Syntheses and characterization of some chiral azoles and pseudodisaccharides as potential biological agents

A Dissertation Submitted to the Quaid-I-Azam University Islamabad in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Organic Chemistry

BY

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2008
Sayings of the Quran

In the Name of Allah, the Most Beneficent, the Most Merciful

(1) Read! In the Name of your Lord, Who has created (all that exists), (2) Has created man from a clot (a piece of thick coagulated blood). (3) Read! And your Lord is the Most Generous, (4) Who has taught (the writing) by the pen (5) Has taught man that which he knew not. (6) Nay! Verily, man does transgress all bounds (in disbelief and evil deed, etc.). (7) Because he considers himself self-sufficient. (8) Surely! Unto your Lord is the return.

(Al-Alaq, Verses 1-8)
DECLARATION

This is to certify that this dissertation entitled “Synthesis and Characterization of Some Chiral Azoles and Pseudodisaccharides as Potential Biological Agents” submitted by Mr. Tashfeen Akhtar is accepted in its present form by the Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan, as satisfying the partial requirement for the degree of Doctor of Philosophy in Organic Chemistry.

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This effort is dedicated to

My

Encouraging and Inspirational Teachers

Dear Friends

And

Loving Family
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Abstract

The azoles are well known biological agents used as antifungal, antibacterial, antiviral and antitumour drugs. Synthesis of chiral azoles in not well known in the heterocyclic literature. The synthesis of pseudodisaccharides (neodisaccharides), on the other hand, is also not very common in carbohydrate chemistry. The pseudodisaccharides are seen as important compounds for the future drug discovery in the field of enzyme inhibition and various bacterial and viral infections. The present work deals with these two classes of compounds and is divided into two parts: synthesis and biological evaluation of chiral azoles (part A) and synthesis of pseudodisaccharides (part B).

The first part (part A) deals with the synthesis and biological evaluation of azoles. The synthesis of azoles was carried out starting from arlyoxyalkanoic acids, L-amino acids and mandelic acid. Besides using literature procedures for the synthesis of azoles, one-pot method for the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles was also developed. The structures of the synthesized compounds were confirmed by IR, $^1$H- and $^{13}$C-NMR, EIMS, FABMS, XRD and elemental analysis. The target compounds were screened for their potential as antibacterial, antifungal, antiviral, antitumour and urease inhibition. Among the tested compounds, $N$-(benzothiazol-2-yl)-2-[5-(1-(3,4-dichlorophenoxy)ethyl)-1,3,4-oxadiazole-2-yl-thio]acetamide (7e) was evaluated as a promising antitumour agent. The urease inhibition assay revealed that compounds 2-(hydroxybenzyl)-5-(4-chlorophenyl)amino-1,3,4-oxadiazole (11b), 3-(hydroxybenzyl)-4-(4-chlorophenyl)-2H-1,2,4-triazole-3-thione (12b) and 3-(Hydroxybenzyl)-4-(4-fluorophenyl)-2H-1,2,4-triazole-3-thione (12c) are more potent than the standard with an IC$_{50}$ = 16.1, 18.9 and 16.7 µM, respectively (compared to thiourea as standard, IC$_{50}$ = 21.0 µM). The triazole 4-(4-Chlorophenyl)-5-[1-(4-methylbenzenesulfonamido)-2-mercaptoethyl]-2H-1,2,4-triazole-3-thione (13f) was found inhibiting HIV-1 replication in cell culture and exhibited an EC$_{50}$ of 23.9 µg/mL against HIV-1 and 9.90 µg/mL against HIV-2. The rest of the compounds tested against different tumour and viral cell lines exhibited non significant activity at non toxic concentrations.
The second part (part B) describes the synthesis of amine and ether-linked pseudodisaccharides. The synthesis of primary-primary (pri-pri) and primary-secondary (pri-sec) amine-linked pseudodisaccharides was carried out using Mitsunobu coupling and epoxide opening reactions, respectively. The secondary-secondary (sec-sec) ether-linked disaccharides were synthesized by direct $S_N2$ substitution reaction on 1,2:5,6-di-O-isopropylidene-α-D-allofuranose-3-triflate under standard alkylation conditions. The sugars used as a source of in situ alkoxide ion include furanoses, axial and equatorial pyranose hydroxyls and a carbasugar. The structures of the disaccharides were established using 1 and 2D NMR, HRMS, X-Ray analysis and polarimetry. Attempted synthesis of sec-sec amine linkage by epoxide opening and pri-sec or sec-sec by Mitsunobu reaction met with failure, probably on steric grounds.
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Meaning</th>
</tr>
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<tbody>
<tr>
<td>ATR</td>
<td>Attenuated total reflectance</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Bt</td>
<td>Butyl</td>
</tr>
<tr>
<td>CSA</td>
<td>Camphorsulfonic acid</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DEAD</td>
<td>Diethyl azodicarboxylate</td>
</tr>
<tr>
<td>DIAD</td>
<td>Diisopropyl azodicarboxylate</td>
</tr>
<tr>
<td>DIPEA</td>
<td>Diisopropyl ethyl amine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-(N,N)-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>(N,N)-Dimethyl formamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPT</td>
<td>Di-(2-pyridyl)thionocarbonate</td>
</tr>
<tr>
<td>EIMS</td>
<td>Electron impact mass spectrometry</td>
</tr>
<tr>
<td>EtOAC</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>FABMS</td>
<td>Fast atom bombardment mass spectrometry</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>LCD</td>
<td>Liquid crystal display</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MHz</td>
<td>Mega hertz</td>
</tr>
<tr>
<td>MsCl</td>
<td>Mesityl chloride</td>
</tr>
<tr>
<td>MTT</td>
<td>Microculture tetrazolium test assay</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>Ns</td>
<td>2-Nitrobenzene sulfonyl</td>
</tr>
<tr>
<td>PDC</td>
<td>Pyridinium dichromate</td>
</tr>
<tr>
<td>Pet. Ether</td>
<td>Petroleum ether</td>
</tr>
<tr>
<td>Ph(_3)P</td>
<td>Triphenyl phosphine</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>pri</td>
<td>Primary</td>
</tr>
<tr>
<td>pri- pri</td>
<td>primary-primary</td>
</tr>
<tr>
<td>pri-sec</td>
<td>primary-secondary</td>
</tr>
<tr>
<td>PTS</td>
<td>4-Methyl benzenesulfonyl</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>sec</td>
<td>Secondary</td>
</tr>
<tr>
<td>sec-sec</td>
<td>secondary-secondary</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethyl amine</td>
</tr>
<tr>
<td>Tf</td>
<td>Trifluoromethane</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoro acetic acid</td>
</tr>
<tr>
<td>TFAA</td>
<td>Trifluoro acetic anhydride</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>Tr</td>
<td>Trityl</td>
</tr>
<tr>
<td>Triflate</td>
<td>Trifluoromethansulfonate</td>
</tr>
</tbody>
</table>
Synthesis

and

Biological Evaluation

of Azoles
Chapter 1

INTRODUCTION
Introduction

An object with a non superimposable mirror image is called chiral and this property of an object is known as chirality. One of the beauties of life is, of being chiral at the molecular level. Although the relationship of chirality to the rotation of plane polarized light was first observed by Jean-Baptiste Biot in 1815\textsuperscript{1}, the molecular basis of this phenomenon was deduced by Louis Pasteur only in 1848 when he isolated two enantiomers of tartaric acid as sodium ammonium tartarate\textsuperscript{2}.

Today chirality is playing an important role in different fields of science. For example, the drug development needs a clear picture of the host-guest stereochemistry as the enzymes interact in different ways with different stereoisomers. The specific binding property of the enzymes has selection for one or the other enantiomer of the drug. As a result, over 50\% of the marketed drugs are chiral (racemates or pure enantiomers) and this percentage is increasing with time\textsuperscript{3}. One enantiomer of a drug may be beneficial and the other harmful at the same time or the two enantiomers may have different biological activities. Therefore, the synthesis and utilization of the enantiopure drugs is gaining much pace. Thalidomide tragedy reminds the importance of chirality in drugs\textsuperscript{4}.

The bioactivities of the molecules depend not only on chirality but on the pharmacophore as well. Different pharmacophores may have different activities depending on their interactions with the biological system. Among various pharmacophores, azoles are an important class of compounds which show diverse biological activities depending on the type of nucleus and its substitution pattern.

The five membered ring compounds containing at least one nitrogen atom are called azoles. The initial attention in the chemistry of azoles was mainly due to the fact that azoles are fungistatic, orally active, and have broad-spectrum activities against most yeasts and filamentous fungi\textsuperscript{5}. Many different azoles like imidazoles, oxazoles, thiazoles, oxadiazoles, thiadiazoles, triazoles, tetrazoles and even pentazoles are known. These azoles have different positional isomers and the number of isomers
increases with increasing heteroatoms in the ring. Some examples of azoles follow, only one of the different possible isomers is being presented here.

\[
\begin{array}{cccc}
\text{N=O} & \text{N=N} & \text{N=N} & \text{N=N} \\
1,3\text{-oxazole} & 1,3\text{-imidazole} & 1,3,4\text{-oxadiazole} & 1,3,4\text{-thiadiazole} \\
\text{N} & \text{N} & \text{N} & \text{N} \\
1,2,3\text{-triazole} & 1,2,4\text{-triazole} & 1,2,3,4\text{-tetrazole} & \text{pentazole} \\
\end{array}
\]

Among various azoles, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles enjoy more importance from the medicinal as well as industrial point of view. Furthermore, these are bioisosters and may be synthesized from a common intermediate (i.e., carboxylic acid hydrazides) following different reaction sequences. A brief overview of these selected azoles, their syntheses along with their biological and industrial applications, is being presented in the following discussion.

1.1 1,3,4-Oxadiazoles

The five-membered ring system with two nitrogen and one oxygen atom gives rise to an important class of biologically active compounds, the oxadiazoles. These three atoms have four different arrangements in the ring giving rise to 1,2,3-, 1,2,4-, 1,2,5-, and 1,3,4-oxadiazoles. The chemistry of 1,3,4-oxadiazoles is well documented in literature.\textsuperscript{6}

The synthesis of 2-amino-1,3,4-oxadiazoles can be accomplished by the reaction of hydrazides with cyanogen bromide in the presence of a base.\textsuperscript{7}

\[
\begin{align*}
\text{RCONHNH}_2 + \text{BrCN} & \xrightarrow{\text{NaHCO}_3} \text{ArN}^{\text{O}}\text{N}^{\text{NH}}_2 \\
\end{align*}
\]

Katritzky et al. used di(benzotriazolyl)methanimine for the synthesis of 2-amino-5-aryl-1,3,4-oxadiazoles.\textsuperscript{8}
1-Acyl-4-alkyl/aryl semicarbazides/thiosemicarbazides may be cyclized to 2-alkyl/arylamino-5-alkyl/aryl-1,3,4-oxadiazoles using special reagents and reaction conditions\(^9\)\(^{10}\). The dehydrating agents used include thionyl chloride\(^{10}\), phosphorous oxychloride\(^{11}\), phosphorous pentoxide\(^{12}\), triphenylphosphine\(^{13}\) and an anhydride\(^{14}\).

\[
\begin{array}{c}
\text{Ar} \quad \text{O} \\
\text{NH} \quad \text{O} \\
\text{HN} \quad \text{O} \\
\text{Ar'} \\
\end{array}
\begin{array}{c}
\overset{\text{specific reagents}}{\xrightarrow{\text{AgNO}_3}} \\
\overset{\text{O}}{\text{N}} \\
\overset{\text{O}}{\text{N}} \\
\text{Ar} \\
\end{array}
\begin{array}{c}
\overset{\text{O}}{\text{N}} \\
\overset{\text{O}}{\text{N}} \\
\text{Ar} \\
\text{Ar'}
\end{array}
\]

\(X = O, S\)

Recently, a new method for the cyclization of 1,4-disubstituted thiosemicarbazides leading to 1,3,4-oxadiazoles has been reported by Feng et al\(^{15}\).

A general method for the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles involves the cyclization of a diacyl hydrazine\(^{16}\).

\[
\begin{array}{c}
\overset{\text{O}}{\text{R-C-NHNH-C-R'}} \\
\end{array}
\overset{\text{POCl}_3}{\xrightarrow{\text{MW}}} 
\begin{array}{c}
\overset{\text{N-N}}{\text{O}} \\
\overset{\text{O}}{\text{O}} \\
\text{R} \\
\text{R'}
\end{array}
\]

Microwave synthesis, along with the conventional methods, has opened a new chapter in the syntheses of various heterocycles\(^{17}\). Using this technology, Khan et al. have reported the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles\(^{18}\).
Solid phase organic synthesis is another important tool in modern synthetic organic chemistry. It has also been successfully applied in the synthesis of substituted 1,3,4-oxadiazoles\textsuperscript{19}.

The 1,3,4-oxadiazole-2-thiols exist in two tautomeric forms\textsuperscript{20} i.e., thione and thiol. The synthesis of 1,3,4-oxadiazole-2-thiones/thiols may be achieved by converting a hydrazide to dithiocarbazate with carbon disulfide in alcoholic potassium/sodium hydroxide solution followed by cyclization in the presence of a base\textsuperscript{21}.

The synthetic applications of 1,3,4-oxadiazole-2-thiols/thiones are diverse, due to its ambient reactivity with either sulphur or nitrogen acting as a nucleophile under different reaction conditions. The ambient reactivity of 1,3,4-oxadiazole-2-thiols/thiones is summarized in the scheme below.
Scheme I: Various derivatives of 1,3,4-oxadiazole-2-thiols/thiones

Over the years, the heterocycles have become a part and parcel of daily life. Be it their medicinal use or agriculture, polymeric and pigment related aspects, 1,3,4-oxadiazoles can not be ruled out of the race. In the field of medicine they are being used as antitumour, antitubercular, antimicrobial, antiinflammatory, anticonvulsant, enzyme inhibitors and hypoglycemic agents. Their insecticidal and fungicidal activities have also been reported. The aromaticity of the nucleus also makes it an important member of the pigment industry, both as dyes and fluorescent whiteners. 1,3,4-Oxadiazoles are also used as fluorescent material in organic light emitting diodes, besides being used as a core nucleus in liquid crystalline molecules. The nucleus is also gaining importance in electrooptical devices. Polymeric oxadiazoles (or polyoxadiazoles) are regarded as high performance materials. Some examples of useful 1,3,4-oxadiazoles follow:
1.2 1,3,4-Thiadiazoles

A five membered ring system containing one sulphur and two nitrogen atoms is called thia
diazole. Thiadiazoles constitute an important class of sulphur containing heterocycles. Differ
ent possible arrangements of heteroatoms in the ring give rise to 1,2,3-, 1,2,4-, 1,2,5- and 1,3,4-thia
diazoles\textsuperscript{43}.

