3,5-Disubstituted-1H-1,2,4-triazoles

The development of new approaches for the synthesis of heterocycles decorated with unique functional groups forms the basis of extensive research activity in synthetic organic chemistry. Justification of much of the chemistry directed to the synthesis of the compounds, possessing nitrogen at the ring fusion is due to the application of compounds having interesting biological properties in the field of medicinal chemistry. The 1,2,4-triazole moiety is a structural element in certain antiasthmatic, antiviral (ribavirin), antifungal (flucanoazole), antibacterial, hypotonic (triazolam) drugs. Certain compounds containing 1,2,4-triazole nucleus have been reported to possess bactericidal, antiviral, insecticidal, anticaner, antinflammatory, anticonvulsant properties. Also some triazole derivatives have been synthesized as plant growth regulators.

Owing to its broad spectrum of biological activity the 1,2,4-triazole ring system represents an attractive target to invent new substrates for their synthesis and production of combinatorial libraries. 3,5-Disubstituted-1,2,4-triazoles are found in several pharmacologically active compounds. Recent examples include selective adenosine A_{2A} receptor antagonist and the phosphodiesterase V inhibitor. Previously, 1,2,4-triazoles were synthesized by hydrazides and nitriles either by Pinner reaction and Pellizzari condensation which involve the cyclodehydrative condensation between nitrile and hydrazide. These procedures (Scheme 2.12) path b-d are usually conducted at elevated temperature and involve the activation of nitrile to acylamidrazone intermediate 2 prior to cyclization.

These conventional procedures not only involve high reaction temperature and long reaction time but also results in low yields of product\textsuperscript{84-86}. Here in we are reporting simple and scalable methodology for the one-pot synthesis of 3,5-disubstituted-1H-1,2,4-triazoles.

Scheme 2.12 Pathways for synthesis of 3,5-disubstituted-1H-1,2,4-triazoles

To the best of our knowledge 3,5-disubstituted-1H-1,2,4-triazoles have not yet been reported from long-chain saturated and olefinic carboxylic acids. The present work is in continuation of study on the synthesis of heterocycles from such acids. Tetrazoles, pyrazolines, tetrazine, spiro [oxathiolane-2, 2'-dihydrotetrazoles], aziridines and benzothiazoles have been previously prepared from fatty acids\textsuperscript{87a-h}. Cyanoethoxy and morpholine derivatives of hydroxy long-chain acids\textsuperscript{87i} and fatty esters\textsuperscript{87j} showed significant antifungal and antibacterial activity. In view of the above mentioned pharmacological applications of 1,2,4-triazoles, the synthesis of this biologically active moiety was successfully carried out bearing long alkyl and alkenyl chain.

2.3 Results and discussion

3,5-Disubstituted-1H-1,2,4-triazoles 5a-o were synthesized by the condensation of long-chain saturated and olefinic carboxylic acid hyrazides 3a-e with nitriles 4 in presence of catalytic amount of K\textsubscript{2}CO\textsubscript{3} in n-BuOH (Scheme 2.13). The
use of catalytic amount of $\text{K}_2\text{CO}_3$ provided the product in reduced reaction time in appreciable yield.

$$
\begin{align*}
\text{R}_3\text{CO} & + \text{NH-NH}_2 \\
\text{R}_5\text{R}_4 & \xrightarrow{\Delta \text{K}_2\text{CO}_3} \\
\text{NH-NH}_2 & \\
\text{N} & \\
\text{R}_3
\end{align*}
$$

\begin{align*}
3\text{a-e} & \quad 4\text{a-c} & \quad 5\text{a-o} \\
\text{a}) \quad \text{R}_4=\text{R}_5=\text{H} & \quad \text{b}) \quad \text{R}_4=\text{H}, \text{R}_5=\text{OH} & \quad \text{c}) \quad \text{R}_4=\text{R}_5=\text{OH}
\end{align*}

Scheme 2.13 Synthesis of 3,5-disubstituted-1H-1,2,4-triazoles

As can be seen from Table 2.1, the scope of the reaction using saturated, olefinic (internal and terminal) and hydroxy acid hydrazides was found to be good. The yield of 3,5-disubstituted-1H-1,2,4-triazoles did not depend on the length of chain of acid hydrazide and was found to be appreciable. The synthesized compounds were identified on the basis of elemental analysis, IR, $^1\text{H}$ NMR $^{13}\text{C}$ NMR and mass spectra.
Table 2.1 3,5-Disubstituted-1H-1,2,4-triazoles

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting from</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3a, 4a</td>
<td></td>
<td>H</td>
<td>H</td>
<td>5a</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>3b, 4a</td>
<td></td>
<td>H</td>
<td>H</td>
<td>5b</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>3c, 4a</td>
<td></td>
<td>H</td>
<td>H</td>
<td>5c</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>3d, 4a</td>
<td></td>
<td>H</td>
<td>H</td>
<td>5d</td>
<td>82</td>
</tr>
<tr>
<td>5</td>
<td>3e, 4a</td>
<td></td>
<td>H</td>
<td>H</td>
<td>5e</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>3a, 4b</td>
<td></td>
<td>H</td>
<td>OH</td>
<td>5f</td>
<td>81</td>
</tr>
<tr>
<td>7</td>
<td>3b, 4b</td>
<td></td>
<td>H</td>
<td>OH</td>
<td>5g</td>
<td>88</td>
</tr>
<tr>
<td>8</td>
<td>3c, 4b</td>
<td></td>
<td>H</td>
<td>OH</td>
<td>5h</td>
<td>85</td>
</tr>
<tr>
<td>9</td>
<td>3d, 4b</td>
<td></td>
<td>H</td>
<td>OH</td>
<td>5i</td>
<td>83</td>
</tr>
<tr>
<td>10</td>
<td>3e, 4b</td>
<td></td>
<td>H</td>
<td>OH</td>
<td>5j</td>
<td>80</td>
</tr>
<tr>
<td>11</td>
<td>3a, 4c</td>
<td></td>
<td>OH</td>
<td>OH</td>
<td>5k</td>
<td>81</td>
</tr>
<tr>
<td>12</td>
<td>3b, 4c</td>
<td></td>
<td>OH</td>
<td>OH</td>
<td>5l</td>
<td>80</td>
</tr>
<tr>
<td>13</td>
<td>3c, 4c</td>
<td></td>
<td>OH</td>
<td>OH</td>
<td>5m</td>
<td>79</td>
</tr>
<tr>
<td>14</td>
<td>3d, 4c</td>
<td></td>
<td>OH</td>
<td>OH</td>
<td>5n</td>
<td>79</td>
</tr>
<tr>
<td>15</td>
<td>3e, 4c</td>
<td></td>
<td>OH</td>
<td>OH</td>
<td>5o</td>
<td>78</td>
</tr>
</tbody>
</table>

'¹H NMR spectrum of 3-(4'-hydroxyphenyl)-5-(dec-9-enyl)-1H-1,2,4-triazole (5g) showed characteristic signals of δ 10.97 of -NH proton, multiplets at δ 7.57-7.53 for four aromatic protons. Methine proton of C-9 showed signals at δ 5.82. C-10 methylene designated as Hₑ and Hₑ displayed two distinct δ values when coupled with adjacent C-9 methine protons. Thus, the '¹H NMR spectrum showed two doublet of doublet at δ 5.02 and 4.90 for Hₑ and Hₑ protons respectively. A triplet for two hydrogens was observed at δ 2.91 for methylene protons alpha to triazole ring. The structure of 5g was further supported by its mass spectral studies, which showed
molecular ion peak at m/z 299 consistent with its molecular formula C_{18}H_{25}N_{3}O. Base peak appears at m/z 160. Detailed spectra of titled compounds are given in the experimental section.

All the newly synthesized compounds were evaluated in vitro against an assortment of two Gram-positive bacteria *Staphylococcus aureus* MSSA 22 and *Bacillus subtilis* ATCC 6051 and two Gram-negative bacteria *Escherichia coli* K 12 and *Salmonella typhimurium* MTCC 98 at a concentration of 100μg/ml. Chloramphenicol was used as standard drug for the comparison of antibacterial results. Screening results are summarized in Table 2.2.

**Table 2.2 In vitro antibacterial activity of compounds 5a-o**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Diameter of zone of inhibition (mm) at 100μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram negative</td>
</tr>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>5a</td>
<td>14.6±0.61</td>
</tr>
<tr>
<td>5b</td>
<td>14.2±0.64</td>
</tr>
<tr>
<td>5c</td>
<td>13.6±0.53</td>
</tr>
<tr>
<td>5d</td>
<td>13.2±0.53</td>
</tr>
<tr>
<td>5e</td>
<td>13.2±0.50</td>
</tr>
<tr>
<td>5f</td>
<td>16.1±0.98</td>
</tr>
<tr>
<td>5g</td>
<td>15.9±0.36</td>
</tr>
<tr>
<td>5h</td>
<td>15.4±0.61</td>
</tr>
<tr>
<td>5i</td>
<td>13.3±0.75</td>
</tr>
<tr>
<td>5j</td>
<td>13.1±0.65</td>
</tr>
<tr>
<td>5k</td>
<td>16.6±0.59</td>
</tr>
<tr>
<td>5l</td>
<td>16.6±0.60</td>
</tr>
<tr>
<td>5m</td>
<td>15.9±0.65</td>
</tr>
<tr>
<td>5n</td>
<td>14.0±0.91</td>
</tr>
<tr>
<td>5o</td>
<td>14.2±0.53</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25</td>
</tr>
<tr>
<td>Control DMSO</td>
<td>---</td>
</tr>
</tbody>
</table>

The newly generated compounds 5a-o have exerted significant inhibitory activity against the growth of the test bacterial strains. The data pertaining to Table 2.2 reveal that 5a-o have significant influence on antibacterial profile of *S. typhimurium* and
S. aureus. The synthesized compounds showed good inhibitory results against B. subtilis and E. coli

In another set of experiments, the above mentioned compounds 5a-o were also examined for antifungal activity. Nystatin was used as standard drug for the comparison of antifungal results. The synthesized compounds showed excellent inhibitory results for Candida albicans IOA-109 and good results against Penicillium sp. (lab isolate) and Helminthosporum oryzae (2537 lab isolate) (Table 2.3).

Table 2.3 In vitro antifungal activity of compounds 5a-o

<table>
<thead>
<tr>
<th>Compound</th>
<th>C.albicans</th>
<th>H.oryzae</th>
<th>A.niger</th>
<th>T.viridae</th>
<th>Penicillium sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>18.4±0.31</td>
<td>12.3±0.36</td>
<td>15.0±0.25</td>
<td>5.1±0.42</td>
<td>12.5±0.5</td>
</tr>
<tr>
<td>5b</td>
<td>18.1±0.32</td>
<td>12.5±0.50</td>
<td>14.7±0.86</td>
<td>5.4±0.46</td>
<td>12.7±0.61</td>
</tr>
<tr>
<td>5c</td>
<td>18.3±0.55</td>
<td>11.2±0.49</td>
<td>15.1±0.42</td>
<td>5.6±0.60</td>
<td>12.9±0.80</td>
</tr>
<tr>
<td>5d</td>
<td>17.2±0.49</td>
<td>10.7±0.61</td>
<td>14.4±0.40</td>
<td>5.5±0.5</td>
<td>13.2±0.53</td>
</tr>
<tr>
<td>5e</td>
<td>17.2±0.35</td>
<td>10.8±0.71</td>
<td>14.8±0.80</td>
<td>5.4±0.58</td>
<td>13.2±0.5</td>
</tr>
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<td>5f</td>
<td>19.0±0.44</td>
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<td>15.1±0.42</td>
<td>6.5±0.50</td>
<td>14.6±0.55</td>
</tr>
<tr>
<td>5g</td>
<td>18.9±0.55</td>
<td>13.2±0.46</td>
<td>14.8±0.91</td>
<td>6.7±0.42</td>
<td>14.7±0.56</td>
</tr>
<tr>
<td>5h</td>
<td>18.0±0.68</td>
<td>14.0±0.47</td>
<td>15.1±0.55</td>
<td>6.7±0.61</td>
<td>15.2±0.43</td>
</tr>
<tr>
<td>5i</td>
<td>17.6±0.38</td>
<td>14.2±0.47</td>
<td>15.7±0.42</td>
<td>6.8±0.50</td>
<td>15.2±0.58</td>
</tr>
<tr>
<td>5j</td>
<td>18.0±0.85</td>
<td>14.2±0.60</td>
<td>15.5±0.50</td>
<td>6.9±0.59</td>
<td>15.6±0.40</td>
</tr>
<tr>
<td>5k</td>
<td>18.9±0.40</td>
<td>13.6±0.40</td>
<td>16.1±0.40</td>
<td>8.1±0.50</td>
<td>16.0±0.30</td>
</tr>
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<td>5l</td>
<td>18.2±0.43</td>
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<td>16.0±0.50</td>
<td>8.2±0.43</td>
<td>16.5±0.55</td>
</tr>
<tr>
<td>5m</td>
<td>17.7±0.44</td>
<td>13.9±0.46</td>
<td>15.7±0.80</td>
<td>8.8±0.25</td>
<td>17.2±0.50</td>
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<tr>
<td>5n</td>
<td>18.4±0.40</td>
<td>14.1±0.60</td>
<td>16.1±0.48</td>
<td>8.7±0.50</td>
<td>17.0±0.36</td>
</tr>
<tr>
<td>5o</td>
<td>17.9±0.57</td>
<td>14.7±0.55</td>
<td>16.0±0.30</td>
<td>9.2±0.62</td>
<td>17.4±0.43</td>
</tr>
<tr>
<td>Nystatin</td>
<td>20</td>
<td>18</td>
<td>18</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Control DMSO</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

All compound showed moderate activity results against Trichoderma viridae (lab isolate) and Aspergillus niger (lab isolate). The data also revealed that 5a-o has produced the marked enhancement in the potency of these analogues as antibacterial and antifungal agents.
2.4 Experimental

Anhydrous conditions were achieved by oven drying flasks and other equipments. Reactions were monitored by TLC on silica gel G. 60-80 mesh silica gel was used for column chromatography. All reagents and solvents were generally used as received from commercial suppliers and when required solvents were dried and distilled before use. Undec-10-enoic, (Z)-octadec-9-enoic and octadecanoic acids and BF₃·OEt₂ were obtained commercially from Fluka Chemicals (Switzerland). The eluent was a mixture of petroleum ether/EtOAc in different proportions for different compounds and visualized under iodine chamber. (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. The concentrate of pure hydroxy acids were obtained by Gunstone’s partitioning of freshly prepared fatty acids and further purified by column chromatography. 'H NMR spectra was 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. The molecular ion peak is designated as (M⁺). IR spectra were obtained on Shimadzu 8201 PC FT-IR using KBr pellet with absorption given in cm⁻¹.

General procedure for the preparation of fatty acid hyrazides (3a-e)

The hydrazides of corresponding fatty acids 3a-e were prepared by refluxing the (0.01mmol) methyl esters with hydrazine hydrate (0.02mmol) in MeOH at about 130oC for 2 hours. The mixture was then cooled to room temperature. The hydrazide was solidified in solvent. Filtered, washed with water and recrystallized in EtOH. The fatty acid hydrazides 3a-e were characterized by melting points. The spectral details of 3d, 3e is given below.
(9Z, 12R)-12-Hydroxooctadec-9-enoic acid hydrazide (3d)

Yield 85%; Mp=108-110 °C.

**IR (KBr):** 3390, 3280, 2960, 2890 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 8.20 (s, 1H, -NH-), 5.46 (m, 2H, -CH=CH-), 4.16 (d, J=6.9 Hz, 2H, -NHz), 3.88 (m, 1H, -CHOH), 2.88 (t, 2H, J = 7.9 Hz, -CH₂ α to ring), 2.31 (m, 1H, -CHOH), 2.04 (m, 4H, -CH₂=CH=CH-CH₂-), 1.93 (m, 2H, -CH₂ β to ring), 1.33 (br.s, 18H, chain CH₂), 0.86 (3H, dist.t, CH₃).

**MS:** 309 (M⁺), 175, 87, 73, 59.

**Anal:** Calcd. for C₁₈H₃₆N₂O₂: C, 69.90; H, 11.65; N, 9.06. Found: C, 69.88; H, 11.62; N, 9.08%.

(9R, 12Z)-9-Hydroxooctadec-12-enoic acid hydrazide (3e)

Yield 80%; Mp=110-112 °C.

**IR (KBr):** 3390, 3280, 2960, 2890 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 8.11 (s, 1H, -NH-), 5.39 (m, 2H, -CH=CH-), 4.11 (d, J=6.9 Hz, 2H, -NHz), 3.76 (m, 1H, -CHOH), 2.90 (t, 2H, J = 7.9 Hz, -CH₂ α to ring), 2.28 (m, 1H, -CHOH), 2.09 (m, 4H, -CH₂=CH=CH-CH₂-), 1.98 (m, 2H, -CH₂ β to ring), 1.31 (br.s, 18H, chain CH₂), 0.86 (3H, dist.t, CH₃).

**MS:** 309 (M⁺), 199, 87, 73, 59.

**Anal:** Calcd. for C₁₈H₃₆N₂O₂: C, 69.90; H, 11.65; N, 9.06. Found: C, 69.88; H, 11.62; N, 9.08%

*General procedure for the preparation of 3,5-disubstituted-1H-1,2,4-triazoles from fatty acid hydrazides (5a-o)*

A mixture of nitrile 4 (3mmol), acid hydrazide (3a-e) (1mmol) and K₂CO₃ (0.5mmol) in n-BuOH (2ml) was stirred and refluxed at 150°C for 4 hours. The progress of reaction was monitored on TLC. After completion of reaction the solvent was removed under reduced pressure and the compounds were adsorbed on silica gel and purified by column chromatography. All the compounds 5a-o were obtained as
oily liquid. The purity of compounds was ascertained by TLC resolution studies using petroleum ether/EtOAc (4:1, v/v) and few drops of MeOH as mobile phase. For column chromatography (Hexane:EtOAc, v:v) 5a, (99:1); 5b, (99:1); 5c, (98:2), 5d, (95:5); 5e, (95:5); 5f, (98:2), 5g, (98:2); 5h, (96:4); 5i, (95:5); 5j, (95:5); 5k, (98:2); 5l, (97:3); 5m, (95:5); 5n, (94:6); 5o, (94:6).

**Antibacterial activity**

The newly synthesized compounds were screened *in vitro* against an assortment of two Gram-positive bacteria *Staphylococcus aureus* MSSA 22 and *Bacillus subtilis* ATCC 6051 and two Gram-negative bacteria *Escherichia coli* K12 and *Salmonella typhimurium* MTCC 98. Screening results are summarized in Table 2.2. All the synthesized compounds were dissolved in DMSO. The antibacterial activity of test compounds and standard chloramphenicol was done by filter paper disc method. Media with DMSO was set up as control. All cultures were routinely maintained on NA (nutrient agar) and incubated at 37°C. The inoculums of bacteria were performed by growing the culture in NA broth at 37°C for overnight. The culture was centrifuged at 1000rpm and pellets was resuspended and diluted in sterile NSS to obtain viable count 10⁵ CFU/ml. 0.1 ml of approximately diluted bacterial culture suspension was spread with the help of spreader on NA plates uniformly. Sterile 8mm discs (Hi-media Pvt. Ltd.) were impregnated with 100μg/ml concentration of the test compounds. Antibiotic disc, chloramphenicol (30μg/disc Hi-Media) was used as control. The disc was placed onto the plate. Each plate had one control disc impregnated with solvent. The plates were then incubated for 24 hours at 37°C. Diameters of the zone of inhibition (mm) were measured and the average diameters for the test samples were calculated in triplicate sets.

**Antifungal activity**

The standard agar disc diffusion method was performed to evaluate the antifungal property of the test compounds and standard nystatin. The newly
synthesized compounds were screened for *Aspergillus niger* (lab isolate), *Candida albicans* IOA-109, *Penicillium* sp. (lab isolate), *Trichoderma viridae* (lab isolate), *Helminthosporum oryzae* (2537 ICAR, Jaipur). The synthesized compounds were dissolved in DMSO. Media with DMSO was set up as control. All cultures were routinely maintained on SDA and incubated at 28°C. Spore formation of filamentous fungi was prepared from 7 day old culture in sterile normal solution (8% NaCl) and approximately diluted to obtain $10^5$CFU/ml. The inoculums of non sporing fungi, *C. albicans* were performed by growing the culture in SD broth at 37°C for overnight. The culture was centrifuged at 1000rpm and pellets was resuspended and diluted in sterile NSS to obtain viable count $10^5$CFU/ml. 0.1 ml of approximately diluted fungal culture suspension was spread with the help of spreader on SDA plates uniformly. Sterile 8mm discs (Hi-media Pvt. Ltd.) were impregnated with test compounds. Antibiotic disc, nystatin (30μg/disc Hi-Media) was used as control. The disc was placed onto the plate. Each plate had one control disc impregnated with solvent. The plates were incubated at 28°C for filamentous fungi for 72 hours or more while for *C. albicans* plates were incubated at 37°C for 18-48 hours. Antifungal activity was determined by measuring the diameters of the inhibition zone (mm) in triplicate sets.

**Spectroscopic data**

3-Phenyl-5-heptadecyl-1H-1,2,4-triazole (5a)

IR (KBr): 3382, 1607, 1123 cm$^{-1}$. 

$^1$H NMR (400 MHz, CDCl$_3$): δ 10.96 (s, 1H, -NH-), 7.69-7.67 (m, 2H, Ar-H), 7.65-7.60 (m, 1H, Ar-H), 7.49-7.42 (m, 2H, Ar-H), 2.96 (t, 2H, J = 7.6 Hz –CH$_2$ α to ring), 2.05 (m, 2H, -CH$_2$ β to ring), 1.72 (br.s, 28H, chain CH$_2$), 0.86 (3H, dist.t, CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 159.15, 147.91, 133.81, 129.15, 127.35, 32.17, 30.39, 29.91, 28.66, 23.17, 14.84.

MS (m/z, %): (M+1)$^+$ 384 (10.5), M$^+$ 383 (36.1), 354 (25.3), 270 (27.7), 214 (63.8), 186 (16.6), 158 (100).
**3,5-Disubstituted-1H-1,2,4-triazoles**

**Anal. Calcd.** for C\textsubscript{25}H\textsubscript{41}N\textsubscript{3} : C, 78.29; H, 10.76; N, 10.95. Found: C, 78.85; H, 10.70; N, 10.90%.

**3-Phenyl-5-(dec-9-enyl)-1H-1,2,4-triazole (5b)**

**IR** (KBr): 3392, 1594, 1122 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CD\textsubscript{3}Cl): \(\delta\) 10.98 (s, 1H, -NH-), 7.66-7.64 (m, 2H, Ar-H), 7.62-7.58 (m, 1H, Ar-H), 7.49-7.45 (m, 2H, Ar-H), 5.82 (1H, tdd, \(J_{H-H_2} = 6.6\) Hz, \(J_{H-H_1} = 10.2\) Hz, \(J_{H-H_2} = 17.1\) Hz, CH\(_2\)=CH\(-\)), 5.02 (1H, dd, \(J_{H_2-H} = 10.2\) Hz, \(J_{H_2-H} = 1.2\) Hz, \(H_2C=CH-\)), 4.90 (1H, dd, \(J_{H-H_2} = 17.1\) Hz, \(J_{H-H_2} = 1.2\) Hz, \(H_2C=CH-\)), 3.10 (t, 2H, \(J = 7.6\) Hz, -CH\(_2\) α to ring), 2.05 (m, 2H, -CH\(_2\)=CH=CH\(_2\)), 1.99 (m, 2H, -CH\(_2\) β to ring), 1.38 (br.s, 10H, chain CH\(_2\)).

\(^{13}\)C NMR (100 MHz, CD\textsubscript{3}Cl): 158.94, 147.24, 139.82, 133.91, 129.66, 127.63, 114.67, 32.81, 30.85, 29.26, 28.32, 22.97, 14.21.

**MS (m/z, %):** (M+1)\(^+\) 284 (14.7), M\(^+\) 283 (10.8), 270 (28.3), 242 (29.9), 228 (11.8), 214 (18.3), 186 (26.2), 144 (100).

**Anal. Calcd.** for C\textsubscript{18}H\textsubscript{25}N\textsubscript{3}: C, 76.29; H, 8.88; N, 14.83. Found: C, 76.75; H, 8.82; N, 14.77%.

**3-Phenyl-5-(heptadec-8-enyl)-1H-1,2,4-triazole (5c)**

**IR** (KBr): 3382, 1597, 1133 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CD\textsubscript{3}Cl): \(\delta\) 10.96 (s, 1H, -NH-), 7.67-7.63 (m, 2H, Ar-H), 7.60-7.56 (m, 1H, Ar-H), 7.49-7.44 (m, 2H, Ar-H), 5.34 (m, 2H, -CH=CH-), 2.97 (t, 2H, \(J = 7.6\) Hz, -CH\(_2\) α to ring), 2.05 (m, 4H, -CH\(_2\)=CH=CH\(_2\)-), 1.96 (m, 2H, -CH\(_2\) β to ring), 1.73 (br.s, 20H, chain CH\(_2\)), 0.86 (3H, dist.t, CH\(_3\)).

\(^{13}\)C NMR (100 MHz, CD\textsubscript{3}Cl): 158.22, 146.91, 134.14, 131.15, 129.01, 128.14, 33.81, 30.86, 29.91, 28.63, 22.62, 14.68.

**MS (m/z, %):** (M+1)\(^+\) 382 (40.5), M\(^+\) 381 (30.1), 338 (38.3), 310 (27.7), 268 (63.8), 242 (16.6), 158 (100), 144 (10.9).

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3,5-Disubstituted-1H-1,2,4-triazoles

**Anal. Calcd.** for C_{25}H_{39}N_{3}: C, 78.70; H, 10.29; N, 11.01. Found: C, 78.28; H, 10.35; N, 11.07%.

3-Phenyl-5-[(8Z, 11R)-11-hydroxyheptadec-8-enyl]-1H-1,2,4-triazole (5d)

**IR** (KBr): 3386, 1590, 1119 cm\(^{-1}\).

**\(^1\)H NMR** (400 MHz, CDCl\(_3\)): 6 10.99 (s, 1H, -NH\(-\)), 7.66-7.64 (m, 2H, Ar-H), 7.60-7.57 (m, 1H, Ar-H), 7.49-7.46 (m, 2H, Ar-H), 3.88 (m, 1H, -CHOH), 3.11 (t, 2H, J = 7.6 Hz, -CH\(_2\) \(\alpha\) to ring), 2.43 (m, 1H, -CHOH), 2.05 (m, 4H, CH\(_2\)-CH=CH-CH\(_2\)), 1.89 (m, 2H, -CH\(_2\) \(\beta\) to ring), 1.73 (br.s, 18H, chain CH\(_2\)), 0.89 (3H, dist.t, CH\(_3\)).

**\(^13\)C NMR** (100 MHz, CDCl\(_3\)): 157.93, 146.82, 134.05, 131.61, 129.24, 127.95, 67.85, 39.22, 34.62, 31.22, 29.43, 28.72, 27.14, 22.75, 14.91.

**MS** (m/z, %): (M+1)\(^+\) 398 (9.8), M\(^+\) 397 (21.2), 340 (13.2), 312 (13.1), 282 (26.8), 200 (39.9), 186 (100), 172 (30.0).

**Anal. Calcd.** for C\(_{25}\)H\(_{39}\)N\(_3\)O: C, 75.53; H, 9.88; N, 10.56. Found: C, 75.01; H, 9.93; N, 10.61%.

3-Phenyl-5-[(8R, 11Z)-8-hydroxyheptadec-11-enyl]-1H-1,2,4-triazole (5e)

**IR** (KBr): 3387, 1594, 1124 cm\(^{-1}\).

**\(^1\)H NMR** (400 MHz, CDCl\(_3\)): 6 10.98 (s, 1H, -NH\(-\)), 7.69-7.65 (m, 2H, Ar-H), 7.62-7.55 (m, 1H, Ar-H), 7.49-7.45 (m, 2H, Ar-H), 3.88 (m, 1H, -CHOH), 3.11 (t, 2H, J = 7.6 Hz, -CH\(_2\) \(\alpha\) to ring), 2.42 (m, 1H, -CHOH), 2.03 (m, 4H, -CH\(_2\)-CH=CH-CH\(_2\)-), 1.98 (m, 2H, -CH\(_2\) \(\beta\) to ring), 1.29 (br.s, 18H, chain CH\(_2\)), 0.88 (3H, dist.t, CH\(_3\)).

**\(^13\)C NMR** (100 MHz, CDCl\(_3\)): 157.27, 146.81, 134.73, 131.52, 129.57, 127.21, 67.36, 39.17, 34.61, 31.81, 29.14, 28.71, 27.15, 22.51, 14.71.

**MS** (m/z, %): (M+1)\(^+\) 398 (5.8), M\(^+\) 397 (31.2), 368 (33.2), 326 (13.1), 272 (26.1), 228 (19.9), 214 (100), 172 (30.0).

**Anal. Calcd.** for C\(_{25}\)H\(_{39}\)N\(_3\)O: C, 75.53; H, 9.88; N, 10.56. Found: C, 75.99; H, 9.82; N, 10.51%.
3-(4'-Hydroxyphenyl)-5-heptadecyl-1H-1,2,4-triazole (5f)

IR (KBr): 3387, 1590, 1132 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 10.97 (s, 1H, -NH-), 7.57-7.53 (m, 4H, Ar-H), 6.95 (Ar-OH), 2.78 (t, 2H, J = 7.6 Hz, -CH₂ α to ring), 1.92 (m, 2H, -CH₂ β to ring), 1.23 (br.s, 20H, chain CH₂), 0.88 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 159.14, 151.84, 148.76, 138.97, 129.75, 117.33, 32.52, 30.72, 22.82, 21.72, 14.56.

MS (m/z, %): (M+1)⁺ 400 (13.8), M⁺ 399 (41.6), 286 (16.6), 272 (83.3), 258 (16.6) 188 (8.3), 174 (44.4), 160 (100).

Anal. Calcd. for C₂₅H₄₁N₃O: C, 75.15; H, 10.33; N, 10.51. Found: C, 75.66; H, 10.39; N, 10.45%.

3-(4'-Hydroxyphenyl)-5-(dec-9-enyl)-1H-1,2,4-triazole (5g)

IR (KBr): 3390, 1590, 1129 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 10.97 (s, 1H, -NH-), 7.57-7.53 (m, 4H, Ar-H), 6.93 (Ar-OH), 5.82 (1H, tdd, J₂-H₂style=ch₂ = 6.6 Hz, J₂-H₂ = 10.2 Hz, J₁-H₁ = 17.1 Hz, CH₂=CH-), 5.02 (1H, dd, J₂-H₂ = 10.2 Hz, J₂-H₂ = 1.2 Hz, H₂C=CH-), 4.90 (1H, dd, J₁-H₁ = 17.1 Hz, J₁-H₁ = 1.2 Hz, H₁C=CH-), 2.91 (t, 2H, J = 7.9 Hz, -CH₂ α to ring), 2.02 (m, 2H, -CH₂=CH=CH₂), 1.93 (m, 2H, -CH₂ β to ring), 1.38 (br.s, 10H, chain CH₂).

