CHAPTER 3

2.5-Di substituted-1,3,4-thiadiazoles
3.1. Theoretical

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

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

The substituted 1,3,4-thiadiazoles have been very useful in agriculture and many fields of technology such as dyes, lubricating compositions, analytical reagents, optically active liquid crystals, photographic materials and many other uses. The 2-amino-5-phenyl-1,3,4-thiadiazole (APT) (iv) and its derivatives are found to inhibit corrosion of steel and copper.
The macrocyclic polyether compounds containing a 1,3,4-thiadiazole moiety (n-MCTH) (v) has been used in the corrosion inhibition of C38 carbon steel in acidic media\textsuperscript{26}.

![Chemical Structure](image)

$v \quad n=1-5$

The mercapto-5-$R$-amino-1,3,4-thiadiazole derivatives have been studied for their electrochemical behaviors at carbon paste electrode\textsuperscript{27}. The poly(2-amino-5-mercapto-1,3,4-thiadiazole) (PMAT) (vi) film has been used for selective determination of L-cysteine\textsuperscript{28} and folic acid\textsuperscript{29}.

![Chemical Structure](image)

$vi$

The 2,5-dimercapto-1,3,4-thiadiazole and its analogs have been found to possess a broad range of potential applications such as solid state organic crystals, bioorganic catalysis etc\textsuperscript{30}. The thiadiazoles containing the imine (-C=N-) group have long been of interest as luminophores for optical applications owing to their electron accepting nature\textsuperscript{31}.

The immobilization of 5-amino-1,3,4-thiadiazole-thiol (ATT) onto analogue of heulandite (vii) for divalent toxic metals removal has also been reported\textsuperscript{32}. 

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The discovery of the diverse pharmacological and material properties of 1,3,4-thiadiazoles and its derivatives has stimulated a substantial interest in the chemistry and synthesis of these important heterocycles and their analogs.

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

\[
\text{ArCOOH} \rightarrow \text{POCl}_3
\]

\[\text{viii} \rightarrow \text{ix}\]

\(\text{Ar} = \text{4-Br-C}_6\text{H}_4, \text{C}_6\text{H}_5, \text{4-NO}_2\text{-C}_6\text{H}_4, \text{4-CH}_3\text{O-C}_6\text{H}_4\)

**Scheme 3.1.1**

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

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

Scheme 3.1.3

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

\[ \text{ArO}^- \text{OH} + \text{H}_2\text{N}^-\text{S}^-\text{NH}_2 \xrightarrow{\text{MW}, 8 \text{ min}} \text{PEG'OP(O)Cl}_2 \rightarrow \text{N}^-\text{N}^-\text{NH}, \]

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

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

**Scheme 3.1.4**

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

\[ \text{Ar}^- \quad \text{Phenyl, Methyl, H.} \]

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

**Scheme 3.1.5**
2,5-Disubstituted-1,3,4-thiadiazoles

\[
\text{Ar'} \quad \text{N—N} \quad \text{II} \\
\text{NH}_2
\]

\[
\text{H} \quad \text{SH} + \text{Ar'}
\]

Toluene sulfonic acid

DMF

\[
\text{N—N} \quad \text{II} \\
\text{N—H}
\]

\[
\text{xxvii} \quad \text{xx} \quad \text{xxi}
\]

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

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

Scheme 3.1.6

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

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

Scheme 3.1.7

\[
\text{xxii} \quad \text{Desulfurization} \quad \text{xxiii}
\]

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

R= NHCH₂CH₂CH₂CH₃, NH-C₆H₁₁.

Scheme 3.1.8

 Aryl-N-[(4-[2-(2-chlorophenyl)-4-oxo-(3-hydroquinazolin-3-yl)]-phenyl)-
carbonylamino)-amino]-thioxomethyl]-amides (xxvi) when stirred in concentrated
sulphuric acid gave aryl-N-5-{4-[2-(2-chlorophenyl)-4-oxo-(3-hydroquinazolin-3-yl)]-
phenyl}-1,3,4-thiadiazole-2-yl-amides (xxvii) in excellent yields⁴⁰ (Scheme 3.1.9).