The discovery of phenyl hydrazine\textsuperscript{4a} by Fischer\textsuperscript{4b} and that of hydrazine by Curtius in
the later half of the nineteenth century prompted the diazole chemistry. Although, Fischer re
ported 1,3,4-thiadiazole nucleus first in 1882, yet Freud and Kuh elaborated the details of these
systems only in 1890\textsuperscript{4c}. The work in the field of thiadiazole chemistry started gaining ap
preciation from 1894, however, the parent unsubstituted 1,3,4-thiadiazole was first synthe
sized by Goerdeler \textit{et al}\textsuperscript{45} in 1956 using the following reaction scheme.
The most common method used for the synthesis of substituted 1,3,4-thiadiazoles involves ferric ammonium sulphate or ferric chloride catalyzed oxidative cyclization of thiosemicarbazones.  

\[
\text{O}_2\text{N}\begin{array}{c}
\text{S}\\
\text{CHO}
\end{array} \xrightarrow{\text{HNO}_3, \text{AcOH}, \text{AC}_2\text{O}} \text{O}_2\text{N}\begin{array}{c}
\text{S}\\
\text{CH(OOCCH}_2\text{CH})_2
\end{array} \xrightarrow{\text{Thiosemicarbazide}} \text{O}_2\text{N}\begin{array}{c}
\text{S}\\
\text{N} - \text{NH}_2
\end{array}
\]

2,5-Disubstituted-1,3,4-thiadiazoles may also be synthesized by cyclodehydration of dithiocarbazates and thiosemicarbazides in the presence of a dehydrating agent like \(\text{H}_2\text{SO}_4\), \(\text{H}_3\text{PO}_4\), etc.

\[\begin{array}{c}
\text{O} \rightarrow \text{RNCS} \\
\text{R-NHNH}_2 \rightarrow \text{RNHNHNNHR} \rightarrow \text{RN'-SNHNR}
\end{array}\]

Molina et al. reported the synthesis of 1,3,4-thiadiazole derivatives from methyl 2-methyldithiocarbazate.

\[
\begin{array}{c}
\text{H}_2\text{N} - \text{N} - \text{SMe} \\
\text{Me} \\
\text{R-N=CS} \\
\text{benzene, reflux}
\end{array}
\rightarrow
\begin{array}{c}
\text{HNN} - \text{N} - \text{SMe} \\
\text{R-N} - \text{SMe} \\
\text{Me}
\end{array}
\rightarrow
\begin{array}{c}
\text{RHN} - \text{S} - \text{N=CS} \\
\text{Me}
\end{array}
\]

Yarovenko et al. synthesized 3-substituted-1,3,4-thiadiazoles from carbamoyl thiohydrazides using a new cyclizing agent.
Golovchenko et al\textsuperscript{50} have reported a new synthetic route for the synthesis of 2,5-disubstituted-1,3,4-thiadiazole derivatives using 2-acylamino-3,3-dichloroacrylonitriles.

\[
\begin{array}{cccccc}
R^1 & \text{HN} & \text{C} & \text{N} & \text{Cl} & \text{O} \\
\text{HN} & \text{C} & \text{N} & \text{NH} & \text{H} & \text{H} \\
\text{R}^1 & \text{N} & \text{C} & \text{S} & \text{N} & \text{R}^2 \\
\text{CN} & \text{NH} & \text{H} & \text{N} & \text{R}^2 \\
\end{array}
\]

Kulburn et al investigated two strategies for the synthesis of substituted 1,3,4-thiadiazoles using resin-bound thiosemicarbazides\textsuperscript{51}.

\[
\begin{array}{cccccc}
\text{R}^1 & \text{CHO} & \text{NH} & \text{R}^1 & \text{S} & \text{R}^2 \\
\text{NH} & \text{R}^1 & \text{N} & \text{HN} & \text{R}^2 & \text{O} \\
\text{DPT, DCM} & \text{R}^1 & \text{S} & \text{NH} & \text{R}^1 & \text{S} \\
\text{FeCl}_3 & \text{H}_2 & \text{N} & \text{R}^1 & \text{O} & \text{N} \\
\text{TFA} & \text{H}_2 & \text{N} & \text{R}^1 & \text{O} & \text{N} \\
\end{array}
\]

The presence of pharmacophor moiety, N-C-S, in 1,3,4-thiadiazoles explains the diverse biological activities associated with this nucleus. Cefazoline; a member of cephalosporin antibiotics, contains 2-methyl-1,3,4-thiadiazole-5-thiol moiety\textsuperscript{52}.

\[
\text{Cefazoline}
\]

8
The carbonic anhydrase inhibitors, acetazolamide and methazolamide, are bifunctional, containing a sulphonamide and 1,3,4-thiadiazole nucleus. Several other potent anticonvulsant agents containing 1,3,4-thiadiazole nucleus have also been reported.

Antibacterial and antimicrobial properties of 1,3,4-thiadiazole ring system have been widely described. These compounds were proved potent in the treatment of mycobacterial infections. 2-Amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole has broad spectrum antimicrobial activity. Its derivatives also exhibit antifungal as well as insecticidal activities.

The anticancer activity of thiadiazole derivatives has attracted great attention in recent years. The anticancer potential of 1,3,4-thiadiazole derivatives is well documented in the current literature.
1,3,4-Thiadiazole derivatives, 2-amino-1,3,4-thiadiazole and 2-ethy lamino-1,3,4-thiadiazole show antileukemic activity. The compounds containing 1,3,4-thiadiazole nucleus also exhibit analgesic/antiinflammatory and antulcerative activities. Some derivatives have been found antidepressant, antileishmanicidal and antimycotic.

Along with the medicinal importance of this particular nucleus, it has wide spread applications in other fields of life. Aminothiadiazoles are one of the first heterocyclic diazo components of the dyes. These aminothidiazoles have found applications in the synthesis of brilliant red polyester dyes. Recent work on this ring system has proved them to be important components of electro-optical devices, the nucleus is also being used as mesogenic core to alter the liquid crystalline properties. Some 1,3,4-thiadiazoles are used as corrosion inhibitors, while others show good lubrication properties because of their low solubility in oils.

1.3 1,2,4-Triazoles

A five membered ring system containing three nitrogen atoms is called a triazole. Two possible arrangements of the nitrogen atoms give rise to 1,2,3-triazoles or u-triazoles and 1,2,4-triazoles or s-triazoles. The s-triazoles also exist in two tautomeric
forms, i.e., 1,2,4-1H-triazoles and 1,2,4-4H-triazoles. The N-substituted triazoles are named with respect to the parent triazole\textsuperscript{75}.

\[
\begin{array}{c}
\text{N} - \text{NH} \\
\text{N} \\
\end{array}
\begin{array}{c}
\text{N} - \text{N} \\
\text{N} \\
\end{array}
\rightleftharpoons
\begin{array}{c}
\text{N} - \text{N} \\
\text{H} \\
\end{array}
\rightleftharpoons
\begin{array}{c}
\text{N} - \text{NH} \\
\text{N} \\
\end{array}
\]

1,2,4-1H-triazole 1,2,4-4H-triazole

The name triazole was first used by Bladin\textsuperscript{76} in 1885, for carbon-nitrogen ring system C\textsubscript{2}N\textsubscript{3}H\textsubscript{3}. It took few more years when Andreocc\textsuperscript{77} and Bladin\textsuperscript{78} independently reported the synthesis of 1,2,4-triazoles from simple substituted 1,2,4-triazoles using two different routes.

The methods used for the synthesis of 1,2,4-triazole derivatives have been reviewed in the literature\textsuperscript{79}. The most common method involves the reaction of a carboxylic acid hydrazide or hydrazine with suitable reagents/substrates\textsuperscript{80,81}.

\[
\begin{array}{c}
\text{R} - \text{C} - \text{NNH}_2 \\
\begin{array}{c}
\text{O} \\
\end{array}
\end{array}
\xrightarrow{\text{i)CS}_2\text{KOH}}
\begin{array}{c}
\text{R} - \text{C} - \text{NNH}_2 \\
\begin{array}{c}
\text{SH} \\
\text{NH}_2 \\
\end{array}
\end{array}
\xrightarrow{\text{ii)NH}_2\text{NH}_2\text{H}_2\text{O}}
\begin{array}{c}
\text{N} - \text{N} \\
\text{R} - \text{C} - \text{NNH}_2 \\
\begin{array}{c}
\text{SH} \\
\text{NH}_2 \\
\end{array}
\end{array}
\]

The condensation of thio-carbohydrazide or carbohydrazide with aliphatic or aromatic carboxylic acids leads to the formation of 3-alkyl/aryl-4-amino-5-mercapto/hydroxy-1,2,4-triazoles\textsuperscript{82}.

\[
\begin{array}{c}
\text{X} = \text{C} - \text{NNH}_2 \\
\text{NNNH}_2 \\
\end{array}
\xrightarrow{\text{RCOOH}}
\begin{array}{c}
\text{X} = \text{C} - \text{NNH}_2 \\
\text{NNNH}_2 \\
\end{array}
\xrightarrow{\text{Heat}}
\begin{array}{c}
\text{R} \\
\text{N} - \text{N} - \text{N} \\
\text{NH}_2 \\
\end{array}
\]

\(X = \text{O, S}; \text{R} = \text{alkyl, aryl}\)
1,2,4-Triazoles may also be synthesized by the reaction of hydrazine or amines with 1,3,4-oxadiazoles\textsuperscript{83}.

\[
\begin{align*}
\text{HN} &\text{-} &\text{N} \\
\text{N} &\text{-} &\text{N} \\
\text{S} &\text{-} &\text{O} &\text{-} &\text{R} &\text{ NH}_2 &\rightarrow &\text{HN} &\text{-} &\text{N} \\
 & & &\text{-} &\text{S} &\text{-} &\text{N} &\text{-} &\text{R} &\text{-} &\text{R} &\text{ R} &= \text{alkyl, aryl} \\
R' &= \text{NH}_2, \text{alkyl, aryl}
\end{align*}
\]

Price \textit{et al.} reported an improved synthesis of 3,5-dimethyl-1,2,4-triazoles by the reaction of \(N\)-acetyl amine with acetic hydrazide\textsuperscript{84}.

\[
\begin{align*}
\text{R} &\text{-} &\text{N} \\
\text{O} &\text{-} &\text{H} &\text{N} &\text{-} &\text{C} &\text{-} &\text{R} &\text{ acetic hydrazide} &\rightarrow &\text{N} &\text{-} &\text{N} &\text{-} &\text{R} &\text{ R}
\end{align*}
\]

The cyclodehydration of 1-acyl-4-substituted thiosemicarbazides / semicarbazides under basic conditions leads to the formation of 3,4-disubstituted-5-mercapto/oxo-1,2,4-triazoles\textsuperscript{85}.

\[
\begin{align*}
\text{R} &\text{-} &\text{C} &\text{-} &\text{N} &\text{NH} &\text{-} &\text{C} &\text{-} &\text{N} &\text{-} &\text{R} &\text{ Base} &\rightarrow &\text{HN} &\text{-} &\text{N} \\
& & & & & & & & & & & & & & &\text{X} = \text{O}, \text{S} \\
& & & & & & & & & & & & & & &\text{X} = \text{O}, \text{S}
\end{align*}
\]

The synthesis of 3,5-disubstituted-1,2,4-triazoles has also been reported by the reaction of diacyl hydrazine and ammonia\textsuperscript{86}.

\[
\begin{align*}
\text{R} &\text{-} &\text{C} &\text{-} &\text{N} &\text{NH} &\text{-} &\text{C} &\text{-} &\text{R} &\text{ NH}_3, \text{ZnCl}_2 &\rightarrow &\text{N} &\text{-} &\text{N} &\text{-} &\text{R} &\text{ R} \\
& & & & & & & & & & & & & & &\text{OR} \\
& & & & & & & & & & & & & & &\text{PCl}_3, \text{NH}_3
\end{align*}
\]

3,5-Disubstituted-1,2,4-triazoles may also be prepared by the reaction of diacyl amine and semicarbazide hydrochloride\textsuperscript{87}.

\[
\begin{align*}
\text{H}_2\text{NN} &\text{-} &\text{C} &\text{-} &\text{NH}_2 &\text{, HCl} &+ &\text{R} &\text{-} &\text{C} &\text{-} &\text{N} &\text{-} &\text{C} &\text{-} &\text{R} &\text{ aq. NaOAc} &\rightarrow &\text{N} &\text{-} &\text{N} &\text{-} &\text{R} &\text{ R}
\end{align*}
\]
Bentiss et al. have reported the use of microwave irradiations in the synthesis of 3,5-disubstituted-1,2,4-triazoles by the reaction of aromatic nitriles with hydrazine hydrochloride in the presence of hydrazine hydrate in ethylene glycol.88

\[
\text{ArCN} \xrightarrow{\text{Ethylene glycol}} \text{Ar} = \text{N} \xrightarrow{\text{NH}_2\text{NH}_2, 2\text{HCl}} \text{NH}_2\text{NH}_2\text{H}_2\text{O} \]

Recently, a convenient and efficient one step base catalysed synthesis of 3,5-disubstituted 1,2,4-triazoles has been reported by Yeung et al. under microwave conditions.89

\[
\text{RCN} + \overset{\overset{\text{O}}{\text{N}}}{\text{R'}} \xrightarrow{\text{K}_2\text{CO}_3, \text{BuOH}} \underset{\text{R}, \text{R}' = \text{aryl, heteroaryl}}{\text{N}} \hat{=} \text{R}' \hat{=} \text{N} \hat{=} \text{R} \]

The reactive intermediates, (chboroalkyl)azohexachloroantimonates, derived from azo compounds at low temperature in the presence of SbCl₅, on treatment with nitriles gave a protonated intermediate, in situ hydrolysis with sodium hydrogen carbonate and ammonia solution affords 1,2,4-triazole derivatives.90

\[
\begin{align*}
\text{R} & \overset{\text{O}}{\text{R'}} \xrightarrow{\text{NH}_2\text{NH}_2} \text{R} \hat{=} \text{N} \hat{=} \text{N} \hat{=} \text{R} \overset{\text{1) Cl}_2}{\underset{\text{2) SbCl}_5, \text{CH}_2\text{Cl}_2}{}} \underset{\text{R'}, \text{CH}_2\text{Cl}_2}{< \text{0°C to RT}} \\
\text{R'} \text{CN}, \text{CH}_2\text{Cl}_2 & \xrightarrow{\text{MeCN, 0°C, 3h}} \text{NaHCO}_3, \text{NH}_3 \xrightarrow{\text{R'}} \text{N} \hat{=} \text{N} \hat{=} \text{R} \overset{\text{SbCl}_6}{\underset{\text{SbCl}_6}{\text{SbCl}_6}}
\end{align*}
\]

Wang et al.91 have reported the synthesis of 2,5-disubstituted-1,2,4-triazoles using polyethylene glycol-bound (PEG-bound) munchnones (1,3-oxazolium-5-oxide). 1,3-Dipolar cycloaddition reaction between diethyl azo dicarboxylate (DEAD) and munchnones, generated from the corresponding carboxylic acid chlorides, afforded PEG-bound 1,2,4-triazoles.
The importance of 1,2,4-triazoles resides in their enormous pharmacological activities. These compounds have also found applications in industries like dyes, photography, cotton, textile, polymers, etc. Because of less efficacy and narrower antifungal spectrum of imidazoles, trials for their replacement were under way. At that time triazole nucleus appeared as a strong compatriot, having broad spectrum of biological activities and at the same time lesser toxicity. The chemistry and pharmacology of azoles is well documented\textsuperscript{92-93}. A common mode of action of azoles involves inhibition of the biosynthesis of an important lipid \textit{i.e.}, ergosterol, a major steroid in fungal membranes. The result is accumulation of 14-\(\alpha\)-methylated steroid and disruption of fungal membrane\textsuperscript{94}.