¹³C NMR (100 MHz, CDCl₃): 158.76, 151.23, 148.15, 139.72, 129.16, 116.27, 114.43, 34.25, 33.76, 29.22, 24.72.

MS (m/z, %): (M+1)⁺ 300 (19.9), M⁺ 299 (12.3), 272 (12.5), 244 (17.3), 230 (3.9), 216 (2.2), 188 (3.9), 174 (4.2), 160 (100).

Anal. Calcd. for C₁₈H₂₅N₃O: C, 72.22; H, 8.41; N, 14.03. Found: C, 72.74; H, 8.46; N, 14.09%.
3-(4'-Hydroxyphenyl)-5-(heptadec-8-enyl)-1H-1,2,4-triazole (5h)

IR (KBr): 3387, 1594, 1130 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): \( \delta \) 10.97 (s, 1H, -NH-), 7.57-7.56 (m, 4H, Ar-H), 6.93 (Ar-OH), 5.39 (2H, m, -CH=CH-), 2.79 (t, 2H, J = 5.7 Hz, -CH₂ α to ring), 2.02 (m, 4H, -CH₂-CH=CH-CH₂-), 1.96 (m, 2H, -CH₂ β to ring), 1.23 (br.s, 20H, chain CH₂), 0.88 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 157.73, 151.24, 148.72, 130.82, 129.17, 118.41, 34.27, 31.83, 29.68, 29.19, 27.04, 24.74, 22.56, 14.61.

MS (m/z, %): (M+1)^+ 398 (13.8), M^+ 397 (41.6), 284 (16.6), 258 (16.6) 188 (8.3), 174 (100), 160 (44.4).

Anal. Calcd. for C₂₅H₃₉N₃O: C, 75.53; H, 9.88; N, 10.56. Found: C, 75.99; H, 9.83; N, 10.50%.

3-(4'-Hydroxyphenyl)-5-{(8Z, 11R)-n-hydroxyheptadec-8-enyl}-1H-1,2,4-triazole (5i)

IR (KBr): 3390, 1590, 1123 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): \( \delta \) 10.98 (s, 1H, -NH-), 7.58-7.52 (m, 4H, Ar-H), 6.98 (Ar-OH), 5.46 (m, 2H, -CH=CH-), 3.88 (m, 1H, -CH-OH), 3.44 (t, 2H, J = 7.5 Hz, -CH₂ α to ring), 2.31 (m, 1H, -CH-OH), 2.04 (m, 4H, -CH₂-CH=CH-CH₂-), 1.91 (m, 2H, -CH₂ β to ring), 1.33 (br.s, 18H, chain CH₂), 0.86 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 157.94, 151.62, 148.52, 130.86, 129.36, 118.74, 67.87, 39.26, 34.17, 31.84, 29.63, 28.96, 27.27, 24.72, 22.54, 14.36.

MS (m/z, %): (M+1)^+ 414 (12.2), M^+ 413 (26.1), 370 (75.8), 356 (60.9), 328 (33.4), 202 (69.8), 174 (14.2), 160 (100).

Anal. Calcd. for C₂₅H₄₀N₃O₂: C, 72.61; H, 9.49; N, 10.16. Found: C, 72.05; H, 9.55; N, 10.20%.
3-(4'-Hydroxyphenyl)-5-[(8R, 11Z)-8-hydroxyheptadec-11-enyl]-1H-1,2,4-triazole

(5j)

IR (KBr): 3379, 1592, 1119 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 10.99 (s, 1H, -NH-), 7.57-7.53 (m, 4H, Ar-H), 6.93 (Ar-OH), 5.39 (m, 2H, -CH=CH-), 3.88 (m, 1H, -CH=CH-), 3.23 (t, 2H, J = 6.4 Hz, -CH₂ α to ring), 2.28 (m, 1H, J = 7.2 Hz, -CH-OH), 2.04 (m, 4H, -CH₂-CH=CH-CH₂), 1.89 (m, 2H, -CH₂ β to ring), 1.27 (br.s, 18H, chain CH₂), 0.86 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 158.94, 151.62, 148.74, 131.11, 129.46, 118.67, 67.36, 39.64, 34.16, 317.8, 29.55, 28.73, 27.22, 24.55, 22.11, 14.42.

MS (m/z, %): (M+1)⁺ 414 (12.5), M⁺ 413 (29.1), 342 (51.2), 316 (33.3), 288 (9.8), 188 (100), 174 (18.8), 160 (80.2).

Anal. Calcd. for C₂₅H₃₉N₃O₂: C, 72.61; H, 9.49; N, 10.16. Found: C, 72.99; H, 9.43; N, 10.11%.

3-(4',5'-Dihydroxyphenyl)-5-(heptadecyl)-1H-1,2,4-triazole (5k)

IR (KBr): 3349, 1604, 1134 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 10.97 (s, 1H, -NH-), 7.16 (d, 1H, J = 1.6 Hz, Ar-H), 7.07 (d, 1H, J = 2 Hz, Ar-H), 7.03 (d, 1H, J = 2 Hz, Ar-H), 6.89 (Ar-OH), 6.87 (Ar-OH), 2.78 (t, 2H, J = 7.6 Hz, -CH₂ α to ring), 1.92 (m, 2H, -CH₂ β to ring), 1.23 (br.s, 28H, chain CH₂), 0.88 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 164.57, 145.16, 144.24, 121.92, 115.91, 32.19, 30.38, 28.91, 22.37, 14.50.

MS (m/z, %): (M+1)⁺ 416 (17.0), M⁺ 415 (66.0), 356 (10.4), 314 (2.1), 300 (31.2), 208 (19.2), 190 (12.5), 176 (100).

Anal. Calcd. for C₂₅H₄₁N₃O₂: C, 72.26; H, 9.93; N, 10.11. Found: C, 72.79; H, 9.99; N, 10.15%.
3-(4',5'-Dihydroxyphenyl)-5-(dec-9-enyl)-1H-1,2,4-triazole (5l)

IR (KBr): 3387, 1596, 1130 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 10.98 (s, 1H, -NHe), 7.11 (d, 1H, J = 1.6 Hz, Ar-H), 7.04 (d, 1H, J = 2 Hz, Ar-H), 7.02 (d, 1H, J = 2 Hz, Ar-H), 6.88 (Ar-OH), 6.87 (Ar-OH), 5.82 (1H, tdd, J_H-H₂ = 6.6 Hz, J_H-H₂ = 10.2 Hz, J_H-H₂ = 17.1 Hz, CH₂=CH-), 5.02 (1H, dd, J_H-H₂ = 10.2 Hz, J_H-H₂ = 1.2 Hz, CH₂C=CH-), 4.90 (1H, dd, J_H-H₂ = 17.1 Hz, J_H-H₂ = 1.2 Hz, CH₂C=CH-), 3.13 (t, 2H, J = 8 Hz, CH₂), 2.02 (m, 2H, CH₂=CH), 1.93 (m, 2H, CH₂=CH), 1.38 (br.s, 1OH, chain CH₂).

³¹C NMR (100 MHz, CDCl₃): 164.91, 145.82, 144.85, 139.94, 121.96, 116.72, 114.67, 34.22, 33.79, 29.24, 28.42, 24.21.

MS (m/z, %): (M+1)^+ 316 (25.7), M^+ 315 (23.8), 288 (44.2), 274 (66.6), 243 (17.1), 208 (36.3), 198 (14.6), 176 (100).

Anal. Calcd. for C₁₈H₂₅N₃O₂: C, 68.55; H, 7.98; N, 13.32. Found: C, 68.05; H, 7.93; N, 13.26%.

3-(4',5'-Dihydroxyphenyl)-5-(heptadec-8-enyl)-1H-1,2,4-triazole (5m)

IR (KBr): 3384, 1594, 1127 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 10.97 (s, 1H, -NHe), 7.11 (d, 1H, J = 1.6 Hz, Ar-H), 7.04 (d, 1H, J = 2 Hz, Ar-H), 7.02 (d, 1H, J = 2 Hz, Ar-H), 6.93 (Ar-OH), 6.89 (Ar-OH), 5.34 (m, 2H, -CH=CH), 2.78 (t, 2H, J = 7.6 Hz, -CH₂α to ring), 2.02 (m, 4H, -CH₂=CH-CH=CH₂), 1.92 (m, 2H, -CH₂β to ring), 1.23 (br.s 20H, chain CH₂), 0.88 (3H, dist.t, CH₃).

³¹C NMR (100 MHz, CDCl₃): 163.42, 146.83, 144.84, 131.91, 121.66, 119.83, 33.18, 29.96, 25.69, 22.76, 14.92.

MS (m/z, %): (M+1)^+ 414 (17.0), M^+ 413 (66.0), 356 (10.4), 314 (12.1), 300 (31.2), 208 (19.2), 190 (100), 176 (57.5).

Anal. Calcd. for C₂₅H₃₉N₃O₂: C, 72.61; H, 9.49; N, 10.16. Found: C, 72.06; H, 9.44; N, 10.21%.
3,5-Disubstituted-1H-1,2,4-triazoles

3-(4',5'-Dihydroxyphenyl)-5-[(8Z,11R)-11-hydroxyheptadec-8-enyl]-1H-1,2,4-triazole (5n)

IR (KBr): 3386, 1597, 1123 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 10.98 (s, 1H, -NH⁻), 7.13 (d, 1H, J = 1.6 Hz, Ar-H), 7.04 (d, 1H, J = 2 Hz, Ar-H), 7.01 (d, 1H, J = 2 Hz, Ar-H), 6.89 (Ar-OH), 6.86 (Ar-OH), 5.46 (m, 2H, -CH=CH-), 3.88 (m, 1H, -CH-OH), 3.44 (m, 1H, -CH=CH-CH₂⁻), 1.91 (m, 2H, -CH₂β to ring), 2.31 (m, 1H, -CH-OH), 2.04 (m, 4H, -CH₂-CH=CH₂-C₆H₆), 1.33 (br.s, 18H, chain CH₂), 0.86 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 163.92, 147.84, 145.15, 131.86, 121.17, 119.23, 68.25, 39.84, 37.25, 33.18, 29.94, 27.6, 24.19, 22.70, 14.35.

MS (m/z, %): (M+1)⁺ 430 (6.6), M⁺ 429 (28.3), 344 (43.3), 260 (13.8), 253 (41.6), 208 (8.3), 190 (47.2), 176 (100).


3-(4',5'-Dihydroxyphenyl)-5-[(8R,11Z)-8-hydroxyheptadec-11-enyl]-1H-1,2,4-triazole (5o)

IR (KBr): 3392, 1600, 1123 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 10.98 (s, 1H, -NH⁻), 7.11 (d, 1H, J = 1.6 Hz, Ar-H), 7.04 (d, 1H, J = 2 Hz, Ar-H), 7.02 (d, 1H, J = 2 Hz, Ar-H), 6.89 (Ar-OH), 6.87 (Ar-OH), 5.39 (m, 2H, -CH=CH-), 3.88 (m, 1H, -CH-OH), 3.23 (m, 1H, -CH=CH-CH₂⁻), 3.23 (t, 1H, J = 6.4 Hz, -CH₂α to ring), 2.28 (m, 1H, -CH-OH), 2.04 (m, 4H, -CH₂-CH=CH₂-C₆H₆), 1.89 (m, 2H, -CH₂β to ring), 1.27 (br.s, 18H, chain CH₂), 0.86 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 163.63, 147.55, 145.16, 131.72, 121.26, 119.68, 68.49, 39.80, 37.41, 33.12, 29.5, 27.33, 24.24, 22.47, 14.21.

MS (m/z, %): (M+1)⁺ 430 (55.5), M⁺ 429 (8.3), 372 (55.5), 332 (41.6), 301 (22.2), 280 (13.6), 208 (8.3), 176 (100).

2.5 References


3,5-Disubstituted-1H-1,2,4-triazoles

50. B. L. Heyes, Microwaves Synthesis; Chemistry at the Speed of Light, CEM Publ: Matthews, NC, 2002.
3,5-Disubstituted-1H-1,2,4-triazoles


CHAPTER-3

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

The widespread use of 1,3,4-oxadiazoles as a scaffold in medicinal chemistry establishes this moiety as an important bio-active class of heterocycles. These molecules are also utilized as pharmacophores due to their favorable metabolic profile and ability to engage in hydrogen bonding. 1,3,4-Oxadiazoles have a wide range of pharmaceutical and biological activities including antimicrobial, antifungal, anti-inflammatory, and antihypertensive\textsuperscript{1-5}. In particular, marketed antihypertensive agents such as tiodazosin\textsuperscript{6} and nesapidil\textsuperscript{7} as well as antibiotics such as furamizole\textsuperscript{8} contain the oxadiazole nucleus. Biologically relevant entities containing the 1,3,4-oxadiazole motif include the HIV integrase inhibitor (i)\textsuperscript{9} angiogenesis inhibitor (ii)\textsuperscript{10}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{images.png}
\captionof{figure}{(i) and (ii)}
\end{figure}

The substituted oxadiazoles serve both as biomimetic and reactive pharmacophores and many are key elements with potential biological activities\textsuperscript{11-13} such as pesticidal\textsuperscript{14}, antiperipheral vasomotility\textsuperscript{15}, CNS stimulant, antiinflammatory, hypotensive\textsuperscript{16}, insecticidal\textsuperscript{17}, bactericidal\textsuperscript{18}, hypoglycemic\textsuperscript{19,20}, analgesic, anticonvulsive, antiemetic, diuretic\textsuperscript{21}, muscle relaxant\textsuperscript{22,23}, herbicidal\textsuperscript{24,25} and fungicidal activity\textsuperscript{26,27}.

N-Substituted 2-amino- and 2-alkylthio-1,3,4-oxadiazoles containing benzothiazole fragments in position 5 possess a wide spectrum of biological activity, including anti-inflammatory (iii)\textsuperscript{28,29}, antimicrobial\textsuperscript{30} and hypotensive\textsuperscript{31} activity.
2,5-Disubstituted-1,3,4-oxadiazoles

(iii)

The 1,3,4-oxadiazole derivatives show leprostatic and tuberculostatic properties and exhibit antibacterial, antiproteolytic and anticonvulsant activities. Also they have analgesic, antipyretic, antiphlogistic, bactericides, insecticides, fungicidical and several other biological activities.

Moreover, 1,3,4-oxadiazoles are becoming an important member in the heterocyclic family not only because of their wide usage as dyes, photosensitive and electrical materials.

3-Methyl-2-({[5-aryl-1,3,4-oxadiazol-2-yl]methyl}aniline)methyl)-4(3H)quinazolinone (iv) was synthesized by Komaraiah et al. The amino alkyl bridge was conceived to provide conformational flexibility and improve binding characteristics to (iv).

(iv)

1,3,4-oxadiazoles have attracted considerable interest in medicinal chemistry as surrogates of carboxylic acids, esters and carboxamides. In particular the 2-amino-1,3,4-oxadiazoles have recently been reported to exhibit promising anti-tumour activity.

Bis-mercapto-1,3,4-oxadiazoles were used as active substances to control *Meloidogyne incognita* in tomato.

The common synthetic route to these compounds involves cyclization of diacylhydrazines with a variety of anhydrous reagents such as thionyl chloride, phosphorus pentoxide, phosphorus oxychloride, triflic anhydride (Scheme 3.1).
2,5-Disubstituted-1,3,4-oxadiazoles

Scheme 3.1

triphenylphosphine\(^\text{42}\), polyphosphoric acid\(^\text{43}\) and sulfuric acid\(^\text{1}\). The acylation of tetrazoles is another synthetic route to these compounds\(^\text{44}\). They have also been prepared by oxidation of acylhydrazones with different oxidizing agents\(^\text{45-47}\). One-pot synthesis of 1,3,4-oxadiazoles from acid hydrazides and hydrazine with an acid chloride\(^\text{48}\) as well as from hydrazines with carboxylic acids\(^\text{49}\) have also been reported.

When terephthalic acid hydrazide (vii) was refluxed with phenyl isocyanate (viii) using the procedure described for the synthesis of bis-semicarbazide (ix) was formed (Scheme 3.2). The compound (ix) on treatment with POCl\(_3\) underwent cyclization to the corresponding 5,5'-\((1,4\text{-phenylene})\)-bis-\((2\text{-phenylamino}-1,3,4\text{-oxadiazole})\) (x)\(^\text{50}\). The compound (x) showed potential antibacterial activity.

Scheme 3.2

The reaction of 1-adamantanecarbonyl chloride (xi) with certain carboxylic acid hydrazides (xii) in pyridine yielded the corresponding N-acyl adamantane-1-carbohydrazide derivatives (xiii), which were cyclized to the corresponding 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles (xiv) via heating with phosphorus...
oxychloride\textsuperscript{51} (Scheme 3.3). The synthesized compounds showed antiinflammatory activity.

\[
\begin{align*}
\text{ClO}_2^- + \text{R} \rightarrow \text{N}\text{O}_2\text{N}^- + \text{C}_6\text{H}_5
\end{align*}
\]

Polshettiwar \textit{et al.}\textsuperscript{52} reported the green protocol for the synthesis of 1,3,4-oxadizoles (xvii) catalyzed efficiently by solid supported Nafion\textsuperscript{\textregistered} NR50 (NF) and phosphorus pentasulfide in alumina (P\textsubscript{4}S\textsubscript{10}/Al\textsubscript{2}O\textsubscript{3}) with excellent yields (Scheme 3.4).

\[
\begin{align*}
\text{C}_6\text{H}_5\text{O}^- + \text{R} \rightarrow \text{C}_6\text{H}_5\text{N}\text{O}_2\text{N}^- + \text{C}_6\text{H}_5
\end{align*}
\]

5-Aryl-2-(2-hydroxy-phenyl)-1,3,4-oxadiazoles (xx) by reaction of salicylic hydrazide (xviii) with carboxylic acids (xix) in the presence of thionyl chloride under neat conditions is described\textsuperscript{53} (Scheme 3.5).

\[
\begin{align*}
\text{OH}^- + \text{R} \rightarrow \text{OH}^- + \text{C}_6\text{H}_5
\end{align*}
\]
2,5-Disubstituted-1,3,4-oxadiazoles (xxiv) were prepared by oxidation of 1-aroyl-2-arylidene hydrazines (xxiii) with potassium permanganate on the surface of silica gel and also in mixtures of acetone and water under microwave irradiation\(^{54}\) (Scheme 3.6).

\[
\begin{align*}
\text{R}_1^\text{O} & + \text{R}_2^\text{NH}_2 & \text{SiO}_2, H^+, MW & \rightarrow & \text{R}_1^\text{NH} & \text{KMO}_2, \text{SiO}_2, MW \\
\text{NH} & \text{H} & \text{or Acetone, Water} & \rightarrow & \text{R}_1^\text{O} & \text{R}_2
\end{align*}
\]

Scheme 3.6

2-(4-Methoxylphenyloxyacetylamido)-5-aryloxymethyl-1,3,4-oxadiazoles (xxvii) were synthesized by cyclization of 1-aryloxyacetyl-4-(4-methoxylphenyloxyacetyl)-thiosemicarbazides (xxvi) in the presence of mercuric acetate under microwave irradiation\(^{55}\) (Scheme 3.7).

\[
\begin{align*}
\text{OCH}_3 & \text{NH}_2, \text{SCN, PEG400CH}_2\text{Cl}_2 & \text{ArOCH}_2\text{CONNH}_2 & \text{Hg(OAc)}_2/\text{ArOH, MW} & \text{ArOH}_2\text{C} \\
\text{OCH}_2\text{COCH}_2\text{CONHNCH}_2\text{COCH}_2\text{OAr} & & & & \text{H}_2\text{CO}
\end{align*}
\]

Scheme 3.7

5,5'-Dimercapto-bis-[1,3,4-oxadiazol-2-yl]octane (xxix) and 5,5'-dibenzylthiobis-[1,3,4-oxadiazol-2-yl]-butane were synthesized by reaction of alkanedioic acids hydrazides (xxviii) with CS\(_2\) in alcoholic KOH solution to give (xxix) which yielded (xxx) with addition of benzyl bromide\(^{56}\) (Scheme 3.8).

\[
\begin{align*}
\text{H}_2\text{N} & \text{NH} & \text{NH}_2 & \text{KOH/EtOH} & \text{HS} & \text{KOH} \\
\text{CS}_2 & & & & \text{PhH}_2\text{CS} & \text{2PhCH}_2\text{Br}
\end{align*}
\]

Scheme 3.8
Shu Wang, Zhitaş and Wenting Hua\textsuperscript{57} have synthesized the macrocycles containing 1,3,4-oxidizole moiety (xxx).}

\begin{center}
\includegraphics[width=0.5\textwidth]{xxx}
\end{center}

\textbf{Scheme 3.9}

2,5-Substituted-1,3,4-oxadiazoles containing 2-benzothiazolylthiomethyl moiety (xxxiv) were synthesized by condensation of (2-benzothiazolylthio)acetic acid imino ester dihydrochloride (xxxii) with hydrazides of various carboxylic acids\textsuperscript{58} (Scheme 3.9).

\begin{center}
\includegraphics[width=0.7\textwidth]{xxxiii}
\end{center}

\textbf{Scheme 3.9}

Brain et al.\textsuperscript{59} reported the synthesis of simple 1,3,4-oxadiazoles via cyclodehydration of 1,2-diacylhydrazines using a polymer-supported Burgess reagent or the polymer-bound phosphazene base PS-BEMP in the presence of toluenesulfonyl chloride as a dehydrating agent\textsuperscript{60}. Brown\textsuperscript{61} and Kilburn\textsuperscript{62} have also shown that 2-amino-1,3,4-oxadiazoles can be prepared in excellent yields on solid phase from the corresponding immobilised 1,4-disubstituted semicarbazides using either 1,3-diisopropylcarbodiimide (DIPC), 1,3-dicyclohexylcarbodiimide (DCC) or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) as the cyclisation mediator.
Wang et al. have reported the one step synthesis of 1,3,4-oxadizoles from diacyl hydrazide in the presence of DMC (2-chloro-1,3-dimethylimidazolinium chloride), 1,3,4-oxadiazoles can be obtained in good yields from carboxylic acids and acid hydrazides in CH$_2$Cl$_2$ by heating under microwave condition (Scheme 3.10).

![Scheme 3.10](image-url)
3.2 Synthesis, Antibacterial and Antifungal Activity of Some New 2,5-Disubstituted-1,3,4-oxadiazoles*

1,3,4-oxadiazoles are a class of heterocycles which have attracted significant interest in medicine, pesticide chemistry and material science. They are of significant interest in medicinal chemistry as ester bioesters in a number of biological targets including 5-HT receptor agonists\(^2\), muscarinic agonists\(^{64}\), 5-HT antagonists\(^{65}\), human NK\(_1\) antagonists\(^{66}\), antirhinoviral compounds\(^{67}\) and anti-inflammatory agents\(^{68}\). Numerous compounds containing 1,3,4-oxadiazoles have been evaluated for their antimicrobial\(^{69}\), psychotropic\(^{70}\), antiaflatoxigenic\(^{71}\), anticonvulsant\(^{72}\), tuberculostatic\(^{73}\), antimitotic\(^{74}\), analgesic\(^{75}\) and CNS activity\(^{76}\). They have been used as peptide mimetics due to their particular geometric and electrostatic properties\(^{77}\). A variety of 2,5-dialkyl, 2,5-diaryl, 2-alkyl,5-aryl-1,3,4-oxadiazoles show herbicidal activity\(^{78}\). Therefore the development of novel synthetic routes to achieve access to these molecules is of prime interest. Further, the increasing number of multidrug resistant pathogens has led us to screen the newly synthesized derivatives against the representative panel of bacterial and fungal strains.

Keeping in mind the practical applications of 1,3,4-oxadiazoles, the design and synthesis of hitherto unknown oxadiazoles bearing long alkyl and alkenyl chain and their in vitro biological screening against Gram positive and Gram negative bacteria and fungi was performed. The symmetrically disubstituted-1,3,4-oxadiazoles have not yet been reported from saturated and olefinic long-chain carboxylic acids. Tetrazoles\(^{79,80}\), pyrazolines\(^{81}\), tetrazine\(^{82,83}\), spiro [oxathiolane-2, 2'-dihydrotetrazoles]\(^{84}\), aziridines\(^{85}\) and benzothiazoles\(^{86}\) have been previously prepared in author’s laboratory. Cyanoethoxy and morpholine derivatives of hydroxy fatty acids\(^{87}\) and long-chain alkenoates\(^{88}\) showed significant antifungal and antibacterial activity.

3.3 Results and discussion

A typical reaction procedure in a single step involves the addition of a solution of hydrazine hydrate to acid chloride 2a-f at 0°C in dry dioxane and stirring at room temperature to furnish corresponding 1,2-diacylhydrazine 3a-f (Scheme 3.11). To the prior acid chloride was synthesized from saturated and olefinic fatty acids by in situ preparation. Since fatty acid chlorides 2a-f are not commercially available the present method has greatly solved the problem by facile and efficient in situ preparation. We have used BF$_3$OEt$_2$ for cyclodehydration of 1,2-diacylhydrazines 3a-f. BF$_3$OEt$_2$ in dry dioxane was added to the 1,2-diacylhydrazine 3a-f without isolating the product and reaction mixture refluxed for 1-2 hours.

![Scheme 3.11 Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles.](image)

Table 3.1 2,5-Disubstituted-1,3,4-oxadiazoles

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,3,4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>[CH$<em>2$]$</em>{11}$</td>
<td>98</td>
</tr>
<tr>
<td>b</td>
<td>[CH$<em>2$]$</em>{13}$</td>
<td>98</td>
</tr>
<tr>
<td>c</td>
<td>[H]</td>
<td>97</td>
</tr>
<tr>
<td>d</td>
<td>[CH$<em>2$]$</em>{6}$</td>
<td>98</td>
</tr>
<tr>
<td>e</td>
<td>[CH$<em>2$]$</em>{5}$</td>
<td>95</td>
</tr>
<tr>
<td>f</td>
<td>[CH$<em>2$]$</em>{6}$</td>
<td>90</td>
</tr>
</tbody>
</table>
As can been seen from Table 3.1 the scope of the reaction using saturated, olefinic (internal and terminal) and hydroxy fatty acid is found to be good. The yields of 1,3,4-oxadiazoles 4a-f are excellent and independent of the substituents present in the precursor. In $^1$H NMR spectra of 2,5-di-(dec-9-enyl)-1,3,4-oxadiazole (4c), multiplet for four hydrogens was observed at $\delta$ 2.54 for methylene protons alpha to oxadiazole moiety. Methine proton of C-9 showed signals at $\delta$ 5.82. C-10 methylene 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 spectrum showed two doublet of doublet at $\delta$ 5.02 and 4.90 for H_Z and H_E protons respectively. The structure of 4c was further supported by its mass spectral studies, which showed molecular ion peak at $m/z$ 346 consistent with its molecular formula C_{22}H_{36}N_2O. Base peak appears at $m/z$ 277. Detailed spectra of titled compounds are given in the experimental section.

All the newly synthesized compounds were evaluated in vitro against an assortment of two Gram-positive bacteria *Staphylococcus aureus* MSSA 22 (SA) and *Bacillus subtilis* ATCC 6051 (BS) and two Gram-negative bacteria *Escherichia coli* K 12 (EC) and *Salmonella typhimurium* MTCC 98 (ST) . Screening results are summarized in Table 3.2.

**Table 3.2 In vitro antibacterial activity of compounds 4a-f**

<table>
<thead>
<tr>
<th>Compound</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>S. typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>27</td>
<td>26</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>4b</td>
<td>29</td>
<td>24</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>4c</td>
<td>24</td>
<td>25</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>4d</td>
<td>24</td>
<td>25</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>4e</td>
<td>20</td>
<td>26</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>4f</td>
<td>20</td>
<td>26</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>40</td>
<td>36</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>Control (DMSO)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

The minimum inhibitory concentrations (MIC) of all the tested compounds were 100$\mu$g/ml. The newly generated compounds 4a-f have exerted significant
inhibitory activity against the growth of the tested bacterial strains. The data pertaining to Table 3.2 reveal that 4a-f have significant influence on antibacterial profile of Gram-positive bacteria. The higher susceptibility of Gram-positive bacteria is due to absence of a unique outer membrane of peptidoglycon, hence the wall of these bacteria is permeable to these derivatives. The compounds 4a-f showed good inhibitory results against *S. typhimurium* and *E. coli*. Fig. 3.1 and 3.2 shows that *in vitro* screening of synthesized compounds gives promising results compared to the reference drug chloramphenicol and nystatin.

![Graph showing % inhibition of antibacterial activity for compounds 4a-f over control drug](image)

**Fig. 3.1** Percent inhibition of antibacterial activity for compounds 4a-f over control drug

All the synthesized compounds were screened for antifungal activity against *Candida albicans* (IOA-109) (CA), *Penicillium* sp. (lab isolate) (PN), *Fusarium oxysporum* (lab isolate from ICAR, Jaipur) (FO), *Trichoderma viridae* ATCC 5170 (TV) and *Aspergillus niger* (lab isolate from ICAR, Jaipur) (AN) at a concentration of 100μg/ml. Nystatin was used as standard drug for the comparison of antifungal results.
Table 3.3  *In vitro* antifungal activity of compounds 4a-f

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>C. albicans</em></th>
<th><em>T. viridae</em></th>
<th><em>Penicillium</em> sp.</th>
<th><em>F. oxysporum</em></th>
<th><em>A. niger</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>27</td>
<td>18</td>
<td>22</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>4b</td>
<td>22</td>
<td>15</td>
<td>20</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>4c</td>
<td>21</td>
<td>14</td>
<td>21</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>4d</td>
<td>22</td>
<td>15</td>
<td>23</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>4e</td>
<td>27</td>
<td>15</td>
<td>18</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>4f</td>
<td>23</td>
<td>13</td>
<td>17</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Nystatin</td>
<td>30</td>
<td>20</td>
<td>25</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Control(DMSO)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Among the tested organisms, compounds 4a, 4e showed excellent inhibitory results for *C. albicans* IOA-109. Compounds 4b, 4c, 4d revealed good results against *Penicillium* sp. and *F. oxysporum*. All compound showed moderate activity results against *T. viridae* ATCC 5170 and *A. niger* (Table 3.3). The data also revealed that 4a, 4c, 4d has produced the marked enhancement in the potency of these analogues as antifungal agents.

![Percent inhibition of antibacterial activity for compounds 4a-f over control drug](image)

Fig. 3.2  *Percent inhibition of antibacterial activity for compounds 4a-f over control drug*
3.4 Experimental

The sources of all the fatty acids and instrumentation details are the same as given in Chapter 1 (page 38).