Scheme 3.1.9

Pintilie et al.⁴¹ synthesized the 2-[1-(3-3-nitrobenzoylamino)-3-(methylthio)]-
propyl-5-(alkyl/phenyl) amino-1,3,4-thiadiazoles (xxix) from 1-[N-(3-nitrobenzoyl)-D,L-
methionyl]-4-alkyl/phenyl-thiosemicarbazides (xxviii) by stirring at room temperature
with concentrated sulphuric acid (Scheme 3.1.10).
2,5-Disubstituted-1,3,4-thiadiazoles

Scheme 3.1.10

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

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

Scheme 3.1.11

The \(N\)-ethyl-5-substituted-1,3,4-thiadiazol-2-amines (xxxiii), with high cytotoxicity were synthesized from \(N\)-ethyl hydrazinecarbothioamides (xxxii) in sulfuric acid medium\(^{43}\) (Scheme 3.1.12).

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The thiosemicarbazide of 6-chloro-2-aminobenzothiazole ([xxxiv]) on cyclization with different carboxylic acids in phosphorus oxy chloride provided the corresponding 2-aryl-5-(6-chloro-1,3-benzothiazole-2-yl-amino)-1,3,4-thiadiazole ([xxxv]) of pharmaceutical importance\(^\text{44}\) ([Scheme 3.1.13]).

Padmavathi et al.\(^\text{45}\) converted 2-arylsulfonylmethyl/arylmethanesulfonylmethyl-5-aryl-1,3,4-oxadiazoles ([xxxvi]) to 2-arylsulfonylmethyl/arylmethanesulfonylmethyl-5-aryl-1,3,4-thiadiazoles ([xxxvii]) by treatment with a two fold excess of thiourea in tetrahydrofuran ([Scheme 3.1.14]).
2,5-Disubstituted-1,3,4-thiadiazoles

The reaction of ester (xxxviii) with hydrazine in ethanol gave the aroyl hydrazides (xxxix) which were converted to 5-substituted-2-mercapto-1,3,4-thiadiazoles (xLi) via potassium aroyl dithiocarbazates (xL)\(^{46}\) (Scheme 3.1.15).

Augustine \textit{et al.}\(^{47}\) have given the propylphosphonic anhydride (T3P) mediated synthesis of 1,3,4-thiadiazoles (xLiv) by the reaction of carboxylic acid (xLii), hydrazide (xLiii) and \(P_2S_5\) in triethylamine (TEA) (Scheme 3.1.16).
Various 1,4-bis(6-substituted)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (xLvi) were synthesized from terphthalic dihydrazide (xLv) via the formation of bis-potassium dithiocarbazinate (xLv) and 1,4-phenylene-bis(4-amino-4H-1,2,4-triazole-3-thiol) (xLvii)\(^{48}\) (Scheme 3.1.17).

Abdelhamid et al.\(^{49}\) reported the synthesis of 2,3-dihydro-1,3,4-thiadiazoles (Li) by stirring a mixture of appropriate hydrazonoyl halide (xLix) with 2-(benzofuran-2-ylcarbonyl)-3-mercapto-3-methylsulfanylacetonitrile (L) in ethanol (Scheme 3.1.18).
2,5-Disubstituted 1,3,4-thiadiazoles

Scheme 3.1.18

A novel approach to 3,6-disubstituted [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (Liv) using silica-supported dichlorophosphate as a recoverable cyclodehydrant, carboxylic acids (Lii) and thiocarbohydrazide (Liii) as starting material is described by Li and Zhao\(^5\) (Scheme 3.1.19).

\[
\begin{align*}
R &= \text{CO}_2\text{C}_2\text{H}_5, \text{CONH}_2\text{C}_6\text{H}_5, \text{COCH}_3, \text{COC}_6\text{H}_5. \\
R &= \text{C}_6\text{H}_5, 4-\text{CH}_3\text{OC}_6\text{H}_4, 2-\text{CH}_3-\text{C}_6\text{H}_4, 2-\text{Cl-}\text{C}_6\text{H}_4, \text{Fur-2-yl}. 
\end{align*}
\]