Some commonly used antifungal 1,2,4-triazoles are fluconazole, itraconazole, ravuconazole and posaconazole\textsuperscript{93}.
Ribavirin\textsuperscript{95a} (1-β-D-ribofuranosyl-[1\textsubscript{H}]-1,2,4-triazole-3-carboxamide) was synthesized in 1970s and is a well-known antiviral agent showing broad spectrum of activity. The broad spectrum activity of this drug is due to its ability to inhibit DNA and RNA polymerase. Ribavirin derivatives with broad spectrum activity have also been prepared\textsuperscript{95b}.

1,2,4-Triazoles and their derivatives also show anticancer,\textsuperscript{96}, anticonvulsant,\textsuperscript{97}, antifungal,\textsuperscript{70,98} antimicrobial,\textsuperscript{99} antiinflammatory\textsuperscript{100} and insecticidal properties\textsuperscript{101}, besides being used as corrosion inhibitors\textsuperscript{102} and energetic materials\textsuperscript{103}. Some examples of important 1,2,4-triazoles follow:

\begin{itemize}
  \item Anticancer\textsuperscript{96a}
  \item Anticonvulsant\textsuperscript{97}
  \item Anti-HIV\textsuperscript{104}
  \item Anti-hypersensitive\textsuperscript{105}
\end{itemize}
1,2,4-Triazoles are also used as intermediates in the synthesis of different condensed heterocycles like triazolothiadiazoles\textsuperscript{107}, triazolothiadiazines\textsuperscript{108} and triazolotriazoles\textsuperscript{109}, etc.

The syntheses and frequent use of 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole derivatives in pharmaceutical and other industries is described in the foregoing text. Several methods have been developed for the synthesis of azoles and various substitution patterns studied. A number of marketed azole drugs like ketoconazole, fluconazole, posaconazole, ribavirin and cefazoline are chiral and are established antifungal, antiviral or antibiotic agents.

It is a well established fact that enzymes have specific binding sites and compounds with only that specific stereochemistry fit into the binding site. Therefore, need of the hour lies in the synthesis of more and more chiral compounds and explore their biological activities, inorder to find more potent drugs. Although, azoles are one of the most potent known pharmacophores as is apparent from their use in the pharmaceutical industry, still only few examples\textsuperscript{110} of synthesis and biological evaluation on chiral azoles are found in the literature.

The synthesis of chiral azoles either by chiral induction or starting from enantiopure precursors, has a wide room in future drug development. A general approach in the synthesis of enantiopure molecules is to start with a readily available, optically pure, starting material. This not only avoids the need for resolutions in case of chiral induction, but also because naturally occurring enantiopure compounds, like amino acids and sugars, are readily available in large quantities.
Plan of Work

As discussed in introduction, the azoles have numerous pharmacological and industrial applications. The interactions of the azole derivatives with the biological system may be enhanced by inducing chirality in the molecules, since chiral drugs are highly selective biological agents because of the specificity associated with the enzyme structure. In the development of azole drugs; Miconazole to posaconazole, chirality is the most important feature. Presently more than 50% of the marketed drugs are chiral and owing to the adverse affects of the second enantiomer, the synthesis of enantiopure drugs is gaining much importance.

In the present work, it was planned to synthesize chiral azole derivatives and explore their pharmacological potential. The selected azoles i.e., 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles are bioisosters and provide very good structural comparison of their biological activities within themselves. The retrosynthetic analysis suggested their synthesis from a common intermediate i.e., carboxylic acid hydrazide.

Based on the retrosynthetic analysis following synthetic scheme was designed.
The main focus of the project will be the synthesis of chiral azoles starting from carboxylic acids. The carboxylic acids may include aryloxyalkanoic acids, mandelic acid and L-amino acids having an $\alpha$-chiral centre. The synthetic strategy involves the protection of $\text{NH}_2$ of the amino acids and synthesis of aryloxyalkanoic acids. The selected acids will be converted to their respective hydrazides via esterification following hydrazinolysis. The hydrazides may be regarded as the main intermediates in the completion of the designed synthetic plan. The hydrazides may be converted to 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles using different sets of reaction conditions.

The syntheses will be confirmed using spectroanalytical techniques. Finally, a selected number of compounds will be tested for their potential as antifungal, antibacterial, antitumor and antiviral agents.
Chapter 2

Results

And

Discussion
Results and Discussion

The development of new antifungal agents, the first and second generation of azoles, opened a new era in the field ofazole chemistry. 1,3,4-Oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles, exhibit prominent biological activities like antifungal, antibacterial, antimicrobial, anti-inflammatory, anticonvulsant, antihypertensive, anti-HIV, antitumor, anti-HCV, hypoglycemic, enzyme inhibitory and plant growth regulation, as mentioned in chapter 1. The importance of these nuclei is not limited only to the pharmaceutical industry but various other industries have also benefited, some examples being their use in dye, cotton, textile and photographic industries. The development in the optical research has found their use in electro optical devices and these nuclei have also been used to enhance the liquid crystalline properties of liquid crystal display (LCD) systems. The potential of these conjugated nuclei is gaining increasing prominence in organic light emitting diodes (OLEDs) as well.

The efficacy of the heterocyclic nuclei in all fields may be enhanced or specified by introducing chirality. This property of the molecules is associated with specific biological activities of two enantiomers of a single compound (for example; darvon and novrad)\textsuperscript{111a}. The specific nature of enzymes allows only one particular orientation of the drug molecule to bind the receptor. A beauty associated with theazole antifungals is their chirality.

The synthesis of chiral five membered heterocycles is not very common and only few examples are found\textsuperscript{110,111c} in the literature. The selected azoles are bioisosters, \textit{i.e.}, these have the same ring construction, the only difference being the variation of a single heteroatom in the ring. The reterosynthetic analysis (plan of work) of these azoles led to their possible synthesis from a common intermediate \textit{i.e.}, a carboxylic acid hydrazide.

2.1 Synthesis of carboxylic acid hydrazides (5a-s)

The carboxylic acid hydrazides are seen as important precursors in the synthesis of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles by either direct cyclization or via the intermediate semicarbazides or thiosemicarbazides under varying reaction
conditions. In addition, a variety of biological activities are associated with carboxylic acid hydrazides.

A comprehensive literature review on chiral starting materials (natural or synthetic) for the synthesis of the key intermediates (carboxylic acid hydrazides) in the synthetic strategy, led us to the selection of aryloxyalkanoic acids (3a-h), L-amino acids (3i-s) and mandelic acid (3t). The acids were converted to their respective methyl esters before being subjected to hydrazinolysis affording carboxylic acid hydrazides. These hydrazides were used as key intermediates in the accomplishment of the synthetic plan.

2.1.1 Synthesis of carboxylic acids

The aryloxyalkanoic acids were synthesized starting from different phenols and (±)-α-bromopropanoic/butanoic acids. The selected L-amino acids before being subjected to hydrazinolysis were N-protected using 4-methylbenzene sulfonyl (PTS) chloride. The selection of PTS group was based on the stability of the resulting sulfonamide linkage under strong basic conditions and biological activities associated with it. The commercially available mandelic acid was also used for the synthesis of corresponding hydrazide.

a) Synthesis of 2-(aryloxy)alkanoic acids

Substituted phenols (1a-d) were treated with (±)-2-bromoalkanoic acids (2a,b) in the presence of aqueous sodium hydroxide\(^{112}\) (scheme I). The neutralization of the reaction mixture with 6N hydrochloric acid afforded (±)-aryloxyalkanoic acids (3a-h).

\[
\begin{align*}
\text{Ar-OH} & \quad + \quad \text{Br} \quad \text{R} \quad \text{CH COOH} \quad \text{i, ii} \quad \text{Ar} \quad \text{O} \quad \text{R} \quad \text{CH COOH} \\
1a-d & \quad 2a,b & \quad 3a-h
\end{align*}
\]

<table>
<thead>
<tr>
<th>Compd</th>
<th>Ar</th>
<th>R</th>
<th>Compd</th>
<th>Ar</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>4-BrC(_6)(_4)</td>
<td>Me</td>
<td>3e</td>
<td>4-BrC(_6)(_4)</td>
<td>Et</td>
</tr>
<tr>
<td>3b</td>
<td>4-ClC(_6)(_4)</td>
<td>Me</td>
<td>3f</td>
<td>4-ClC(_6)(_4)</td>
<td>Et</td>
</tr>
<tr>
<td>3c</td>
<td>3,4-Cl(_2)C(_6)(_4)</td>
<td>Me</td>
<td>3g</td>
<td>3,4-Cl(_2)C(_6)(_4)</td>
<td>Et</td>
</tr>
<tr>
<td>3d</td>
<td>4-MeC(_6)(_4)</td>
<td>Me</td>
<td>3h</td>
<td>4-MeC(_6)(_4)</td>
<td>Et</td>
</tr>
</tbody>
</table>

Reagents and conditions: i) Aq. NaOH, reflux ii) dil HCl

Scheme I: Synthesis of aryloxyalkanoic acids
The acids were characterized by their physical constants and IR spectral data. In the IR spectra, the formation of aryloxyalkanoic acids was indicated by the appearance of a strong carbonyl absorption in the range of 1714-1698 cm\(^{-1}\) and a broad and irregular absorption for the OH of carboxylic acid (3200-2500 cm\(^{-1}\)). The broad absorption for the OH suggested the hydrogen bonding and probable existence of the carboxylic acids in the dimeric form. The dimeric form of the acids was further confirmed by the crystal structure of 2-(3,4-dichlorophenoxy)propanoic acid (3c: Figure 2.1). The crystal structure also helped in assigning absolute configuration \(i.e., R\) to the compound (3c). The crystal data is given below and selected bond lengths and bond angles are presented in Table 2.1.

![Molecular structure](image)

**Figure 2.1:** a) The molecular structure of 3c showing the atom labelling and displacement ellipsoids drawn at the 50% probability level. b) View of the hydrogen bonds (dashed lines) forming a centrosymmetric dimer. The unit cell has been omitted for clarity.

**Crystal data**

\[
\begin{align*}
\text{C}_9\text{H}_8\text{Cl}_2\text{O}_3 & \quad V = 976 (2) \text{ Å}^3 \\
M_r = 235.07 & \quad Z = 4 \\
\text{Monoclinic, } P2_1 & \quad \text{Mo } K\alpha \text{ radiation} \\
\alpha = 4.585 (5) \text{ Å} & \quad \mu = 0.64 \text{ mm}^{-1} \\
\beta = 6.412 (8) \text{ Å} & \quad T = 113 (2) \text{ K} \\
\gamma = 33.21 (4) \text{ Å} & \quad 0.40 \times 0.30 \times 0.22 \text{ mm} \\
\beta = 90.471 (14)^\circ & \\
\end{align*}
\]

**Table 2.1:** Selected bond lengths (Å) and bond angles (°)

<table>
<thead>
<tr>
<th>Atom</th>
<th>Bond length</th>
<th>Atom</th>
<th>Bond length</th>
</tr>
</thead>
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<td>C2—O1</td>
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<tr>
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<td>1.727 (4)</td>
<td>C7—Cl2</td>
<td>1.731 (4)</td>
</tr>
<tr>
<td>Atom</td>
<td>Angle</td>
<td>Atom</td>
<td>Angle</td>
</tr>
<tr>
<td>O3—C4—C5</td>
<td>124.3 (3)</td>
<td>O3—C4—C9</td>
<td>115.2 (3)</td>
</tr>
</tbody>
</table>
b) **Synthesis of N-(4-methylbenzenesulfonyl)amino acids**

L-Amino acids (1i-s) with different structural features were selected and the amino group protected using PTS chloride\(^{113}\) in aqueous sodium hydrogen carbonate and diethyl ether (scheme II). The etheral layer was removed and aqueous phase neutralized with dil. HCl to afford N-PTS amino acids (3i-s). The protected amino acids were characterized by their physical constants and IR spectral data.

\[
\begin{align*}
& \text{Compd} \quad R \quad \text{Compd} \quad R \\
& 3i \quad \text{CH}_3 \quad 3j \quad \text{CH}(_2)\text{CH}_3 \\
& 3k \quad \text{CH}_2\text{CH}(_2)\text{H} \quad 3l \quad \text{CH}(_2)\text{CH}_2\text{CH}_3 \\
& 3m \quad \text{CH}_3\text{OH} \quad 3n \quad \text{CH}_2\text{C} (_2)\text{H}_3 \\
& 3o \quad \text{CH}_2\text{CH}_2\text{SCH}_3 \quad 3p \quad \text{CH}_2\text{SH} \\
& 3q \quad \text{CH}(_2)\text{OH} \quad 3r \quad \text{CH}(_2)\text{N}(_2)\text{CH}(_2)\text{NH}(_2) \\
\end{align*}
\]

Reagents and conditions: i) Diethyl ether, Aq. Na\(_2\)CO\(_3\); ii) dil HCl

**Scheme II:** Protection of L-amino acids

The N-sulfonylation was indicated in the IR spectra by the appearance of symmetric and unsymmetric sulphonamide stretchings in the regions of 1160-1135 and 1375-1350 cm\(^{-1}\), respectively. The absorptions for carbonyl stretching were observed in the region of 1720-1700 cm\(^{-1}\). The presence of carboxylic acid was further confirmed by the broad OH absorptions in the region of 3200-2500 cm\(^{-1}\).