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

Thionyl chloride (3 mmol) was added to fatty acid 1a-f (2.5 mmol) at 80°C for about 2 hours to form the corresponding acid chloride 2a-f. The progress of reaction was monitored by TLC. The excess of thionyl chloride was distilled off. To a stirred solution of acid chloride (2.5 mmol) in dry dioxane (25ml) was added hydrazine hydrate (1.25 mmole) at 0°C and stirred further for 30 minutes at room temperature. BF₃·OEt₂ (6.25 mmole) in dry dioxane (5ml) was added dropwise to the above stirred reaction mixture in 15 minutes and reaction mixture further refluxed for 1 to 2 hours at 130°C. The resulting solution was concentrated in vacuum, saturated NH₄Cl solution added till the pH becomes 6, extracted with EtOAc, dried over anhydrous Na₂SO₄ and concentrated in vacuum to give the crude product 4a-f as oily liquids, which is further purified by column chromatography (Hexane:EtOAc, v:v) 4a, (99:1); 4b, (99:1); 4c, (98:2); 4d, (97:3); 4e, (95:5); 4f, (95:5).

**Antibacterial Activity**

The antibacterial activity of test compounds and standard chloramphenicol was done by filter paper disc method against *Staphylococcus aureus* MSSA 22, *Escherichia coli* K12, *Bacillus subtilis* ATCC 6051, *Salmonella typhimurium* MTCC 98 at a concentration of 100μg/ml. Media with DMSO was set up as control.

**Antifungal activity**

The standard agar disc diffusion method was performed to evaluate the antifungal property of the test compounds and standard nystatin. *Aspergillus niger* (lab isolate from ICAR, Jaipur), *Candida albicans* IOA-109, *Penicillium* sp (lab isolate),...
Fusarium oxysporum (lab isolate from ICAR, Jaipur), Trichoderma viridae ATCC 5170 were used in this study. Solvent control DMSO was also run. Percent inhibition (% inhibition) of antibacterial and antifungal activity for different derivatives over control drug, was calculated by using the following formula:

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

where, \( \alpha \) and \( \beta \) stand for zone of inhibition of control drug and synthesized compounds, respectively.

**Spectroscopic data**

**2,5-Dipentadecyl-1,3,4-oxadiazole (4a)**

IR (KBr): 1024, 1247, 1639 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \& 2.54 (m, 4H, \( \alpha \) to oxadiazole ring), 1.63 (m, 4H, \( \beta \) to oxadiazole ring), 1.30 (br.s, 48H, chain CH\(_2\)), 0.88 (6H, dist. t, CH\(_3\)).

\(^13\)C NMR (100 MHz, CDCl\(_3\)): 172.63, 34.15, 31.98, 29.45, 24.73, 22.75, 14.17.

MS (m/z, %): (M+1)\(^+\) 491 (8), (M\(^+\)) 490 (15), 475 (60), 433 (100), 405 (50), 377 (100), 349 (25), 335 (10), 321 (85), 279 (22).

Anal. Calcd. for C\(_{32}\)H\(_{62}\)N\(_2\)O: C, 78.36; H, 12.65; N, 5.71. Found: C, 78.39; H, 12.99; N, 5.66%.

**2,5-Deheptadecyl-1,3,4-oxadiazole (4b)**

IR (KBr): 1030, 1250, 1645 cm\(^{-1}\).

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \& 2.56 (m, 4H, \( \alpha \) to oxadiazole ring), 1.64 (m, 4H, \( \beta \) to oxadiazole ring), 1.33 (br.s, 56H, chain CH\(_2\)), 0.88 (6H, dist. t, CH\(_3\)).

\(^13\)C NMR (100 MHz, CDCl\(_3\)): 173.26, 34.09, 31.95, 29.42, 24.71, 22.72, 14.15.

MS (m/z, %): (M+1)\(^+\) 547 (5), (M\(^+\)) 546 (32), 517 (68), 489 (100), 447 (40), 419 (12), 363 (22), 321 (60), 298 (10), 235 (31), 214 (21).

Anal. Calcd. for C\(_{36}\)H\(_{70}\)N\(_2\)O: C, 79.12; H, 12.82; N, 5.12. Found: C, 79.28; H, 12.80; N, 5.32%.
2,5-Disubstituted-1,3,4-oxadiazoles

2,5-Disubstituted-1,3,4-oxadiazole (4c)

**IR** (KBr): 1040, 1240, 1640 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 5.82 (tdd, 1H, J_H-eCH₂ = 6.6 Hz, J_H-H₂ = 10.2 Hz, J_H-H_e = 17.1 Hz, CH₂=CH-H), 5.02 (dd, 1H, J_H-eH = 10.2 Hz, J_H₂-H_e = 1.2 Hz, H₂C=CH), 4.90 (dd, 1H, J_H-eH = 17.1 Hz, J_H₂-H_e = 1.2 Hz, H₂C=CH), 2.55 (m, 4H, α to oxadiazole ring), 2.03 (m, 4H, -CH₂=CH(CH₂)₂, 1.62 (m, 4H, β to oxadiazole ring), 1.29 (br.s, 20H, chain CH₂).

**¹³C NMR** (100 MHz, CDCl₃): 172.73, 139.19, 114.22, 34.20, 33.84, 29.15, 24.72.

**MS** (m/z, %): (M+1)⁺ 347 (10), (M⁺) 346 (18), 305 (39), 291 (16), 277 (100), 263 (46), 249 (48), 235 (82), 207 (8).

**Anal. Calcd.** for C₂₂H₃₈N₂O: C, 76.30; H, 10.98; N, 8.09. Found: C, 76.33; H, 10.86; N, 8.17%.

2,5-Di-(heptadec-8-enyl)-1,3,4-oxadiazole (4d)

**IR** (KBr): 1024, 1300, 1639 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 5.38 (m, 4H, -CH=CH-), 2.60 (m, 4H, α to oxadiazole ring), 2.01 (m, 8H, -CH₂=CH-CH₂=CH₂-), 1.51 (m, 4H, β to oxadiazole ring), 1.13 (br.s, 40H, chain CH₂), 0.92 (6H, dist. t, CH₃).

**¹³C NMR** (100 MHz, CDCl₃): 172.63, 129.82, 40.53, 34.00, 29.39, 31.64, 26.33, 24.74, 14.14.

**MS** (m/z, %): (M+1)⁺ 545 (15), (M⁺) 544 (44), 529 (100), 515 (8), 501 (71), 487 (39), 473 (26), 391 (67), 391 (37), 307 (5), 139 (62).

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

2,5-Di-[(8Z,11R)-11-hydroxy-heptadec-8-enyl]-1,3,4-oxadiazole (4e)

**IR** (KBr): 1025, 1280, 1650 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 5.37 (m, 4H, -C=CH-), 3.63 (m, 2H, -CH=O), 2.54 (m, 4H, a to oxadiazole ring), 2.29 (m, 2H, -CH=O), 2.02 (m, 8H, -CH2-CH=CH-CH₂-), 1.54 (m, 4H, β to oxadiazole ring), 1.37 (br.s, 36H, chain CH₂), 0.88 (6H, dist. t, CH₃).

**¹³C NMR** (100 MHz, CDCl₃): 174.67, 130.51, 71.67, 37.06, 34.30, 31.64, 28.88, 25.47, 23.42, 14.53.

**MS** (m/z, %): (M+1)⁺ 559 (12), (M⁺) 558 (40), 543 (100), 501 (17), 487 (16), 443 (37), 403 (58), 361 (41), 347 (10), 333 (61), 155 (82).

**Anal. Calcd.** for C₃₆H₆₆N₂O₂: C, 77.41; H, 11.82; N, 5.01. Found: C, 77.49; H, 11.72; N, 5.09%.

2,5-Di-[(8R,11Z)-8-hydroxy-heptadec-11-enyl]-1,3,4-oxadiazole (4f)

**IR** (KBr): 1024, 1240, 1640 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 5.39 (m, 4H, -CH=CH-), 3.68 (m, 2H, -CH=O), 2.47 (m, 4H, α to oxadiazole ring), 2.27 (m, 2H, -CH=O), 2.04 (m, 8H, -CH₂-CH=CH-CH₂-), 1.57 (m, 4H, β to oxadiazole ring), 1.37 (br.s, 36H, chain CH₂), 0.88 (6H, dist. t, CH₃).

**¹³C NMR** (100 MHz, CDCl₃): 174.65, 130.56, 71.67, 37.06 34.36, 31.63, 28.82, 25.48, 23.44, 14.58.

**MS** (m/z, %): (M+1)⁺ 559 (13), (M⁺) 558 (40), 529 (28), 487 (100), 461 (56), 449 (37), 389 (13), 375 (10), 333 (61), 97 (82).

**Anal. Calcd.** for C₃₆H₆₆N₂O₂: C, 77.41; H, 11.82; N, 5.01. Found: C, 77.44; H, 11.76; N, 5.12%.

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3.5 References


CHAPTER-4
3,5-Disubstituted-
1,2,4-oxadiazoles
4.1 Theoretical

Nitrogen-oxygen heterocycles are of synthetic interest because they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities\(^1\). 3,5-Functionalized-1,2,4-oxadiazoles have received considerable attention in the pharmaceutical industry as heterocyclic amide and ester biosteres\(^2\). The oxadiazole nucleus is well studied pharmacophoric scaffold that has emerged as a core structural unit of various muscarinic agonists\(^3\), benzodiazepine receptor partial agonists\(^4\), dopamine transporters\(^5\), tyrosine kinase inhibitor\(^6\), a growth hormone secretagogue\(^7\) and antiinflammatory agents\(^8\).

The widespread interest in 1,2,4-oxadiazoles containing structures has prompted extensive studies for their synthesis. Irrespective of the steric and electronic nature of acylating agents (chloroanhydrides and anhydrides of carboxylic acids), the primary products of the acylation of amidoximes in equimolar mixtures of reactants are the O-acylated amidoximes\(^9\)–\(^16\).

The acylation of amidoximes of (2-oxopyrrolidino)-alkane acids (i) in dioxane leads to O-alkoylamidoximes (ii) which on subsequent thermolysis leads to 1,2,4-oxadiazole (iii)\(^9\) (Scheme 4.1).

\[\text{Scheme 4.1}\]

Hikmet and Dogan\(^17\) reported that the reaction of acetamidoxime (iv) with monochloroacetic acid chloroanhydride on boiling in chloroform led to the formation
of 1,2,4-oxadiazole (v) and 3-methyl-4H-1,2,4-oxadiazin-5(6H)-one (vi) (Scheme 4.2).

\[
\begin{align*}
\text{(iv)} & \quad \text{H}_3\text{C}-\text{NOH} \quad \text{CH}_{\text{Cl}}\text{COCl} \quad \text{CH}_3 \quad \text{H}_3\text{C}-\text{N=CCH}_3\text{NH}_2 \\
\text{(v)} & \quad \text{CH}_2\text{ON}=\text{CCH}_3\text{NH}_2 \\
\text{(vi)} & \quad \text{H}_3\text{C}-\text{N} \quad \text{O}
\end{align*}
\]

\text{Scheme 4.2}

It was also reported that the interaction of a mixture of E/Z isomers of NCC(=NOH)NHAr with BrCOCH₂Br in CH₂Cl₂ at 20°C in the presence of Et₃N led to the formation of 3-cyano-4-Ar-1,2,4-oxadiazin-5(4H)-ones with 10–84% yields¹⁸, while the reactions between N-para-tolylamidoximes of substituted benzoic acids (vii) with chloroacetyl chloride led to 3-aryl-4-(para-tolyl)-1,2,4-oxadiazin-5(4H)-ones (viii)¹⁹ (Scheme 4.3).

\[
\begin{align*}
\text{(vii)} & \quad \text{XC}_6\text{H}_4\text{NOH} \quad \text{ClCH}_2\text{COCl} \quad \text{p-CH}_3\text{H}_6\text{C}_4 \\
\text{(viii)} & \quad \text{XC}_6\text{H}_4\text{NHC}_6\text{H}_4\text{p-CH}_3
\end{align*}
\]

\text{Scheme 4.3}

According to Goddard²⁰, regioisomeric 5-thienyl-1,2,4-oxadiazoles (xi) were synthesized using the following Scheme 4.4.

\[
\begin{align*}
\text{(ix)} & \quad \text{H}_3\text{COOC} \quad \text{NH}_2 \\
\text{(x)} & \quad \text{H}_3\text{COOC} \quad \text{N=CCH}_3\text{NH}_2 \\
\text{(xi)} & \quad \text{H}_3\text{C}-\text{N} \quad \text{O}
\end{align*}
\]

\text{Scheme 4.4}
Potential muscarinic receptor agonists — quinuclidines (xiii) containing 1,2,4-oxadiazole rings — were synthesized in single-stage reactions between methylquinuclidine-3-carboxylate (xii) and the corresponding amidoxime or hydroxyguanidine. The reactions were carried out on boiling in THF in the presence of sodium hydride or on boiling in ethanol in the presence of dispersed molecular sieves\(^1\) (Scheme 4.5).

![Scheme 4.5](image)

Methyloxadiazole (xv) was obtained with a 65% yield by treating the muscarine agonist arecoline (xiv) with acetamidoxime sodium salt (Scheme 4.6).

![Scheme 4.6](image)

The same method was used for obtaining methyl (xvii) and amino (xviii) derivatives in the quinuclidine series\(^2\) (Scheme 4.7).

![Scheme 4.7](image)
The ability of the 1,2,4-oxadiazole ring to play the role of a bioisosteric ester moiety in the development of stable muscarinic receptor agonists (possessing activity with respect to the central nervous system) stimulated the synthesis of a series of 5-[(3-alkyl)-1,2,4-oxadiazol-5-yl]-1,4,5,6-tetrahydropyrimidines (xx). The removal of the trityl protection in the initial esters (xix) in trifluoroacetic acid led to the formation of the corresponding trifluoroacetates (xx)

![Scheme 4.8](image)

The notion that 1,2,4-oxadiazoles are perfect bioisosteric moieties for the ester groups of well-known cocaine analogs involved in dopamine transport also inspired the synthesis of 3-β-(substituted phenyl)-2-β-(3-substituted-1,2,4-oxadiazole-5-yl) tropanes (xxii) (Scheme 4.9).

![Scheme 4.9](image)

A single-stage condensation of R₁C₆H₄I (xxiii) with NH₂CR₂=NOH in CO atmosphere in the presence of a catalyst [Cl₂Pd(PPh₃)₂, (Ph₃P)₄P, or Cl₂Pd(dppe)] and a base (Et₃N) in an appropriate solvent (DMF, N-methylpyrrolidone, or toluene) was used for the synthesis of substituted-1,2,4-oxadiazoles (xxiv) (Scheme 4.10).
Substituted 1,2,4-oxadiazoles (xxvii) were also obtained with good yields using a single-stage condensation of the corresponding amidoximes (xxv) with carboxylic acids (xxvi) in the presence of a peptide condensation agent. The process was conducted by heating the reaction mixture in diglyme for several hours at 100°C (Scheme 4.11).

The several groups of 1,2,4-oxadiazoles (xxix) were obtained via the interaction of amidoximes (xxviii) with nitriles in the presence of ZnCl₂ and HCl in butyl- and iso-butylacetate (Scheme 4.12).
The reaction of thiocarbamoylformamidoxime \( \text{xxx} \) with orthoformic ester at room temperature in the presence of a catalyst (boron trifluoride etherate) leads to the formation of 1,2,4-oxadiazole-3-carboxylic acid thioamide \( \text{xxxi} \) (Scheme 4.13).

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{NH}_2 \\
\text{S} & \quad \text{NOH} \\
\text{S} & \quad \text{NH}_2 \\
\text{(xxx)} & \quad \text{(xxxi)}
\end{align*}
\]

Scheme 4.13

A convenient method for the synthesis of five-membered heterocycles is offered by 1,3-dipolar cycloaddition. The formation of 1,2,4-oxadiazole systems in the course of cycloaddition of N-oxides to nitriles was studied. In particular, 1,2,4-oxadiazoles \( \text{xxxv} \) were obtained as cycloadducts formed as a result of the reaction of liberated N-oxide \( \text{xxxiii} \) with dipolarophilic nitrile \( \text{xxxiv} \) (Scheme 4.14).

\[
\begin{align*}
\text{(CH}_3\text{OCO)\text{O}_2CHNO}_2 & \quad \Delta \\
\text{[CH}_3\text{OCO-C=N-O]} & \quad \text{+ RCN} \\
\text{(xxxii)} & \quad \text{(xxxiii)} & \quad \text{(xxxiv)} & \quad \text{(xxxv)}
\end{align*}
\]

Scheme 4.14

Oxadiazoles \( \text{xxxviii} \) were obtained via the reaction of benzonitrile N-oxide \( \text{xxxvi} \) with aromatic nitriles \( \text{xxxvii} \) (Scheme 4.15).

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{Y} & \quad \text{Y} \\
\text{X} & \quad \text{X} \\
\text{(xxxvi)} & \quad \text{(xxxvii)} & \quad \text{(xxxviii)}
\end{align*}
\]

(\( X = 2, 3, 4-\text{OH}, 2-\text{OCH}_3; \ Y = \text{H}, 5-\text{OCH}_3, 5-\text{NO}_2 \))

Scheme 4.15
The interaction of ethynylnitrochlorocyanooacetate (xxxix) with N-oxides of 4-, 3-, and 2-methoxybenzonitriles (xl) leads to the formation of 3-aryl-5-nitrochloroethoxycarbonylmethyl-1,2,4-oxadiazoles (xli)\(^\text{31}\) (Scheme 4.16).

![Scheme 4.16](image)

Itoh \textit{et al.}\(^\text{32}\) have synthesized the 3-benzoyl and 3-acetyl-1,2,4-oxadiazole derivatives (xliv) by the reaction of nitriles (xlii) with iron (III) nitrate at 80°C in benzophenone and acetophenone respectively (Scheme 4.17).

![Scheme 4.17](image)
4.2 Efficient, One-pot Synthesis of Novel 3,5-Disubstituted-1,2,4-oxadiazoles from Long-Chain Carboxylic Acid Derivatives Under Conventional and Microwave Conditions

Among oxadiazoles, 1,2,4-oxadiazoles derivatives have gained importance in medicinal chemistry. In the literature, 1,2,4-oxadiazoles have shown affinities for serotonin and norepinephrine transporters. 1,2,4-Oxadiazole ring system has also been used as a urea bioisostere in β3 adrenergic receptor agonists, as biosteres of ester and amide and as antitumor agents. Furthermore, derivatives containing 1,2,4-oxadiazoles ring systems have been employed as serotoninergic (5-HT3) anti-inflammatory agents, antitumor agents, monoamine oxidase inhibitors, coronary artery dilaters, anesthetic agents, muscle relaxants, antischistosomal agents, aldose reductase inhibitors and histamine H3 antagonists.

Knowing the pharmacological importance of the oxadiazole ring systems and in continuation of study on the derivatization of fatty acids in author’s laboratory, this chapter focused on the synthesis of this nucleus bearing long-alkyl and alkenyl chains at C-5. It describes the facile synthesis of 1,2,4-oxadiazoles by conventional heating and by application of microwave energy in solvent-free conditions. The interest in one pot conversion of carboxylic acid derivatives and amidoximes to 3,5-disubstituted-1,2,4-oxadiazoles prompted to utilize derivatives of carboxylic acid-esters and acid chlorides for the synthesis. To the best of my awareness this contribution reports for the first time a simple and straightforward synthesis of 1,2,4-oxadiazoles under microwave condition having long hydrocarbon chain attached at C-5.

The methods reported in chapter tolerates variety of functional groups on both the reaction partners and presents a straightforward procedures for the efficient and facile synthesis of 3,5-disubstituted-1,2,4-oxadiazoles in moderate to excellent yields. The reactions described in this chapter have a remarkable synthetic utility and are valuable addition to the synthesis and manipulation of fatty acid derivatives because
they make compounds available in one step from acid derivatives with or without microwave irradiation.

4.3 Results and discussion

The following methods of 1,2,4-oxadiazole synthesis shows that there are broad possibilities for the synthesis of new compounds offering highly effective agents bioisosteric with ester and amide groups. Modern trends in the synthesis of new 1,2,4-oxadiazoles are the search for highly effective pathways of room-temperature synthesis (excluding undesired high-temperature rearrangements) and the use of effective catalysts and microwave-stimulated processes.

At first the investigation of one-pot synthesis of 3,5-disubstituted-1,2,4-oxadiazoles from easily synthesized fatty esters 1a-d and amidoximes 2a-e was carried out. To the solution of fatty ester in toluene was added amidoxime followed by addition of K$_2$CO$_3$ and allowed to reflux for 9-13 hours (Scheme 4.18). The one-pot procedure provided 3a-t in 50-58% yield (Table 4.1). The need for higher yield of products prompted to employ more reactive acid chloride 1'a-d (Scheme 4.19). The 1'a-d was added dropwise to a stirred solution of the amidoxime 2a-e in pyridine and reaction was refluxed for 1-2.5 hours. In most of the cases the yield of product was 60-62% (Table 4.1).

![Scheme 4.18](image)

Scheme 4.18 Synthesis of 3,5-disubstituted-1,2,4-oxadizoles from long-chain esters and amidoximes
### Table 4.1 3,5-Disubstituted-1,2,4-oxadiazoles under conventional heating conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>(R)</th>
<th>Product</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Method 1</td>
<td>Method 2</td>
</tr>
<tr>
<td>1</td>
<td>H-H</td>
<td>CH₃</td>
<td>3a</td>
<td>10</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td>CH₃</td>
<td>3b</td>
<td>10.5</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td>CH₃</td>
<td>3c</td>
<td>10.5</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td>CH₃</td>
<td>3d</td>
<td>11</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-OH</td>
<td></td>
<td>3e</td>
<td>11</td>
<td>1.3</td>
</tr>
<tr>
<td>6</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td></td>
<td>3f</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td></td>
<td>3g</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;-</td>
<td></td>
<td>3h</td>
<td>11</td>
<td>2.3</td>
</tr>
<tr>
<td>9</td>
<td>Cl-CH₂</td>
<td></td>
<td>3i</td>
<td>10</td>
<td>2.4</td>
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<tr>
<td>10</td>
<td>Cl-CH₂</td>
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<td>3j</td>
<td>11</td>
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<td>11</td>
<td>Cl-CH₂</td>
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<td>12</td>
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<td>3l</td>
<td>12</td>
<td>2</td>
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<tr>
<td>13</td>
<td>-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td></td>
<td>3m</td>
<td>9</td>
<td>1.4</td>
</tr>
<tr>
<td>14</td>
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<td></td>
<td>3n</td>
<td>9.5</td>
<td>1.5</td>
</tr>
<tr>
<td>15</td>
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<td></td>
<td>3o</td>
<td>10</td>
<td>1.2</td>
</tr>
<tr>
<td>16</td>
<td>-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td></td>
<td>3p</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>4-OH-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td></td>
<td>3q</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>4-OH-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td></td>
<td>3r</td>
<td>13</td>
<td>2.5</td>
</tr>
<tr>
<td>19</td>
<td>4-OH-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td></td>
<td>3s</td>
<td>13</td>
<td>2.5</td>
</tr>
<tr>
<td>20</td>
<td>4-OH-C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td></td>
<td>3t</td>
<td>13</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Scheme 4.19 Synthesis of 3,5-disubstituted-1,2,4-oxadiazoles from long-chain acid chlorides and amidoximes

To explore the probability of getting the pharmacophoric important moiety in higher yields and in shorter reaction time, our attention has turned to employ 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 later years, microwave have become popular among synthetic organic chemists both to improve classical organic reactions, shortening reaction time and/or improving yields, as well as to promote new reactions. The esters 1a-d, acid chlorides 1'a-d and amidoximes 2a-e were supported on neutral alumina in same molar ratio as in conventional reaction method and subjected to microwave irradiation in open pyrex glassware for appropriate time (Scheme 4.20).

Scheme 4.20 Synthesis of 3,5-disubstituted-1,2,4-oxadiazoles under microwave irradiation

At first the condensation reaction of amidoxime (2a) with undec-10-enoic acid derivatives was chosen as a model to optimize the conditions for the preparation of compounds 3a-t (Table 4.2). In order to determine the optimum conditions for the synthesis of oxadiazoles, variations in molar ratios of reagents and the irradiation time and power level of microwave set-up were investigated.
Table 4.2 Optimization of the microwave-assisted condensation of 1a (a') and 2a on neutral alumina

![Reaction Scheme](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Support</th>
<th>molar ratios</th>
<th>Time (min)</th>
<th>Power (%)</th>
<th>Yield (%)</th>
<th>molar ratios</th>
<th>Time (min)</th>
<th>Power (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neutral alumina</td>
<td>0.5/1</td>
<td>5</td>
<td>20</td>
<td>30</td>
<td>0.5/0.8</td>
<td>5</td>
<td>10</td>
<td>30</td>
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<tr>
<td>2</td>
<td>Neutral alumina</td>
<td>0.8/1</td>
<td>7</td>
<td>30</td>
<td>40</td>
<td>0.8/1</td>
<td>6</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>Neutral alumina</td>
<td>1/1</td>
<td>9</td>
<td>40</td>
<td>48</td>
<td>1/1.1</td>
<td>7</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>Neutral alumina</td>
<td>1/1.1</td>
<td>11</td>
<td>50</td>
<td>55</td>
<td>1/1.2</td>
<td>8</td>
<td>40</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>Neutral alumina</td>
<td>1/1.2</td>
<td>13</td>
<td>60</td>
<td>78</td>
<td>1/1.4</td>
<td>9</td>
<td>40</td>
<td>92</td>
</tr>
</tbody>
</table>

After some experimentation, we found a set of conditions that generally provide products in good yield. The optimum conditions for molar ratio of fatty esters, fatty acid chlorides and amidoxime, irradiation time and power level of microwave set up are given in Table 4.3.
Table 4.3 Reaction of long chain carboxylic acid derivatives with amidoximes to synthesize 3,5-disubstituted-1,2,4-oxadiazole under microwave-assisted, solvent-free conditions.

<table>
<thead>
<tr>
<th>Product</th>
<th>Power&lt;sub&gt;a&lt;/sub&gt; (%)</th>
<th>Time&lt;sub&gt;c&lt;/sub&gt; (min)</th>
<th>Yield&lt;sub&gt;d&lt;/sub&gt; (%)</th>
<th>Power&lt;sub&gt;b&lt;/sub&gt; (%)</th>
<th>Time&lt;sub&gt;e&lt;/sub&gt; (min)</th>
<th>Yield&lt;sub&gt;d&lt;/sub&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>1/1.2</td>
<td>60</td>
<td>13</td>
<td>78</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3b</td>
<td>1/1.2</td>
<td>60</td>
<td>13</td>
<td>78</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3c</td>
<td>1/1.2</td>
<td>60</td>
<td>14</td>
<td>77</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3d</td>
<td>1/1.2</td>
<td>60</td>
<td>14</td>
<td>75</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3e</td>
<td>1/1.2</td>
<td>60</td>
<td>15</td>
<td>78</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3f</td>
<td>1/1.2</td>
<td>60</td>
<td>15</td>
<td>78</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3g</td>
<td>1/1.2</td>
<td>60</td>
<td>15</td>
<td>75</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3h</td>
<td>1/1.2</td>
<td>60</td>
<td>16</td>
<td>71</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3i</td>
<td>1/1.2</td>
<td>60</td>
<td>16</td>
<td>77</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3j</td>
<td>1/1.2</td>
<td>60</td>
<td>16</td>
<td>72</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3k</td>
<td>1/1.2</td>
<td>60</td>
<td>17</td>
<td>70</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3l</td>
<td>1/1.2</td>
<td>60</td>
<td>18</td>
<td>78</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3m</td>
<td>1/1.2</td>
<td>60</td>
<td>18</td>
<td>75</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3n</td>
<td>1/1.2</td>
<td>60</td>
<td>18</td>
<td>70</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3o</td>
<td>1/1.2</td>
<td>60</td>
<td>17</td>
<td>71</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3p</td>
<td>1/1.2</td>
<td>60</td>
<td>17</td>
<td>70</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3q</td>
<td>1/1.2</td>
<td>60</td>
<td>19</td>
<td>72</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3r</td>
<td>1/1.2</td>
<td>60</td>
<td>19</td>
<td>70</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3s</td>
<td>1/1.2</td>
<td>60</td>
<td>19</td>
<td>71</td>
<td>1/1.1</td>
<td>40</td>
</tr>
<tr>
<td>3t</td>
<td>1/1.2</td>
<td>60</td>
<td>19</td>
<td>71</td>
<td>1/1.1</td>
<td>40</td>
</tr>
</tbody>
</table>

<sup>a</sup> All reactions were carried out using esters (1a-d) (1eq), amidoxime (2a-e) (1.2eq) and K₂CO₃ under microwave irradiations.

<sup>b</sup> All reactions were carried out using acid chlorides (1'a-d) (1eq) and amidoxime (2a-e) (1.1eq) under microwave irradiations.

<sup>c</sup> Microwave equipment multimode was used. Full power means 100% and is 1.35 KW.

<sup>d</sup> Monitored by TLC.

<sup>e</sup> All yields refer to isolated products and the products were characterized by IR, ¹H NMR, MS and elemental analysis.

Multimode microwave irradiation at variable power was used. As per our requirement, the strategy was successfully worked out for both esters and acid chlorides. In both the cases the reaction proceeded in shorter reaction time and appreciable increment in product yield was obtained as compared to conventional reaction conditions. The maximum yield was obtained in case of acid chlorides in 7-9 minutes whereas esters were obtained in 70-78% in 13-19 minutes. The generality and scope of the synthetic procedures was demonstrated by subjecting aliphatic, vinyl and
aromatic amidoximes with olefinic (terminal and internal) and hydroxy olefinic carboxylic acid derivatives.

It was observed that when reaction was allowed to proceed for time longer than mentioned in Table 4.3 no further increase in yield of product was observed. The compounds were characterized by IR, $^1$H NMR, $^{13}$C NMR, Mass and elemental analysis. IR absorptions characteristic of C=N and C-O was observed in all the newly synthesized compounds. The characteristic signals for NCO and NCN in $^{13}$C NMR at 180.7-178.7 and 169.8-168.1 further strengthen the synthesis of oxadiazole moiety in 3a-t. Detailed spectral values of 3a-t are given in experimental section.
4.4 Experimental

The sources of all the fatty acids and instrumentation details are the same as given in Chapter 1 (page 38).

**General procedure for the synthesis of amidoximes (2a-e)**

Amidoximes 2a-e were prepared by heating under reflux 2-4 days a solution of the appropriate nitrile with, in 10-20% excess, equimolar amounts of hydroxylamine hydrochloride and sodium hydroxide in aqueous EtOH. The mixture was then concentrated under vacuum, diluted with cold water and left overnight. The solid that formed was recovered, washed with cold water, dried under vacuum and recrystallized with EtOH. \(2a\) mp 127-128 °C, \(2b\) mp 90-92 °C, \(2c\) mp 110-112 °C, \(2d\) mp 76-78 °C, \(2e\) mp 130-132 °C.