Scheme 3.1.19

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

4H-1,2,4-triazole-3-thiol (Lv), aryl/aroyl acid and phosphorus oxy chloride for 8-12 h\(^5\) (Scheme 3.1.20).

\[
\begin{array}{c}
\text{Lv} \\
\begin{array}{c}
\text{R} \\
\text{NH}_{2}
\end{array}
\end{array}
\xrightarrow{\text{R\textsubscript{1}COOH, PCl\textsubscript{3}}} 
\begin{array}{c}
\text{Lvi} \\
\begin{array}{c}
\text{R}\textsubscript{1} \\
\text{S}
\end{array}
\end{array}
\]

R = C\textsubscript{6}H\textsubscript{5}-CH\textsubscript{2}, C\textsubscript{6}H\textsubscript{5}-OCH\textsubscript{2}, 2-OHC\textsubscript{6}H\textsubscript{4}.

R\textsubscript{1} = C\textsubscript{6}H\textsubscript{5}CONHCH\textsubscript{2}, 2-Br-C\textsubscript{6}H\textsubscript{4}, 2-C\textsubscript{6}H\textsubscript{5}CO-C\textsubscript{6}H\textsubscript{4}, C\textsubscript{10}H\textsubscript{7}CH\textsubscript{2}, C\textsubscript{8}H\textsubscript{6}NCH\textsubscript{2}, C\textsubscript{6}H\textsubscript{5}COCH\textsubscript{2}CH\textsubscript{2}.

Scheme 3.1.20

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

\[
\begin{array}{c}
\text{Lvii} \quad \text{Lviii} \\
\begin{array}{c}
\text{R} \\
\text{S}
\end{array} & \begin{array}{c}
\text{R\textsubscript{2}} \\
\text{N}
\end{array}
\end{array}
\xrightarrow{\text{Et\textsubscript{3}N}} 
\begin{array}{c}
\text{Lix} \\
\begin{array}{c}
\text{R\textsubscript{1}} \\
\text{R\textsubscript{2}}
\end{array}
\end{array}
\]

R = CH\textsubscript{2}, CH\textsubscript{2}Ph.

R\textsubscript{1} = C\textsubscript{6}H\textsubscript{5}, o-CH\textsubscript{3}C\textsubscript{6}H\textsubscript{4}, p-ClC\textsubscript{6}H\textsubscript{4}.

R\textsubscript{2} = COCH\textsubscript{3}, COOEt, C\textsubscript{6}H\textsubscript{5}CO.

X = Cl, Br.

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

\begin{center}
\begin{tikzpicture}
\node (Lx) at (0,0) {$\text{Lx}$};
\node (Lxi) at (2,0) {$\text{Lxi}$};
\node (Lxii) at (4,0) {$\text{Lxii}$};
\node (arrow) at (2,1) {$\rightarrow$};
\draw (Lx) -- (arrow) -- (Lxii);
\draw (Lx) -- (0,1) node[above] {MW};
\draw (Lxi) -- (0,1.5) node[above] {170 °C, 0.5 h};
\end{tikzpicture}
\end{center}

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

\textbf{Scheme 3.1.22}

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

\begin{center}
\begin{tikzpicture}
\node (Lxiii) at (0,0) {$\text{Lxiii}$};
\node (Lxiv) at (2,0) {$\text{Lxiv}$};
\node (Lxv) at (4,0) {$\text{Lxv}$};
\node (arrow) at (2,1) {$\rightarrow$};
\draw (Lxiii) -- (arrow) -- (Lxv);
\draw (Lxiv) -- (0,1) node[above] {Br–Y};
\draw (Lxiv) -- (0,1.5) node[above] {KOH/EtOH};
\end{tikzpicture}
\end{center}

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

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

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

\textbf{Scheme 3.1.24}

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

\textbf{Scheme 3.1.25}

\textbf{R= CH\textsubscript{3}, CH\textsubscript{2}Ph, CH\textsubscript{2}-O-Ph(p-Cl)}. 