### 2.1.2 Synthesis of hydrazides

The carboxylic acids (3a-t) were converted to their respective methyl esters (4a-t) using methanol and a catalytic amount of conc. sulphuric acid under reflux\(^{114a}\). The esters were pure enough to be used in the next step. The hydrazine hydrate (80%) treatment of the esters afforded the corresponding hydrazides\(^{114b}\) (scheme III, 5a-t). The characterization of hydrazides (5a-h) was carried out using IR, \(^1\)H- and \(^13\)C-NMR
spectroscopic data, while the structures of hydrazides (5i-t) were confirmed by comparison of their physical constants and IR spectral data with the literature values\textsuperscript{114a-c}.

\[
\begin{array}{cccccc}
R-C\overset{\text{H}}{\overset{\text{O}}{\overset{\text{I}}{C}}-C-OH} & i & H\overset{\text{O}}{\overset{\text{II}}{\overset{\text{II}}{C}}-C-OMe} & ii & H\overset{\text{O}}{\overset{\text{R}}{\overset{\text{R}}{C}}-C-\overset{\text{NH}_{3}}{\overset{\text{R}}{\overset{\text{R}}{C}}-NH_{3}}}
\end{array}
\]

<table>
<thead>
<tr>
<th>Compd</th>
<th>R</th>
<th>R'</th>
<th>Compd</th>
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<th>R'</th>
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<tr>
<td>a</td>
<td>4-BrC\textsubscript{6}H\textsubscript{4}O</td>
<td>Me</td>
<td>b</td>
<td>4-ClC\textsubscript{6}H\textsubscript{4}O</td>
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</tr>
<tr>
<td>c</td>
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<td>Me</td>
<td>d</td>
<td>4-MeC\textsubscript{6}H\textsubscript{4}O</td>
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<tr>
<td>e</td>
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<td>f</td>
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<tr>
<td>g</td>
<td>3,4-Cl\textsubscript{2}C\textsubscript{6}H\textsubscript{4}O</td>
<td>Et</td>
<td>h</td>
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<tr>
<td>i</td>
<td>CH\textsubscript{3}</td>
<td>NHPTS</td>
<td>j</td>
<td>CH(CH\textsubscript{2})\textsubscript{2}</td>
<td>NHPTS</td>
</tr>
<tr>
<td>k</td>
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<td>l</td>
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<tr>
<td>m</td>
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<td>NHPTS</td>
<td>n</td>
<td>CH\textsubscript{2}C\textsubscript{6}H\textsubscript{5}</td>
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<tr>
<td>o</td>
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<td>p</td>
<td>CH\textsubscript{3}SH</td>
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<tr>
<td>3q</td>
<td>CH(CH\textsubscript{2})OH</td>
<td>NHPTS</td>
<td>r</td>
<td>$\overset{\text{H}}{\overset{\text{H}}{\overset{\text{R}}{C}}-C-N_{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{R}}{\overset{\text{R}}{C}}}}}}$</td>
<td>NHPTS</td>
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<tr>
<td>3s</td>
<td>$\overset{\text{H}}{\overset{\text{H}}{\overset{\text{R}}{C}}-C-N_{\overset{\text{H}}{\overset{\text{H}}{\overset{\text{R}}{\overset{\text{R}}{C}}}}}}$</td>
<td>NHPTS</td>
<td>t</td>
<td>Ph</td>
<td>OH</td>
</tr>
</tbody>
</table>

Reagents and conditions: i) H\textsuperscript{+} / MeOH, reflux; ii) NH\textsubscript{2}NH\textsubscript{2}H\textsubscript{2}O, MeOH, reflux

**Scheme III: Synthesis of hydrazides**

The synthesis of hydrazides was indicated in the IR spectra by the appearance of N–H absorptions in the region of 3400-3200 cm\textsuperscript{-1} and disappearance of the broad OH absorptions for carboxylic acids. The strong carbonyl absorptions shifted towards the lower wave number in the region 1670-1650 cm\textsuperscript{-1} and are also indicative of the formation of hydrazides.

In \textsuperscript{1}H-NMR spectra, separate signals for NH and NH\textsubscript{2} protons were observed in all the cases. The upfield signal, a broad singlet in the range 4.11-4.01 ppm integrating to two protons, was assigned to NH\textsubscript{2} protons. The downfield signal in the range 7.40-7.13 ppm, with one proton integral, was assigned to NH proton. The downfield shift of NH proton in comparison to NH\textsubscript{2} protons is attributed to the acidic nature of the amide proton. In \textsuperscript{13}C-NMR the signal in the range of 165-170 ppm was assigned to the carbonyl carbon. The signals for other carbons were shielded or deshielded depending...
on the nature of the functional group (OC<sub>3</sub>H<sub>2</sub>X e.g., X = Cl, Br, Me) in the neighbourhood affecting the electron density around a particular carbon atom.

The hydrazides may be cyclized to 1,3,4-oxadiazoles, 1,3,4-thiadiazoles or 1,2,4-triazoles under varying reaction conditions, either directly or via intermediate semicarbazides or thiosemicarbazides.

2.2 Synthesis of 5-(1-aryloxyalkyl)-1,3,4-oxadiazole-2-thiones/thiols (6a-h) and benzothiazole derivatives (7a-h)

2.2.1 Synthesis of 5-(1-aryloxyalkyl)-1,3,4-oxadiazole-2-thiones/thiols (6a-h)

The hydrazides (5a-h) were converted to 5-(1-aryloxyalkyl)-1,3,4-oxadiazole-2-thiones/thiols (scheme IV, 6a-h) on treatment with carbon disulfide and potassium hydroxide in methanol using a literature procedure<sup>20d</sup>. The products were obtained in good yields (67-84 %) and characterized by their spectral data.

![Chemical structure](image)

**Scheme IV:** Synthesis of 1,3,4-oxadiazole-2-thiones/thiols

The comparison of the IR spectra of the products with that of the corresponding hydrazides indicated the formation of the oxadiazole nucleus. The disappearance of sharp carbonyl peak in the range of 1670-1650 cm<sup>-1</sup> and NH, NH<sub>2</sub> absorptions in the range of 3400-3200 cm<sup>-1</sup> and appearance of weak C=N absorptions in the region 1620-1580 cm<sup>-1</sup> indicated the formation of the expected nucleus.
In $^1$H-NMR spectra, the disappearance of two signals for three NH protons of the hydrazides and appearance of a broad singlet for NH was observed in compounds 6a, 6d-f and 6h in the range of 11.62-9.91 ppm, while in case of compounds 6b-c and 6g it was observed at 1.27 ppm for the SH proton, indicating the thione form in the former and thiol form in the latter case. The $^{13}$C-NMR spectra exhibited signals for C-5 (C=N) and C-2 (C=S) in the range of 161.6-163.2 and 178.5-187.7 ppm, respectively, at the expense of the signal for the carbonyl carbon. The synthesis of oxadiazoles was further confirmed by the mass spectral analysis. In EIMS, the appearance of molecular ion peak confirmed the synthesis of the desired nucleus. In bromo- and chloro-substituted compounds specific patterns of X and X+2 peaks for these halogens further confirmed their presence in the compounds. The fragments corresponding to the arylxooxalky and phenoxy parts were also observed along with fragments resulting from the cleavage of the 1,3,4-oxadiazole nucleus (Figure 3.2). The elemental compositions were also found in good agreement with the calculated values for carbon, hydrogen, nitrogen and sulfur.

![Figure 2.2: Suggested modes of fragmentation of 1,3,4-oxadiazole-2-thiones (6a-h)](image)

In addition to all these spectroscopic characterizations, attempted crystal growth on the oxadiazoles resulted in fine crystals of 5-[1-(3,4-dichlorophenoxy)ethyl]-1,3,4-oxadiazole-2(3H)-thione (6c), suitable for the collection of crystallographic data. This solved the structure in an unambiguous manner for (6c). In this case the solid and solution state structures are thione and thiol tautomers, respectively. Probably the thiol form is stabilized through hydrogen bonding with the solvent molecules. The crystal structure established S configuration at the chiral center.
A single crystal of dimensions 0.40 x 0.30 x 0.25 mm was selected for X-ray diffraction. The crystal data revealed that there are eight molecules of oxadiazole and four molecules of water in the unit cell and shows two fold axis of symmetry passing through water molecule. The crystal structure is stabilized by the N···H···O as well as C=S···H hydrogen bonding. The crystal data and selected bond lengths and bond angles are given below.

**Crystal Data**

C\textsubscript{10} H\textsubscript{9} C\textsubscript{12} N\textsubscript{2} O\textsubscript{2.50} S  
Mr = 300.15  
$T = 273(2)$ K  
Monoclinic, $C2/c$  
$a = 11.8725(2)$ Å  
$\alpha = 90^\circ$  
$b = 7.89320(10)$ Å  
$\beta = 92.91(10)^\circ$  
$c = 26.6092(4)$ Å  
$\gamma = 90^\circ$  
$V = 2490.38(6)$ Å$^3$  
$Z = 1.601$ mg/m$^3$

Table 2.2: Selected bond lengths (Å) and bond angles (°)

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<thead>
<tr>
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<td>C7—O3</td>
<td>1.4360(17)</td>
<td>C3—O3</td>
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<td>1.2762(17)</td>
<td>C9—O2</td>
<td>1.3732(15)</td>
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<tr>
<td>N3—C10</td>
<td>1.3298(17)</td>
<td>C7—C9</td>
<td>1.4966(19)</td>
<td>N3—N4</td>
<td>1.3818(16)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Atom</th>
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<th>Atom</th>
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<tbody>
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<td>114.04(12)</td>
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<td>106.20(9)</td>
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<td>112.60(11)</td>
<td>C9—N4—N3</td>
<td>103.48(11)</td>
</tr>
<tr>
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<td>N3—C10—O2</td>
<td>104.76(11)</td>
<td>N3—C10—S1</td>
<td>131.54(11)</td>
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</table>
2.2.2  **Synthesis of benzothiazole derivatives (7a-h) of 5-(1-aryloxyalkyl)-1,3,4-oxadiazole-2-thiones/thiols**

For the coupling of oxadiazoles (6a-h) with benzothiazole nucleus (scheme IV), N-(2-benzothiazolyl)chloroacetamide (A) was prepared by the reaction of chloroacetyl chloride and 2-aminobenzothiazole\(^{115}\). The chloroacetamide (A) was coupled with 1,3,4-oxadiazoles (6a-h) using anhydrous potassium carbonate (K\(_2\)CO\(_3\)) in acetone to afford benzothiazole derivatives of 5-(1-aryloxyalkyl)-1,3,4-oxadiazole-2-thiones/thiols (6a-h) (scheme V, 7a-h).

\[
\begin{align*}
\text{6a-h} & \quad + \quad \text{A} \quad \xrightarrow{i} \quad \text{7a-h} \\
\text{a} & \quad R = 4-\text{BrC}_2\text{H}_4\text{O} ; \quad R' = \text{Me} & \quad \text{b} & \quad R = 4-\text{ClC}_2\text{H}_4\text{O} ; \quad R' = \text{Me} \\
\text{c} & \quad R = 3,4-\text{Cl}_2\text{C}_2\text{H}_5\text{O} ; \quad R' = \text{Me} & \quad \text{d} & \quad R = 4-\text{MeC}_2\text{H}_5\text{O} ; \quad R' = \text{Me} \\
\text{e} & \quad R = 4-\text{BrC}_2\text{H}_4\text{O} ; \quad R' = \text{Et} & \quad \text{f} & \quad R = 4-\text{ClC}_2\text{H}_4\text{O} ; \quad R' = \text{Et} \\
\text{g} & \quad R = 3,4-\text{Cl}_2\text{C}_2\text{H}_5\text{O} ; \quad R' = \text{Et} & \quad \text{h} & \quad R = 4-\text{MeC}_2\text{H}_5\text{O} ; \quad R' = \text{Et}
\end{align*}
\]

Reagents and conditions: i) K\(_2\)CO\(_3\), acetone

**Scheme V:** Synthesis of benzothiazole derivatives (7a-h)

The coupling was indicated in the IR spectra by the appearance of a sharp amidic carbonyl peak in the range of 1697-1687 cm\(^{-1}\). In \(^1\)H-NMR spectra, four new signals, each integrating to one proton were observed, in addition to the signals corresponding to the starting oxadiazoles. The splitting pattern of the new signals observed in the aromatic region *i.e.*, two doublets and two apparent triplets, is typical for o-disubstituted benzene ring. In the aliphatic region, a downfield singlet (4.63-4.42 ppm), with two protons integral, was assigned to the methylene protons sandwiched between the thio- and carbonyl groups, justifying the downfield shift of the signal. The appearance of a singlet at \(\delta 12.77\) (7d) and 12.78 in all other compounds at the expense of a singlet in the region \(\delta 11.62-9.91\) (6a-e) and at \(\delta 1.27\) for compounds (6f-h) supported the successful thioetherification. This downfield singlet was assigned to primary amidic proton. In \(^13\)C-NMR, new signals corresponding to the benzothiazolyl acetamide part of the molecule were observed in addition to the signals for starting oxadiazole moiety. The signal observed in the range of 35.9-36.2 ppm was
assigned to methylene carbon in compound (7a-h). The electron impact (EI) mass fragmentation pattern showed the fragments in agreement with the structure (Figure 2.4). However, inorder to determine the molecular ion peaks FABMS had to be carried out. In FABMS protonated molecular ion peaks were observed for all the compounds.

![Figure 2.4: Suggested modes of mass fragmentation for compounds (7a-h)](image)

The most abundant fragments were observed at m/z 191 and 177 as a result of cleavage of the bonds α- and β-to the amide linkage. The fragments of the ether cleavage as well as hydrogen shift to aryloxy oxygen were also observed. The fragments observed at m/z 291 and 104/105+R,R' were formed by the cleavage of molecule into two parts. The elemental analysis was found in good agreement with the calculated values for C, H, N and S.