**General procedure for the synthesis of 3,5-disubstituted-1,2,4-oxadiazoles (3a-t) from long-chain esters 1a-d with amidoximes (2a-e) (Method 1)**

To the solution of ester 1a-d (3 mmol) in toluene (5 ml) was added amidoxime 2a-e (3.6 mmol) followed by \(K_2CO_3\) (3.3 mmol). The reaction mixture was stirred for suitable time (9-13 hours) under refluxed conditions and product formation is detected by TLC. The reaction mixture was cooled to room temperature, diluted with EtOAc (25 ml) and washed successively with brine (10 ml) and water. The organic phase was dried over anhydrous \(Na_2SO_4\), filtered and concentrated. The crude product was further purified by column chromatography. \((\text{Hexane:EtOAc, v:v})\) \(3a, (99:1)\); \(3b, (98:2)\); \(3c, (97:3)\); \(3d, (97:3)\); \(3e, (99:1)\); \(3f, (98:2)\); \(3g, (97:3)\); \(3h, (97:3)\); \(3i, (99:1)\); \(3j, (98:2)\); \(3k, (97:3)\); \(3l, (97:3)\); \(3m, (98:2)\); \(3n, (97:3)\); \(3o, (95:5)\); \(3p, (95:5)\); \(3q, (99:1)\); \(3r, (98:2)\); \(3s, (94:6)\); \(3t, (94:6)\).
General procedure for the synthesis of 3,5-disubstituted-1,2,4-oxadiazoles (3a-t) from long-chain acid chloride (1'a-d) with amidoximes (2a-e) (Method 2)

The acid chlorides 1'a-d were synthesized as reported earlier in Chapter 3 (page 67). An acid chloride 1'a-d (3 mmol) was added dropwise to a stirred solution of the amidoxime 2a-e (3.3 mmol) in pyridine. An exothermic reaction occurred after which in most cases, the solution was heated under reflux for 1-2.5 hour, after which the synthesis of product could be detected by TLC. The reaction mixture was cooled to room temperature, diluted with EtOAc (25 ml) and washed successively with brine (10 ml) and water. The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated. The crude product was further purified by column chromatography.

General procedure for the synthesis of 3,5-disubstituted-1,2,4-oxadiazoles (3a-t) under solvent-free microwave condition

The acid chloride 1'a-d (2mmol) and amidoxime 2a-e (2.2mmol) were mixed with neutral alumina in a 4:1 ratio. The mixture was shacked for 4-5 minutes. To have a complete mixture CH₂Cl₂ was added in a small quantity to solubilize the reagent and then solvent was evaporated. The reaction mixture was irradiated in microwave for required time (Table 4.3). After irradiation crude product was extracted in CH₂Cl₂ diluted (25 ml) and washed successively with brine (10 ml) and water. The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated and purified by silica gel chromatography. The same procedure was employed in case of ester derivative 1a-d (2 mmol) and amidoxime 2a-e (2.4 mmol) and K₂CO₃ (2.4 mmol).

Spectroscopic data

3-Methyl-5-(dec-9-enyl)-1,2,4-oxadiazole (3a)

Colorless oily liquid

IR (KBr): 2926, 2854, 1660, 1440 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 5.82 (tdd, 1H, Jₓ⁻ₓ⁻CHₓ = 6.6 Hz, JₓB = 10.2 Hz,
3,5-Disubstituted-1,2,4-oxadiazoles

$J_{xa} = 17.1$ Hz, $CH_2=CH_N$-, 5.02 (1H, dd, $J_{rx} = 10.2$ Hz, $J_{RA} = 1.2$ Hz, $H_B C=CH$),
4.90 (1H, dd, $J_{AX} = 17.1$ Hz, $J_{AB} = 1.2$ Hz, $H_A C=CH$), 2.35 (t, 2H, $J = 7.56$ Hz,
$CH_2 \alpha$ to ring), 2.20 (s, 3H, $CH_3$ at ring), 2.05 (m, 2H, $-CH_2-CH=CH_2$), 1.65 (m, 2H,
$CH_2 \beta$ to ring), 1.29 (br.s, 10H, chain $CH_2$).

$^1^C NMR$ (100 MHz, CDCl$_3$): 179.71, 168.72, 139.22, 114.26, 34.17, 33.82, 29.42,
28.64, 28.12, 24.53, 22.12, 15.02.

$MS$ (m/z, %): (M$^+$)$^+$ 223 (7), (M$^-$) 222 (25), 139 (43), 126 (32), 112 (61), 27 (15).

$Anal. Calcd.$ for C$_{13}$H$_{22}$N$_2$O: C, 70.24; H, 9.97; N, 12.59. Found: C, 70.85; H, 9.91;
N, 12.62.

3-Methyl-5-(heptadec-8-enyl)-1,2,4-oxadiazole (3b)

Colorless oily liquid

$IR$ (KBr): 2928, 2859, 1661, 1440 cm$^{-1}$.

$^1^H NMR$ (400 MHz, CDCl$_3$): 5 5.80 (m, 2H, $-CH=CH$), 2.34 (t, 2H, $J = 7.48$ Hz
$CH_2$, $\alpha$ to ring), 2.21 (s, 3H, $CH_3$ at ring), 2.04 (m, 4H, $-CH_2-CH=CH-CH_2$), 1.64 (m, 2H,
$CH_2$ $\beta$ to ring), 1.27 (br.s, 20H, chain $CH_2$), 0.88 (3H, dist. t, $CH_3$).

$^1^3^C NMR$ (100 MHz, CDCl$_3$): 180.62, 168.12, 130.13, 40.55, 34.91, 33.64, 29.75,
29.55, 29.32, 29.16, 27.12, 25.54, 24.82, 14.95.

$MS$ (m/z, %): (M$^+$)$^+$ 321 (18), (M$^-$) 320 (28), 237 (55), 139 (71), 113 (14), 86 (35).

$Anal. Calcd.$ for C$_{20}$H$_{36}$N$_2$O: C, 74.96; H, 11.31; N, 7.34. Found: C, 74.90; H, 11.37;
N, 7.39.

3-Methyl-5-[8Z, 11R)-11-hydroxyheptadec-8-enyl]-1,2,4-oxadiazole (3c)

Colorless oily liquid

$IR$ (KBr): 2930, 2853, 1664, 1443 cm$^{-1}$.

$^1^H NMR$ (400 MHz, CDCl$_3$): 5 5.82 (m, 2H, $-CH=CH$), 3.81 (m,1H, $-CH-OH$), 2.33
(t, 2H, $J = 7.51$ Hz, $CH_2$ $\alpha$ to ring), 2.19 (s, 3H, $CH_3$ at ring), 2.35 (m, 1H, CH-OH),
2.04 (m, 4H, $-CH_2-CH=CH-CH_2$), 1.65 (m, 2H, $CH_2$ $\beta$ to ring), 1.27 (br.s, 18H, chain
$CH_2$), 0.88 (3H, dist. t, $CH_3$).
3,5-Disubstituted-1,2,4-oxadiazoles

$^1$H NMR (100 MHz, CDCl$_3$): 180.25, 169.16, 130.52, 71.61, 37.16, 34.61, 33.82, 31.86, 31.47, 29.68, 29.22, 28.97, 27.16, 25.97, 15.06.

$^13$C NMR (100 MHz, CDCl$_3$): 180.51, 168.92, 131.53, 71.84, 37.85, 34.86, 33.51, 31.71, 30.63, 29.54, 28.92, 27.74, 25.41, 15.05.

MS (m/z, %): (M+1)$^+$ 337 (10), (M$^+$) 336 (15), 251 (30), 251 (54), 207 (25), 183 (19).

Anal. Calcd. for C$_{20}$H$_{36}$N$_2$O$_2$: C, 71.39; H, 10.77; N, 8.33. Found: C, 71.90; H, 10.71; N, 8.38.

3-Methyl-5-([8R, 11Z]-8-hydroxyheptadec-11-enyl)-1,2,4-oxadiazole (3d)
Colorless oily liquid

IR (KBr): 2926, 2850, 1665, 1445 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): δ 5.81 (m, 2H, -CH=CH-), 3.80 (m, 1H, -OH), 2.36 (t, 2H, J = 7.55 Hz, CH$_2$ α to ring), 2.21 (s, 3H, CH$_3$ at ring), 2.35 (m, 1H, CH-OH), 2.03 (m, 4H, -CH$_2$-CH=CH-CH$_2$), 1.64 (m, 2H, CH$_2$ β to ring), 1.29 (br.s, 18H, chain CH$_2$), 0.88 (3H, dist.t, CH$_3$).

$^13$C NMR (100 MHz, CDCl$_3$): 180.25, 169.16, 130.52, 71.61, 37.16, 34.61, 33.82, 31.86, 31.47, 29.68, 29.22, 28.97, 27.16, 25.97, 15.06.

MS (m/z, %): (M+1)$^+$ 337 (5), (M$^+$) 336 (30), 225 (54), 182 (43), 128 (37), 177 (45).

Anal. Calcd. for C$_{20}$H$_{36}$N$_2$O$_2$: C, 71.39; H, 10.77; N, 8.33. Found: C, 71.93; H, 10.82; N, 8.37.

3-Viny-5-(dec-9-enyl)-1,2,4-oxadiazole (3e)
Yellow oily liquid

IR (KBr): 2930, 2859, 1669, 1442 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): δ 6.24 (1H, dd, $J_{X'X''}$ = 17.8 Hz, $J_{B'A'}$ = 0.88 Hz, $H_A$C$_2'$ = C$_1$H$_X'$), 6.08 (1H, dd, $J_{X'B}$ = 11.7 Hz, $J_{B'A'}$ = 0.88 Hz, $H_B$C$_2''$ = C$_1$H$_X''$), 5.67 (1H, dd, $J_{X'A'}$ = 17.8 Hz, $J_{X'B'}$ = 11.7 Hz, $C_2''$H$_2''$ = C$_1''$H$_X''$), 5.82 (tdd, 1H, $J_{X''-CH_2}$ = 6.6 Hz, $J_{XB}$ = 10.2 Hz, $J_{XA}$ = 17.1 Hz, CH$_2$ = CH$_X$), 5.02 (1H, dd, $J_{BA}$ = 10.2 Hz, $J_{B'A}$ = 1.2 Hz, $H_B$C = CH), 4.90 (1H, dd, $J_{AX}$ = 17.1 Hz, $J_{AB}$ = 1.2 Hz, $H_A$C = CH$'$), 2.35 (t, 2H, $J$ = 7.44 Hz, CH$_2$ α to ring), 2.06 (m, 2H, -CH$_2$-CH = CH$_2$), 1.64 (m, 2H, CH$_2$ β to ring), 1.29 (br.s, 10H, chain CH$_2$).
3,5-Disubstituted-1,2,4-oxadiazoles

$^{13}C \text{ NMR}$ (100 MHz, CDCl$_3$): 179.81, 168.34, 135.20, 117.92, 139.62, 114.63, 34.76, 33.17, 29.29, 28.77, 28.15, 24.66, 22.51.

$MS$ (m/z, %): (M+1)$^+$ 235 (15), (M$^+$) 234 (70), 139 (10), 111 (40), 97 (23), 95 (32).

$\text{Anal. Calcd.}$ for C$_{14}$H$_{22}$N$_2$O: C, 71.44; H, 9.45; N, 11.95. Found: C, 71.93; H, 9.40; N, 11.99.

3- Vinyl -5-(heptadec-8-enyl)-1,2,4-oxadiazole (3f)

Colorless oily liquid

$IR$ (KBr): 2931, 2857, 1666, 1444 cm$^{-1}$.

$^1H \text{ NMR}$ (400 MHz, CDCl$_3$): $\delta$ 6.24 (1H, dd, $J_{X'A'} = 17.8$ Hz, $J_{B'A'} = 0.88$ Hz, $H_A' C_2 = C_1 H_X$), 6.09 (1H, dd, $J_{X'B} = 11.7$ Hz, $J_{B'A} = 0.88$ Hz, $H_B' C_2 = C_1 H_X$), 5.68 (1H, dd, $J_{X'A'} = 17.8$ Hz, $J_{X'B} = 11.7$ Hz, $C_2 H_2 = C_1 H_X$), 5.81 (m, 2H, $-CH=CH-$), 2.33 (t, 2H, $J = 7.56$ Hz, $CH_2 \alpha$ to ring), 2.04 (m, 4H, $-CH_2-CH=CH-CH_2$), 1.65 (m, 2H, $CH_2 \beta$ to ring), 1.30 (br.s, 20H, chain $CH_2$), 0.88 (3H, dist.t, $CH_3$).

$^{13}C \text{ NMR}$ (100 MHz, CDCl$_3$): 180.13, 169.35, 132.73, 118.56, 131.17, 33.83, 31.68, 29.93, 29.64, 29.21, 27.63, 25.74, 24.16, 14.91.

$MS$ (m/z, %): (M+1)$^+$ 333 (12), (M$^+$) 332 (66), 237 (10), 211 (60), 169 (56), 113 (19).

$\text{Anal. Calcd.}$ for C$_{21}$H$_{36}$N$_2$O: C, 75.86; H, 10.91; N, 8.42. Found: C, 75.30; H, 10.96; N, 8.38.

3- Vinyl -5-[(8Z, 11R)-11-hydroxyheptadec-8-enyl]-1,2,4-oxadiazole (3g)

Colorless oily liquid

$IR$ (KBr): 2933, 2850, 1663, 1449 cm$^{-1}$.

$^1H \text{ NMR}$ (400 MHz, CDCl$_3$): $\delta$ 6.24 (1H, dd, $J_{X'A'} = 17.8$ Hz, $J_{B'A'} = 0.88$ Hz, $H_A' C_2 = C_1 H_X$), 6.08 (1H, dd, $J_{X'B} = 11.7$ Hz, $J_{B'A} = 0.88$ Hz, $H_B' C_2 = C_1 H_X$), 5.67 (1H, dd, $J_{X'A'} = 17.8$ Hz, $J_{X'B} = 11.7$ Hz, $C_2 H_2 = C_1 H_X$), 5.82 (m, 2H, $-CH=CH-$), 3.82 (m, 1H, $-CH-OH$), 2.57 (t, 2H, $J = 7.51$ Hz, $CH_2 \alpha$ to ring), 2.32 (m, 1H, $CH-CH_2$), 2.04 (m, 4H, $-CH_2-CH=CH-CH_2$), 1.67 (m, 2H, $\beta$ to ring), 1.29 (br.s, 18H, chain $CH_2$), 0.88 (3H, dist.t, $CH_3$).
3,5-Disubstituted-1,2,4-oxadiazoles

$^{13}$C NMR (100 MHz, CDCl$_3$): 179.93, 168.14, 136.22, 118.15, 130.52, 71.51, 37.66, 34.17, 33.53, 31.82, 31.44, 29.66, 29.57, 28.38, 27.81, 25.23, 14.61.

MS (m/z, %): (M+1)$^+$ 349 (20), (M$^+$) 348 (32), 263 (62), 227 (30), 219 (77), 170 (15).

**Anal. Calcd.** for C$_{21}$H$_{36}$N$_2$O$_2$: C, 72.38; H, 10.40; N, 8.04. Found: C, 72.90; H, 10.44; N, 8.09.

3- Vinyl-5-[(8R,11Z)-8-hydroxyheptadec-11-enyl]-1,2,4-oxadiazole (3h)

Colorless oily liquid

IR (KBr): 2928, 2860, 1665, 1446 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 6.24 (1H, dd, $J_{X'A'}$ = 17.8 Hz, $J_{B'A'}$ = 0.88 Hz, $H_A C_2^r$ = C$_1$-H$_X$), 6.08 (1H, dd, $J_{X'A'}$ = 17.8 Hz, $J_{XB'}$ = 11.7 Hz, $C_2$-H$_2$ = C$_1$-H$_X^-$), 5.67 (1H, dd, $J_{X'A'}$ = 17.8 Hz, $J_{XB'}$ = 11.7 Hz, $C_2$-H$_2$ = C$_1$-H$_X^-$), 5.82 (m, 2H, -CH=CH$^-$), 3.81 (m, 1H, -CH$_2$OH), 2.36 (t, 2H, J = 7.56 Hz, CH$_2$ a to ring), 2.36 (m, 1H, CH=OH), 2.04 (m, 4H, -CH$_2$- CH=CH-CH$_2$), 1.66 (m, 2H, CH$_2$ b to ring), 1.30 (br.s, 18H, chain CH$_2$), 0.89 (3H, dist.t, CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 179.91, 168.16, 136.27, 118.18, 130.54, 71.52, 37.66, 34.15, 33.82, 31.85, 31.43, 29.68, 29.59, 28.33, 27.84, 25.22, 14.64.

MS (m/z, %): (M+1)$^+$ 349 (14), (M$^+$) 348 (54), 277 (30), 253 (44), 223 (25), 127 (17).

**Anal. Calcd.** for C$_{21}$H$_{36}$N$_2$O$_2$: C, 72.38; H, 10.40; N, 8.04. Found: C, 72.89; H, 10.35; N, 8.08.

3-Chloromethyl-5-(dec-9-enyl)-1,2,4-oxadiazole (3i)

Colorless oily liquid

IR (KBr): 2932, 2854, 1660, 1449 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.82 (tdd, 1H, $J_{X'-CH_3}$ = 6.6 Hz, $J_{XB}$ = 10.2 Hz, $J_{X'A}$ = 17.1 Hz, CH$_2$=CH$^-$), 5.02 (1H, dd, $J_{BX}$ = 10.2 Hz, $J_{BA}$ = 1.2 Hz, $H_B$=CH), 4.90 (1H, dd, $J_{AX}$ = 17.1 Hz, $J_{AB}$ = 1.2 Hz, $H_A$=CH), 4.13 (s, 2H, Cl-CH$_2$), 2.34 (t, 2H, $J$ = 7.23 Hz, CH$_2$ a to ring), 2.05 (m, 2H, -CH$_2$- CH=CH$_2$), 1.66 (m, 2H, CH$_2$ b to ring), 1.29 (br.s, 10H, chain CH$_2$).
$^{13}$C NMR (100 MHz, CDCl$_3$): 180.52, 168.15, 139.64, 114.42, 47.81, 35.73, 32.71, 29.53, 28.12, 25.13, 24.15, 22.41.

$^{13}$C NMR (100 MHz, CDCl$_3$): 180.43, 169.64, 31.74, 71.66, 47.82, 37.84, 34.56, 33.17, 31.65, 31.43, 29.62, 29.56, 28.24, 26.87, 24.29, 14.56.

3-Chloromethyl-5-(heptadec-8-enyl)-1,2,4-oxadiazole (3j)

Colorless oily liquid

IR (KBr): 2927, 2855, 1667, 1441 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.82 (m, 2H, -CH=CH-), 4.17 (s, 2H, Cl-CH$_2$), 2.35 (t, 2H, $J = 7.53$ Hz, CH$_2$ α to ring), 2.17 (m, 1H, CH-CH$_2$-CH$_3$), 2.04 (m, 4H, -CH$_2$-CH=CH$_2$-CH$_3$), 1.67 (m, 2H, CH$_2$ β to ring), 1.29 (br.s, 18H, chain CH$_2$), 0.88 (3H, dist.t, CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 180.51, 168.12, 131.91, 48.86, 34.73, 33.91, 31.55, 29.92, 29.74, 29.15, 27.67, 25.14, 24.12, 15.19.

$^{13}$C NMR (100 MHz, CDCl$_3$): 180.43, 169.64, 31.74, 71.66, 47.82, 37.84, 34.56, 33.17, 31.65, 31.43, 29.62, 29.56, 28.24, 26.87, 24.29, 14.56.

3-Chloromethyl-5-[(8Z, 11R)-11-hydroxyheptadec-8-enyl]-1,2,4-oxadiazole (3k)

Colorless oily liquid

IR (KBr): 2926, 2858, 1669, 1450 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.82 (m, 2H, -CH=CH-), 4.17 (s, 2H, Cl-CH$_2$), 3.82 (m, 1H, -CH-OH), 2.35 (t, 2H, $J = 7.53$ Hz, CH$_2$ α to ring), 2.17 (m, 1H, CH-OH), 2.04 (m, 4H, -CH$_2$-CH=CH$_2$-CH$_3$), 1.67 (m, 2H, CH$_2$ β to ring), 1.29 (br.s, 18H, chain CH$_2$), 0.88 (3H, dist.t, CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 180.43, 169.64, 31.74, 71.66, 47.82, 37.84, 34.56, 33.17, 31.65, 31.43, 29.62, 29.56, 28.24, 26.87, 24.29, 14.56.
3,5-Disubstituted-1,2,4-oxadiazoles

**MS** (m/z, %): (M+2)^+ 372 (12), (M+1)^+ 371 (22), 370 (36), 287 (10), 285 (30), 241 (10), 215 (20), 207 (28).

**Anal. Calcld.** for C_{20}H_{35}ClN_2O_2: C, 64.76; H, 9.50; N, 7.55. Found: C, 64.26; H, 9.54; N, 7.60.

**3-Chloromethyl-5-[(8R, 11Z)-8-hydroxyheptadec-11-enyl]-1,2,4-oxadiazole (3l)**
Colorless oily liquid

**IR** (KBr): 2930, 2857, 1661, 1450 cm\(^{-1}\).

**\(^1\)H NMR** (400 MHz, CDCl\(_3\)): \(\delta\) 5.82 (m, 2H, \(-\text{CH=CH-}\)), 4.14 (s, 2H, Cl-CH\(_2\)), 3.82 (m, 1H, \(-\text{CH-OH}\)), 2.32 (t, 2H, \(J = 7.52\) Hz, CH\(_2\) \(\alpha\) to ring), 2.15 (m, 1H, CH-OH), 2.04 (m, 4H, \(-\text{CH}_2\text{-CH=CH-CH}_2\)), 1.66 (m, 2H, CH\(_2\) \(\beta\) to ring), 1.30 (br.s, 18H, chain CH\(_2\)), 0.89 (3H, dist.t, CH\(_3\)).

**\(^13\)C NMR** (100 MHz, CDCl\(_3\)): 180.76, 169.85, 130.71, 71.24, 47.86, 37.87, 34.86, 33.43, 31.74, 31.62, 29.51, 28.92, 28.23, 25.84, 24.20, 14.05.

**MS** (m/z, %): (M+2)^+ 372 (15), (M+1)^+ 371 (25), (M^+) 370 (45), 273 (50), 247 (6), 245 (18), 301 (11), 299 (33).

**Anal. Calcld.** for C_{20}H_{35}ClN_2O_2: C, 64.76; H, 9.50; N, 7.55. Found: C, 64.30.; H, 9.47; N, 7.59.

**3-Phenyl-5-(dec-9-enyl)-1,2,4-oxadiazole (3m)**
Colorless oily liquid

**IR** (KBr): 2926, 2855, 1660, 1443 cm\(^{-1}\).

**\(^1\)H NMR** (400 MHz, CDCl\(_3\)): \(\delta\) 7.68 (m, 2H, Ar-\(\text{H}\)), 7.59 (m, 1H, Ar-\(\text{H}\)), 6.90 (m, 2H, Ar-\(\text{H}\)), 5.82 (tdd, 1H, \(J_{\text{X}-\text{CH}} = 6.6\) Hz, \(J_{\text{XB}} = 10.2\) Hz, \(J_{\text{XA}} = 17.2\) Hz, CH\(_2\)=CH\(_X\)-), 5.02 (1H, dd, \(J_{\text{RX}} = 10.2\) Hz, \(J_{\text{RD}} = 1.2\) Hz, H\(_9\)C=CH\(-\)), 4.90 (1H, dd, \(J_{\text{AX}} = 17.1\) Hz, \(J_{\text{AB}} = 1.2\) Hz, H\(_9\)C=CH\(-\)), 2.33 (t, 2H, \(J = 7.23\) Hz, CH\(_2\) \(\alpha\) to ring), 2.05 (m, 2H, -CH\(_2\)-CH=CH\(_2\)), 1.61 (m, 2H, CH\(_2\) \(\beta\) to ring), 1.29 (br.s, 10H, chain CH\(_2\)).

**\(^13\)C NMR** (100 MHz, CDCl\(_3\)): 179.51, 168.32, 139.25, 129.96, 129.17, 128.68, 127.92, 114.27, 34.22, 33.86, 29.87, 29.18, 28.74, 28.23, 24.52, 22.41.
3,5-Disubstituted-1,2,4-oxadiazoles

**MS (m/z, %):** (M+1)$^+$ 285 (16), (M$^+$) 284 (54), 257 (25), 229 (70), 145 (58), 97 (45).

**Anal. Calcd.** for C$_{18}$H$_{24}$N$_2$O: C, 76.03; H, 8.49; N, 9.85. Found: C, 76.55; H, 8.44; N, 9.83.

3-Phenyl-5-(heptadec-8-enyl)-1,2,4-oxadiazole (3n)

Colorless oily liquid

**IR** (KBr): 2929, 2859, 1666, 1451 cm$^{-1}$.

$^1$H **NMR** (400 MHz, CDCl$_3$): $\delta$ 7.67 (m, 2H, Ar-H), 7.59 (m, 1H, Ar-H), 6.92 (m, 2H, Ar-H), 5.82 (m, 2H, -CH=CH-), 2.34 (t, 2H, $J = 7.61$ Hz, CH$_2$ α to ring), 2.04 (m, 4H, -CH$_2$-CH=CH-CH$_2$), 1.65 (m, 2H, CH$_2$ β to ring), 1.30 (br.s, 20H, chain CH$_2$), 0.88 (3H, dist.t, CH$_3$).

$^{13}$C **NMR** (100 MHz, CDCl$_3$): 179.91, 169.52, 131.93, 129.34, 128.95, 128.76, 128.17, 34.58, 31.91, 29.92, 25.83, 25.24, 24.75, 23.76, 14.77.

**MS (m/z, %):** (M+1)$^+$ 383 (9), (M$^+$) 382 (56), 311 (42), 269 (65), 243 (70), 223 (52).

**Anal. Calcd.** for C$_{25}$H$_{38}$N$_2$O: C, 78.49; H, 10.01; N, 7.32. Found: C, 78.01; H, 10.06; N, 7.36.

3-Phenyl-5-[(8Z,11R)-11-hydroxyheptadec-8-enyl]-1,2,4-oxadiazole (3o)

Colorless oily liquid

**IR** (KBr): 2933, 2854, 1665, 1446 cm$^{-1}$.

$^1$H **NMR** (400 MHz, CDCl$_3$): $\delta$ 7.66 (m, 2H, Ar-H), 7.57 (m, 1H, Ar-H), 6.93 (m, 2H, Ar-H), 5.82 (m, 2H, -CH=CH-), 3.82 (m, 1H, -CH=OH), 2.35 (t, 2H, $J = 7.54$ Hz, CH$_2$ α to ring), 2.19 (m, 1H, CH=OH), 2.04 (m, 4H, -CH$_2$-CH=CH-CH$_2$), 1.67 (m, 2H, CH$_2$ β to ring), 1.29 (br.s, 18H, chain CH$_2$), 0.88 (3H, dist.t, CH$_3$).

$^{13}$C **NMR** (100 MHz, CDCl$_3$): 180.61, 169.72, 132.63, 129.74, 129.55, 129.26, 128.97, 65.61, 37.09, 34.38, 33.67, 31.66, 28.95, 25.54, 23.43, 22.52, 14.51.

**MS (m/z, %):** (M+1)$^+$ 399 (8), (M$^+$) 398 (80), 313 (75), 269 (60), 155 (56), 130 (13).

**Anal. Calcd.** for C$_{25}$H$_{38}$N$_2$O$_2$: C, 75.34; H, 9.60; N, 7.03. Found: C, 75.80; H, 9.64; N, 7.06.
3-Pheny-5-((8R, 11Z)-8-hydroxyheptadec-11-enyl)-1,2,4-oxadiazole (3p)

Colorless oily liquid

**IR** (KBr): 2931, 2854, 1660, 1440 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): 8 7.67 (m, 2H, Ar-H), 7.57 (m, 1H, Ar-H), 6.95 (m, 2H, Ar-H), 5.82 (m, 2H, -CH=CH-), 3.82 (m, 1H, -CH-OH), 2.36 (t, 2H, J = 7.51 Hz, CH₂ α to ring), 2.17 (m, 1H, CH-OH), 2.04 (m, 4H, -CH₂-CH=CH-CH₂), 1.66 (m, 2H, CH₂ β to ring), 1.30 (br.s, 18H, chain CH₂), 0.89 (3H, dist.t, CH₃).

**¹³C NMR** (100 MHz, CDCl₃): 180.41, 168.32, 132.93, 129.84, 129.64, 128.76, 128.37, 71.21, 36.82, 34.63, 33.23, 31.62, 28.91, 25.51, 23.42, 22.52, 14.52.

**MS (m/z, %):** (M⁺) 399 (13), (M⁺) 398 (34), 327 (29), 273 (45), 256 (77), 130 (35).

**Anal. Calcd.** for C₂₅H₃₈N₂O₂: C, 75.34; H, 9.60; N, 7.03. Found: C, 75.82; H, 9.66; N, 7.00.

3-(4'-Hydroxyphenyl)-5-(dec-9-enyl)-1,2,4-oxadiazole (3q)

Colorless oily liquid

**IR** (KBr): 2926, 2854, 1660, 1440 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): 8 7.72 (m, 2H, Ar-H), 7.43 (m, 2H, Ar-H), 5.82 (tdd, 1H, Jₓ-x‴ₓ‴ₓ‴ = 6.6 Hz, Jₓ-B = 10.2 Hz, Jₓ-A = 17.1 Hz, CH₂=CH₂), 4.02 (1H, dd, Jₓ-B = 10.2 Hz, Jₓ-A = 1.2 Hz, HₓC=CH), 3.49 (m, 1H, Ar-OH), 2.33 (t, 2H, J = 7.34 Hz, CH₂ α to ring), 2.05 (m, 2H, -CH₂-CH=CH₂), 1.66 (m, 2H, CH₂ β to ring), 1.29 (br.s, 10H, chain CH₂).

**¹³C NMR** (100 MHz, CDCl₃): 178.73, 169.83, 160.21, 139.72, 116.42, 128.83, 114.55, 33.74, 32.12, 29.92, 28.71, 27.36, 23.53, 21.42.

**MS (m/z, %):** (M⁺) 301 (10), (M⁺) 300 (78), 272 (35), 259 (60), 217 (72), 175 (28).

**Anal. Calcd.** for C₁₈H₂₄N₂O₂: C, 71.98; H, 8.05; N, 9.32. Found: C, 71.50; H, 8.09; N, 9.37.
3-(4'-Hydroxyphenyl)-5-(heptadec-8-enyl)-1,2,4-oxadiazole (3r)

Colorless oily liquid

**IR** (KBr): 2930, 2854, 1668, 1447 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 7.73 (m, 2H, Ar-H), 7.52 (m, 2H, Ar-H), 5.82 (m, 2H, -CH=CH-), 2.36 (t, 2H, J = 7.63 Hz, CH₂ α to ring), 3.55 (m, 1H, Ar-OH), 2.04 (m, 4H, -CH₂-CH=CH₂-CH₂), 1.65 (m, 2H, CH₂ β to ring), 1.30 (br.s, 20H, chain CH₂), 0.88 (3H, dist.t, CH₃).