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The refluxing reaction of 1,2-bis(4-chloro-5H-1,2,3-dithiazol-5-ylidene)hydrazine (Lxxii) in PhCl and benzyltriethylammonium iodide (Lxxiii) under an argon atmosphere afforded the 1,3,4-thiadiazole-2,5-dicarbonitrile (Lxxiv)\(^5^7\) (Scheme 3.1.26).

\[
\begin{align*}
\text{Lxxii} & \quad \text{Lxxiii} & \quad \text{Lxxiv} \\
\begin{array}{c}
\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{N} \\
\text{Cl} \\
\text{S} \\
\text{S}
\end{array}
\end{array} & + & \begin{array}{c}
\text{BnEt}_3\text{NI}
\end{array} & \text{PhCl} & \text{Argon} & \begin{array}{c}
\text{NC}
\end{array} & \begin{array}{c}
\text{S}
\end{array} & \begin{array}{c}
\text{NC}
\end{array}
\end{align*}
\]

Scheme 3.1.26

Umamatheswari \textit{et al.}\(^5^8\) reported the synthesis of 5-spiro-(3-methyl-2,6-diphenyl tetrahydropyran-4-yl)-4,5-dihydro-[1,3,4]-thiadiazole (Lxxvii) by the treatment of respective thiosemicarbazone (Lxxv) with freshly distilled acetic anhydride (Lxxvi) (Scheme 3.1.27).

\[
\begin{align*}
\text{Lxxv} & \quad \text{Lxxvi} & \quad \text{Lxxvii} \\
\begin{array}{c}
\text{S} \\
\text{N} \\
\text{NH}_2
\end{array} & + & \begin{array}{c}
\text{HC} \begin{array}{c}
\text{CO} \\
\text{CH}_3
\end{array}
\end{array} & \text{Reflux, 10h} & \begin{array}{c}
\text{HC} \begin{array}{c}
\text{CO} \\
\text{CH}_3
\end{array}
\end{array}
\end{align*}
\]

Scheme 3.1.27

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The 2,5-diphenyl-1,3,4-thiadiazole containing vinyl monomers [PTBEMA (Lxxviii), PVPT (Lxxix), PTPEMA (Lxxx)] and their polymers with considerable fluorescent properties have been synthesized\textsuperscript{59}.

\[ \text{Lxxviii} \]

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

\[ \text{Lxxix} \]

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

\[ \text{Lxxx} \]

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

\[
\text{Aromatic acid} \xrightarrow{P\text{OCI}_3} \text{Y= C}_6\text{H}_5, \text{CH}_3\text{C}_6\text{H}_4, \text{CH}_3\text{OC}_6\text{H}_4, \text{ClC}_6\text{H}_4, \text{F}\text{C}_6\text{H}_4, \text{O}_2\text{NC}_6\text{H}_4,}
\]

Scheme 3.1.28

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

\[
\begin{align*}
\text{Lxxxv} & \quad \text{Lxxxvi} \quad \text{Lxxxvii} \\
R_1= & \quad \text{Ph, 3,4-Cl}_2\text{C}_6\text{H}_3, \text{n-Bu, PhCH}_2, (R)-1-\text{Phenylethyl, Adamantyl.} \\
R_2= & \quad \text{CH}_2\text{CH}_2\text{CN, Et.} \\
R_3= & \quad \text{Ph, PhCH}_2, 4-\text{MeC}_6\text{H}_4, 4-\text{Pyridine, 2-HOC}_6\text{H}_4. 
\end{align*}
\]
The ever increasing demand for novel medicinally active compounds and the laborious process of lead discovery and optimization have resulted in the continuous search for simple and efficient compounds containing such useful scaffolds. Among the well known heterocyclic systems the derivatives of thiadiazoles possess broad-spectrum biological activities and many other uses. The therapeutic effects of compounds containing 1,3,4-thiadiazole rings have been studied for a number of pathological conditions including inflammation, pain and depression. This scaffold represents a pharmacophore itself as it has been observed in several substances showing activity against a broad range of therapeutic targets. Furthermore, the synthesis of thiadiazoles has attracted wide attention due to the diversity of their applications as antimicrobial, anti-tubercular, anticancer and anticonvulsant agents.