2.2.3 Biological screening

2.2.3.1 In vitro antitumor activity

Compounds 6a-h and 7a-h were tested for their in vitro antitumor activity against a panel of tumor cell lines consisting of CD4 human T-cells containing an integrated leukaemia (CCRF-CEM), human acute T-lymphoblastic leukaemia (WIL-2NS), human splenic B-lymphoblastoid cells (CCRF-SB), human acute B-lymphoblastic leukaemia (SK-MEL-28), human skin melanoma (SK-MEL-28), human breast adenocarcinoma (MCF-7), human lung squamous carcinoma (SK-MES-1), human
hepatocellular carcinoma (HepG2), human prostate carcinoma (DU-145), human foreskin fibroblast (CRL.7065) and human lung fibroblast (MRC-5). The Microculture Tetrazolium Assay (MTT) method\textsuperscript{116} was used for the estimation of \textit{in vitro} tumor-inhibiting activity of the tested compounds. The cell lines of tumor sub panels were incubated within five concentrations (0.01-100 \, \mu M) of each tested compound for 48 h. The results are being displayed in Table 2.3. The oxadiazoles (6a-h) exhibited non significant activity against all the cancer cell lines with CC\textsubscript{50} >100 \, \mu M. However, coupling of the oxadiazoles with the benzothiazole via thioetether linkage resulted into an increase in the anticancer activity. The benzothiazole derivatives, 7c and 7f exhibited prominent cytotoxicity and selectivity towards leukemia cell lines CCRF-CEM, with CC\textsubscript{50} (\mu M) of 12±2, and 8±1, respectively. The compounds 7a, 7d, 7e, 7g and 7h were also found active against leukemia cell lines CCRF-CEM, with CC\textsubscript{50} (\mu M) of 18±4, 17±0.2, 14±3, 15±0.7 and 19±2, respectively. The least active compound against leukemia cell lines CCRF-CEM with CC\textsubscript{50} (\mu M) >100 \, \mu M was 7b. The compound 7c (CC\textsubscript{50} 14±0.7 \, \mu M) along with 7d, 7f, 7g and 7h exhibited good activity against human splenic B-lymphoblastoid cells WIL-2NS, with CC\textsubscript{50} (\mu M) of 64±2, 46±5, 58±5 and 40±7, respectively. The compounds 7a-h exhibited non significant activity against other cancer cell lines with CC\textsubscript{50} >100 \, \mu M.

The antitumor screening results revealed that the most interesting compounds were those combining two chloro substituents at 3 and 4-positions (compound 7e), which generally enhanced its antileukemic CCRF-CEM potency. The potency would have decreased for the tested derivatives by structural change with a single chloro substituent (e.g.: compound 7f, CC\textsubscript{50} 12±2 \, \mu M)). On the other hand, replacement of the chloro substituent of 7b or 7f with a bromo substituent (7a,7e) essentially decreased the leukemia inhibitory activity (CC\textsubscript{50} 14±3 \, \mu M). In summary, \textit{in vitro} screening led to the identification of the N-(benzothiazol-2-yl)-2-(5-(1-(3,4-dichlorophenoxy)ethyl)-1,3,4-oxadiazole-2-yllthio)acetamide (7e) as a new antitumor candidate, being the promising agent for further structural modifications, and pharmacological evaluation.
Table 2.3: Anti tumour activity of oxadiazoles (6a-h) and benzothiazole derivatives (7a-h)

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<tr>
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<th>MT-4</th>
<th>CCRF-CEM</th>
<th>WIL-2NS</th>
<th>CCRF-SB</th>
<th>SK-MEL</th>
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*Compd. conc. (µM) required to reduce the viability of mock-infected MT-4 (CD4+ Human T-cells containing an integrated HTLV-1 genome), CCRF-CEM (CD4+ human acute T-lymphoblastic leukaemia), WIL-2NS (Human splenic B-lymphoblastoid cells), CCRF-SB (Human acute B-lymphoblastic leukaemia), SK-MEL-28 (Human skin melanoma), MCF7 (Human breast adenocarcinoma), SK-MES-1 (Human lung squamous carcinoma), HepG2 (Human hepatocellular carcinoma), DU-145 (Human prostate carcinoma), CRL7065 (Human foreskin fibroblast), MRC-5 (Human lung fibroblast) by 50%, as determined by the colorimetric MTT method.
2.2.3.2 *In vitro* antiviral activity

Compounds 6a-h and 7a-h were also tested for their *in vitro* anti-HIV-1 activity, using IIIb strain in human T-lymphocyte (MT-4) cells, based on a Microculture Tetrazolium (MTT) assay\(^{116}\).

Cytotoxicity was also measured on MT-4 cells. None of the *in vitro* tested compounds was found to inhibit HIV-replication, at EC\(_{50}\) lower than the CC\(_{50}\) compared to the antiviral agent efavirenz (EFV)\(^{117}\). Thus, no selective anti-HIV activity could be witnessed (EC\(_{50}\) > 100 \(\mu\)M).

Compounds 6a-h and 7a-h (Table 2.4) were also evaluated against various viruses: mock-infected MDBK (bovine normal kidney); BVDV (bovine viral diarrhoea virus); mock-infected BHK (Hamster normal kidney fibroblast); BHK (kidney fibroblast) cells from the YFV (yellow fever virus) and Reo (reo virus 1); mock-infected VERO-76 (monkey normal kidney); HSV-1 (herpes virus 1), VV (vaccinia virus); VSV (vesicular stomatitis virus), CVB-2 (coxsackie virus B2), Sb-1(polio virus 1) and RSV (respiratory syncytial virus) by 50% in VERO-76 monolayers. As compared to the known antiviral agent Cidofovir\(^{118}\), no activity for any of the compounds at non-toxic concentrations was observed.
Table 2.4: Antiviral screening of oxadiazoles (6a-h) and benzothiazole derivatives (7a-h)

<table>
<thead>
<tr>
<th>Compd</th>
<th>^aMT-4 CC50 [µM]</th>
<th>^bHIV-1 EC50 [µM]</th>
<th>^cMDBK CC50 [µM]</th>
<th>^dBVDN EC50 [µM]</th>
<th>^eBHK-21 CC50 [µM]</th>
<th>^fYFV EC50 [µM]</th>
<th>^gReo-1 CC50 [µM]</th>
<th>^hVero-76 EC50 [µM]</th>
<th>^iHSV-1 CC50 [µM]</th>
<th>^jVV EC50 [µM]</th>
<th>^kVSV EC50 [µM]</th>
<th>^lCVB-2 EC50 [µM]</th>
<th>^mSB-1 EC50 [µM]</th>
<th>^nRSV EC50 [µM]</th>
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^a Compound conc. (µM) required to reduce the viability of mock-infected MT-4 (CD4+ Human T-cells containing an integrated HTLV-1 genome) cells by 50%, as determined by the colorimetric MTT method. ^b Compound conc. (µM) required to achieve 50% protection of MT-4 cells from the HIV-1-induced cytopathogenicity, as determined by the MTT method. ^c Compound conc. (µM) required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. ^d Compound conc. (µM) required to achieve 50% protection of MDBK cells from the BVDV (Bovine Viral Diarrhea Virus)-induced cytopathogenicity, as determined by the MTT method. ^e Compound conc. (µM) required to achieve 50% protection of BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. ^f Compound conc. (µM) required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. ^g Compound conc. (µM) required to achieve 50% protection of BHK (Kidney fibroblast) cells from the YFV (Yellow Fever Virus) and Reo (Reovirus 1)-induced cytopathogenicity, as determined by the MTT method. ^h Compound conc. (µM) required to reduce the viability of mock-infected VERO-76 (Monkey normal kidney) monolayers by 50%, as determined by the MTT method. ^i Compound conc. (µM) required to reduce the plaque number of HSV-1 (Herpesvirus 1), VV (Vaccinia Virus), VSV (Vesicular Stomatitis Virus), CVB-2 (Coxsackievirus B2), Sh-I (Poliovirus 1) and RSV (Respiratory Syncytial Virus) by 50% in VERO-76 monolayers.
2.3 Synthesis of 3-alkyl-4-amino-1,2,4-triazole-5-thiones (8a-i)

The hydrazides (Si-o, r, s) were converted to 4-amino-1,2,4-triazoles (Scheme VI, 8a-i) using a published procedure. The completion of the reaction was monitored by TLC and lead acetate test. The triazoles were obtained from moderate to very good yields (52-75%).

\[
\begin{align*}
\text{R} & \quad \text{HC} - \text{CONHNH}_2 & \quad \text{i, ii, iii} & \quad \text{R}_1 \quad \text{HC} \quad \text{N} = \text{S} \\
\text{HN} & \quad \text{PTS} & & \text{NH} \\
\text{5i-o, r, s} & & & \text{8a-i} \\
\end{align*}
\]

Reagents and conditions: i) CS$_2$ + KOH; ii) N$_2$H$_4$H$_2$O; iii) 6N HCl

Scheme VI: Synthesis of 4-amino-1,2,4-triazoles

The cyclization of the hydrazides (5i-o, r, s) to triazoles (8a-i) was indicated in the IR spectra by the appearance of weak absorption bands for C=O in the range of 1597 to 1553 cm$^{-1}$ at the expense of the strong carbonyl absorption in the region of 1670 to 1650 cm$^{-1}$. The structures of triazoles (8a-i) were confirmed by NMR spectroscopy. In $^1$H-NMR spectra, the appearance of a down field singlet in the range $\delta$ 13.32-12.08 integrating to one proton was assigned to N–H proton of the triazole ring. The signals for 4-methylbenzene ring protons were observed as two doublets. The pronounced diastereostopic effect was observed for the aliphatic protons, resulting into the complex splitting patterns for the influenced protons. In $^{13}$C-NMR spectra of triazoles (8a-i), the downfield signals in the range 155-160 and 167-178 ppm were assigned to the C-2 and C-5 of the triazole ring, respectively. The synthesis was further confirmed by mass spectral analysis. The common fragment observed in all the cases was at m/z 155 resulting by the cleavage of sulfonamide linkage. The loss of SO$_2$ from this fragment generated a fragment at m/z 91, observed as the base peak in most
of the cases. The elemental analysis was also found in good agreement with the calculated values for C, H, N and S.

![Chemical structure diagram](image)

**Figure 2.5:** Suggested modes of fragmentation for 1,2,4-triazoles (8a-i)

### 2.3.1 Biological Studies

#### 2.3.1.1 Urease Inhibition Activity

The urease inhibition activity of the selected compounds (8a-8c, 8e, 8g, 8i) was carried out according to the literature protocol using thiourea as the standard inhibitor having an IC$_{50}$ value of 21 μM. The results are summarised in the Table 2.5.

**Table 2.5:** The urease inhibitory activity of compounds (8a-c, e, g, i) against jack bean urease

<table>
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<tr>
<th>Compound</th>
<th>R</th>
<th>IC$_{50}$ ± SEM (μM)</th>
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<tbody>
<tr>
<td>8a</td>
<td>CH$_3$</td>
<td>35.9 ± 0.5</td>
</tr>
<tr>
<td>8b</td>
<td>CH(CH$_3$)$_2$</td>
<td>22.0 ± 1.6</td>
</tr>
<tr>
<td>8c</td>
<td>CH$_3$CH(CH$_3$)$_2$</td>
<td>33.5 ± 3.0</td>
</tr>
<tr>
<td>8e</td>
<td>CH$_2$OH</td>
<td>33.6 ± 0.9</td>
</tr>
<tr>
<td>8g</td>
<td>CH$_3$CH$_2$SCH$_3$</td>
<td>43.8 ± 0.3</td>
</tr>
<tr>
<td>8i</td>
<td></td>
<td>29.7 ± 0.2</td>
</tr>
<tr>
<td>Thiourea (NH$_2$CSNH$_2$)</td>
<td></td>
<td>21.0 ± 0.01</td>
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</table>

All the six scanned compounds exhibited good urease inhibition activity. Compound 8b proved to be the most potent showing good enzyme inhibition activity with an IC$_{50}$ = 22.0 μM which is comparable to 21.0 μM of the standard. The compound 8i
also exhibited a good activity with an IC₅₀ value of 29.7 μM. The least active compound (8g) had an IC₅₀ = 43.8 μM, the activity of the rest of the compounds fall in the range 33.5 – 35.9 μM. From these results it appears that there is no effect of the bulk of ‘R’ group (at chiral centre) on the activity of these compounds. The most active compounds of the series i.e., 8b may act as a potential molecule for the structural modifications. It is suggested that the substitution either on mercapto or amino or on both may reveal a potent lead molecule for future research in the field of urease inhibition.

2.3.1.2 Antimicrobial Activity

The synthesized compounds were tested for their antibacterial activity using agar well diffusion method at a sample concentration of 5mg/mL of DMSO against five bacterial strains for 24 and 48 hours. The activities of synthesised compounds were found to be non-significant as compared to the standard cefexime and roxithromycin, against the tested bacterial strains: Escheria Coli, Micrococcus Luteus, Pseudomonas Picketii, Bordetella Bronchiseptica and Enterococcus avium.

These compounds were also tested for their antifungal activity at sample concentration of 200μg/mL of DMSO for 7 days at 27 °C against Trichphyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani and Candida glabrata, but no significant activity was observed at non toxic concentrations.

2.4 Synthesis of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles through thiosemicarbazides

The disubstituted 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles were synthesized under varying cyclization conditions, using thiosemicarbazides as intermediates.
2.4.1 Thiosemicarbazides

2.4.1.1 Synthesis

The synthesis of thiosemicarbazides is divided into two groups for the ease of understanding. The synthesis was carried out using a literature procedure.\textsuperscript{121}

a) Synthesis of 1-(2-hydroxy-2-phenylacetyl)-4-aryl thiosemicarbazides (10a-e)

The thiosemicarbazides (10a-e) were prepared by the reaction of mandelic acid hydrazide (5t) with arylisothiocyanates (9a-e) in refluxing methanol (scheme VII). The completion of the reaction was indicated by TLC and resulting thiosemicarbazides were recrystallized from aqueous ethanol.

\[ \text{Scheme VII: Synthesis of arythiosemicarbazides using mandelic acid hydrazide} \]

b) Synthesis of 1-[2-(4-methylbenzenesulfonamido)alkanoyl]-4-(4-chlorophenyl)thiosemicarbazides (10f-m)

The thiosemicarbazides (10f-m) were synthesized by the reaction of hydrazides (5j-m, o-q,s) with 4-chlorophenyl isothiocyanate (9c) in methanol under reflux (scheme VIII). The thiosemicarbazides were recrystallized from aqueous ethanol. The thiosemicarbazides (11a-h) were characterized using spectroscopic techniques.
Scheme VIII: Synthesis of thiosemicarbazides using amino acid hydrazides

2.4.1.2 Characterization of thiosemicarbazides (10f-m)

The IR spectra of thiosemicarbazides exhibited broad absorptions for NH peaks in the region of 3480-3260 cm\(^{-1}\) at the expense of strong NH\(_2\) absorptions. The shift in the carbonyl stretching was also observed along with the appearance of the thiocarbonyl stretchings in the region 1250-1200 cm\(^{-1}\). The peaks for the unsymmetrical and symmetrical O=S=O stretchings were observed in the regions 1360-1330 and 1170-1145 cm\(^{-1}\), respectively.