**¹³C NMR** (100 MHz, CDCl₃): 180.15, 169.21, 159.11, 131.56, 117.16, 129.17, 35.91, 33.25, 29.12, 28.63, 28.42, 27.82, 24.74, 23.13, 14.52.

**MS** (m/z, %): (M+1)^+ 399 (8), (M⁺) 398 (36), 369 (70), 285 (30), 217 (10), 161 (12).

**Anal. Calcd.** for C₂₅H₃₈N₂O₂: C, 75.34; H, 9.60; N, 7.03. Found: C, 75.85; H, 9.55; N, 7.08.

3-(4'-Hydroxyphenyl)-5-[(8Z, 11R)-11-hydroxyheptadec-8-enyl]-1,2,4-oxadiazole (3s)

Colorless oily liquid

**IR** (KBr): 2932, 2860, 1666, 1450 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 7.75 (m, 2H, Ar-H), 7.55 (m, 2H, Ar-H), 5.82 (m, 2H, -CH=CH-), 3.82 (m, 1H, -CH-OH), 3.50 (m, 1H, Ar-OH), 2.36 (t, 2H, J = 7.53 Hz, CH₂ α to ring), 2.15 (m, 1H, CH-OH), 2.04 (m, 4H, -CH₂-CH=CH₂-CH₂), 1.67 (m, 2H, CH₂ β to ring), 1.29 (br.s, 18H, chain CH₂), 0.88 (3H, dist.t, CH₃).

**¹³C NMR** (100 MHz, CDCl₃): 179.72, 168.82, 159.83, 131.84, 116.97, 128.17, 71.28, 37.71, 34.56, 33.71, 31.62, 28.91, 25.51, 23.42, 22.55, 14.51.

**MS** (m/z, %): (M+1)^+ 415 (15), (M⁺) 414 (18), 399 (67), 329 (80), 285 (33), 213 (43).

**Anal. Calcd.** for C₂₅H₃₈N₂O₃: C, 72.44; H, 9.23; N, 6.75. Found: C, 72.95; H, 9.28; N, 6.79.
3,5-Disubstituted-1,2,4-oxadiazoles

3-(4'-Hydroxyphenyl)-5-[(8R, 11Z)-8-hydroxyheptadec-11-enyl]-1,2,4-oxadiazole

(3t)

Colorless oily liquid

IR (KBr): 2933, 2858, 1670, 1448 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): 7.76 (m, 2H, Ar-H), 7.54 (m, 2H, Ar-H), 5.82 (m, 2H, -CH=CH-), 3.82 (m, 1H, -OH), 3.48 (m, 1H, Ar-OH), 2.36 (t, 2H, J = 7.52 Hz, α to ring), 2.16 (m, 1H, CH-OH), 2.04 (m, 4H, -CH₂-CH=CH₂-CH₂), 1.65 (m, 2H, CH₂ β to ring), 1.30 (br.s, 18H, chain CH₂), 0.89 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 178.7, 168.9, 159.8, 131.6, 116.7, 129.9, 65.9, 37.5, 33.9, 32.7, 31.6, 29.9, 27.5, 26.4, 23.4, 22.5, 14.7.

MS (m/z, %): (M+1)⁺ 415 (14), (M⁺) 414 (22), 371 (65), 357 (40), 303 (57), 217 (20).

Anal. Calcd. for C₂₅H₃₈N₂O₃: C, 72.44; H, 9.23; N, 6.75. Found: C, 72.01; H, 9.29; N, 6.70.
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CHAPTER-5

2-Substituted-benzimidazoles,
tetrahydrobenzimidazoles
and imidazoles
5.1 Theoretical

The incorporation of the imidazole and benzimidazole nuclei is an important synthetic strategy in drug discovery\(^1\). The high therapeutic properties of the related drugs have encouraged the medicinal chemists to synthesize the large number of novel chemotherapeutic agents. Imidazole and benzimidazole drugs have broaden scope in remedying various dispositions in clinical medicine\(^2\). Optimization of benzimidazole based structures has resulted in marketed medicines such as Omeprazole\(^3\) and Pimobendan\(^4\) and lead compounds in a wide range of therapeutic areas (e.g. casein kinase \(2^5\), factor Xa\(^6\), hepatitis C virus\(^7\)). Pharmaceutical properties including; antifungal and antimycotic\(^8\), antiprotozoal and trichomonas infection\(^9\), antineoplastic\(^10\), antiulcer \(^11\), antihistaminic, antiallergic\(^12\), anesthetic, hypnotic\(^13\), antihypertensive\(^14\), anthelmintic\(^15\), neuroleptic, antipsychotic\(^16\) and thromboxane synthetase inhibitor\(^17\), all are unique characteristics known from imidazole and benzimidazole derivatives.

Benzimidazole is a versatile core contained in several substances possessing a broad spectrum of pharmacological activity such as anticancer, antimicrobial, pesticide, and anthelmintic properties\(^18\). This class of molecules has also found commercial applications in several therapeutic areas such as antiulcerative, antiviral, and antihistaminic agents\(^19\). Benzimidazoles exhibit significant activity against several viruses including HIV\(^20\), herpes (HSV-1)\(^21\), RNA\(^22\), influenza\(^23\) and human cytomegalovirus (HCMV)\(^20a\). In recent years benzimidazoles have been reported to act as topoisomerase I inhibitors\(^24\), selective neuropeptide YY1 receptor antagonists\(^25\), angiotensin II (All) inhibitors\(^26\), inhibition of HCMV replication\(^20a\), HT3 antagonists in isolated guinea pig ileum\(^27\). Hence, the development of new synthetic methods for benzimidazoles, which are currently not easily attainable by existing methods, is considered important by organic chemists.

Several biologically active synthetic compounds possess five-membered nitrogen-containing heterocycles in their structures\(^28\). The imidazole core is a
common moiety in a large number of natural products and pharmacologically active compounds\textsuperscript{29}. Recently, there has been considerable amount of progress in imidazole chemistry due to the recognition of the importance of the imidazole structure in biological processes and the increasing application of imidazole-containing compounds, such as etomidate, cimetidine, omeprazole and lansoprazole, in drug therapy\textsuperscript{30}. The imidazole ring system is a key structural fragment found in many natural products\textsuperscript{31}. It also serves as a good ligand for various metal ions. Metal binding properties of imidazole-based ligands have been explored in detail because of their presence at the active site of metallo-proteins or enzymes involved in several important metabolic processes\textsuperscript{32}.

N,N-Dimethylchlorosulfitemethaniminium chloride has been found to be an efficient reagent for the one-pot synthesis of benzimidazoles (iii) in excellent yield by condensation of carboxylic acids (i) with o-phenylenediamine (ii)\textsuperscript{33} (Scheme 5.1).

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\textbf{Scheme 5.1}};
\end{tikzpicture}
\end{center}

Vlaskina and Perevalov\textsuperscript{34} have synthesized substituted benzimidazoles (vi) by reduction of esters of 4-alkylamino-3,5-dinitrobenzoic acids (v) by tin chloride (Scheme 5.2).

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\textbf{Scheme 5.2}};
\end{tikzpicture}
\end{center}
In an effort to find an orally bioavailable antiviral for the treatment of rhino/enteroviral infections, a series of vinylacetylene benzimidazoles (viii) were made by Tebbe et al.\textsuperscript{35} (Scheme 5.3).

![Scheme 5.3](image)

Several 2'-aryl-5-substituted-2,5'-bi-1\textsubscript{H}-benzimidazole derivatives were synthesized\textsuperscript{36} and evaluated as topoisomerase I poisons and for their cytotoxicity toward the human lymphoblast cell line RPMI 8402 (Scheme 5.4).

![Scheme 5.4](image)

The preparation and anthelmintic properties of methyl 5(6)-phenylsulfinyl-2-benzimidazolecarbamate (xii) were described by Averkin et al.\textsuperscript{37}

![xii](image)

R= PhS-O, PhS-, PhS-O\textsubscript{2}

Walker et al.\textsuperscript{38} have synthesized the 1-[4-(4-chlorophenyl)-2-(2,6-dichlorophenylthio) -n -butyl]-1-\textsubscript{H}-imidazole nitrate (xiii), as potent antifungal agent.
2-(N-Substituted)-aminobenzimidazoles are widely used structural motifs in medicinal chemistry as well as in drug discovery and can be found in a number of biologically active molecules. Several compounds from this class have been used as anticancer, antihistamine and antiviral agents. Some examples of pharmaceutical interest are given below.

Norastemizole : $R = H$

Mebendazole (anticancer)

Astemizole : $R = \text{antiallergic, antihistamine}$

Das et al. have reported the highly efficient synthesis of 2-aminobenzimidazoles using various diamines and substituted dithiocarbamates. The reaction is promoted by dithiocarbamate and catalytic amount of CuO (Scheme 5.5).
2-Substituted-benzimidazoles, tetrahydrobenzimidazoles and imidazoles

Scheme 5.5

Antibacterial\textsuperscript{42-44}, anti-inflammatory\textsuperscript{45}, antiulcer\textsuperscript{46,47} and antiviral\textsuperscript{48,49} effects have been shown with various thiazolo-[3,2-a]-benzimidazole derivatives. Furthermore, certain thiazolo-[3,2-a]-benzimidazole derivatives, such as tilomisole (WY-18,251) (xix) was largely studied\textsuperscript{50,51} demonstrating their anti-inflammatory\textsuperscript{52} and immunomodulatory\textsuperscript{53} activities.

Also, some thiazolo-[3,2-a]-benzimidazole derivatives were used for treatment of cancer\textsuperscript{54}, cerebral infarction\textsuperscript{55}, neurogenic pain\textsuperscript{56}, and bone diseases\textsuperscript{57}.

A series of 2-(substituted phenyl)-1H-benzimidazole (xxii) derivatives with various substituents were synthesized via microwave irradiation using a short synthetic route and Na\textsubscript{2}S\textsubscript{2}O\textsubscript{5} as oxidant\textsuperscript{58} (Scheme 5.6).

\begin{align*}
\text{(xx)} & \quad \text{(xxi)} & \quad \text{(xxii)} \\
R_1 = \text{H, CH}_3; & \quad CF_3, & \quad \text{R}_4 = \text{H, OCH}_3, \text{NO}_2, \text{OCH}_2\text{CH}_3; & \quad R_5 = \text{H, OCH}_3, \text{OH, OCH}_3, \text{N(CH}_3)_2; & \quad R_5 = \text{H, OCH}_3
\end{align*}

Scheme 5.6
The interaction of 2-acetyl-(aroyl)-methyl-1H-benzimidazoles (xxiv) with phenylhydrazine on heating in trifluoroacetic acid proceeds by a type of Fischer reaction with the formation of 2-[2-methyl(aryl)-3-indolyl]-1H-benzimidazoles (xxv) (Scheme 5.7).

\[ \text{CH}_2\text{COR} \xrightarrow{\text{NH}_2\text{NPh, CF}_3\text{COOH, NH}_4\text{OH}} \text{Scheme 5.7} \]

Dzvinchuk et al.\(^{60}\) developed a convenient preparative method for synthesis of 2-(1,2,3-thiadiazol-5-yl)-1H-benzimidazoles (xxvi) based on reaction of benzoylhydrazones of 2-acylmethyl-1H-benzimidazoles (xxv) with thionyl chloride (Scheme 5.8).

\[ \text{ Scheme 5.8 } \]

Some chloroaryloxoyalkyl imidazole and benzimidazole derivatives were synthesized\(^{61}\). The relevant step in the synthetic sequence was the initial condensation of 4-chloro or 2,4-dichlorophenol with 1, n-dibromoalkanes (n = 2, 4, 5) (xxvii) to provide (xxviii) in sufficient yields. The subsequent condensation of (xxviii) with some imidazole derivatives and benzimidazole afforded products (xxix) and (xxx) in good yields (Scheme 5.9).
2-Substituted-benzimidazoles, tetrahydrobenzimidazoles and imidazoles

A simple, high-yielding synthesis of 2,4,5-trisubstituted imidazoles (xxxiii) from 1,2-diketones (xxxi) and aldehydes (xxxii) in the presence of NH₄OAc under microwave irradiation in described⁶² (Scheme 5.10).

Frantz et al.⁶³ has described synthesis of substituted imidazoles (xxxvii) via organocatalysis (Scheme 5.11).

Synthesis of 1,5-disubstituted and 1,4,5-trisubstituted imidazoles (xxxix) from aldimines and imidoyl chlorides was given by van Leusen et al.⁶⁴ (Scheme 5.12).
2-Substituted-benzimidazoles, tetrahydrobenzimidazoles and imidazoles

Scheme 5.12

Iizuka et al.\(^{65}\) have reported the synthesis of imidazole derivatives (xlv) as highly selective inhibitors of thromboxane synthetase (Scheme 5.13).

Scheme 5.13

Synthesis of N-alkylated derivatives of imidazoles (xlvii) as antibacterial agents was reported by Khabnadideh et al.\(^{66}\) (Scheme 5.14).

Scheme 5.14

Van Vliet et al.\(^{67}\) have reported the synthesis of 2-substituted-benzimidazoles (xlix) from nitroanilines using microwave conditions (Scheme 5.15).
Lin et al.\textsuperscript{68} reported the high-throughput synthesis of benzimidazoles (li) derivatives under microwave conditions (Scheme 5.16).
5.2 Convenient One-pot synthesis of Novel 2-Substituted-benzimidazoles, Tetrahydrobenzimidazoles and Imidazoles and Evaluation of their in vitro Antibacterial and Antifungal Activities*

The benzimidazole has been an important pharmacophore and privileged structure in medicinal chemistry, encompassing a diverse range of biological activities including antiarrhythmic, antiulcer, anthelmintical, inotropic, antihistamine, antifungal, antiviral, and cytotoxicity displaying diverse range of biological activities. Benzimidazoles exhibit significant activity as potential antitumor agents, smooth muscle cell proliferation inhibitors, a treatment for intestinal cystitis, and in diverse area of chemistry. The imidazole core is a common moiety in a large number of natural products and pharmacologically active compounds. Recently, there has been considerable amount of progress in imidazole chemistry due to the recognition of importance of the imidazole structure in biological processes and the increasing application of imidazole containing compounds, such asetomidate, cimetidine, omeprazole and lansoprazole, in drug therapy. Therefore the development of facile synthetic routes to achieve access to these molecules is of prime interest. In view of the above mentioned pharmacological applications of benzimidazoles and imidazoles and in continuation of the synthesis of biologically active molecules, the design and synthesis of hitherto unknown benzimidazoles and imidazoles bearing long alkenyl chain was carried out.

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

5.3 Results and discussion

A typical one-pot procedure for the synthesis of \(4a-1\) involves the addition of 1,2-phenylenediamine derivatives \(2\) to the acid chloride \(1a-d\) (Scheme 5.17) at 0 °C in dry dioxane and stirring for 30 minutes at room temperature to furnish the corresponding N-acyl-1,2-phenylenediamine derivatives \(3\). To the prior, acid chloride \(1a-d\) was synthesized from olefinic and hydroxy olefinic long-chain acids by \textit{in situ} preparation.

\[
\begin{align*}
R & \quad \text{R,} = H \\
2a & \quad \text{R,} = \text{NO}_2 \\
2c & \quad \text{R,} = \text{Cl}
\end{align*}
\]

\[
\begin{align*}
1a-d & \quad 3a-d \\
2a & \quad 3'a-d \\
2b & \quad 3''a-d \\
2c & \quad 3''a-d
\end{align*}
\]

\textbf{Scheme 5.17 Synthesis of 2-substituted-benzimidazoles}

Since acid chlorides \(1a-d\) are not commercially available the present method has greatly solved the problem by facile and efficient \textit{in situ} preparation. We have used BF\(_3\)OEt\(_2\) for cyclization of \(3\). BF\(_3\)OEt\(_2\) in dry dioxane was added to the \(3\) without isolating the product and reaction mixture refluxed for 1-2 hours at 130 °C.
Table 5.1 2-substituted-benzimidazoles

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>R₂</th>
<th>Starting from</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>1a, 2a</td>
<td>4a</td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>H</td>
<td>1b, 2a</td>
<td>4b</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>H</td>
<td>1c, 2a</td>
<td>4c</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>H</td>
<td>1d, 2a</td>
<td>4d</td>
</tr>
<tr>
<td>5</td>
<td>NO₂</td>
<td>H</td>
<td>1a</td>
<td>2b</td>
</tr>
<tr>
<td>6</td>
<td>NO₂</td>
<td>H</td>
<td>1b</td>
<td>2b</td>
</tr>
<tr>
<td>7</td>
<td>NO₂</td>
<td>H</td>
<td>1c</td>
<td>2b</td>
</tr>
<tr>
<td>8</td>
<td>NO₂</td>
<td>H</td>
<td>1d</td>
<td>2b</td>
</tr>
<tr>
<td>9</td>
<td>Cl</td>
<td>H</td>
<td>1a</td>
<td>2c</td>
</tr>
<tr>
<td>10</td>
<td>Cl</td>
<td>H</td>
<td>1b</td>
<td>2c</td>
</tr>
<tr>
<td>11</td>
<td>Cl</td>
<td>H</td>
<td>1c</td>
<td>2c</td>
</tr>
<tr>
<td>12</td>
<td>Cl</td>
<td>H</td>
<td>1d</td>
<td>2c</td>
</tr>
</tbody>
</table>

The yields of products 4a-l are excellent and independent of the substituents present in the precursor. The scope of the reaction using olefinic (internal and terminal) and hydroxy acids is found to be good. This strategy was also be used to increase the structural diversity of the member library through the synthesis of benzimidazole 4m-p (Scheme 5.18).

Scheme 5.18 Synthesis of 2-substituted-imidazoles

Similarly 4q-t (Scheme 5.19) have been prepared by the reaction of ethylenediamine 7 to the acid chloride 1a-d at 0 °C in dry dioxane and stirring for 30
minutes at room temperature to furnish the corresponding 8a-d which undergo cyclization in presence of BF$_3$OEt$_2$ to form the 4q-t.

![Scheme 5.19 Synthesis of 2-substituted-tetrahydrobenzimidazoles](image)

The yield of products was found to be appreciable. The preliminary study of 4a, 4b, 4r has been reported earlier. The newly synthesized compounds were analyzed for C, H and N content and structures were confirmed by IR, $^1$H NMR, $^{13}$C NMR and mass spectral data. IR absorptions at 1630 cm$^{-1}$ (C=N) was obtained for 2-(dec-9-enyl)-1H-benzimidazole 4a. The $^1$H NMR showed signal of methine proton of C-9 at $\delta$ 5.82. C-10 methylene designated as H$_E$ and H$_Z$ displayed two distinct $\delta$ values when coupled with adjacent C-9 methine protons. The spectrum showed two doublets of doublet at $\delta$ 5.02 and 4.90 for H$_Z$ and H$_E$ protons respectively. The structure of 4a was further supported by its mass spectral studies, which showed molecular ion peak at m/z 256 consistent with its molecular formula C$_{17}$H$_{24}$N$_2$.

The antibacterial and antifungal screening revealed that all the tested compounds 4a-l, 4m-p, 4q-t showed moderate to good inhibition.
Table 5.1 *In vitro* antibacterial activity of compounds 4a-t

<table>
<thead>
<tr>
<th>Compound</th>
<th>E.coli</th>
<th><em>S</em>.typhimurium</th>
<th>B.subtilis</th>
<th><em>S</em>.aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>18(150)</td>
<td>16(75)</td>
<td>22(75)</td>
<td>20(150)</td>
</tr>
<tr>
<td>4b</td>
<td>16(150)</td>
<td>14(75)</td>
<td>21(75)</td>
<td>19(150)</td>
</tr>
<tr>
<td>4c</td>
<td>17(150)</td>
<td>12(75)</td>
<td>19(75)</td>
<td>19(150)</td>
</tr>
<tr>
<td>4d</td>
<td>17(150)</td>
<td>12(75)</td>
<td>19(75)</td>
<td>19(150)</td>
</tr>
<tr>
<td>4e</td>
<td>20(75)</td>
<td>12(37.5)</td>
<td>19(37.5)</td>
<td>22(150)</td>
</tr>
<tr>
<td>4f</td>
<td>20(75)</td>
<td>11(37.5)</td>
<td>20(37.5)</td>
<td>21(37.5)</td>
</tr>
<tr>
<td>4g</td>
<td>16(37.5)</td>
<td>12(37.5)</td>
<td>21(37.5)</td>
<td>19(37.5)</td>
</tr>
<tr>
<td>4h</td>
<td>18(37.5)</td>
<td>13(37.5)</td>
<td>22(37.5)</td>
<td>19(37.5)</td>
</tr>
<tr>
<td>4i</td>
<td>20(75)</td>
<td>14(37.5)</td>
<td>19(75)</td>
<td>22(37.5)</td>
</tr>
<tr>
<td>4j</td>
<td>18(75)</td>
<td>15(37.5)</td>
<td>18(75)</td>
<td>20(37.5)</td>
</tr>
<tr>
<td>4k</td>
<td>18(75)</td>
<td>17(37.5)</td>
<td>19(75)</td>
<td>21(37.5)</td>
</tr>
<tr>
<td>4l</td>
<td>19(37.5)</td>
<td>17(37.5)</td>
<td>20(75)</td>
<td>21(37.5)</td>
</tr>
<tr>
<td>4m</td>
<td>17(37.5)</td>
<td>11(75)</td>
<td>18(75)</td>
<td>18(75)</td>
</tr>
<tr>
<td>4n</td>
<td>17(150)</td>
<td>10(75)</td>
<td>19(75)</td>
<td>16(75)</td>
</tr>
<tr>
<td>4o</td>
<td>15(150)</td>
<td>12(75)</td>
<td>19(75)</td>
<td>17(75)</td>
</tr>
<tr>
<td>4p</td>
<td>16(150)</td>
<td>13(75)</td>
<td>23(75)</td>
<td>16(75)</td>
</tr>
<tr>
<td>4q</td>
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<td>14(74)</td>
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<td>16(150)</td>
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</tr>
<tr>
<td>4s</td>
<td>12(150)</td>
<td>12(75)</td>
<td>18(37.5)</td>
<td>15(150)</td>
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<tr>
<td>4t</td>
<td>12(150)</td>
<td>12(75)</td>
<td>19(37.5)</td>
<td>15(150)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25(12.5)</td>
<td>20(6)</td>
<td>24(6)</td>
<td>26(12.5)</td>
</tr>
<tr>
<td>Control DMSO</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Values in brackets are MIC values (μg/ml)

The antibacterial screening indicated that among the tested bacterial strains, good inhibitory results were obtained against *Salmonella typhimurium* and *Escherichia coli*. The structural activity study showed that benzimidazoles and their substituted derivatives 4a-l have varying degrees of microbial inhibition. The antibacterial and antifungal activity seemed to be dependent on the heterocyclic moiety as well as on the nature of substituents. Although the benzimidazoles 4a-l itself are observed active but the activity was further enhanced by the presence of nitro and chloro substituent on the benzimidazoles moiety (Table 5.1).

The nitro substituted derivatives 4e, 4f have shown good activity against *E. coli* and *S. aureus*. The maximum inhibition was observed in 4h against *B. subtilis*. The chloro substituted derivatives 4i-l have shown maximum inhibition against *S. aureus*. The tetrahydrobenzimidazoles 4m-p are moderately active against *S. typhimurium* and 4m, 4p are best active against *E. coli* and *B. subtilis* whereas the imidazoles 4q-t showed moderate activity results against test bacterial strains.
In another set of experiments, the above mentioned compounds were also examined for antifungal activity (Table 5.2). Nystatin was used as standard drug for the comparison of antifungal results. The nitro and chloro substituted compounds 4e-1 showed same trend in case of fungal strains.

Table 5.2 *In vitro antifungal activity of compounds 4a-t*

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>C. albicans</em></th>
<th><em>H. Oryzae</em></th>
<th><em>A. niger</em></th>
<th><em>T. viridae</em></th>
<th><em>Penicillium sp.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>19(75)</td>
<td>13(150)</td>
<td>14(150)</td>
<td>7(75)</td>
<td>18(75)</td>
</tr>
<tr>
<td>4b</td>
<td>19(75)</td>
<td>12(150)</td>
<td>16(150)</td>
<td>6(75)</td>
<td>18(75)</td>
</tr>
<tr>
<td>4c</td>
<td>19(75)</td>
<td>13(150)</td>
<td>16(150)</td>
<td>8(75)</td>
<td>19(75)</td>
</tr>
<tr>
<td>4d</td>
<td>18(75)</td>
<td>11(150)</td>
<td>14(150)</td>
<td>8(75)</td>
<td>17(75)</td>
</tr>
<tr>
<td>4e</td>
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<td>10(75)</td>
<td>15(37.5)</td>
<td>8(37.5)</td>
<td>18(37.5)</td>
</tr>
<tr>
<td>4f</td>
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<td>12(75)</td>
<td>15(37.5)</td>
<td>8(37.5)</td>
<td>18(37.5)</td>
</tr>
<tr>
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<td>15(75)</td>
<td>16(37.5)</td>
<td>9(37.5)</td>
<td>19(37.5)</td>
</tr>
<tr>
<td>4h</td>
<td>19(37.5)</td>
<td>14(75)</td>
<td>14(37.5)</td>
<td>9(37.5)</td>
<td>19(37.5)</td>
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<td>16(75)</td>
<td>15(37.5)</td>
<td>10(37.5)</td>
<td>19(37.5)</td>
</tr>
<tr>
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<td>14(37.5)</td>
<td>9(37.5)</td>
<td>19(37.5)</td>
</tr>
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<td>4l</td>
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<td>14(37.5)</td>
<td>8(37.5)</td>
<td>19(37.5)</td>
</tr>
<tr>
<td>4m</td>
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<td>11(150)</td>
<td>12(37.5)</td>
<td>7(37.5)</td>
<td>18(75)</td>
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<td>17(75)</td>
</tr>
<tr>
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<td>7(37.5)</td>
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<td>11(150)</td>
<td>12(37.5)</td>
<td>8(37.5)</td>
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</tr>
<tr>
<td>4q</td>
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<td>12(150)</td>
<td>11(150)</td>
<td>6(75)</td>
<td>16(150)</td>
</tr>
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<td>13(150)</td>
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<td>6(75)</td>
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<td>4t</td>
<td>18(75)</td>
<td>12(150)</td>
<td>14(150)</td>
<td>6(75)</td>
<td>19(150)</td>
</tr>
<tr>
<td>Nystatin</td>
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<td>18(12.5)</td>
<td>15(6)</td>
<td>20(6)</td>
</tr>
</tbody>
</table>

Control DMSO --- --- --- --- ---

Values in brackets are MIC values (µg/ml)

The excellent inhibition results were obtained against *C. albicans* and *Penicillium* sp. by 4e-h. The moderate activity was obtained in *H. oryzae* and *A. niger*. The inhibitory activity against the *T. viridae* was significantly lower than the other tested microorganisms. Hence we conclude that higher activity of compounds 4e-h and 4i-l may be attributed to the presence of nitro and chloro groups. The inhibition by 4a-d, 4m-p and 4q-t is due to the presence of benzimidazole, tetrahydrobenzimidazole and imidazole respectively. Thus the nature of substituents and basic skeleton of molecule have strong influence on the extent of antibacterial and
antifungal activities. Thus data revealed that all compounds have produced the marked enhancement in the potency of these analogues as antifungal and antifungal agents.
5.4 Experimental

The sources of all the fatty acids and instrumentation details are the same as given in Chapter 1 (page 38).

**General procedure for the synthesis of 2-substituted benzimidazoles, tetrahydrobenzimidazoles and imidazoles (4a-t)**

The acid chlorides 1a-d were prepared as reported earlier in Chapter 3 (page 67). Then, 1,2-phenylenediamine derivatives (0.01 mmol) 2a-c and 5 was added to the stirred solution of acid chloride at 0°C in dry dioxane and keep stirring for 30 minutes at room temperature to furnish the corresponding 3 and 6a-d respectively. Similarly 2-substituted imidazoles 4q-t was synthesized by utilizing cis cyclohexanediamine to furnish 8a-d. BF₃ OEt₂ (0.015 mol) in dry dioxane (10 ml) was added dropwise to the above stirred reaction mixture in 10 minutes and reaction mixture further refluxed for 1 to 2 hours at 130 °C. The resulting solution was concentrated in vacuum, saturated NH₄Cl solution added at till the pH becomes 6, extracted with EtOAc, dried over anhydrous Na₂SO₄ and concentrated in vacuum to give the crude product 4a-t, which was further purified by column chromatography (Hexane:EtOAc, v:v) 4a, (99:1); 4b, (98:2); 4c, (97:3); 4d, (97:3); 4e, (99:1); 4f, (98:2); 4g, (97:3); 4h, (97:3); 4i, (99:1); 4j, (98:2); 4k, (97:3); 4l, (97:3); 4m, (99:1); 4n, (98:2); 4o, (97:3); 4p, (97:3); 4q, (99:1); 4r, (98:2); 4s (94:6); 4t, (94:6).

**Antibacterial Activity**

The antibacterial activity of test compounds and standard chloramphenicol was done by filter paper disc method against *Staphylococcus aureus* MSSA 22, *Escherichia coli* K12, *Bacillus subtilis* ATCC 6501, *Salmonella typhimurium* MTCC 98 at a concentration of 100μg/ml. Media with DMSO was set up as control.
Antifungal activity

The standard agar disc diffusion method was performed to evaluate the antifungal property of the test compounds and standard nystatin. *Aspergillus niger* (lab isolate from ICAR, Jaipur), *Candida albicans* IOA-109, *Penicillium* sp (laboratory isolate), *Helminthosporum oryzae* (lab isolate from ICAR, Jaipur), *Trichoderma viridae* ATCC 5170 were used in this study. Solvent control DMSO was also run.

SPECTROSCOPIC DATA

2-(Dec-9-enyl)-1H-benzimidazole (4a)

Colorless oily liquid; yield: 89%.

**IR** (KBr): 3350, 1630, 1585 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 9.01 (s, 1H, N-H), 6.73-6.71 (m, 4H, Ar-H), 5.82 (tdd, 1H, $J_{CH_2} = 6.6$ Hz, $J_{H-H} = 10.2$ Hz, $J_{H-H} = 17.1$ Hz, CH₂=CH⁻), 5.02 (dd, 1H, $J_{H} = 10.2$ Hz, $J_{H} = 1.2$ Hz, CH⁻C=CH), 4.90 (dd, 1H, $J_{H} = 17.1$ Hz, $J_{H} = 1.2$ Hz, CH⁻C=CH⁻), 2.34 (t, 2H, $J = 7.12$ Hz, α to benzimidazole ring), 2.03 (m, 2H, -CH₂=CH=CH₂), 1.63 (m, 2H, β to benzimidazole ring), 1.30 (br.s, 10H, chain CH₂).