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

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

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

The typical reaction procedure for the synthesis of 3a-d involved the reaction of long chain alkenoic acid hydrazide (1a-d) with phenyl isothiocyanate to furnish the corresponding thiosemicarbazides (2a-d) which on dehydrative cyclization by AC₂O produced 2,5-disubstituted-1,3,4-thiadiazoles (3a-d) in excellent yields. To the prior, the long chain acid hydrazides (1a-d) were synthesized from corresponding long chain alkenoic acids by esterification and further treatment with hydrazine hydrate. The reaction sequence is outlined in Scheme 3.3.1.

\[
\begin{align*}
1a-d & \quad \text{PhNCS} \quad \rightarrow \quad 2a-d \quad \overset{\text{Ac}_2\text{O}}{\longrightarrow} \quad 3a-d \\
\text{R} & \quad \text{O} \quad \text{NH} \quad \text{NH} \quad \text{S} \quad \text{NH} \quad \text{N} \quad \text{N} \quad \text{R} \quad \text{NH} \quad \text{H} \quad \text{N} \quad \text{C} \quad \text{H} \quad \text{H} \quad \text{R}
\end{align*}
\]

*Scheme 3.3.1. Synthesis of 2,5-disubstituted-1,3,4-thiadiazoles 3a-d*

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

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

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

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

**Antibacterial studies**

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

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

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

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

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

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

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

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

The results have been reported in Fig. 3.3.1.

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

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

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

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

**Antifungal studies**

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

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA</td>
</tr>
<tr>
<td>3a</td>
<td>19.5±0.5</td>
</tr>
<tr>
<td>3b</td>
<td>27.2±0.2</td>
</tr>
<tr>
<td>3c</td>
<td>25.1±0.7</td>
</tr>
<tr>
<td>3d</td>
<td>24.9±1.4</td>
</tr>
<tr>
<td>Standard</td>
<td>30.0±0.2</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MIC (µg/ml)</th>
<th>MFC (µg/ml)</th>
<th>MIC (µg/ml)</th>
<th>MFC (µg/ml)</th>
<th>MIC (µg/ml)</th>
<th>MFC (µg/ml)</th>
<th>MIC (µg/ml)</th>
<th>MFC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>25.5</td>
<td>50.0</td>
<td>25.0</td>
<td>100</td>
<td>25.0</td>
<td>50.0</td>
<td>25.0</td>
<td>100</td>
</tr>
<tr>
<td>3b</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>25.0</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>3c</td>
<td>12.5</td>
<td>25.0</td>
<td>12.5</td>
<td>50.0</td>
<td>12.5</td>
<td>25.0</td>
<td>25.0</td>
<td>50.0</td>
</tr>
<tr>
<td>3d</td>
<td>25.0</td>
<td>50.0</td>
<td>25.0</td>
<td>50.0</td>
<td>25.0</td>
<td>100</td>
<td>25.0</td>
<td>100</td>
</tr>
<tr>
<td>St.</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
</tr>
</tbody>
</table>

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

The antifungal screening data showed moderate to good activity. The excellent inhibition results were obtained against all the test strains by compound 3b. Moderate activity was shown by compounds 3c and 3d against the tested fungal strains. The compound 3a showed significantly lower inhibitory activity. The compound 3b showed maximum activity.

CA = C. albicans, AF = A. fumigatus, TM = T. mentagrophytes and PM = P. marneffei.

Fig. 3.3.2 MFC/MIC of compound 3a-d compared to control drug Greseofulvin (Gr)
2,5-Disubstituted-1,3,4-thiadiazoles

against *C. albicans*, *A. fumigates* and *T. metagrophyte* strains. The MFC of most of the compounds was two or three folds higher than the corresponding MIC results. Most of the synthesized 1,3,4-thiadiazoles showed good fungistatic activity against the fungal strain *C. albicans*. Hence it could be concluded that the higher anti-fungal potency of the compound 3b may be attributed to the presence of an internal double bond in the long chain alkenyl substituent of the synthesized 1,3,4-thiadiazoles. Contrary to the antibacterial studies, the presence of the hydroxy on the alkenyl side chain turns out to be detrimental for the anti-fungal activity perhaps due to pharmacokinetic reasons.