In \(^1\)H-NMR spectra, formation of the product was confirmed by the appearance of two new doublets/multiplets in the aromatic region, corresponding to the chlorophenyl moiety in the molecule. The presence of three apparently broad downfield singlets integrating to one proton each was a strong evidence for the formation of thiosemicarbazides. These singlets were assigned to three NH protons of the thiosemicarbazide functionality, as compared to only two signals in the spectra of hydrazides.

2.4.2 Synthesis of 2-arylamo-5-(hydroxybenzyl)-1,3,4-oxadiazole (11a-e) and 4-aryl-5-(hydroxybenzyl)-1,2,4-triazole-3-thiones (12a-e)

The 1,3,4-oxadiazoles (11a-e) and 1,2,4-triazoles (12a-e) were synthesized from thiosemicarbazides (10a-e) using different sets of reaction conditions. The 1,3,4-oxadiazoles were synthesized by the cyclization of thiosemicarbazides (10-a-e) in the
presence of mercuric acetate\textsuperscript{122} and 1,2,4-triazoles by cyclodehydration under basic conditions\textsuperscript{123} and (scheme VIII).

\begin{center}
\begin{align*}
\text{10a-e} & \\
\text{11a-e} & \\
\text{12a-e} & \\
\text{Reagents and conditions: i) Hg(OAc)\textsubscript{2}; ii) 5\% NaOH}
\end{align*}
\end{center}

Scheme IX: Cyclization of thiosemicarbazides to 1,3,4-oxadiazoles and 1,2,4-triazoles

The syntheses of oxadiazoles (11a-e) and triazoles (12a-e) were indicated in their IR spectra by disappearance of the strong carbonyl absorptions in the spectra of thiosemicarbazides from 1670 to 1650 cm\textsuperscript{-1} and appearance of weak bands in the region of 1620 to 1580 cm\textsuperscript{-1} for C=N stretchings. The disappearance of broad NH absorption bands in the spectra of thiosemicarbazides in the region of 3480-3260 cm\textsuperscript{-1} also indicated the cyclization into the respective nuclei.

In \textsuperscript{1}H-NMR spectra, the disappearance of three signals for NH protons confirmed the cyclization of thiosemicarbazides to the respective nucleus. A downfield singlet observed in the range of 5.90-5.59 ppm was assigned to the CH proton in all the compounds (11a-e, 12a-e). The signals for the aromatic protons were observed as three sets. The phenyl ring protons appeared as a multiplet integrating for five protons and the signals for the p-disubstituted benzene ring were observed as two doublets. In \textsuperscript{13}C-NMR spectra the carbon signal observed in the range of 63.6-67.6 ppm was assigned to the carbinol carbon (CHOH). The aromatic carbons were observed between 113-126 ppm. In the spectra of triazoles (12a-e) the carbon signals in the range 169.7-170.4 ppm and 153.5-155.6 ppm were assigned to the C-3 (C=S) and C-5 (C=N) of the triazole ring, respectively. The appearance of a carbon signal near 170 ppm confirmed the existence of triazole nucleus in the thione form. In case of oxadiazoles (11a-e), the C-2 and C-5 (C=N) carbons were observed between 160.1-
161.1 ppm. The mass spectra further confirmed the formation of the desired nuclei. The molecular ion peak was observed in all the compounds. In case of chloro and bromo substituents characteristic isotopic patterns were observed. The fragments corresponding to both aromatic rings were also observed along with the fragments showing the cleavage of oxadiazole and triazole moieties.

2.4.2.1 Biological Studies

a) Urease Inhibition Studies

The synthesized compounds (11a-e, 12a-e) were tested for their *in vitro* urease inhibition activities at 0.2mM concentration against jack beans urease. The urease inhibition activity was carried out according to the literature protocols\(^1\) using thiourea as the standard inhibitor having an IC\(_{50}\) value of 21 ± 0.01 μM. The results are presented in Table 2.6.

**Table 2.6: Urease Inhibition studies of oxadiazoles (11a-e) and triazoles (12a-e)**

<table>
<thead>
<tr>
<th>Compd</th>
<th>Inhibition (%)</th>
<th>IC(_{50}) ±SEM (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11a</td>
<td>6.7</td>
<td>---</td>
</tr>
<tr>
<td>11b</td>
<td>-ive</td>
<td>---</td>
</tr>
<tr>
<td>11c</td>
<td>3.1</td>
<td>---</td>
</tr>
<tr>
<td>11d</td>
<td>91.5</td>
<td>16.1 ± 0.12</td>
</tr>
<tr>
<td>11e</td>
<td>86.2</td>
<td>80.3 ± 1.97</td>
</tr>
<tr>
<td>12a</td>
<td>99.3</td>
<td>32.0 ± 0.0272</td>
</tr>
<tr>
<td>12b</td>
<td>98.0</td>
<td>18.9 ± 0.188</td>
</tr>
<tr>
<td>12c</td>
<td>2.1</td>
<td>---</td>
</tr>
<tr>
<td>12d</td>
<td>99.6</td>
<td>16.7 ± 0.178</td>
</tr>
<tr>
<td>12e</td>
<td>61.2</td>
<td>131.7 ± 1.546</td>
</tr>
<tr>
<td>Thiourea</td>
<td></td>
<td>21 ± 0.011</td>
</tr>
</tbody>
</table>

The tested compounds exhibited negative to moderate and excellent inhibition. The compounds 11d, 12b and 12d were found more potent than the standard with IC\(_{50}\) =
16.1, 18.9 and 16.7 µM, respectively (compared to thiourea with IC$_{50} = 21.0$ µM). The most active compound was the one which has methyl group on the aminophenyl part in both 1,3,4-oxadiazole (11d) and 1,2,4-triazole (12d) nuclei. The other compound having greater inhibition than the standard was the 1,2,4-triazole (12b) with 4-chloro substituent on the aminophenyl part but its corresponding oxadiazole (11b) revealed negative inhibition. The compound with $p$-fluoro substitution (11c, 11e) exhibited no observable activity. From these results it can be concluded that the compounds with electron donating group, i.e., methyl, has enhanced the activity while those with the strong electron withdrawing groups like NO$_2$ (11e, 12e) lowered the activity. The compounds with chloro (11b, 12b) and bromo (11a, 12a) substitution showed a wide difference in activity with change of heterocyclic nucleus i.e., from oxadiazole to triazole the activity is appreciably increased.

The higher activity of the compounds against urease can be explained on the basis of the substrate like inhibition mechanism$^{124}$ in which the compound has structural similarity with the natural substrate of the enzyme, i.e., urea (Figure 2.6). The compounds have binding site with urea like structure and bind the active site of the enzyme. The enzyme fails to catalyze the hydrolysis and as a result the activity of the enzyme is retarded.

![Figure 2.6](image)

Figure 2.6: Representation of the possible binding to urease (a) natural substrate of urease i.e., urea (b) 1,2,4-triazole-5-thiones (c) 2-aminoaryl-1,3,4-oxadiazoles

b) Antibacterial Assay

In vitro antibacterial assay was performed using agar well diffusion method$^{120}$ on six different bacterial strains: *Escheria coli*, *Bacillus subtilis*, *Shingella flexenari*, *Staphylococcus aureu*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. The
synthesized 1,3,4-oxadiazoles (11a-e) and 1,2,4-triazoles (12a-e) were found inactive against these bacterial strains.

2.4.3 Synthesis of 1,2,4-triazoles (13a-g) and 1,3,4-thiadiazoles (14a-e,h)

The amino acid derived thiosemicarbazides (10f-m) were converted to the respective 1,2,4-triazoles (13a-g) and 1,3,4-thiadiazoles (14a-e,h) on cyclodehydration under basic and acidic conditions, respectively (scheme IX).

\[ \text{Reagents and conditions: i) aq. NaOH; ii) Conc. H}_2\text{SO}_4 \]

**Scheme IX: Basic and acidic cyclodehydration of thiosemicarbazides**

The synthesis of 1,2,4-triazoles (13a-g) was indicated in their IR spectra by the absence of strong carbonyl absorption. The absence of SH stretching in the characteristic region of 2550-2650 cm\(^{-1}\) and presence of C=S stretching in the region of 1250-1200 cm\(^{-1}\) indicated the thione form of triazoles. In \(^1\)H-NMR spectra, four doublets were observed in the aromatic region; two each for the methylphenyl and 4-chlorophenyl moiety. The signal for NH of sulfonamide moiety was observed as doublet in some cases and as a broad singlet in others in the range of \(\delta 8.34-6.74\). The NH of the triazole ring appeared deshielded as expected in the range of \(\delta 13.87-12.61\),
further confirming the existence of structures in thione form. The complex signals of aliphatic protons were the result of diastereotopic effects.

In the $^{13}$C-NMR spectra of 1,2,4-triazole-3-thiones (13a-g), the values near to $\delta$ 168 and between 145-160 ppm were reported for C=S (C-3) and C=N (C-5), respectively\textsuperscript{125}. With reference to literature values the $^{13}$C-NMR shifts in the range of $\delta$ 168.0-171.2 and $\delta$ 150.3-154.9 observed for compounds (14a-g) may be assigned to (C=S) C-3 and C=N (C-5), respectively.

The synthesis of 1,3,4-thiadiazoles (14a-e, h) was indicated in their IR spectra by the disappearance of the thiocarbonyl, the strong carbonyl and broad NH absorptions observed for starting thiosemicarbazides (10f-m). In $^1$H-NMR spectrum of each compound, $p$-disubstituted benzene rings exhibited four doublets, integrating to eight protons, in the aromatic region, two for the methylphenyl part and two for 4-chlorophenyl part of the molecule. The diastereotopic effects resulted into the complex splitting patterns of the aliphatic protons. The signal for NH of sulfonamide was observed as doublet in some cases and as a broad singlet in others in the range of $\delta$ 8.34-6.74. The signal for arylamino proton appeared in the range of $\delta$ 10.13-8.14. The aliphatic and aromatic protons did not show much shift in their chemical shift values when compared to the corresponding thiosemicarbazides.

In $^{13}$C-NMR spectra of compounds (14a-e,h), the signals in the range of $\delta$ 155-160 were assigned to the thiadiazole ring carbons; C-2 and C-5 (C=N). As a result of symmetrical substitution on benzene rings eight signals for twelve carbons were observed in the aromatic region.

In EIMS spectra, molecular ion peaks were observed in all the compounds except for 13d, 13f, 13g, 14a, 14d and 14e. However, the fragments involving the cleavage of the triazole and thiadiazole nucleus were observed. The most common fragments resulted by the cleavage of sulphonamide linkage and were observed at m/z 155 and 171. Further cleavage leading to the formation of tropylium ion at m/z 91 was observed as base peak in most of the case. The elemental compositions for C, H, N and S were found in good agreement with the calculated values.
2.4.3.1 *In vitro* Anti-HIV screening

Compounds 13a-f, 14a-e and 14h were tested for their *in vitro* anti-HIV-1 (strain IIIb) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells. The results are summarized in Table 2.7 and 2.8, where the data for efavirenz and capravirine were included for comparison purposes. Compound 13f was found to be the only compound from the series, inhibiting HIV-1 replication in cell culture. Compound 13f exhibited an EC$_{50}$ of 23.9 µg/mL against HIV-1 and 9.90 µg/mL against HIV-2 at CC$_{50}$ of 72.7 ± 1.4 µg/mL, resulting in selectivity index of 3 and 7, respectively.

Table 2.7: *In vitro* anti-HIV-1$^{a}$ and HIV-2$^{b}$ of 1,2,4-triazoles (13a-f)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Virus strain</th>
<th>EC$_{50}$ (µg/ml)$^{c}$</th>
<th>CC$_{50}$ (µg/ml)$^{d}$</th>
<th>SI$^{e}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>IIIb</td>
<td>&gt;50.9</td>
<td>58.2 ± 10.1</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;48.1</td>
<td>58.2 ± 10.1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>13b</td>
<td>IIIb</td>
<td>&gt;17.5</td>
<td>19.2 ± 1.2</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;19.4</td>
<td>19.2 ± 1.2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>13c</td>
<td>IIIb</td>
<td>&gt;17.2</td>
<td>25.0 ± 8.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;18.1</td>
<td>25.0 ± 8.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>13d</td>
<td>IIIb</td>
<td>&gt;105.0</td>
<td>≥105.0</td>
<td>≤1</td>
</tr>
<tr>
<td></td>
<td>IIIb</td>
<td>&gt;105.0</td>
<td>≥105.0</td>
<td>≤1</td>
</tr>
<tr>
<td>13e</td>
<td>IIIb</td>
<td>&gt;66.0</td>
<td>74.3 ± 8.6</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;67.7</td>
<td>74.3 ± 8.6</td>
<td>&lt;1</td>
</tr>
<tr>
<td>13f</td>
<td>IIIb</td>
<td>23.9</td>
<td>72.7 ± 1.4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>9.9</td>
<td>72.7 ± 1.4</td>
<td>7</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>IIIb</td>
<td>0.003</td>
<td>40</td>
<td>13,333</td>
</tr>
<tr>
<td>Capravirine</td>
<td>IIIb</td>
<td>0.0014</td>
<td>11</td>
<td>7,857</td>
</tr>
</tbody>
</table>

$^{a}$Anti-HIV-1 activity measured with strain IIIb. $^{b}$Anti-HIV-2 activity measured with strain ROD. $^{c}$Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1- and 2-induced cytopathogenic effect. $^{d}$Compound concentration that reduces the viability of mock-infected MT-4 cells by 50%. $^{e}$SI: Selectivity index (CC$_{50}$/EC$_{50}$)
Based on the chemical structure and the fact that compounds 13f inhibited HIV-2, it may be proposed that this molecule and its derivatives can act as a non-nucleoside reverse transcriptase inhibitor (NNRTI).