**¹³C NMR** (100 MHz, CDCl₃): 143.44, 139.01, 133.56, 121.45, 118.17, 114.14, 34.25, 33.77, 29.20, 28.88, 24.73.

**MS** (m/z, %): (M+1)⁺ 257 (56), M⁺ 256 (45), 229 (34), 145 (40), 117 (100).

**Anal. Calcd.** for C₁₇H₂₄N₂: C, 79.65; H, 9.43; N, 10.92. Found: C, 79.06; H, 9.49; N, 10.87%.

2-(Heptadec-8-enyl)-1H-benzimidazole (4b)

Pale yellow oily liquid; yield: 89%.

**IR** (KBr): 3360, 1640, 1590 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 9.12 (s, 1H, N-H), 6.73-6.70 (m, 4H, Ar-H), 5.38 (m, 2H, -CH=CH⁻), 2.35 (t, 2H, $J = 7.22$ Hz, α to benzimidazole ring), 2.02 (m, 4H, -
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CH₂-CH=CH-(CH₂)₂, 1.61 (m, 2H, β to benzimidazole ring), 1.30 (br.s, 18H, chain CH₂), 0.89 (3H, dist. t, CH₃).

\(^{13}C\) NMR (100 MHz, CDCl₃): 143.22, 133.66, 122.77, 118.41, 34.20, 31.81, 29.65, 29.49, 29.09, 27.05, 24.72, 22.65, 22.45, 14.92.

MS (m/z, %): (M+1)^+ 355 (40), M^+ 354 (34), 237 (25), 181 (25), 117 (100).

Anal. Calcd. for C₂₄H₃₈N₂: C, 81.31; H, 10.79; N, 7.90. Found: C, 81.88; H, 10.73; N, 7.94%.

2-[(8Z, 11R)-11-Hydroxyheptadec-8-enyl]-1H-benzimidazole (4c)

Yellow oily liquid; yield: 88%.

IR (KBr): 3390, 1650, 1580 cm⁻¹.

\(^1H\) NMR (400 MHz, CDCl₃): δ 9.25 (s, 1H, N-H), 6.72-6.70 (m, 4H, Ar-H), 5.42 (m, 2H, -CH=CH-), 3.66 (m, 1H, -CH-OH), 2.32 (t, 2H, J = 7.19 Hz, α to benzimidazole ring), 2.27 (m, 1H, -OH), 2.02 (m, 4H, -CH₂-CH=CH-CH₂-), 1.57 (m, 2H, β to benzimidazole ring), 1.39 (br.s, 18H, chain CH₂), 0.88 (3H, dist. t, CH₃).

\(^{13}C\) NMR (100 MHz, CDCl₃): 143.44, 134.09, 123.22, 117.81, 34.02, 31.81, 29.56, 29.94, 29.90, 27.51, 24.27, 22.65, 22.54, 14.17.

MS (m/z, %): (M+1)^+ 371 (38), M^+ 370 (34), 255 (20), 241 (100), 173 (15).

Anal. Calcd. for C₂₄H₃₈N₂O: C, 77.79; H, 10.33; N, 7.56. Found: C, 77.16; H, 10.39; N, 7.51%.

2-[(8R, 11Z)-8-Hydroxyheptadec-11-enyl]-1H-benzimidazole (4d)

Yellow oily liquid; yield: 88%.

IR (KBr): 3355, 1626, 1596 cm⁻¹.

\(^1H\) NMR (400 MHz, CDCl₃): δ 9.04 (s, 1H, N-H), 6.78-6.76 (m, 4H, Ar-H), 5.44 (m, 2H, -CH=CH-), 3.64 (m, 1H, -CH-OH), 2.31 (t, 2H, J = 7.20 Hz, α to benzimidazole ring), 2.11 (m, 1H, -OH), 2.04 (m, 4H, -CH₂-CH=CH-CH₂-), 1.61 (m, 2H, β to benzimidazole ring), 1.40 (br.s, 18H, chain CH₂), 0.88 (3H, dist. t, CH₃).
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$^{13}$C NMR (100 MHz, CDCl₃): 143.66, 133.17, 123.22, 118.12, 34.25, 31.41, 29.58, 29.39, 29.23, 27.26, 24.72, 24.22, 14.91.

MS (m/z, %): (M+1)$^{+}$ 371 (20), M$^{+}$ 370 (45), 299 (10), 245 (100), 131 (15).

Anal. Calcd. for C$_{24}$H$_{38}$N$_{2}$O: C, 77.79; H, 10.33; N, 7.56. Found: C, 77.21; H, 10.27 N, 7.53%.

2-(Dec-9-enyl)-5-nitro-1H-benzimidazole (4e)

Light yellow oily liquid; yield: 85%.

IR (KBr): 3375, 1660, 1575 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): δ 9.22 (s, 1H, N-H), 7.12 (d, 1H, Ar-H), 7.04 (m, 1H, Ar-H), 6.91 (m, 1H, Ar-H), 5.82 (tdd, 1H, $J_{H-H_1}$ = 6.6 Hz, $J_{H-H_2}$ = 10.2 Hz, $J_{H-H_3}$ = 17.1 Hz, CH$_2$=CH-), 5.02 (dd, 1H, $J_{H-H_1}$ = 10.2 Hz, $J_{H-H_2}$ = 1.2 Hz, $H_2$C=CH), 4.90 (dd, 1H, $J_{H-H_1}$ = 17.1 Hz, $J_{H-H_2}$ = 1.2 Hz, $H_2$C=CH), 2.28 (t, 2H, $J$ = 7.17 Hz, α to benzimidazole ring), 2.04 (m, 2H, -CH$_2$-CH=CH$_2$), 1.66 (m, 2H, β to benzimidazole ring), 1.31 (br.s, 10H, chain CH$_2$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 143.12, 139.01, 136.22, 131.65, 122.26, 117.31, 33.91, 31.61, 28.22, 27.19, 22.33.

MS (m/z, %): (M+1)$^{+}$ 302 (10), M$^{+}$ 301 (82), 274 (10), 176 (100), 162 (60).

Anal. Calcd. for C$_{17}$H$_{23}$N$_{3}$O$_2$: C, 67.76; H, 7.68; N, 13.94. Found: C, 67.30; H, 7.73; N, 13.99%.

2-(Heptadec-8-enyl)-5-nitro-1H-benzimidazole (4f)

Pale yellow oily liquid; yield: 84%.

IR (KBr): 3352, 1636, 1580 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): δ 9.21 (s, 1H, N-H), 7.13 (d, 1H, Ar-H), 7.04 (m, 1H, Ar-H), 6.90 (m, 1H, Ar-H), 5.38 (m, 2H, -CH=CH-), 2.34 (t, 2H, $J$ = 7.24 Hz, α to benzimidazole ring), 2.02 (m, 4H, -CH$_2$-CH=CH-CH$_2$), 1.63 (m, 2H, β to benzimidazole ring), 1.30 (br.s, 18H, chain CH$_2$), 0.89 (3H, dist. t, CH$_3$).

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$^{13}$C NMR (100 MHz, CDCl₃): 143.62, 136.06, 131.33, 129.71, 123.44, 118.13, 34.19, 31.41, 29.53, 28.99, 27.41, 22.32, 22.45, 14.15.

**MS (m/z, %):** (M+1)^+ 400 (20), M^+ 399 (25), 286 (75), 190 (70), 162 (100).

**Anal. Calcd.** for C_{24}H_{37}N_{3}O_{2}: C, 72.15; H, 9.33; N, 10.51. Found: C, 72.70; H, 9.28; N, 10.56%.

2-[(8Z, 11R)-11-Hydroxyheptadec-8-enyl]-5-nitro-1H-benzimidazole (4g)

Yellow oily liquid; yield: 82%.

**IR (KBr):** 3355, 1640, 1580 cm⁻¹.

$^1$H NMR (400 MHz, CDCl₃): δ 9.25 (s, 1H, N-H), 7.13 (d, 1H, Ar-H), 7.04 (m, 1H, Ar-H), 6.91 (m, 1H, Ar-H), 5.45 (m, 2H, -CH=CH-), 3.68 (m, 1H, -CH-OH), 2.33 (t, 2H, J = 7.31 Hz, α to benzimidazole ring), 2.23 (m, 1H, -OH), 2.04 (m, 4H, -CH₂-CH=CH-CH₂-), 1.61 (m, 2H, β to benzimidazole ring), 1.36 (br.s, 18H, chain CH₂), 0.88 (3H, dist. t, CH₃).

$^{13}$C NMR (100 MHz, CDCl₃): 144.13, 135.44, 131.19, 129.31, 123.33, 117.71, 37.11, 34.26, 31.83, 29.61, 27.10, 25.81, 23.11, 22.15, 15.06.

**MS (m/z, %):** (M+1)^+ 416 (35), M^+ 415 (70), 330 (100), 300 (70), 260 (20).

**Anal. Calcd.** for C_{24}H_{37}N_{3}O_{3}: C, 69.37; H, 8.97; N, 10.11. Found: C, 69.79; H, 8.92; N, 10.16%.

2-[(8R, 11Z)-8-Hydroxyheptadec-11-enyl]-5-nitro-1H-benzimidazole (4h)

Yellow oily liquid; yield: 80%.

**IR (KBr):** 3390, 1630, 1584 cm⁻¹.

$^1$H NMR (400 MHz, CDCl₃): δ 9.20 (s, 1H, N-H), 7.16 (d, 1H, Ar-H), 7.04 (m, 1H, Ar-H), 6.93 (m, 1H, Ar-H), 5.43 (m, 2H, -CH=CH-), 3.67 (m, 1H, CH-OH), 2.36 (t, 2H, J = 7.29 Hz, α to benzimidazole ring), 2.21 (m, 1H, -OH), 2.04 (m, 4H, -CH₂-CH=CH-CH₂-), 1.64 (m, 2H, β to benzimidazole ring), 1.42 (br.s, 18H, chain CH₂), 0.87 (3H, dist. t, CH₃).
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$^{13}$C NMR (100 MHz, CDCl₃): 144.23, 135.64, 131.39, 129.41, 123.83, 117.71, 37.51, 34.66, 31.38, 29.51, 27.43, 25.44, 23.11, 22.15, 15.16.

MS (m/z, %): (M+1)$^+$ 416 (10), M$^+$ 415 (70), 344 (100), 290 (25), 246 (20).

Anal. Calcd. for C$_{24}$H$_{37}$N$_3$O$_3$: C, 69.37; H, 8.97; N, 10.11. Found: C, 69.84; H, 8.92; N, 10.13%.

2-(Dec-9-enyl)-5-chloro-1H-benzimidazole (4i)

Colorless oily liquid; yield: 88%.

IR (KBr): 3368, 1640, 1578 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl₃): δ 9.01 (s, 1H, N-H), 7.43-7.31 (m, 3H, Ar-H), 5.82 (tdd, 1H, $J_{H-H} = 6.6$ Hz, $J_{HCH} = 10.2$ Hz, $J_{HCH} = 17.1$ Hz, CH$_2$=CH-), 5.02 (dd, 1H, $J_{H-H} = 10.2$ Hz, $J_{HCH} = 1.2$ Hz, H$_2$C=CH), 4.90 (dd, 1H, $J_{H-H} = 1.2$ Hz, H$_2$C=CH), 2.31 (t, 2H, $J = 7.33$ Hz, α to benzimidazole ring), 2.04 (m, 2H, -CH$_2$-CH=CH$_2$), 1.63 (m, 2H, β to benzimidazole ring), 1.33 (br.s, 10H, chain CH$_2$).

$^{13}$C NMR (100 MHz, CDCl₃): 142.81, 141.31, 132.91, 131.61, 129.71, 128.91, 124.71, 123.46, 29.71, 28.36, 27.91, 23.91, 22.45, 14.81.

MS (m/z, %): (M+1)$^+$ 291 (10), M$^+$ 290 (85), 263 (100), 179 (25), 151 (40).

Anal. Calcd. for C$_{17}$H$_{33}$ClN$_2$: C, 70.22; H, 7.96; N, 9.63. Found: 70.80; H, 7.92; N, 9.58%.

2-(Heptadec-8-enyl)-5-chloro-1H-benzimidazole (4j)

Light yellow oily liquid; yield: 87%.

IR (KBr): 3370, 1655, 1589 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl₃): δ 9.12 (s, 1H, N-H), 7.55-7.20 (m, 3H, Ar-H), 5.44 (m, 2H, -CH=CH-), 2.24 (t, 2H, $J = 7.44$ Hz, α to benzimidazole ring), 2.04 (m, 4H, -CH$_2$-CH=CH-CH$_2$), 1.56 (m, 2H, β to benzimidazole ring), 1.29 (br.s, 18H, chain CH$_2$), 0.87 (3H, dist. t, CH$_3$).

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$^{13}$C NMR (100 MHz, CDCl$_3$): 143.48, 133.85, 131.24, 129.57, 127.71, 121.61, 117.81, 37.41, 34.21, 33.92, 29.56, 29.13, 25.84, 23.13, 22.35, 14.12.

MS (m/z, %): (M+1)$^+$ 389 (30), M$^+$ 388 (75), 275 (15), 165 (100), 151 (80).

Anal. Calcd. for C$_{28}$H$_{37}$CIN$_2$: C, 74.11; H, 9.58; N, 7.20. Found: C, 74.68; H, 9.63; N, 7.16%.

2-[(8Z, 11R)-11-Hydroxyheptadec-8-enyl]-5-chloro-1H-benzimidazole (4k)
Yellow oily liquid; yield: 87%.

IR (KBr): 3412, 1648, 1572 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): δ 9.25 (s, 1H, N-//), 7.43-7.31 (m, 3H, Ar-H), 5.46 (m, 2H, -CH=CH-), 3.67 (m, 1H, -CH(OH)), 2.33 (t, 2H, $J = 7.12$ Hz, α to benzimidazole ring), 2.27 (m, 1H, -OH), 2.02 (m, 4H, CH$_2$-CH=CH-CH$_2$), 1.57 (m, 2H, β to benzimidazole ring), 1.39 (br.s, 18H, chain CH$_2$), 0.88 (3H, dist. t, C//3).

$^{13}$C NMR (100 MHz, CDCl$_3$): 142.98, 133.76, 130.99, 129.76, 127.80, 121.53, 119.01, 37.03, 34.12, 33.57, 29.34, 28.37, 25.71, 23.91, 22.11, 15.17.

MS (m/z, %): (M+1)$^+$ 405 (15), M$^+$ 404 (70), 319 (100), 249 (85), 207 (90).

Anal. Calcd. for C$_{28}$H$_{37}$CIN$_2$: C, 71.18; H, 9.20; N, 6.91. Found: C, 71.66; H, 9.23; N, 6.87%.

2-[(8R, 11Z)-8-Hydroxyheptadec-11-enyl]-5-chloro-1H-benzimidazole (4l)
Yellow oily liquid; yield: 86%.

IR (KBr): 3349, 1629, 1590 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): δ 9.29 (s, 1H, N-//), 7.46-7.33 (m, 3H, Ar-H), 5.45 (m, 2H, -CH=CH-), 3.68 (m, 1H, -CH(OH)), 2.36(t, 2H, $J = 7.23$ Hz, α to benzimidazole ring), 2.29 (m, 1H, -OH), 2.04 (m, 4H, -CH$_2$-CH=CH-CH$_2$-), 1.62 (m, 2H, β to benzimidazole ring), 1.28 (br.s, 18H, chain CH$_2$), 0.88 (3H, dist. t, CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 142.68, 133.67, 130.89, 129.67, 127.82, 121.43, 119.01, 37.53, 34.41, 33.51, 29.43, 28.32, 25.17, 23.22, 22.13, 15.19.

MS (m/z, %): (M+1)$^+$ 405 (10), M$^+$ 404 (80), 333 (40), 279 (85), 179 (65).
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*Anal. Calcd.* for C_{24}H_{37}ClN_{2}O: C, 71.18; H, 9.20; N, 6.91. Found: C, 71.71; H, 9.16; N, 6.94%.

2-(Dec-9-enyl)-4,5,6,7-tetrahydro-1H-benzimidazole (4m)

Pale yellow oily liquid; yield: 90%.

**IR** (KBr): 3338, 1632, 1580 cm\(^{-1}\).

**\(^{1}\)H NMR** (400 MHz, CDCl\(_3\)): \(\delta\) 9.01 (s, 1H, N=H), 5.82 (tdd, 1H, \(J_{H-H} = 6.6\) Hz, \(J_{H-H} = 10.2\) Hz, \(J_{H-H} = 3.6\) Hz, \(\alpha\) to benzimidazole ring), 4.90 (dd, 1H, \(J_{H-H} = 10.2\) Hz, \(J_{H-H} = 17.1\) Hz, \(J_{H-H} = 3.6\) Hz, \(\alpha\) to benzimidazole ring), 2.34 (t, 2H, \(J = 7.36\) Hz, \(\alpha\) to benzimidazole ring), 2.03 (m, 2H, -CH\(_2\)-CH=CH\(_2\)), 1.87 (m, 2H, -CH\(_3\)-ring), 1.69 (m, 2H, \(\beta\) to imidazole ring), 1.50 (m, 8H, -CH\(_2\)-ring), 1.30 (br.s, 10H, chain CH\(_2\)).

**\(^{13}\)C NMR** (100 MHz, CDCl\(_3\)): 170.33, 139.81, 117.61, 64.01, 37.62, 31.91, 29.88, 28.35, 24.57, 22.50, 22.21.

**MS** (m/z, %): (M+1)\(^+\) 263 (30), M\(^+\) 262 (70), 235 (100), 179 (70), 123 (60).

*Anal. Calcd.* for C\(_{17}\)H\(_{30}\)N\(_2\): C, 77.82; H, 11.51; N, 10.67. Found: C, 77.30; H, 11.47; N, 10.70%.

2-(Heptadec-8-enyl)-4,5,6,7-tetrahydro-1H-benzimidazole (4n)

Light yellow oily liquid; yield: 89%.

**IR** (KBr): 3356, 1641, 1593 cm\(^{-1}\).

**\(^{1}\)H NMR** (400 MHz, CDCl\(_3\)): \(\delta\) 9.12 (s, 1H, N=H), 5.38 (m, 2H, -CH=CH\(_2\)), 2.34 (t, 2H, \(J = 7.30\) Hz, \(\alpha\) to benzimidazole ring), 2.02 (m, 4H, -CH\(_2\)-CH=CH-CH\(_2\)-), 1.86 (m, 2H, -CH\(_3\)-ring), 1.66 (m, 2H, \(\beta\) to imidazole ring), 1.50 (m, 8H, -CH\(_2\)-ring), 1.32 (br.s, 18H, chain CH\(_2\)), 0.88 (3H, dist. t, CH\(_3\)).

**\(^{13}\)C NMR** (100 MHz, CDCl\(_3\)): 169.97, 63.91, 36.92, 31.88, 28.22, 27.36, 24.63, 22.49, 22.12, 21.90, 14.91.

**MS** (m/z, %): (M+1)\(^+\) 361 (40), M\(^+\) 360 (85), 247 (45), 207 (50), 193 (67).
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**Anal. Calcd.** for C$_{24}$H$_{44}$N$_2$: C, 79.95; H, 12.29; N, 7.76. Found: C, 79.40; H, 12.36; N, 7.72%.

2-[(8Z, 11R)-11-Hydroxyheptadec-8-enyl]-4,5,6,7-tetrahydro-1H-benzimidazole (4o)
Yellow oily liquid; yield: 88%.

**IR** (KBr): 3350, 1620, 1592 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 9.25 (s, 1H, N-H), 5.42 (m, 2H, -CH=CH-), 3.66 (m, 1H, -CH-OH), 2.32 (t, 2H, $J = 7.28$ Hz, $\alpha$ to benzimidazole ring), 2.27 (m, 1H, -OH), 2.02 (m, 4H, -CH$_2$-CH=CH-CH$_2$), 1.88 (m, 2H, -CH- ring), 1.67 (m, 2H, $\beta$ to imidazole ring), 1.50 (m, 8H, -CH$_2$- ring), 1.39 (br.s, 18H, chain CH$_2$), 0.88 (3H, dist. t, CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 169.99, 71.72, 63.89, 36.98, 34.22, 33.63, 31.66, 28.61, 27.63, 24.56, 22.91, 22.11, 21.81, 14.73.

**MS** (m/z %): (M+1)$^+$ 377 (15), M$^+$ 376 (70), 291 (10), 247 (85), 123 (100).

**Anal. Calcd.** for C$_{24}$H$_{44}$N$_2$O: C, 76.55; H, 11.77; N, 7.43. Found: C, 76.01; H, 11.82; N, 7.40%.

2-[(8R, 11Z)-8-Hydroxyheptadec-11-enyl]-4,5,6,7-tetrahydro-1H-benzimidazole (4p)
Yellow oily liquid; yield: 87%.

**IR** (KBr): 3358, 1658, 1577 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 9.72 (s, 1H, N-H), 5.44 (m, 2H, -CH=CH-), 3.68 (m, 1H, -CH-OH), 2.28 (t, 2H, $J = 7.39$ Hz, $\alpha$ to benzimidazole ring), 2.21 (m, 1H, -OH), 2.03 (m, 4H, -CH$_2$-CH=CH-CH$_2$), 1.87 (m, 2H, -CH- ring), 1.69 (m, 2H, $\beta$ to imidazole ring), 1.52 (m, 2H, -CH$_2$- ring), 1.36 (br.s, 18H, chain CH$_2$), 0.88 (3H, dist. t, CH$_3$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 169.87, 71.73, 64.01, 36.89, 34.32, 33.36, 31.66, 28.64, 27.53, 24.65, 22.98, 22.11, 21.81, 14.69.

**MS** (m/z, %): (M+1)$^+$ 377 (30), M$^+$ 376 (50), 305 (10), 251 (75), 123 (100).
2-Substituted-benzimidazoles, tetrahydrobenzimidazoles and imidazoles

**Anal. calcd.** for C_{24}H_{44}N_{2}O: C, 76.55; H, 11.77; N, 7.43. Found: C, 76.98; H, 11.72; N, 7.47%.

**2-(Dec-9-enyl)-1H-imidazole (4q)**

Colorless oily liquid: yield: 89%.

**IR** (KBr): 3354, 1642, 1598 cm\(^{-1}\).

\(^1\)H **NMR** (400 MHz, CDCl\(_3\)): \(\delta\) 9.81 (s, 1H, N-H), 5.82 (tdd, 2H, \(J_{H_1-H_2} = 6.6\) Hz, \(J_{H_1-H} = 10.2\) Hz, \(J_{H_2-H} = 17.1\) Hz, CH\(_2\)-CH), 5.02 (2H, dd, \(J_{H_1-H} = 10.2\) Hz, \(J_{H_2-H} = 3.6\) Hz, CH\(_2\)-CH), 4.90 (2H, dd, \(J_{H_1-H} = 17.1\) Hz, \(J_{H_2-H} = 3.6\) Hz, CH=CH), 2.31 (t, 2H, \(J = 7.16\) Hz, \(\alpha\) to imidazole ring), 2.04 (m, 2H, -CH\(_2\)-CH=CH\(_2\)), 1.66 (m, \(\beta\) to imidazole ring), 1.36 (br.s, 10H, chain CH\(_2\)).

\(^{13}\)C **NMR** (100 MHz, CDCl\(_3\)): 152.31, 130.96, 125.93, 33.66, 32.81, 30.14, 23.26, 14.31.

**MS** (m/z, %): (M+1)^+ 207 (20), M^+ 208 (22), 176 (36), 109 (100), 81 (100).

**Anal. Calcd.** for C\(_{13}\)H\(_{22}\)N\(_2\): C, 75.69; H, 10.74; N, 13.57. C, 75.22; H, 10.70; N, 13.63%.

**2-(Heptadec-8-enyl)-1H-imidazole (4r)**

Pale yellow oily liquid: yield: 88%.

**IR** (KBr): 3367, 1644, 1590 cm\(^{-1}\).

\(^1\)H **NMR** (400 MHz, CDCl\(_3\)): \(\delta\) 9.77 (s, 1H, N-H), 5.62 (m, 2H, -CH=CH\(_2\)), 2.33 (t, 2H, \(J = 7.35\) Hz, \(\alpha\) to imidazole ring), 2.05 (m, 4H, -CH\(_2\)-CH=CH\(_2\)-CH=CH\(_2\)), 1.62 (m, 2H, \(\beta\) to imidazole ring), 1.29 (br.s, 18H, chain CH\(_2\)), 0.87 (3H, dist. t, CH\(_3\)).

\(^{13}\)C **NMR** (100 MHz, CDCl\(_3\)): 152.71, 131.76, 126.83, 33.38, 32.25, 30.44, 23.62, 14.38.

**MS** (m/z, %): (M+1)^+ 305 (40), M^+ 304 (22), 191 (31), 165 (31), 67 (100).

**Anal. Calcd.** for C\(_{20}\)H\(_{36}\)N\(_2\): C, 78.89; H, 11.91; N, 9.20. C, 78.35; H, 11.86; N, 9.15%.

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2-[(8Z, 11R)-11-Hydroxyheptadec-8-enyl]-1H-imidazole (4s)

Yellow oily liquid: yield: 86%.

IR (KBr): 3378, 1655, 1597 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 9.81 (s, 1H, N-H), 5.66 (m, 2H, -CH=CH-), 3.59 (m, 1H, -CH-OH), 2.36 (t, 2H, J = 7.39 Hz, α to imidazole ring), 2.32 (m, 1H, -OH), 2.03 (m, 4H, -CH₂=CH=CH-CH₂-), 1.62 (m, 2H, β to imidazole ring), 1.29 (br.s, 18H, chain CH₂), 0.88 (3H, dist. t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 152.91, 131.46, 126.57, 71.93, 36.97, 34.21, 33.62, 31.64, 28.51, 27.35, 24.62, 22.28, 22.11, 21.82, 14.36.

MS (m/z, %): (M+1)^+ 321 (40), M⁺ 320 (22), 235 (100), 191 (31), 165 (100).

Anal. Calcd. for C₂₀H₃₆N₂O: C, 74.96; H, 11.31; N, 8.74.

2-[(8R, 11Z)-11-Hydroxyheptadec-11-enyl]-1H-imidazole (4t)

Yellow oily liquid: yield: 86%.

IR (KBr): 3354, 1628, 1578 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 9.81 (s, 1H, N-H), 5.59 (m, 2H, -CH=CH-), 3.61 (m, 1H, -CH-OH), 2.41 (t, 2H, J = 7.44 Hz, α to imidazole ring), 2.33 (m, 1H, -OH), 2.05 (m, 4H, -CH₂-CH=CH-CH₂-), 1.65 (m, 2H, β to imidazole ring), 1.30 (br.s, 18H, chain CH₂), 0.89 (3H, dist. t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 152.81, 131.46, 126.33, 71.93, 36.97, 34.21, 33.62, 31.44, 28.45, 27.63, 24.52, 22.91, 22.27, 21.73, 14.34.

MS (m/z, %): (M+1)^+ 321 (38), M⁺ 320 (46), 249 (100), 195 (18), 165 (73).

Anal. Calcd. for C₂₀H₃₆N₂O: C, 74.96; H, 11.31; N, 8.74.
5.5 References


2-Substituted-benzimidazoles, tetrahydrobenzimidazoles and imidazoles


2-Substituted-benzimidazoles, tetrahydrobenzimidazoles and imidazoles


2-Substituted-benzimidazoles, tetrahydrobenzimidazoles and imidazoles


2-Substituted-benzimidazoles, tetrahydrobenzimidazoles and imidazoles


CHAPTER-6

3,5,6-Trisubstituted-
1,2,4-Triazines
6.1 Theoretical

1,2,4-Triazines are an important class of compounds, which act as anti-inflammatory\(^1\)\(^-\)\(^3\), and antimalarial\(^4\) agents. Some of them are used as antibacterial\(^5\)\(^-\)\(^7\) and antidiabetics\(^8\). 3-Sulfanilamido-5-dimethylthyl-1,2,4-triazine is manufactured and used as a drug\(^9\). 6-Azacytidine derivatives show antiviral effects on the adenovirus genome\(^10\), whereas some triazinone derivatives are used as antiulcer agents\(^11\). Fluorene containing substituted 3-thioxo-1,2,4-triazin-5-ones exhibit antihuman immune virus activity\(^12\).

The 1,2,4-triazine moiety plays a vital role in many biological activities including antiviral\(^13\), antihypertensive\(^13\)\(^,\)\(^14\), blood-platelet aggregation inhibitory\(^14\)\(^,\)\(^15\), analgesic\(^16\), and antibacterial properties\(^17\)\(^,\)\(^18\) as well as some of new anti-HIV and anticancer agents\(^19\).

Microwave assisted organic synthesis (MAOS) continues to affect synthetic chemistry significantly by enabling rapid, reproducible and scalable chemistry development\(^20\). The use of microwave irradiation has been an established tool in organic synthesis for achieving better selectivity, rate enhancement and reduction of thermally degradative by products\(^21\)\(^,\)\(^22\). However these procedures are practically limited as the solvents in microwave oven at elevated temperatures create high pressure, which may cause explosion. One of the advances to overcome this problem is the inorganic solid supported\(^23\)\(^,\)\(^24\) organic synthesis which attracted attention because of enhanced selectivity, milder reaction conditions and associated ease of manipulation. Moreover they also provide an opportunity to work with open vessels and enhanced possibility of upscaling the reactions on preparative scale\(^25\)\(^,\)\(^26\).

Numerous reactions including condensations, cycloadditions, heterocycle formation, metal catalyzed cross coupling have been explored under microwave conditions, some of which have been applied to medicinal chemistry and total synthesis of natural products\(^27\).
Safieh et al.\textsuperscript{28} have described novel methods for the synthesis of a new series of 5-substituted 1,3-dimethyl pyrazolo-[4,3-e][1,2,4]-triazines (v) (Scheme 6.1).

Scheme 6.1

A number of 6-aryl and 3-substituted-6-aryl-1,2,4-triazines (ix) have been prepared by heating a mixture of α-haloacetophenone (vi) and two equivalents of acid hydrazide (vii) in alcohol or acetic acid in the presence of slight excess of NaOAc, KOAc or AgOAc for few minutes\textsuperscript{29} (Scheme 6.2).