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

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

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

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

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

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

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

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

**IR** (KBr): 3236, 1667, 1236 cm⁻¹.

**¹H NMR** (400 MHz, CDCl₃): δ 11.04 (s, 1H, CS-NH-Ar), 9.35 (br. s, 2H, CO-NHNH-CS), 8.14 (d, 2H, J= 7.2 Hz, Ar-H-2"/6"), 7.56 (t, 1H, J= 7.4 Hz, Ar-H-4"), 7.48 (t, 2H, J= 7.4 Hz, Ar-H-3"/5"), 5.85 (tdd, 1H, J_H-CH₂= 6.8 Hz, J_H-H₂=10.0 Hz, J_H-H₃=17.8 Hz, CH₂=CH-), 5.04 (dd, 1H, J_H-H₂=10.0 Hz, J_H-H₃=2.8 Hz, H₂C=CH), 4.91 (dd, 1H,
2,5-Disubstituted-1,3,4-thiadiazoles

$J_{H_1-H_2} = 17.8$ Hz, $J_{H_1-H_2} = 2.8$ Hz, $H_2C=CH_2$, 2.42 (t, 2H, $J = 7.4$ Hz, $CH_2CO$), 2.03 (m, 2H, $CH_2CH_2$), 1.85 (m, 2H, $CH_2CH_2CO$), 1.45-1.25 (br. s, 10H, $(CH_2)_5$).

$^{13}C$ NMR (100 MHz, CDCl$_3$): $\delta$ 168.2, 165.4, 139.2, 133.2, 131.3, 128.9, 128.6, 114.2, 33.8, 29.9, 29.6, 29.3, 29.2, 29.1, 29.0, 28.9.

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

Off-white powder; Yield 77%; Mp 148-150 °C.

IR (KBr): 3343, 3233, 1661, 1237 cm$^{-1}$.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 11.07 (s, 1H, CS-NH-Ar), 9.32 (br. s, 2H, CO-NH-NHNH-CS), 8.14 (d, 2H, $J = 7.4$ Hz, Ar-H-2"/6"), 7.59 (t, 1H, $J = 7.4$ Hz, Ar-H-4"), 7.50 (t, 2H, $J = 7.2$ Hz, Ar-H-3"/5"), 5.32 (m, 2H, CH$_2$-CH=CH-CH$_2$), 4.83 (m, 1H, CH-OH), 2.51 (t, 2H, $J = 7.6$ Hz, CH$_2$-CO), 2.36 (m, 4H, CH$_2$-CH$_2$-CH=CH-CH$_2$), 1.87 (m, 2H, CH$_2$-CH$_2$-CO), 1.71 (m, 1H, CH-OH), 1.44-1.28 (br. s, 18H, $(CH_2)_9$), 0.86 (dist. t, 3H, terminus CH$_3$).

$^{13}C$ NMR (100 MHz, CDCl$_3$): $\delta$ 166.4, 163.7, 138.7, 136.3, 132.2, 128.1, 128.0, 127.8, 70.7, 40.1, 39.9, 39.7, 39.4, 39.2, 31.3, 30.4, 29.4, 29.2, 29.0, 28.8, 26.1, 22.0, 14.1.

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

One mmole of compounds (2a-d) in acetic anhydride (Ac$_2$O) (6.0 ml) was refluxed for 5 h. The resulting mixture was poured into crushed ice (100gm) with stirring.
2,5-Disubstituted-1,3,4-thiadiazoles

The product thus obtained was filtered, washed with cold water, dried and recrystallized from aqueous ethanol and acetone (1:4 ml v/v) to give analytically pure compounds 3a-d. These novel compounds 3a-d, prepared according to the above procedure were characterized from their spectral data.

Spectroscopic Data

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

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

IR (KBr): 3221, 1488, 707 cm⁻¹.