Table 2.8: *In vitro* anti-HIV-1\(^a\) and HIV-2\(^b\) of 1,3,4-thiadiazoles (14a-e,h)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Virus strain</th>
<th>(\text{EC}_{50}) (µg/ml)(^c)</th>
<th>(\text{CC}_{50}) (µg/ml)(^d)</th>
<th>SI(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14a</td>
<td>III(_B)</td>
<td>&gt;14.0</td>
<td>14.0 ± 1.2</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;12.4</td>
<td>14.0 ± 1.2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>14b</td>
<td>III(_B)</td>
<td>&gt;125.0</td>
<td>&gt;125.0</td>
<td>=1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;125.0</td>
<td>&gt;125.0</td>
<td>=1</td>
</tr>
<tr>
<td>14c</td>
<td>III(_B)</td>
<td>&gt;47.4</td>
<td>56.8 ± 13.9</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;67.5</td>
<td>56.8 ± 13.9</td>
<td>&lt;1</td>
</tr>
<tr>
<td>14d</td>
<td>III(_B)</td>
<td>&gt;73.3</td>
<td>74.2 ± 2.8</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;70.6</td>
<td>74.2 ± 2.8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>14e</td>
<td>III(_B)</td>
<td>&gt;12.6</td>
<td>13.3 ± 0.8</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;12.5</td>
<td>13.3 ± 0.8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>14h</td>
<td>III(_B)</td>
<td>&gt;62.7</td>
<td>68.9 ± 8.2</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;65.9</td>
<td>68.9 ± 8.2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>III(_B)</td>
<td>0.003</td>
<td>40</td>
<td>13,333</td>
</tr>
<tr>
<td>Captoprivine</td>
<td>III(_B)</td>
<td>0.0014</td>
<td>11</td>
<td>7,857</td>
</tr>
</tbody>
</table>

\(^a\)Anti-HIV-1 activity measured with strain III\(_B\). \(^b\)Anti-HIV-2 activity measured with strain ROD. \(^c\)Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1- and 2-induced cytopathogenic effect. \(^d\)Compound concentration that reduces the viability of mock-infected MT-4 cells by 50%. \(^e\)SI: Selectivity index (\(\text{CC}_{50}\)/\(\text{EC}_{50}\))

2.5 Synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (15a-e')

The synthesis of 1,3,4-oxadiazoles (15a-d', scheme X) was also carried out by adopting a one pot-two step procedure. The respective hydrazide (5a-h) and isothiocyanates (9a-e) were refluxed and the consumption of hydrazide followed through TLC (2-3 hr). The required quantity of mercuric acetate [Hg(OAc)]\(_2\)] was added and the reaction monitored again by T.L.C. After the completion of reaction (4-5 hr), the isolated product was purified by recrystallization from aqueous ethanol.
Reagents and conditions: i) MeOH, reflux; ii) Hg(OAc)$_2$, reflux

Scheme X: One pot-two step synthesis of 2,5-disubstituted-1,3,4-oxadiazoles

The one pot procedure has advantage over the step wise method in that it is less time consuming, has a simple work up and gives better purified yields. The IR spectra of compounds (15a-d') indicated the formation of 1,3,4-oxadiazole ring by the appearance of weak C=N absorptions in region of 1620-1580 cm$^{-1}$ at the expense of strong carbonyl absorptions in the region from 1670 to 1650 cm$^{-1}$ observed in the spectra of hydrazides. The broad NH absorptions in the region of 3400-3200 cm$^{-1}$ for –NHNH$_2$ also disappeared and absorption for NH was observed in the range 3270-3250 cm$^{-1}$.

In $^1$H-NMR spectra, the cyclization to 1,3,4-oxadiazoles was confirmed by the appearance of only one downfield singlet in the range of $\delta$ 11.67-8.47 with one proton.
integral, assigned to the NH proton at the expense of two signals integrating to three protons in the spectrum of the corresponding hydrazide. The integration of the aromatic protons confirmed that the cyclization has taken place and two benzene rings are present with varied substitution on the ring. The signals for the aromatic protons were observed as two multiplets for the p-substituted aminophenyl moiety and similarly two multiplets for p-substituted phenoxy moiety. The $^1$H-NMR spectra of 3,4-dichlorophenoxy moiety, compounds (15 e, i, k, o, r, v, y, d') contained three signals in the aromatic region, a doublet ($^4J = 3.0$ Hz) assigned to H-2 residing between chloro and oxygen, the second doublet ($^3J = 9.0$ Hz) for proton (H-6) ortho to oxygen and a double doublet ($^4J = 3.0$, $^3J = 9.0$ Hz) for the proton (H-5) ortho to the chlorine atom. In aliphatic region the signal for CH was observed downfield as a quartet (15a-q) or a triplet (15r-d'). The signal for methyl protons was observed as a doublet (15a-q) or triplet (15r-d') and methylene protons (15r-d') were observed as a multiplet with a slight down filed shift due to the electron withdrawing oxygen on the adjacent carbon.

In $^{13}$C-NMR spectra, an upfield shift of 5-10 ppm for the signal of carbonyl carbon in the region of 165-170 ppm in the spectra of hydrazides, was a confirmation of C=N bond formation in the oxadiazoles. The signals for all other aliphatic carbons did not show any significant shifts as compared to the hydrazides. Four new signals in aromatic region corresponding to the 4-aminophenyl moiety were observed. The carbon signals for the compounds (15c-f, x-z) bearing 4-fluorophenoxy moiety were observed as doublets with different $J_{CF}$ values depending on the number of intervening bonds.

The structures of the compounds (15a-d') were further confirmed by the mass spectral analysis. The mass fragmentation patterns were in accordance with the structural features of the molecules. The most important fragments for the cleavage of oxadiazole nucleus were observed at m/z 119 + X and m/z 132/133 + R, R' corresponding to the O–C=N and N–N–C–O cleavage (Figure 3.5). Other fragments containing aminoaryl and aryloxy moieties were also observed. The observed fragments led to the confirmation of the suggested structure for 2,5-disubstituted-1,3,4-oxadiazoles.
2.5.1 Urease inhibition studies

The compounds (15h, 15l, 15n, 15o, 15a', 15b' and 15c') were identified to inhibit the urease on the basis of their structure and may be regarded as substrate like inhibitors as shown in figure 2.6. A selected number of compounds were tested for the potential to inhibit jack bean urease. The IC$_{50}$ values and percent inhibitions of the compounds are presented in Table 2.9.

Table 2.9: Urease inhibition activity of selected oxadiazoles of the series

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition (%)</th>
<th>IC$_{50}$ ±SEM (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15h</td>
<td>5.3</td>
<td>---</td>
</tr>
<tr>
<td>15l</td>
<td>63.4</td>
<td>---</td>
</tr>
<tr>
<td>15n</td>
<td>15.8</td>
<td>---</td>
</tr>
<tr>
<td>15o</td>
<td>27.6</td>
<td>---</td>
</tr>
<tr>
<td>15a'</td>
<td>9.5</td>
<td>---</td>
</tr>
<tr>
<td>15b'</td>
<td>95.9</td>
<td>7.76±0.27</td>
</tr>
<tr>
<td>15c'</td>
<td>11.4</td>
<td>---</td>
</tr>
<tr>
<td>Thiourea</td>
<td></td>
<td>21 ± 0.011</td>
</tr>
</tbody>
</table>

Among the tested compounds 15b' emerged as the most active compound, exhibiting 95.9 percent inhibition at sample concentration of 0.1mM. The compound 15b' also had a very low IC$_{50}$ value of 7.76 µM as compared to the standard used (thiourea, IC$_{50}$= 21µM). The other tested compounds exhibited non significant activity even at a sample concentration of 0.5 mM. The compound 15b' was identified as a future candidate for antiulcer drugs on the basis of its very good urease inhibition and low IC$_{50}$ values.
Conclusions

The azoles *i.e.*, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles were synthesized either by direct cyclization of hydrazides or through intermediate thiosemicarbazides. Besides, a one step method for the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles using hydrazides and arylisothiocyanates was also investigated successfully. The hydrazides remained the key intermediate in the synthetic scheme. The hydrazides were synthesized from the selected carboxylic acids via esterification followed by hydrazinolysis. The selected acids included L-amino acids, aryloxyalkanoic acids and mandelic acid, all having chiral centre at position α to the carboxylic functional group. The syntheses of azoles *i.e.*, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles was confirmed by spectroanalytical techniques.

The azoles were tested for their potential as urease inhibiting, anticancer, antiviral and antimicrobial agents. Some of the compounds exhibited promising urease inhibition, anticancer and antiviral activities. Compound 7c exhibited very good anticancer activity with CC₅₀ of 8±1 (µM) and was identified as a promising agent for further structural modifications and pharmacological evaluations. Among other compounds 7d, 7f, 7g and 7h exhibited significant anticancer activity. Compounds 11d, 12b, 12d and 15b' were found urease inhibitors and more potent than the standard (thiourea IC₅₀ = 21±0.011) with IC₅₀ values of 16.1±0.12, 18.9±0.18, 16.7±0.17 and 7.76±0.27 µM, respectively. These preliminary results of excellent urease inhibition and lower IC₅₀ values incite further investigations of these compounds as antiulcer drugs. The antiviral screening of the compounds revealed 13f as a promising antiviral agent. Based on the structure it may be proposed that 13f or its derivatives may act as non-nucleoside reverse transcriptase inhibitors. The antimicrobial activities of the tested compounds were found non-significant at non-toxic concentrations.
Chapter 3

EXPERIMENTAL
Experimental

3.1 Materials and Methods

The reaction conditions are described in the detailed procedure. The reagents used were of high purity grade. The solvents were purified before use. The synthesis of phenoxy acids was carried out by opting standard procedure for the synthesis of phenoxy alkanoic acids\textsuperscript{112}. The acids were converted to hydrazides via Fischer esterification followed by hydrazinolysis\textsuperscript{112}. 1,3,4-Oxadiazole-2-thiones/thiols were synthesized by literature protocol\textsuperscript{20d} and converted to their benzothiazole derivatives\textsuperscript{115} using anhydrous potassium carbonate and actone. 1,2,4-Triazole-5-thiones/thiols were synthesized by a method reported by Reid et al.\textsuperscript{21} The thiosemicarbazides were subjected to different cyclization conditions to afford 1,3,4-oxadiazoles\textsuperscript{122}, 1,3,4-thiadiazoles\textsuperscript{123} and 1,2,4-triazoles\textsuperscript{123}.

The compounds were characterized by their physical constants and spectroanalytical techniques. Thin layer chromatography (TLC) was carried out using precoated silica gel 60 HF\textsubscript{254} aluminum sheets (Merek). The melting points were determined on Sanyo Gallenkamp digital melting point apparatus in open capillaries and are uncorrected. UV spectra were recorded on Lambda20, Perkin Elmer spectrophotometer. Specific rotation $[\alpha]$ was measured on ATAGO AP-100 automatic polarimeter. IR spectra were recorded on FTX 3000 MX BioRad Excalibur Series IR spectrophotometer using KBr pellets and Perkin Elmer-ATR IR. $^1$H and $^{13}$C-NMR spectra were recorded on Bruker 300 MHz and 500 MHz spectrometers and calibrated to residual solvent peaks. The multiplicities are abbreviated as s = singlet, d = doublet, dd = double doublet, ad = apparent doublet, t = triplet, at = apparent triplet, dt = doulet of triplet, td = triplet of doublet, q = quartet, m = multiplet and br = broad. Mass spectra were recorded on a MAT-112-S spectrometer at 70 eV.

The antimicrobial activities were carried out using agar well diffusion method\textsuperscript{120}. Urease inhibition studies were performed on jack bean urease using indephenol method\textsuperscript{119}. The anticancer and antiviral studies were carried out by microculture tetrazolium test (MTT) method\textsuperscript{116}. 

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3.2 General Procedure for the Synthesis of aryloxyalkanoic acids

In a typical experiment a solution of 2-bromopropanoic/butanoic acid (1.2 eq) in 2 M NaOH (5.0 ml) was added to a solution of 3,4-dichlorophenol (1.0 eq) in 2 M NaOH (5.0 ml), and the mixture refluxed. On completion of the reaction (thin-layer chromatography), the reaction mixture was cooled to room temperature, acidified with 6 M HCl and extracted with diethyl ether (3 x 25 ml). The combined ether extracts were washed with brine (25 ml), dried (anhydrous MgSO₄) and the solvent evaporated in vacuo after filtration. The product was recrystallized from aqueous ethanol.

2-(4-Bromophenoxy)propanoic acid (3a). Yield: 62%; Yellow oil; IR (ATR, cm⁻¹): νᵢ₅ 3250-2530, 1712, 1595, 1483.

2-(4-Chlorophenoxy)propanoic acid (3b). Yield: 67%; m.p. 116-117°C; IR (ATR, cm⁻¹): νᵢ₅ 3200-2550, 1713, 1593, 1487.

2-(3,4-Dichlorophenoxy)propanoic acid (3c). Yield: 91%; m.p. 130-131°C; IR (ATR, cm⁻¹): νᵢ₅ 3270-2490, 1708, 1597, 1482.

2-(4-Methylphenoxy)propanoic acid (3d). Yield: 73%; m.p. 74-76°C; IR (ATR, cm⁻¹): νᵢ₅ 3270-2580, 1698, 1591, 1474.

2-(4-Bromophenoxy)butanoic acid (3e). Yield: 87%; Brownish oil; IR (neat, cm⁻¹): νᵢ₅ 3260-2510, 1712, 1591, 1487.

2-(4-Chlorophenoxy)butanoic acid (3f). Yield: 79%; Brownish oil; IR (neat, cm⁻¹): νᵢ₅ 3280-2570, 1714, 1592, 1488.

2-(3,4-Dichlorophenoxy)butanoic acid (3g). Yield: 96%; Brownish oil; IR (neat, cm⁻¹): νᵢ₅ 3200-2480, 1712, 1595, 1486.
2-(4-Methylphenoxy)butanoic acid (3h). Yield: 85%; Brownish oil; IR (neat, cm\(^{-1}\)): \(\nu_{\text{max}} \) 3220-2510, 1702, 1593, 1478.

3.3 Synthesis of \(N\)-(4-methylbenzenesulfonyl) amino acids

The respective amino acid (0.01 mol) was dissolved in aqueous solution of sodium carbonate (0.02 mol, 50 mL) and added a solution of PTS chloride (0.012 mol) in diethyl ether (25 mL). The reaction mixture was stirred vigorously for 6-8 hours. The ether layer was separated and the aqueous layer was acidified with dilute hydrochloric acid. The precipitated solid was filtered and recrystallized from aqueous ethanol.