Scheme 6.2

Synthesis of thiadiazine and triazino [3,4-b] thiadiazine derivatives were reported by Nami et al.\textsuperscript{30} The addition of dimethyl acetylenedicarboxylate (DMAD)
to dithizon (x) afforded dimethyl-2-methoxycarbonylmethylene-4,8-diphenyl-1,2,4-triazino[3,4-b]-1,3,4-thiadiazin-3-one-6,7-dicarboxylate (xi) (Scheme 6.3).

\[ \text{Ph} \overset{^\text{SN}_r}{\text{NH}} \overset{^\text{SN}_r}{\text{NH}} \text{Ph} \]

Scheme 6.3

Ali\textsuperscript{31} has synthesized 4-(4-[(6-Chloro-4-oxo-4H-chromen-3-yl)methylene]amino)phenyl)-5,6-dihydro-5-phenyl-3-thioxo-1,2,4-triazine and 4-(4-[(6-Chloro-4-oxo-4H-chromen-3-yl)methylene]-amino)phenyl)-2,5-dihydro-5,6-diphenyl-3-thioxo-1,2,4-triazine (xiii) as potential antifungal agents (Scheme 6.4).

\[ \text{R}^\text{a} \text{NH} \text{R}^\text{b} \overset{^\text{SN}_r}{\text{R}^\text{c} \text{COOH}} \overset{^\text{SN}_r}{\text{R}^\text{d} \text{S}^\text{N}_r} \text{S}^\text{N}_r \text{S}^\text{N}_r \text{NH} \text{R}^\text{e} \text{R}^\text{f} \]

Scheme 6.4

Heravi \textit{et al.}\textsuperscript{32} have reported the synthesis of [1,3,4]thiadiazolo [2,3-c][1,2,4]triazin-4-ones (xv) using sulfuric acid supported onto silica gel in solventless system (Scheme 6.5).

\[ \text{NH}_2 \overset{^\text{SN}_r}{\text{NH}} \overset{^\text{SN}_r}{\text{NH}} \text{NH} \text{N} \text{N} \text{N} \overset{^\text{SN}_r}{\text{NH}} \overset{^\text{SN}_r}{\text{NH}} \overset{^\text{SN}_r}{\text{NH}} \text{O} \text{R}^1 \text{COOH} \]

Scheme 6.5
Broadening the scope of 1,2,4-triazine synthesis by the application of microwave technology Zhao et al.\textsuperscript{33} have developed the rapid synthesis of diverse 3,5,6-trisubstituted-1,2,4-triazines (xviii) in excellent yield and purity, including many previously unknown 3-heterocyclic-1,2,4-triazines (Scheme 6.6).

\[
\text{Ph} -\text{O} + \text{Ph} -\text{O} + \text{R} -\text{NH}_2 \xrightarrow{\text{NH}_4\text{OAc (10 equiv.) \text{AcOH}}} \text{Ph} -\text{N} -\text{N} -\text{Ph} \quad (\text{xvi}) \quad (\text{xvii}) \quad (\text{xviii})
\]

Scheme 6.6

4-Amino-6-(\text{tert}-butyl)-3-methylthio-4,5-dihydro-1,2,4-triazin-5-one (xix) was used in nucleophilic substitution reaction with carboxylic acid hydrazides\textsuperscript{34} (Scheme 6.7).

\[
\text{Me}_2\text{C} -\text{N} -\text{Me}_3\text{C} \quad (\text{xix})
\]

Scheme 6.7

In a one pot microwave reaction, an acyl hydrazide-tethered indole underwent a 3-component condensation to form a triazine (xxiii), followed by an inverse-electron demand Diels–Alder reaction and subsequent chelotropic expulsion of nitrogen to deliver novel, unnatural $\beta$-carboline alkaloids (xxiv) in good isolated yields\textsuperscript{35} (Scheme 6.8).
Scheme 6.8
1,2,4-Triazines are a representative class of heterocyclic compounds with a wide variety of interesting properties which are used in medicine and agriculture\textsuperscript{36-39}. It has been associated with diverse pharmacological activities such as hypertension and inhibition of platelets\textsuperscript{40}, antileukemic\textsuperscript{41}, anti-inflammatory\textsuperscript{42} and potent neuroprotective agents\textsuperscript{43}. The 1,2,4-triazine moiety is a structural element in antimalarial\textsuperscript{44}, anticancer\textsuperscript{45}, antifungal\textsuperscript{46}, anticonvulsant\textsuperscript{47}, antibacterial\textsuperscript{48} and antiviral\textsuperscript{49} compounds. Certain compounds containing 1,2,4-triazines nucleus have been reported to possess pesticidal\textsuperscript{50}, neuropharmacological\textsuperscript{51}, analgesic and antidepressant\textsuperscript{52} properties. Also some 1,2,4-triazine derivatives are used for the determination of metal ions and as dyes\textsuperscript{39}. The N-methyl derivatives of 1,2,4-triazines are the naturally occurring antibiotics fervenulin (planomycin), toxoflavin (xanthothricin) and neomycin\textsuperscript{39}.

In view of above mentioned pharmacological importance of heterocycles and our ongoing efforts to synthesize heterocycles from fatty acids\textsuperscript{53-56} we now describe a expeditious ecofriendly solvent free microwave accelerated solid state approach for the rapid synthesis of 3,5,6-trisubstituted-1,2,4-triazines from saturated and olefinic fatty acid hydrazides wherein several disadvantages such as long reaction time and tedious work up has overcome.

\textsuperscript{*}Research paper entitled "Microwave assisted efficient one-pot synthesis of 3,5,6-trisubstituted-1,2,4-triazines from fatty acid hydrazides under solvent-free conditions and their antimicrobial activity" is published. (A. Rauf, S. Sharma, S. Gangal, ARKIVOC 2007 (xvi) 137-147).
6.3 Results and discussion

Considering that MW irradiations using commercial domestic ovens have been used to accelerate organic reactions, the high heating efficiency giving remarkable rate enhancement and dramatic reduction in reaction times and better yields, we became interested to synthesize heterocycles from fatty acids under microwave irradiations. We now report the synthesis of 3,5,6-trisubstituted-1,2,4-triazine 3a-l by condensation of 1,2-diketone 2a-b with various saturated and olefinic (internal and terminal) fatty acids hydrazides 1a-f under MW and solvent free conditions in short time (Scheme 6.9).

\[
\begin{align*}
\text{1a-f} & \quad + \quad \text{2a-b} \\
& \quad \xrightarrow{\text{NH}_4\text{OAc, Et}_3\text{N, SiO}_2, \text{MW}} \\
& \quad \text{3a-l}
\end{align*}
\]

(a) \( R_1=R_3=\text{CH}_3 \)
(b) \( R_2=R_3=\text{C}_6\text{H}_5 \)

Scheme 6.9 Synthesis of 3,5,6-trisubstituted-1,2,4-triazines

As can been seen from Table 6.1 and Table 6.2 the scope of the reaction using saturated, olefinic (internal and terminal) and hydroxy fatty acid hydrazides was found to be good.
Table 6.1 3,5,6-Trisubstituted-1,2,4-triazines

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a,3a</td>
<td></td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>2</td>
<td>1b,3b</td>
<td></td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>3</td>
<td>1c,3c</td>
<td></td>
<td>CH₃</td>
<td>CH₃</td>
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<tr>
<td>4</td>
<td>1d,3d</td>
<td></td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>5</td>
<td>1e,3e</td>
<td></td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>6</td>
<td>1f,3f</td>
<td></td>
<td>CH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>7</td>
<td>1a,3g</td>
<td></td>
<td>C₆H₅</td>
<td>C₆H₅</td>
</tr>
<tr>
<td>8</td>
<td>1b,3h</td>
<td></td>
<td>C₆H₅</td>
<td>C₆H₅</td>
</tr>
<tr>
<td>9</td>
<td>1c,3i</td>
<td></td>
<td>C₆H₅</td>
<td>C₆H₅</td>
</tr>
<tr>
<td>10</td>
<td>1d,3j</td>
<td></td>
<td>C₆H₅</td>
<td>C₆H₅</td>
</tr>
<tr>
<td>11</td>
<td>1e,3k</td>
<td></td>
<td>C₆H₅</td>
<td>C₆H₅</td>
</tr>
<tr>
<td>12</td>
<td>1f,3l</td>
<td></td>
<td>C₆H₅</td>
<td>C₆H₅</td>
</tr>
</tbody>
</table>

The yield of 3,5,6-trisubstituted-1,2,4-triazine did not depend on the length of chain of fatty acid hydrazide and was found to be appreciable (Table 6.2). In order to determine the optimum conditions for the synthesis of triazines in faster and efficient way, molar ratios of reagents and the irradiation time and power level of microwave set-up was investigated. After some experimentation, we found a set of conditions that generally provides products in good yield. The optimum conditions employed are that the molar ratio of fatty acid hydrazides and 1,2-diketone is 1:1 and irradiation time and power level of microwave set up are 10-15 minutes, 60 and 100% power.
Table 6.2 Optimization for the reaction of fatty acid hydrazides with 1,2-diketones under microwave irradiation

<table>
<thead>
<tr>
<th>Entry</th>
<th>1</th>
<th>2</th>
<th>Ratio 1/2</th>
<th>Microwave Equipment</th>
<th>Power (%)</th>
<th>Time (minutes)</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>2a</td>
<td>1:1</td>
<td>Multimode</td>
<td>60</td>
<td>7</td>
<td>3a</td>
<td>92</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>2a</td>
<td>1:1</td>
<td>Multimode</td>
<td>60</td>
<td>7</td>
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</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>2a</td>
<td>1:1</td>
<td>Multimode</td>
<td>60</td>
<td>8</td>
<td>3c</td>
<td>92</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>2a</td>
<td>1:1</td>
<td>Multimode</td>
<td>100</td>
<td>8</td>
<td>3d</td>
<td>89</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>2a</td>
<td>1:1</td>
<td>Multimode</td>
<td>100</td>
<td>8</td>
<td>3e</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>1f</td>
<td>2a</td>
<td>1:1</td>
<td>Multimode</td>
<td>100</td>
<td>9</td>
<td>3f</td>
<td>89</td>
</tr>
<tr>
<td>7</td>
<td>1a</td>
<td>2b</td>
<td>1:1</td>
<td>Multimode</td>
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<td>7</td>
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<td>90</td>
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<tr>
<td>8</td>
<td>1b</td>
<td>2b</td>
<td>1:1</td>
<td>Multimode</td>
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<td>8</td>
<td>3h</td>
<td>91</td>
</tr>
<tr>
<td>9</td>
<td>1c</td>
<td>2b</td>
<td>1:1</td>
<td>Multimode</td>
<td>60</td>
<td>8</td>
<td>3i</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>1d</td>
<td>2b</td>
<td>1:1</td>
<td>Multimode</td>
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<td>10</td>
<td>3j</td>
<td>89</td>
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<td>11</td>
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<td>2b</td>
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<td>100</td>
<td>12</td>
<td>3k</td>
<td>89</td>
</tr>
<tr>
<td>12</td>
<td>1f</td>
<td>2b</td>
<td>1:1</td>
<td>Multimode</td>
<td>100</td>
<td>12</td>
<td>3l</td>
<td>88</td>
</tr>
</tbody>
</table>

(a) All reactions were carried out using fatty acid hydrazides (1 eq) with respect to 1,3-diketone under microwave irradiation.
(b) Microwave equipment multimode was used.
(c) Full power is 1.35 kHz.
(d) Monitored by TLC.
(e) All yields refer to isolated products and the products were characterized by IR, $^1$H NMR, $^{13}$CNMR, MS and elemental analysis.

$^1$H NMR spectrum of 3-(heptadec-8-enyl)-5,6-dimethyl-1,2,4-triazine 3d showed characteristic signal of triplet for two hydrogens at δ 2.77 for methylene protons alpha to triazine ring and multiplet of two hydrogens at 1.63 for -CH$_2$β to ring. The structure of 3d was further supported by its mass spectral studies, which showed molecular ion peak at m/z 345 consistent with its molecular formula C$_{15}$H$_{25}$N$_3$. Base peak appears at m/z 108. Detailed spectra of titled compounds are given in the experimental section.

To check the biological activity of the compounds, the series of the compounds (3a-1) were screened for the antimicrobial activity against bacteria (e.g. *Escherichia*
coli, Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium), fungus (Penicillium sp, Helminthosporum oryzae, Aspergillus niger) and Candida albicans. The results are shown in Table 6.3 and Table 6.4. The compounds showed good activity against H. oryzae, S. aureus, E. coli and better results were obtained against C. albicans, Penicillium, A. niger. The moderate activity of compounds was observed against S. typhimurium and B. subtilis.

**Table 6.3 In vitro antibacterial activity of 3,5,6-trisubstituted-1,2,4-triazines**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>E. coli</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>S. typhimurium</th>
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<tbody>
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<td>+</td>
<td>++</td>
<td>+</td>
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<tr>
<td>3b</td>
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<td>++</td>
<td>+</td>
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<td>+++</td>
</tr>
</tbody>
</table>

Zone of diameter of growth inhibition: Zone of diameter of growth inhibition; <10 mm (-), 10-12 mm (+), 13-15 mm (++), 16-20 mm (+++).

**Table 6.4 In vitro antifungal activity of 3,5,6-trisubstituted-1,2,4-triazines**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>C. albicans</th>
<th>Penicillium sp</th>
<th>A. niger</th>
<th>H. oryzae</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
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<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3b</td>
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<td>++</td>
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<td>++</td>
</tr>
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<td>++</td>
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<td>3j</td>
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<td>++</td>
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</table>

Zone of diameter of of growth inhibition: Zone of diameter of growth inhibition; <10 mm (-), 10-12 mm (+), 13-15 mm (++), 16-20 mm (+++).
6.4 Experimental

The sources of all the fatty acids and instrumentation details are the same as given in Chapter 1 (page 38). The microwave irradiations were carried out using an unmodified domestic oven (LG, Model MC-808WAR, 1.35 KW, 2450MHz).

General procedure for the synthesis of 3,5,6-trisubstituted-1,2,4-triazines (3a-l)

The hydrazides of corresponding fatty acids were prepared as reported earlier in Chapter 2 (page 38)\(^5\). A mixture of fatty acid hydrazide 1a-f (2mmol), diketone 2a-b (2mmol) and silica gel was ground with a pestle, NH\(_4\)OAc and Et\(_3\)N were added in catalytic amount and the prepared mixture was introduced into microwave irradiation in open pyrex beaker for appropriate time (Table 6.2). After complete conversion as indicated by TLC, the mixture was extracted with petroleum ether and washed with water. Then the solvent was extracted in vacuum and the product was purified by column chromatography (Hexane:EtOAc, v:v) 3a, (99:1); 3b, (99:1); 3c, (99:1), 3d, (98:2); 3e, (98:2); 3f, (98:2); 3g, (99:1); 3h, (99:1); 3i, (99:1); 3j, (98:2); 3k, (97:3); 3l, (97:3).

Antibacterial Activity

The newly synthesized compounds were screened in vitro against an assortment of *S. aureus* MSSA 22, *B. subtilis* ATCC 6051, *E. coli* K12 and *S. typhimurium* MTCC 98. Screening results are summarized in Table 6.3. All the synthesized compounds were dissolved in DMF. The antibacterial activity of test compounds and standard chloramphenicol was done by filter paper disc method\(^5\). Media with DMF was set up as control.

Antifungal Activity

The standard agar disc diffusion method\(^5\) was performed to evaluate the antifungal property of the test compounds and standard nystatin. The newly synthesized compounds were screened for *A. niger* (lab isolate), *C. albicans* IOA-109, *Penicillium*...
sp. (lab isolate) and *H. oryzae* (2537 ICAR, Jaipur). The synthesized compounds were dissolved in DMF. Media with DMF was set up as control.

**Spectroscopic data**

**3-Pentadecyl-5,6-dimethyl-1,2,4-triazine (3a)**

*IR* (KBr): 2930, 2859, 1594, 778 cm\(^{-1}\).

*\(^1\)H NMR* (400 MHz, CDCl\(_3\)): \(\delta\) 2.88 (t, 2H, \(J= 6.84\) Hz, -CH\(_2\) \(\alpha\) to ring), 2.35 (m, 6H, ring CH\(_3\)), 1.62 (m, 2H, -CH\(_2\) \(\beta\) to ring), 1.28 (br.s, 24H, chain CH\(_2\)), 0.88 (3H, dist.t, CH\(_3\)).

*\(^{13}\)C NMR* (100 MHz, CDCl\(_3\)): 178.71, 176.77, 174.62, 34.62, 33.99, 31.64, 29.32, 28.01, 25.47, 23.42, 14.17.

*MS* (m/z, %): (M+1)\(^+\) 320 (12), M\(^+\) 319 (42), 150 (18), 136 (20), 122 (55), 108 (100).

*Anal: Calcd.* for C\(_{20}\)H\(_{37}\)N\(_3\): C, 75.23; H, 11.59; N, 13.16. Found: C, 75.16; H, 11.60; N, 13.20%.

**3-Heptadecyl-5,6-dimethyl-1,2,4-triazine (3b)**

*IR* (KBr): 2930, 2860, 1590, 770 cm\(^{-1}\).

*\(^1\)H NMR* (400 MHz, CDCl\(_3\)): \(\delta\) 2.80 (t, 2H, \(J= 6.80\) Hz, -CH\(_2\) \(\alpha\) to ring), 2.35 (m, 6H, ring CH\(_3\)), 1.63 (m, 2H, -CH\(_2\) \(\beta\) to ring), 1.28 (28H, br.s, chain CH\(_2\)), 0.88 (3H, dist.t, CH\(_3\)).

*\(^{13}\)C NMR* (100 MHz, CDCl\(_3\)): 180.10, 179.41, 177.09, 34.09, 31.95, 29.42, 24.71, 22.72, 14.15.

*MS* (m/z, %): (M+1)\(^+\) 348 (10), M\(^+\) 347 (32), 330 (66), 232 (36), 122 (48), 108 (100).

*Anal: Calcd.* for C\(_{22}\)H\(_{41}\)N\(_3\): C, 76.08; H, 12.10; N, 11.81. Found: C, 76.11; H, 12.13; N, 11.89%.
3-(Dec-9-enyl)-5,6-dimethyl-1,2,4-triazine (3c)

**IR** (KBr): 2928, 2866, 1580, 775 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 5.82 (1H, tdd, J₇-Hₓ=CH₂ = 6.6 Hz, J₇-Hₓ = 10.2 Hz, J₇-Hₓ = 17.1 Hz, CH₂=CH-H), 5.02 (1H, dd, J₇-Hₓ = 10.2 Hz, J₇-Hₓ = 1.2 Hz, H₋C=CH-), 4.90 (1H, dd, J₇-Hₓ = 17.1 Hz, J₇-Hₓ = 1.2 Hz, H₋C=CH-), 2.80 (t, 2H, J = 6.8 Hz, -CH₂ α to ring), 2.36 (m, 6H, ring CH₃), 2.03 (m, 2H, -CH₂-CH=CH₂), 1.63 (m, 2H, -CH₂ β to ring), 1.29 (br.s, 10H, chain CH₂).

**¹³C NMR** (100 MHz, CDCl₃): 180.64, 179.76, 178.45, 139.19, 114.22, 34.02, 29.15, 24.72.

**MS** (m/z, %): (M+1)⁺ 248 (15), M⁺ 247 (35), 220 (24), 139 (74), 108 (100).

**Anal: Calcd.** for C₁₅H₂₅N₃: C, 72.87; H, 10.12; N, 17.00. Found: C, 72.99; H, 10.16; N, 17.12 %

3-(Heptadec-8-enyl)-5,6-dimethyl-1,2,4-triazine (3d)

**IR** (KBr): 2930, 2856, 1585, 775 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 5.35 (m, 2H, -CH=CH-H), 2.77 (t, 2H, J = 6.52 Hz, -CH₂ α to ring), 2.34 (m, 6H, ring CH₃), 2.03 (m, 4H, -CH₂-CH=CH-CH₂-), 1.63 (m, 2H, -CH₂ β to ring), 1.30 (br.s, 18H, chain CH₂), 0.88 (3H, dist.t, CH₃).

**¹³C NMR** (100 MHz, CDCl₃): 179.76, 176.77, 174.37, 130.01, 129.10, 44.37, 34.00, 31.64, 29.39, 27.13, 25.54, 24.74, 14.17.

**MS** (m/z, %): (M+1)⁺ 346 (23), M⁺ 345 (75), 330 (63), 232 (46), 122 (29), 108 (100).

**Anal: Calcd.** for C₂₃H₃₈N₃: C, 76.52; H, 11.30; N, 12.17. Found: C, 76.50; H, 11.33; N, 12.19 %

3-(8Z,11R)-11-Hydroxyheptadec-8-enyl]-5,6-dimethyl-1,2,4-triazine (3e)

**IR** (KBr): 2940, 2866, 1580, 778 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 5.38 (m, 2H, -CH=CH-H), 3.64 (m, 1H, -CHOH), 2.88 (t, 2H, J = 6.82 Hz, -CH₂ α to ring), 2.30 (m, 1H, -CHOH), 2.21 (m, 6H, ring CH₃),
3,5,6-Trisubstituted-1,2,4-triazines

2.03 (m, 4H, -CH₂-CH=CH-CH₂-), 1.54 (m, 2H, -CH₂ β to ring), 1.37 (br.s, 18H, chain CH₂), 0.88 (3H, dist.t, CH₃).

^1^JC NMR (100 MHz, CDCl₃): 178.73, 177.0, 175.02, 127.98, 127.83, 77.67, 37.05, 34.63, 32.41, 29.16, 24.29, 14.15.

MS (m/z, %): (M+1)^ + 362 (12), M^ + 361 (24), 276 (82), 136 (78), 122 (64), 108 (108).

Anal: Calcd. for C₂₂H₃₉N₃O: C, 73.13; H, 11.63; N, 10.80. Found: C, 73.11; H, 11.70; N, 10.89%.

3-[(8R, 11Z)-8-Hydroxyheptadec-11-enyl]-5,6-dimethyl-1,2,4-triazine (3f)

IR (KBr): 2930, 2859, 1594, 778 cm⁻¹.

^1^HNMR (400 MHz, CDCl₃): δ 5.38 (m, 2H, -CH=CH-), 3.64 (m, IH, -C=OH), 2.83 (t, 2H, J = 6.82 Hz, -CH₂ α to ring), 2.24 (m, 6H, ring CH₃), 2.31 (m, 1H, -CHOH), 2.04 (m, 4H, -CH₂-CH=CH-CH₂-), 1.63 (m, 2H, -CH₂ β to ring), 1.36 (br.s, 18H, chain CH₂), 0.88 (3H, dist.t, CH₃).

^1^JC NMR (100 MHz, CDCl₃): 178.71, 176.77, 174.62, 130.04, 129.67, 127.86, 71.67, 37.06, 34.60, 33.98, 31.64, 29.28, 24.29, 14.50.

MS (m/z, %): (M+1)^ + 362 (14), M^ + 361 (78), 236 (32), 136 (62), 122 (18), 108 (100).

Anal: Calcd. for C₂₂H₃₉N₃O: C, 73.13; H, 11.63; N, 10.80. Found: C, 73.19; H, 11.80; N, 10.99%.

3-Pentadecyl-5,6-diphenyl-1,2,4-triazine (3g)

IR (KBr): 2930, 1590, 2860, 770 cm⁻¹.

^1^HNMR (300 MHz, CDCl₃): δ 7.53-7.26 (m, 10 H, Ar-H), 2.52 (t, 2H, J = 7.2Hz, -CH₂ α to ring), 1.96 (m, 2H, -CH₂ β to ring), 1.47 (br.s, 18H, chain CH₂), 0.88 (3H, dist.t, CH₃).

^1^JC NMR (100 MHz, CDCl₃): 180.33, 179.76, 178.45, 134.94, 133.04, 129.51, 34.15, 31.98, 29.45, 23.74, 14.17.

MS (m/z, %): (M+1)^ + 444 (9), M^ + 443 (12), 428 (82), 260 (80), 246 (34), 232 (100).
3,5,6-Trisubstituted 1,2,4-triazines

**Anal:** Calcd. for C$_{30}$H$_{41}$N$_3$: C, 81.26; H, 9.25; N, 9.48. Found: C, 81.30; H, 11.70; N, 9.09%.

3-Heptadecyl-5,6-diphenyl-1,2,4-triazine (3h)

**IR** (KBr): 2930, 2859, 1594, 778 cm$^{-1}$.

**$^1$H NMR** (300 MHz, CDCl$_3$): $\delta$ 7.53-7.26 (m, 10 H, Ar-$H$), 2.52 (t, 2H, $J = 6.9$ Hz, -CH$_2$ to ring), 1.96 (m, 2H, -CH$_2$ to ring), 1.47 (br.s, 28H, chain CH$_2$), 0.88 (3H, dist.t, CH$_3$).

**$^{13}$C NMR** (100 MHz, CDCl$_3$): 180.10, 179.76, 173.83, 134.92, 133.01, 129.49, 34.09, 31.95, 29.42, 23.71, 14.15.

**MS** (m/z, %): (M+1)$^+$ 470 (12), M$^+$ 471 (28), 456 (39), 260 (44), 246 (26), 232 (100).

**Anal:** Calcd. for C$_{32}$H$_{45}$N$_3$: C, 81.52; H, 9.55; N, 8.91. Found: C, 81.30; H, 9.66; N, 8.70%.

3-(Dec-9-enyl)-5,6-diphenyl-1,2,4-triazine (3i)

**IR** (KBr): 2933, 2856, 1580, 776 cm$^{-1}$.

**$^1$H NMR** (300 MHz, CDCl$_3$): $\delta$ 8.03-7.25 (m, 10 H, Ar-$H$), 5.82 (1H, tdd, $J_{H-H_2} = 6.6$ Hz, $J_{H-H_2} = 10.2$ Hz, $J_{H_2-C} = 17.1$ Hz, CH$_2$=CH-), 5.02 (1H, dd, $J_{H_2-H} = 10.2$ Hz, H$_2$C=CH-), 4.90 (1H, dd, $J_{H_2-H} = 17.1$ Hz, $J_{H_2-H} = 1.2$ Hz, H$_2$C=CH-), 2.81 (t, 2H, $J = 7.6$ Hz, -CH$_2$ to ring), 2.02 (m, 2H, -CH$_2$-CH=CH$_2$), 1.68 (m, 2H, -CH$_2$ to ring), 1.29 (br.s, 10H, chain CH$_2$).


**MS** (m/z, %): (M+1)$^+$ 374 (13), M$^+$ 373 (35), 346 (66), 260 (70), 246 (82), 232 (100).

**Anal:** Calcd. for C$_{25}$H$_{29}$N$_3$: C, 80.42; H, 8.31; N, 11.26. Found: C, 80.36; H, 8.39; N, 11.22%.
3,5,6-Trisubstituted-1,2,4-triazines

3-(Heptadec-8-enyl)-5,6-diphenyl-1,2,4-triazine (3j)

IR (KBr): 2933, 2855, 1580, 770 cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ 7.85-7.33 (m, 10 H, Ar-H), 5.35 (m, 2H, -CH=CH-), 2.79 (t, 2H, J = 7.2 Hz, -CH₂ α to ring), 2.02 (m, 4H, -CH₂-CH=CH-CH₂-), 1.73 (m, 2H, -CH₂ β to ring), 1.26 (br.s, 20H, chain CH₂), 0.88 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 180.33, 178.95, 176.77, 134.94, 133.04, 129.51, 34.15, 31.98, 29.45, 23.74, 14.17.

MS (m/z, %): (M⁺) 470 (8), M⁺ 469 (48), 356 (15), 260 (38), 246 (23), 232 (100).

Anal: Calcd. for C₃₂H₄₃N₃: C, 81.87; H, 9.16; N, 8.95. Found: C, 81.36; H, 9.39; N, 8.82%.

3-[(8Z,11R)-11-Hydroxyheptadec-8-enyl]-5,6-diphenyl-1,2,4-triazine (3k)

IR (KBr): 2930, 2850, 1585, 776 cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ 8.03-7.21 (m, 10 H, Ar-H), 5.36 (m, 2H, -CH=CH-), 3.89 (m, 1H, -CHOH), 2.61 (t, 2H, J = 7.5 Hz, -CH₂ α to ring), 2.27 (m, 1H, -CHOH), 2.04 (m, 4H, -CH₂-CH=CH-CH₂-), 1.51 (m, 2H, -CH₂ β to ring), 1.25 (br.s, 18H, chain CH₂), 0.86 (3H, dist.t, CH₃).

¹³C NMR (100 MHz, CDCl₃): 178.71, 177.09, 175.09, 175.06, 130.56, 130.47, 128.78, 71.67, 37.06, 34.30, 31.64, 28.88, 24.30, 14.15.

MS (m/z, %): (M⁺) 486 (22), M⁺ 485 (38), 400 (28), 260 (44), 246 (23), 232 (100).

Anal: Calcd. for C₃₂H₄₃N₃O: C, 79.17; H, 8.86; N, 8.65. Found: C, 79.20; H, 8.89; N, 8.60%.

3-[(8R,11Z)-8-Hydroxyheptadec-11-enyl]-5,6-diphenyl-1,2,4-triazine (3l)

IR (KBr): 2940, 2866, 1585, 778 cm⁻¹.

¹H NMR (300 MHz, CDCl₃): δ 7.35-7.00 (m, 10 H, Ar-H), 5.46 (m, 2H, -CH=CH-), 3.91 (m, 1H, -CHOH), 2.54 (t, 2H, J = 7.5 Hz, -CH₂ α to ring), 2.36 (m, 1H, -CHOH), 2.02 (m, 4H, -CH₂-CH=CH-CH₂-), 1.73 (m, 2H, -CH₂ β to ring), 1.38 (br.s, 18H, chain CH₂), 0.88 (3H, dist.t, CH₃).

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$^{13}$C NMR (100 MHz, CDCl$_3$): 178.71, 176.77, 174.62, 130.56, 130.47, 128.78, 71.67, 37.06, 34.60, 33.96, 31.68, 28.90, 24.29, 14.50.

MS (m/z, %): (M+1)$^+$ 486 (24), M$^+$ 485 (34), 360 (56), 260 (67), 246 (82), 232 (100).

Anal. Calcd. for C$_{32}$H$_{43}$N$_3$O: C, 79.17; H, 8.86; N, 8.65. Found: C, 79.16; H, 8.90; N, 8.59 %.
6.5 References


3.5.6-Trisubstituted-1,2,4-triazines


3,5,6-Trisubstituted-1,2,4-triazines


Chapter-7

Isoxazoles and triazoles
7.1 Theoretical

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. Isoxazole derivatives have been reported to possess interesting biological activities and pharmacological activities such as analgesic, anti-inflammatory and hypoglycemic\textsuperscript{1,2}. The alkoxy carbonyl isoxazoles derivatives showed marked action as a diuretic\textsuperscript{3}. Also, alkoxy carbonyl isoxazoles are important precursors for the synthesis of other compounds such as agrochemicals and microbicides\textsuperscript{4}. In addition, 4,5-dihydroisoxazoles are recognized as useful intermediates in organic synthesis. For example they can be converted into various important synthetic units such as \(\beta\)-hydroxy ketones\textsuperscript{5}, \(\gamma\)-amino alcohols\textsuperscript{6}, \(\beta\), \(\gamma\)-unsaturated ketones\textsuperscript{7} and \(\beta\)-hydroxy nitriles\textsuperscript{8}.

1,2,3-triazoles derivatives have received much attention because of their wide range of applications\textsuperscript{9} and biological activities such as anti-HIV\textsuperscript{10}, antimicrobial agents\textsuperscript{11}, \(\beta_3\)-adrenergic receptor agonist\textsuperscript{12} and are important five-membered nitrogen heterocycles involved in a wide range of industrial applications\textsuperscript{13}. 1,3-Dipolar cycloaddition of azides and alkynes is one of the most useful method for the synthesis of 1,2,3-triazoles.