¹H NMR (CDCl₃): δ 12.20 (s, 1H, NH), 8.16 (d, 2H, J= 8.5 Hz, Ar-H-2"/6"), 7.55 (t, 1H, J= 7.3 Hz, Ar-H-4"), 7.44 (t, 2H, J= 7.8 Hz, Ar-H-3"/5"), 5.73 (tdd, 1H, J₆//=CH₂= 6.7 Hz, J₆//=CH₂= 10.1 Hz, J₆//=CH₂= 2.1 Hz, H₂C=CH-), 4.85 (dd, 1H, J₆//=CH₂=16.9 Hz, CH₂=CH-), 4.98 (dd, 1H, J₆//=CH₂=10.1 Hz, J₆//=CH₂= 2.1 Hz, H₂C=CH-), 2.98 (t, 2H, J = 7.5 Hz, CH₂ α to ring), 1.96 (m, 2H, CH₂=CH-CH₂), 1.76 (m, 2H, CH₂ β to ring), 1.25 (br. s, 10H, (CH₂)₅).

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

ESI-MS found [M+Na]⁺ 338.2; C₁₈H₂₅SN₃ [M+Na]⁺ requires 338.47.
2-Phenylamine-5-(Z)(heptadec-8'-enoic)-1,3,4-thiadiazole (3b)

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

**IR** (KBr): 3219, 1468, 701 cm⁻¹.

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

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

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

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

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

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

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

CH$_2$=CH-CH$_2$), 1.83 (m, 2H, CH$_2$ β to ring), 1.71 (m, 1H, CH-OH), 1.26 (br. s, 18H, (CH$_2)_9$), 0.87 (dist. t, 3H, terminus CH$_3$).

$^{13}$C NMR (CDCl$_3$): δ 170.9, 165.0, 133.3, 131.1, 130.7, 130.2, 128.8, 128.5, 73.9, 34.1, 31.5, 29.8, 29.7, 29.5, 29.3, 29.1, 28.9, 27.1, 25.2, 23.1, 22.5, 21.3, 14.6.

ESI-MS found [M+Na]$^+$ 452.4; C$_{18}$H$_{39}$OSN$_3$ [M+Na]$^+$ requires 452.6

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

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

IR (KBr): 3322, 3236, 1461, 699 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): δ 12.17 (s, 1H, NH), 8.31 (d, 2H, J= 8.4 Hz, Ar-H-2"/6"), 7.43 (t, 1H, J= 7.4 Hz, Ar-H-4"), 7.25 (t, 2H, J= 7.4 Hz, Ar-H-3"/5"), 5.36 (m, 2H, CH$_2$-CH=CH-CH$_2$), 3.57 (s, 1H, CH-OH), 2.81 (t, 2H, J= 7.5 Hz, CH$_2$ α to ring), 2.23 (m, 4H, CH$_2$-CH$_2$=CH-CH$_2$), 2.04 (m, 2H, CH$_2$ β to ring), 1.71 (m, 1H, CH-OH), 1.33 (br. s, 18H, (CH$_2)_9$), 0.90 (dist. t, 3H, terminus CH$_3$).

$^{13}$C NMR (CDCl$_3$): δ 168.9, 163.1, 137.6, 133.2, 131.2, 128.7, 128.3, 125.5, 70.56, 40.1, 39.9, 36.3, 24.8, 31.3, 29.1, 29.0, 28.9, 28.8, 28.5, 28.4, 25.1, 22.3, 14.01.

ESI-MS found [M+Na]$^+$ 452.5; C$_{18}$H$_{39}$OSN$_3$ [M+Na]$^+$ requires 452.6.

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-25922), *Staphylococcus aureus* (ATCC-25923),
2,5-Disubstituted-1,3,4-thiadiazoles

*Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* and *Klebsiella pneumoniae* (Clinical isolate) bacterial strains by disc diffusion method\textsuperscript{66,67}. Chloramphenicol (30 \(\mu\)g) was used as positive control and the disk poured in DMSO was used as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition zones were measured and compared with the controls. The bacterial zones of inhibition values are given in Table 3.3.2.

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

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

The results have been reported in Fig. 3.3.1.

**Antifungal studies**

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

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


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