Galactosides and lactosides bearing isoxazoles (iii), regiospecifically prepared by Gigue're \textit{et al.}\textsuperscript{14} by [1,3]-dipolar cycloadditions between alkynes (i), azides or nitrile oxides, provided specific galectin-1 and -3 inhibitors with potencies as low as 20 \(\mu\)M (Scheme 7.1).
Isoxazoles and triazoles

1,3-Dipolar cycloaddition reactions of carbohydrate dipolarophiles (iv) with cerium (IV) ammonium nitrate CAN (IV) in acetone, acetophenone, or pinacolone yielded the corresponding 3,5-disubstituted-isoxazoles (v), stable pharmacophores for glycomimetic syntheses (Scheme 7.2).

Heteropolyanions (HPA) with different structures, including Keggin, Dawson, Preyssler, mixed addenda, and sandwich types, catalyzed the formation of 1,3- diphenyl-isoxazole (viii) from the condensation of 1,3-diphenyl-propane-1,3-dione (vi) and hydroxylamine hydrochloride (vii) in different solvents and under heating conditions (Scheme 7.3). The study indicates that H₃PW₁₁CuO₄₀ is the catalyst of choice and could
catalyze the synthesis of other isoxazole derivatives in high yields and good selectivities\(^\text{16}\).

\[
\begin{align*}
\text{(vi)} & \quad \text{(vii)} & \quad \text{(viii)} \\
\end{align*}
\]

Scheme 7.3

The one-pot synthesis of 5-carboxyisoxazoles (x) from the cyclocondensation of \(\beta\)-alkoxyvinyl trichloromethyl ketones (ix) \([\text{CCl}_3\text{C}(\text{O})\text{C}(\text{R}_2)=\text{C}(\text{R}_1)\text{OR}], \text{where } \text{R}_1, \text{R}_2=\text{H}, \text{Me and } \text{R}=\text{Me, Et}\) and 2-trichloroacetyl cyclohexanone with hydroxylamine is reported\(^\text{17}\). This work shows that the trichloromethyl group attached to \(\beta\)-alkoxyvinyl trichloromethyl ketones (a heterocyclic CCC building block) is an excellent carboxyl group precursor (Scheme 7.4).

\[
\begin{align*}
\text{(ix)} & \quad \text{(x)} \\
\end{align*}
\]

Scheme 7.4

A practical and efficient liquid-phase synthesis of 3,4,5-trisubstituted isoxazoles using poly(ethylene glycol) as support is described. Soluble polymer-supported nitrile oxide generated \textit{in situ} reacted with chalcones to afford polymer-supported isoxazolines (xvi) which were cleaved by sodium methoxide to generate 3,4,5-trisubstituted
Isoxazoles and triazoles

Isoxazoles\textsuperscript{18} (xvii) (Scheme 7.5). This sequential process provided a novel method to synthesize 3,4,5-trisubstituted isoxazoles.

Scheme 7.5

3-Hydroxy benzofuran substituted-isoxazoles (xx) were synthesized by Swamy and Agasimundin\textsuperscript{19} by the reaction of 2-acetyl-3-hydroxybenzofuran (xviii) with different aromatic aldehydes in the presence of strong base, which undergoes cyclocondensation with hydroxylamine hydrochloride (Scheme 7.6).

Scheme 7.6
7.2 Synthesis of Novel 3,5-Disubstituted-dihydroisoxazoles, Isoxazoles, Triazole and Triazolo-tetrazole from Methyl undec-10-enoate and Methyl undec-10-ynoate

The azole ring has also attracted considerable attention in the design of biologically active molecules and advanced organic materials since it can be readily transformed into various bioactive agents\(^\text{20}\). From this point of view, isoxazole appears well suited possessing three positions to be exploited for diversity. This scaffold (and the related isoxazoline) represents a pharmacophore itself as it has been observed in several substances showing activity against a broad range of therapeutic targets. Several methods have been reported for the synthesis of isoxazoles\(^\text{21-24}\). The reaction of dipolarophiles (alkene and alkyne) with cerium (IV) ammonium nitrate, Ce\((\text{NH}_4)_2(\text{NO}_3)_6\), in different ketones under reflux gave isoxazole derivatives containing alkoxy carbonyl. CAN (IV) has been used for a variety of oxidative transformations including \(\alpha\)-nitration of ketones to nitrile oxides under acidic conditions.

The synthesis of heterocyclic moieties from fatty acids under various conditions has been reported earlier\(^\text{25}\). To further extend efforts towards this goal, the synthesis of isoxazole, bis-isoxazole, triazole and triazolo-tetrazole compounds from methyl undec-10-enoate and methyl undec-10-ynoate utilizing CAN (IV) is described.

7.3 Results and discussion

In this chapter, the straightforward synthesis of 3,5-disubstituted-dihydroisoxazoles possessing ester moiety by one-pot process is reported. The cycloaddition of dipolarophiles- methyl undec-10-enoate (1) and methyl undec-10-ynoate (4) with CAN (IV) and several ketones 2 under reflux gave the corresponding isoxazoles efficiently. On refluxing, 1 and CAN (IV) in acetone as solvent 3-acetyl-4,5-dihydroxazole (3a) was obtained in appreciable yield (Scheme 7.7) (Table 7.1). Beside bands for normal fatty ester bond, IR spectra of 3a showed strong absorption bands at
Isoxazoles and triazoles

1640 (C=N), 1587 (C-O) cm\(^{-1}\). In \(^1\)H NMR spectrum appearance of signal at \(\delta 3.14\) attributed to one methyne proton of dihydroisoxazole ring. The product formation 3a was further strengthened by characteristic signal in \(^{13}\)C NMR at 167.98 and 66.71 for C-3 and C-5 respectively. When 2-chloro-3-butanoate (2c) and alkenoate 1 was used in ratio of 2:1 3c was obtained in 78% yield. The bis-isoxazole 3d was obtained in 70% yield as cycloaddition product of alkenoate 1, 1,4-diacetylbenzene (2d) and CAN (IV) in dichloromethane as solvent. The compound 3d was characterized by IR bands at 1657 (C=N) and 1584 (C-O) cm\(^{-1}\). The M\(^+\) at 612 in mass spectrum and \(^{13}\)C NMR values at 168.45 and 65.78 for C-3 and C-5 establish the structure of 3d. The \(^1\)H NMR, mass spectra and elemental analysis data further support the synthesis.

\[
\begin{align*}
\text{H}_2\text{C}=\text{C}-\text{CH}_3 & \quad + \quad \text{H}_2\text{C}=-\text{CO}-\text{CH}_3 \\
\text{CAN} & \quad \rightarrow \quad \text{R} \quad \text{C}_5\text{H}_4\text{COCH}_3
\end{align*}
\]

\(R = 2a, -\text{CH}_3, 2b, -\text{C}_6\text{H}_5, 2c, -\text{CHCl}-\text{CH}_3, 2d, -\text{C}_6\text{H}_4\text{COCH}_3\)

Scheme 7.7 Synthesis of 3,5-disubstituted-dihydroisoxazoles and bis-isoxazole

Moreover, in similar reactions using methyl undec-10-ynoate (4) and CAN (IV) in acetophenone the corresponding isoxazoles 5b was obtained (Scheme 7.8). On refluxing, 4 and CAN (IV) in acetone as solvent 3-acetyl-4,5-isoxazole (5a) was obtained in appreciable yield (Table 7.1). The compound 5c was obtained in appreciable yield on refluxing 2-chloro-3-butanoate (2c) and alkyanoate 4 for 18 hours. The bands at 1642 (C=N), 1590 (C-O) cm\(^{-1}\) in IR and \(\delta 5.41\) for single proton of ring and signals at 148.12,
Isoxazoles and triazoles

109.45, 158.85 in $^{13}$C NMR established the structure of 5c. The IR spectra of 5d showed strong absorption bands at 1658 (C=N), 1585 (C-O) cm\(^{-1}\). The $^1$H NMR spectra peak at $\delta$ 5.39 and $^{13}$C NMR signal at 150.01, 105.54 and 158.45 for C-3, C-4 and C-5 support the formation of bis-isoxazole. The structure of 5d was further established by its mass spectral studies, which showed molecular ion peak at $m/z$ 608 consistent with its molecular formula C\(_{34}\)H\(_{44}\)N\(_2\)O\(_8\).

Scheme 7.8 Synthesis of 3,5-disubstituted-isoxazoles and bis-isoxazole

Table 7.1 3,5-Disubstituted-isoxazoles

<table>
<thead>
<tr>
<th>Entry</th>
<th>Dipolarophile</th>
<th>Ketone (2a-d)</th>
<th>Time (h)</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2a</td>
<td>14</td>
<td>3a</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2b</td>
<td>16</td>
<td>3b</td>
<td>80</td>
</tr>
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<td>17</td>
<td>3c</td>
<td>77</td>
</tr>
<tr>
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<td>1</td>
<td>2d</td>
<td>19</td>
<td>3d</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>2a</td>
<td>17</td>
<td>5a</td>
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<td>2b</td>
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<td>7</td>
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<td>2c</td>
<td>18</td>
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<td>70</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>2d</td>
<td>20</td>
<td>5d</td>
<td>72</td>
</tr>
</tbody>
</table>

To enrich the scanty field of heterocycles from fatty acids by the synthesis of novel triazoles from medium-chain terminal alkyne fatty acid is carried out. The cycloaddition of azidoacetonitrile (6) with terminal alkyne gave corresponding triazole 7 which on further reaction with sodium azide and ammonium chloride in DMF gave tetrazole 8 (Scheme 7.9). The $^1$H NMR value at $\delta$ 6.99, 4.09 was obtained for single proton at ring and -CH\(_2\)CN respectively. The characteristic signals in $^{13}$CNMR at $\delta$
139.01 (C-4), 133.56 (C-5), 114.14 (C-2\textsuperscript{"{n}}), 44.71(C-1\textsuperscript{"{n}}) established the formation of triazole 7. The formation of tetrazole ring in compound 8 was exhibited by additional peak of (N-H) at 3381 cm\textsuperscript{-1}. The \textsuperscript{1}H NMR, \textsuperscript{13}CNMR, elemental analysis and mass spectral data further proved the synthesized compound.

\begin{align*}
\text{H} & \quad \text{CH} \\
\text{N} & \quad \text{C} \\
\text{O} & \quad \text{CH}_3 \\
\end{align*}

Scheme 7.9 Synthesis of 1-cyanomethyl-4-(carbomethoxyoctyl)-1,2,3-triazole and [1-(1H-tetrazol-5-yl-methyl)-4-carbomethoxyoctyl]-(1,2,3)-triazole

One more representative of 1,2,3-triazole 10 was synthesized in appreciable yield by utilizing dipolarophile 4, benzyl chloride 9, sodium azide in DMF (Scheme 7.10). The synthesis of all the newly synthesized compounds was fully supported by mass spectral data and elemental analysis. The detailed spectra are given in experimental section.

\begin{align*}
\text{H} & \quad \text{CH} \\
\text{C} & \quad \text{N} \\
\text{O} & \quad \text{CH}_3 \\
\text{Cl} & \quad \text{Cl} \\
\end{align*}

Scheme 7.10 Synthesis of 1-benzyl-4-(carbomethoxyoctyl)-1,2,3-triazole
7.4 Experimental

The source of undec-10-enoic acid and instrumentation details are the same as given in Chapter 1 (page 38).

*General procedure for the synthesis of 3,5-disubstituted-dihydroisoxazole (3a-c)*

A mixture of methyl undec-10-enoate 1 (0.6 mmol) and ammonium cerium (IV) nitrate (0.6 mmol) in ketone (2a-b) (5 ml) was stirred under reflux for appropriate time. For the synthesis of 3d, alkenoate 1(0.6 mmol), diacetylbenzene 2d (1.2 mmol) and cerium (IV) ammonium nitrate (0.6 mmol) in CH$_2$Cl$_2$ (5 ml) was stirred under reflux for 19 hours. The formation of product was analyzed by thin layer chromatography. The mixture was extracted with Et$_2$O (10 x 3 ml) and washed with 10% aq. NaHCO$_3$ solution (2 x 2 ml), saturated aqueous NaCl (2 x 2 ml) and water. The ethereal solution was dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuum. The resulting oily crude product was further purified by column chromatography.

*Preparation of methyl undec-10-ynoate (4)*

To an acid solution of commercial 10-undecenoic acid (0.022 mole) in CCl$_4$ (15 ml), bromine (0.015mole), was added dropwise. When the addition was over, the mixture was stirred for 3 hours and left overnight. Distillation of CCl$_4$ and work up with Et$_2$O furnished 10, 11-dibromoundecanoic acid as a thick viscous liquid. Dibromide, KOH, water, ethanol were taken in a flask and refluxed for 10 hours. Most of the alcohol was removed under vacuum. The product was dissolved in water, acidified with cold dil. H$_2$SO$_4$, extracted with Et$_2$O. The extracts were washed with water and dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuum. The removal of solvent yields the product which was recrystallized from petroleum-ether. (M.p. 41-42 °C). The acetylenic acid was esterified with dimethyl metherate to form methyl undec-10-ynoate 4.
**General procedure for the synthesis of 3,5-disubstituted-isoxazole (5a-c)**

A mixture of alkynoate 4 (0.6 mmol) and CAN (IV) (0.6 mmol) in ketone (2a-b) (5 ml) was stirred under reflux for appropriate time. The 5d was obtained on refluxing 4 (0.6 mmol), CAN (IV) (0.6 mmol) and 2d (1.2 mmol) in CH₂Cl₂ for 20 hours. The progress of reaction was monitored by thin layer chromatography. The mixture was extracted with Et₂O and washed with aqueous 10% NaHCO₃ solution (2 x 2 ml), saturated aqueous NaCl (2 x 2 ml) and water. The ethereal solution was dried over anhydrous Na₂SO₄ and concentrated in vacuum. The resulting oily crude product was further purified by column chromatography.

**Procedure for the synthesis of azidoacetonitrile (6)**

To an emulsion chloroacetonitrile (0.7 mmol) in 10 ml of water was added NaN₃ (0.8 mmol) at vigorous stirring, and the mixture was slowly heated to 70°C then the reaction mixture was refluxed at 80-85°C for 2 hours. Then it was cooled to 20-25°C and poured into 5 ml EtOAc. The organic layer was separated, the water layer was extracted with EtOAc, and the extract was dried on anhydrous Na₂SO₄ and concentrated in vacuum.

**Synthesis of l-cyanomethyl-4-(carbomethoxyoctyl)-1,2,3-triazole (7)**

A mixture of alkynoate 4 (0.4 mmol) and azidoacetonitrile 6 (0.6 mmol) in 5 ml of alcohol was stirred under reflux for 10 hours and product formation was detected by TLC. The crude product was concentrated in vacuum and purified by column chromatography as viscous oily compound in 70% yield.

**Synthesis of [1-(1H-tetrazol-5-yl-methyl)-4-carbomethoxyoctyl]-1,2,3-triazole (8)**

A suspension of 4.5 mmol of NaN₃ and 4.5 mmol of ammonium chloride was dissolved at stirring and heating in 5 ml of DMF, then the solution was cooled, and 4mmol of
compound 7 was added. The mixture was stirred for 20 hours at 120°C. The reaction mixture was cooled, the precipitate was filtered off, and the filtrate was evaporated in vacuum. The residue after removal was poured in water and wash with ether, acidified with HCl till the pH becomes 2, then the solution was extracted with EtOAc dried on anhydrous Na₂SO₄ and concentrated in vacuum and obtained in 61% yield as yellow oily compound.

**Synthesis of 1-benzyl-4-(carbomethoxyoctyl)-1,2,3-triazole (10)**

The alkynoate 4 (0.4 mmol), benzyl chloride (0.4 mmol), and NaN₃ (0.5 mmol), was taken in 5 ml DMF and stirred under refluxing for 16 hours and product formation was detected by TLC. Then it was cooled to 20-25 °C and poured into 5 ml CHCl₃ and wash with water. The organic layer was separated, and the extract was dried on anhydrous Na₂SO₄ and concentrated in vacuum. The crude product was purified by column chromatography and obtained as oily liquid in 64% yield.

**Spectroscopic data**

3-Acetyl-5-(carbomethoxyoctyl)-4,5-dihydroisoxazole (3a)

**IR (KBr):** 1739, 1710, 1640, 1587 cm⁻¹.

**¹H NMR (400 MHz, CDCl₃):** δ 3.66 (s, 3H, -COOC₂H₃), 3.14 (m, 1H, -CH- ring), 2.35 (t, 2H, CH₂-COOCH₃), 2.12 (s, 3H, COCH₃), 1.97-1.19 (br.s 16H chain and ring CH₂).

**¹³C NMR (100 MHz, CDCl₃):** 196.95, 172.31, 167.98, 66.71, 50.37, 34.00, 31.86, 29.70, 29.50, 29.22, 27.13, 24.85, 24.62, 14.15.

**MS (m/z, %):** (M+1)^+ 284 (14), (M⁺) 283 (60), 224 (14), 209 (48), 210 (26), 168 (63).

**Anal. Calcd.** for C₁₅H₂₅N0₄: C, 63.59; H, 8.88; N, 4.94. **Found:** C, 63.01; H, 8.83; N, 4.98.
3-Benzoyl-5-(carbomethoxyoctyl)-4,5-dihydroisoxazole (3b)

IR (KBr): 1740, 1712, 1655, 1580 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 7.83 (m, 2H, Ar-H), 7.51 (m, 3H, Ar-H), 3.67 (s, 3H, -COOC₂H₃), 3.14 (m, 1H, -CH- ring), 2.33 (t, 2H, CH₂-COOCH₃), 1.66-1.21 (br.s 16H, chain and ring CH₂).

¹³C NMR (100 MHz, CDCl₃): 194.60, 172.46, 167.83, 134.92, 133.01, 129.93, 129.05, 67.10, 50.62, 34.09, 31.95, 29.72, 29.09, 22.72, 14.17.

MS (m/z, %): (M+1)⁺ 346 (30), (M⁺) 345 (12), 286 (86), 258 (40), 244 (55), 174 (48).

Anal. Calcd. for C₂₀H₂₇NO₄: C, 69.55; H, 7.87; N, 4.05. Found: C, 69.98; H, 7.81; N, 4.09.

3-(2'-Chloropropanoyl)-5-(carbomethoxyoctyl)-4,5-dihydroisoxazole (3c)

IR (KBr): 1738, 1715, 1667, 1590 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 4.39 (q, 1H, CH-Cl), 3.66 (s, 3H, -COOC₂H₃), 3.16 (m, 1H, -CH- ring), 2.34 (t, 2H, -CH₂-COOCH₃), 1.86-1.20 (br.s, 19H, chain, ring CH₂ and -CH₃).

¹³C NMR (100 MHz, CDCl₃): 196.67, 172.43, 167.98, 66.78, 54.71, 50.62, 34.39, 33.82, 29.74, 29.27, 28.92, 14.16.

MS (m/z, %): (M+1)⁺ 333 (13), (M⁺) 332 (58), 245 (72), 231 (20), 203 (36), 175 (61).

Anal. Calcd. for C₁₆H₂₆ClNO₄: C, 57.92; H, 7.89; N, 4.22. Found: C, 57.21; H, 7.85; N, 4.27.

Bis-(3-Benzoyl-5-(carbomethoxyoctyl))-4,5-dihydroisoxazole (3d)

IR (KBr): 1745, 1715, 1657, 1584 cm⁻¹.

¹H NMR (400 MHz, CDCl₃): δ 7.64-7.49 (m, 4H, Ar-H), 3.67 (s, 6H, -COOC₂H₃), 3.26 (m, 2H, -CH- ring), 2.33 (t, 4H, -CH₂-COOCH₃), 1.92-1.13 (br.s 32H, chain and ring CH₂).
$^{13}$C NMR (100 MHz, CDCl$_3$): 196.95, 180.10, 172.76, 168.45, 134.92, 133.01, 129.93, 129.05, 65.78, 52.62, 34.09, 31.95, 29.72, 29.09, 22.72, 14.19.

$MS$ (m/z, %): (M+1)$^+$ 613 (17), (M$^+$) 612 (22), 524 (34), 488 (28), 392 (10), 178 (29).

$Anal. Calcd.$ for C$_{34}$H$_{48}$N$_2$O$_8$: C, 66.87; H, 7.59; N, 4.58. Found: C, 66.50; H, 7.55; N, 4.62.

3-Acetyl-5-(carbomethoxyoctyl)-isoxazole (5a)

IR (KBr): 1742, 1720, 1662, 1590 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.44 (s, 1H, -CH- ring), 3.66 (s, 3H, -COOCH$_3$), 2.14 (s, 3H, -CO-CH$_3$), 2.32 (t, 2H, -CH$_2$-COOCH$_3$), 1.78-1.08 (br.s 14H, chain CH$_2$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 196.95, 171.92, 159.07, 149.77, 106.43, 52.51, 34.25, 33.77, 29.05, 28.88, 24.73, 14.17.

$MS$ (m/z, %): (M+1)$^+$ 282 (13), (M$^+$) 281 (37), 222 (18), 207 (70), 199 (14), 166 (26).

$Anal. Calcd.$ for C$_{15}$H$_{23}$NO$_4$: C, 64.04; H, 8.23; N, 4.98. Found: C, 64.68; H, 8.29; N, 4.95.

3-Benzoyl-5-(carbomethoxyoctyl)-isoxazole (5b)

IR (KBr): 1744, 1718, 1646, 1593, cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.82 (m, 2H, Ar-H), 7.49 (m, 3H, Ar-H), 5.31 (s, 1H-CH-ring), 3.66 (s, 3H, COOCH$_3$), 2.34 (t, 2H, -CH$_2$-COOCH$_3$), 1.92-1.14 (br.s, 14H, chain CH$_2$).

$^{13}$C NMR (100 MHz, CDCl$_3$): 196.96, 172.74, 159.96, 147.95, 133.58, 129.90, 127.77, 121.05, 117.81, 102.29, 52.62, 34.45, 31.41, 29.65, 29.09, 28.99, 22.45, 14.16.

$MS$ (m/z, %): (M+1)$^+$ 344 (18), (M$^+$) 343 (25), 284 (52), 269 (74), 200 (39), 172 (44).

$Anal. Calcd.$ for C$_{20}$H$_{25}$NO$_4$: C, 69.96; H, 7.33; N, 4.07. Found: C, 69.31; H, 7.38; N, 4.03.
3-(2'-Chloropropanoyl)-5-(carbomethoxyoctyl)-isoxazole (5c)

**IR** (KBr): 1747, 1722, 1642, 1590 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 5.41 (s, 1H, -CH- ring), 4.32 (q, 1H, -CH-Cl), 3.66 (s, 3H, -COOCH₃), 2.33 (t, 2H, -CH₂-COOCH₃), 1.80-1.25 (br.s 17H, chain CH₂ and -CH₃).

**¹³C NMR** (100 MHz, CDCl₃): 189.20, 172.48, 158.85, 148.12, 109.45, 60.95, 37.22, 34.12, 31.88, 29.16, 25.08, 23.13, 22.53, 14.76, 15.02.

**MS** (m/z, %): (M⁺) 331 (23), (M⁺) 330 (64), 256 (77), 242 (80), 186 (54), 158 (28).


Bis-(3-Benzoyl-5-(carbomethoxyoctyl))-isoxazole (5d)

**IR** (KBr): 1738, 1717, 1658, 1585 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 7.52-7.31 (m, 4H, Ar-H), 5.39 (s, 2H, -CH₂ ring), 3.66 (s, 6H, COOCH₃), 2.35 (t, 4H, CH₂-COOCH₃), 1.14 (br.s 28H, chain CH₂).

**¹³C NMR** (100 MHz, CDCl₃): 190.21, 172.99, 158.45, 150.01, 134.66, 129.59, 128.32, 105.54, 50.62, 33.31, 32.19, 29.7, 24.91, 15.13.

**MS** (m/z, %): 609 (M⁺+1, 13), 608 (M⁺) (88), 490 (49), 432 (88), 348 (67), 288 (72).

**Anal. Calcd.** for C₃₄H₄₄N₂O₈: C, 67.09; H, 7.28; N, 4.60. Found: C, 67.56; H, 7.24; N, 4.65.

1-Cyanomethyl-4-(carbomethoxyoctyl)-1,2,3-triazole (7)

**IR** (KBr): 1740, 1540 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 6.99 (s, 1H, -CH ring), 4.09 (s, 2H, -CH₂-CN), 3.66 (s, 3H, -COOCH₃), 2.34 (t, 2H, CH₂-COOCH₃), 2.51 (m, 2H, -CH₂α to ring), 1.23 (br.s, 12 H, chain CH₂).

**¹³C NMR** (100 MHz, CDCl₃): 172.44, 139.01, 133.56, 114.14, 50.62, 44.71, 34.71, 29.20, 28.88, 24.73, 14.01.

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**MS (m/z, %):** (M+1)^+ 279 (23), (M^) 278 (60), 219 (68), 204 (80), 191 (46), 121 (65).

**Anal. Calcd.** for C_{14}H_{22}N_{4}O_{2}: C, 60.43; H, 7.96; N, 20.12. Found: C, 60.01; H, 7.92; N, 20.17.

[1-(1H-Tetrazol-5-yl-methyl)-4-carbomethoxyoctyl]-(1,2,3) triazole (8)

**IR (KBr):** 3381, 1745, 1538 cm^{-1}.

**^1H NMR (400 MHz, CDCl₃):** δ 8.31 (s, 1H, N-H), 6.89 (s, 1H, -CH ring), 4.86 (s, 2H, CH₂-Ar), 3.67 (s, 3H, COOCH₃), 2.33 (t, 2H, CH₂-COOCH₃), 2.07 (m, 2H, -CH₂ α to ring), 1.20 (br.s, 12 H, chain CH₃).

**^13C NMR (100 MHz, CDCl₃):** 172.66, 144.31, 130.57, 130.49, 129.12, 128.53, 52.16, 49.88, 37.22, 33.76, 31.88, 29.36, 25.68, 23.13. 15.12.

**MS (m/z, %):** (M+1)^+ 322 (17), (M^) 321 (30), 262 (49), 220 (66), 178 (38), 150 (33).

**Anal. Calcd.** for C_{14}H_{23}N_{7}O_{2}: C, 52.34; H, 7.21; N, 30.49. Found: C, 52.71; H, 7, 26; N, 30.01.

1-Benzyl-4-(carbomethoxyoctyl)-1,2,3-triazole (10)

**IR (KBr):** 1734, 1535 cm^{-1}.

**^1H NMR (400 MHz, CDCl₃):** δ 7.10-7.01 (m, 5H, Ar-H), 6.88 (s, 1H, -CH ring), 4.31 (s, 2H, CH₂-Ar), 3.67 (s, 3H, COOCH₃), 2.34 (t, 2H, CH₂-COOCH₃), 1.99 (m, 2H, -CH₂ α to ring), 1.42 (br.s, 12 H, chain CH₃).

**^13C NMR (100 MHz, CDCl₃):** 172.10, 141.80, 133.58, 129.90, 129.75, 127.89, 59.10, 50.81, 33.76, 31.88, 29.36, 25.68, 23.13. 15.12.

**MS (m/z, %):** (M+1)^+ 330 (19), (M^) 329 (47), 270 (67), 255 (79), 214 (41), 158 (26).

**Anal. Calcd.** for C_{19}H_{27}N_{3}O_{2}: C, 69.28; H, 8.25; N, 12.75. Found: C, 69.70; H, 8.61; N, 12.71.
7.5 References


Publications & Presentations
Publications and Presentations
Papers published/Accepted/Communicated

1 Microwave assisted efficient one-pot synthesis of 3,5,6-trisubstituted-1,2,4-triazines from fatty acid hydrazides under solvent-free conditions and their antimicrobial activity.

2 One-pot synthesis, antibacterial and antifungal activities of novel 2,5-disubstituted-1,3,4-oxadiazoles.

3 Synthesis, antibacterial and antifungal activity of some novel 3,5-disubstituted-1H-1,2,4-triazoles

4 Convenient one-pot synthesis of novel 2-substituted benzimidazoles, tetrahydrobenzimidazoles and imidazoles and evaluation of their in vitro antibacterial and antifungal activities

5 Green Chemistry approach to the sustainable advancement to the synthesis of heterocyclic chemistry
6 A simple, rapid and efficient one-pot protocol for the synthesis of 2-substituted benzothiazole derivatives and their antimicrobial screening.

7 Solvent free synthesis of 2-alkyl and 2-alkenylbenzothiazoles from fatty acids under microwave irradiation.

8 Silica-gel-supported phosphorus pentoxide: a simple and efficient solid-supported reagent for esterification of long-chain carboxylic acids and their antimicrobial screening

9 Microwave assisted synthesis of 2-long alkenyl chain benzoxazoles and naphtho[2,3-d]-oxazoles and their antimicrobial evaluation
S. Gangal, S. Sharma, A. Rauf, Phosphorus, Sulphur, Silicon (Accepted)

10 Isolation and characterization of unusual fatty acids in Prosopis cineraria seed oil
S. Gangal, S. Sharma, A. Rauf, Chemistry of Natural Compounds (Accepted)

11 An efficient, one-pot synthesis of novel 3,5-disubstituted-1,2,4-oxadiazoles from long-chain carboxylic acid derivatives
S. Sharma, S. Gangal, A. Rauf, Acta Chimica Slovenica (Communicated)
Synthesis of novel 3,5-disubstituted-dihydroisoxazoles, isoxazoles, triazole and tetrazolo-triazole from methyl undec-10-enoate and methyl undec-10-ynoate

*S. Sharma, A. Rauf, Turkish Journal of Chemistry (Communicated)*

### Papers Presented

1. **A. Rauf and S. Sharma**
   
   Efficient one-pot synthesis of 5 alkyl-3 phenyl-1,2,4 triazoles from fatty acids hydrazides in 25th Silver Jubilee Conference, Indian Council of Chemists held on December, 2006 at Birla College, Kalyan, Mumbai.

2. **A. Rauf and S. Sharma**
   
   A base catalysed synthesis of 3,5-disubstituted-1,2,4-triazoles from fatty acid hydrazides, 9th CRSI National Symposium in Chemistry, 1-4, Feb’ 2007, Department of Chemistry, University of Delhi.

3. **A. Rauf and S. Sharma**
   
   Microwave assisted efficient one-pot synthesis of 3,5,6-trisubstituted-1,2,4-triazines from fatty acid hydrazides under solvent free conditions and their antimicrobial activity, National Seminar on Green Chemistry and Natural Products, 26-27 Nov’ 2007, Department of Chemistry, University of Delhi.

4. **A. Rauf and S. Sharma**
   
   An efficient one-pot protocol for the synthesis of novel 2,5-disubstituted-1,3,4-oxadiazoles from long-chain alkanoic and alkenoic acids and their antimicrobial screening, 26th, Annual Conference, Indian Council of Chemists, Department of Chemistry, Dr. H. S. Gour University, Sagar, Madhya Pradesh.