3.4 General procedure for the synthesis of carboxylic acid hydrazides (5a-h)

The respective carboxylic acid (0.2 mol) was dissolved in methanol (50 mL), concentrated sulfuric acid (2.0 mmol) was added and the reaction mixture subjected to reflux for 8-10 hours. After completion of the reaction (TLC), the excess alcohol was removed \textit{in vacuo}, poured into water, neutralized with sodium carbonate and extracted with diethyl ether (3 x 50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated \textit{in vacuo}. The esters were sufficiently pure to be subjected to hydrazinolysis.

The respective ester (0.02 mol) was dissolved in methanol (30 mL), hydrazine hydrate (80%) (0.06 mol) was added slowly and the reaction monitored by thin layer chromatography (Silicagel, petroleum ether:acetone, 8:2). After completion of reaction, the reaction mixture was concentrated \textit{in vacuo}. The resulting crude solid was filtered, washed with water and recrystallized from aqueous ethanol.

2-(4-Bromophenoxy)propanoic acid hydrazide (5a). Yield: 78%; m.p. 143-144 °C; IR (KBr, cm\(^{-1}\)): \(\nu_{\text{max}} \) 3332, 3295, 1655; \(^1\)H-NMR (300 MHz, CDCl\(_3\)): \(\delta\) 1.57 (3H, d, \(J = 6.5\) Hz, OCHMe), 3.88 (2H, br s, \(NH_2\)), 4.71 (1H, d, \(J = 6.5\) Hz, OCHMe), 6.76 (2H, m, Ar-H), 7.39 (2H, m, Ar-H), 7.75 (1H, br s, \(NH\)); \(^{13}\)C-NMR (75 MHz, CDCl\(_3\)): \(\delta\) 18.6, 74.8, 114.5, 117.2, 132.7, 155.8, 172.0.
2-(4-Chlorophenoxo)propanoic acid hydrazide (5b). Yield: 76%; m.p. 97-98 °C; IR (KBr, cm⁻¹): νmax 3346, 3281, 1663; ¹H-NMR (CDCl₃, 300 MHz): δ 1.58 (3H, d, J = 6.6 Hz, OCHMe), 3.87 (2H, br s, NH₂), 4.72 (1H, q, J = 6.6 Hz, OCHMe), 6.82 (2H, m, Ar-H), 7.26 (2H, m, Ar-H), 7.67 (1H, br s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 18.6, 74.9, 116.7, 127.2, 129.8, 155.8, 172.0.

2-(3,4-Dichlorophenoxo)propanoic acid hydrazide (5c). Yield: 80%; m.p. 140-141 °C; IR (KBr, cm⁻¹): νmax 3345, 3276, 1653; ¹H-NMR (CDCl₃, 300 MHz): δ 1.42 (3H, d, J = 6.6 Hz, OCHMe), 4.30 (2H, br s, NH₂), 4.78 (1H, q, J = 6.6 Hz, OCHMe), 6.94 (1H, dd, J = 9.8, 2.7 Hz, Ar-H-6), 7.19 (1H, d, J = 2.7 Hz, Ar-H-2), 7.51 (1H, d, J = 9.0 Hz, Ar-H-5), 9.38 (1H, br s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 19.0, 73.6, 116.5, 117.7, 123.4, 131.4, 131.9, 157.1, 169.8.

2-(4-Methylphenoxo)propanoic acid hydrazide (5d). Yield: 93%; m.p. 70-71 °C; IR (KBr, cm⁻¹): νmax 3342, 3287, 1664; ¹H-NMR (CDCl₃, 300 MHz): δ 1.57 (3H, d, J = 6.9 Hz, OCHMe), 2.30 (3H, s, Me-Ar), 3.87 (2H, br s, NH₂), 4.73 (1H, q, J = 6.9 Hz, OCHMe), 6.79 (2H, J = 8.1 Hz, Ar-H), 7.09 (2H, d, J = 8.1 Hz, Ar-H), 7.77 (1H, br s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 18.7, 20.6, 74.7, 115.3, 130.2, 131.6, 154.6, 172.6.

2-(4-Bromophenoxo)butanoic acid hydrazide (5e). Yield: 79%; m.p. 121-123 °C; IR (KBr, cm⁻¹): νmax 3337, 3289, 1659; ¹H-NMR (CDCl₃, 300 MHz): δ 1.02 (t, 3H, J = 7.3 Hz, CH₂CH₃), 1.86-2.03 (2H, m, CH₂CH₃), 3.87 (2H, br s, NH₂), 4.57 (1H, dd, J = 4.8, 6.6 Hz, OCHCH₂CH₃), 6.78 (2H, m, Ar-H), 7.39 (2H, m, Ar-H), 7.60 (1H, br s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 9.18, 26.0, 79.7, 114.5, 117.2, 132.7, 156.4, 171.4.

2-(4-Chlorophenoxo)butanoic acid hydrazide (5f). Yield: 73%; m.p. 142-143 °C; IR (KBr, cm⁻¹): νmax 3329, 3283, 1665; ¹H-NMR (300 MHz, CDCl₃): δ 1.02 (3H, t, J = 7.3 Hz, CH₂CH₃), 1.88-2.03 (2H, m, CH₂CH₃), 3.73 (2H, br s, NH₂), 4.57 (1H, dd, J = 6.4, 4.6 Hz, OCHCH₂CH₃), 6.83 (2H, m, Ar-H), 7.25 (2H, m, Ar-H), 7.62 (1H, br s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 9.2, 26.0, 79.8, 116.7, 127.2, 129.7, 155.8, 171.4.
2-(3,4-Dichlorophenoxy)butanoic acid hydrazide (5g). Yield: 76%; m.p. 98-99 °C; IR (KBr, cm⁻¹): νₚₓₐₓ 3338, 3281, 1656; ¹H-NMR (300 MHz, CDCl₃): δ 0.91 (3H, t, J = 7.5 Hz, CH₂CH₃), 1.79-1.83 (2H, m, CH₂CH₂), 4.31 (2H, br s, NH₂), 4.59 (1H, t, J = 6.0 Hz, OCH₂CH₂), 6.94 (1H, dd, J = 9.0, 3.0 Hz, Ar-H-6), 7.19 (1H, d, J = 3.0 Hz, Ar-H-2), 7.51 (1H, d, J = 9.0 Hz, Ar-H-5), 9.38 (1H, br s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 9.7, 26.0, 78.7, 116.6, 117.8, 123.4, 131.4, 131.9, 157.5, 169.1.

2-(4-Methylphenoxy)butanoic acid hydrazide (5h). Yield: 80%; m.p. 56-57 °C; IR (KBr, cm⁻¹): νₚₓₐₓ 3337, 3289, 1659; ¹H-NMR (300 MHz, CDCl₃): δ 1.02 (3H, t, J = 7.5 Hz, CH₂CH₃), 1.87-2.03 (2H, m, CH₂CH₂), 2.29 (3H, Me-Ar), 3.85 (2H, br s, NH₂), 4.58 (1H, dd, J = 7.5, 6.6 Hz, OCH₂CH₂), 6.79 (2H, m, Ar-H), 7.09 (2H, m, Ar-H), 7.73 (1H, br s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 9.3, 20.5, 26.1, 79.6, 115.3, 130.2, 131.5, 155.2, 172.0.

The syntheses of hydrazides (5i-t) were confirmed by comparison of their physical constants with the literature values¹¹⁶a-c.

3.5 Synthesis of 1,3,4-oxadiazole-2-thiones (6a-h) and benzothiazole derivatives (7a-h)

3.5.1 General Procedure for the synthesis of 1,3,4-oxadiazole-2-thiones (6a-h)

To a mixture of potassium hydroxide (12.37 mmol) and respective hydrazide (10.30 mmol) in methanol (30 mL), carbon disulfide (12.37 mmol, 0.75 mL) was added drop wise with stirring. The yellow solution so obtained was refluxed till the evolution of hydrogen sulfide ceased (18-20 hr). The reaction mixture was cooled to room temperature and filtered. The filtrate was poured into ice-cooled water and acidified with 6N hydrochloric acid (HCl) till congo red. The precipitated solid was filtered, dried and recrystallized from ethanol water pair to afford pure oxadiazoles.

5-[1-(4-Bromophenoxy)ethyl]-1,3,4-oxadiazole-2-thione (6a). Yield: 82%; m.p. 124-125°C; IR (KBr, cm⁻¹): νₚₓₐₓ 3179, 3093, 2964, 1605, 1587, 1483, 1265;
$^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 1.78 (3H, d, $J$ = 6.6 Hz, OCH$_3$E), 5.32 (1H, d, $J$ = 6.6 Hz, OCH$_3$E), 6.86 (2H, m, Ar-H), 7.37 (2H, m, Ar-H), 11.08 (1H, s, NH$_2$); $^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 18.7, 67.7, 109.8, 117.9, 132.9, 155.9, 162.6, 178.7; EIMS: m/z 299/301 [M$^+$], 174, 173, 172, 171, 157, 155, 145, 129, 77, 75; Anal. Caled for C$_{10}$H$_5$BrN$_2$O$_2$S (299.96): C, 39.88; H, 3.01; N, 9.30. Found: C, 39.62; H, 2.93; N, 9.02.

5-[1-(4-Chlorophenoxy)ethyl]-1,3,4-oxadiazole-2-thione (6b). Yield: 83%; m.p. 113-115°C; IR (KBr, cm$^{-1}$): $\nu$ max 3088, 2994, 2936, 1611, 1585, 1495, 1490, 1277; $^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 1.27 (1H, s, SH), 1.77 (3H, d, $J$ = 6.6 Hz, OCH$_3$E), 5.32 (1H, d, $J$ = 6.6 Hz, OCH$_3$E), 6.92 (2H, m, Ar-H), 7.26 (2H, m, Ar-H); $^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 18.6, 67.7, 117.4, 129.7, 130.1, 155.1, 162.5, 178.6; EIMS: m/z 256/258 [M$^+$], 130, 129, 128, 127, 111, 101, 77; Anal. Caled. for C$_{10}$H$_5$ClN$_2$O$_2$S (256.71): C, 46.79; H, 3.53; N, 10.91. Found: C, 46.53; H, 3.43; N, 10.79.

5-[1-(3,4-Dichlorophenoxy)ethyl]-1,3,4-oxadiazole-2-thione (6c). Yield: 76%; m.p. 108-110°C; IR (KBr, cm$^{-1}$): $\nu$ max 3086, 2996, 2937, 1611, 1584, 1506, 1489, 1279; $^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 1.27 (1H, s, SH), 1.83 (3H, d, $J$ = 6.3 Hz, OCH$_3$E), 5.39 (1H, q, $J$ = 6.3 Hz, OCH$_3$E), 6.93 (1H, dd, $J$ = 8.1, 3.0 Hz, Ar-H-6), 7.10 (1H, d, $J$ = 3.0 Hz, Ar-H-2), 7.43 (1H, d, $J$ = 8.1 Hz, Ar-H-5); $^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 18.7, 67.9, 115.7, 118.3, 126.4, 131.0, 133.0, 155.7, 162.2, 178.7; EIMS: m/z 290/292 [M$^+$], 189, 164, 163, 162, 161, 147, 145, 128, 101, 75; Anal. Caled. for C$_{10}$H$_5$ClN$_2$O$_2$S (291.15): C, 41.25; H, 2.77; N, 9.62. Found: C, 40.94; H, 2.59; N, 9.45.

5-[1-(4-Methylphenox)ethyl]-1,3,4-oxadiazole-2-thione (6d). Yield: 67%, m.p. 86-88°C; IR (KBr, cm$^{-1}$): $\nu$ max 3120, 3082, 2999, 2938, 1611, 1489, 1265; $^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 1.76 (3H, d, $J$ = 6.6 Hz, OCH$_3$E), 2.30 (3H, s, Me-Ar), 5.33 (1H, d, $J$ = 6.6 Hz, OCH$_3$E), 6.87 (2H, d, $J$ = 8.7 Hz, Ar-H), 7.10 (2H, d, $J$ = 8.4 Hz, Ar-H), 9.91 (1H, s, NH$_2$); $^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 18.7, 20.6, 67.6, 116.1, 130.2, 132.2, 154.5, 163.2, 178.6; EIMS: m/z 236 [M$^+$], 235, 135, 108, 107, 91, 77, 75, 65; Anal. Caled. for C$_{10}$H$_{12}$N$_2$O$_2$S (236.29): C, 55.91; H, 5.12; N, 11.86. Found: C, 55.70; H, 5.03; N, 11.66.
5-[1-(4-Bromophenoxy)propyl]-1,3,4-oxadiazole-2-thione (6e). Yield: 84%; m.p. 84-86°C; IR (KBr, cm⁻¹): νmax 3177, 3098, 2963, 1615, 1591, 1497, 1270; ¹H-NMR (300 MHz, CDCl₃): δ 0.95 (3H, t, J = 7.5 Hz, CH₂CH₃), 2.03-2.24 (2H, m, CH₂CH₃), 5.08 (1H, t, J = 6.6 Hz, OCHCH₂CH₃), 6.86 (2H, m, Ar-H), 7.40 (2H, m, Ar-H), 10.83 (1H, br s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 9.4, 26.3, 72.7, 115.0, 117.7, 132.7, 155.9, 162.0, 178.5; EIMS: m/z 314/316 [M⁺], 174, 173, 172, 171, 159, 157, 155, 129, 77, 75; Anal. Calcd. for C₁₁H₁₁BrN₂O₂S (315.19): C, 41.92; H, 3.52; N, 8.89. Found: C, 41.71; H, 3.47; N, 8.67.

5-[1-(4-Chlorophenoxy)propyl]-1,3,4-oxadiazole-2-thione (6f). Yield: 79%; m.p. 84-86°C; IR (KBr, cm⁻¹): νmax 3082, 2936, 2884, 1612, 1585, 1502, 1491, 1278; ¹H-NMR (300 MHz, CDCl₃): δ 1.09 (3H, t, J = 7.5 Hz, CH₂CH₃), 2.23-2.63 (2H, m, CH₂CH₃), 5.08 (1H, t, J = 6.6 Hz, OCHCH₂CH₃), 6.91 (2H, m, Ar-H), 7.25 (2H, m, Ar-H), 10.65 (1H, s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 9.4, 18.6, 72.8, 117.3, 127.7, 129.2, 155, 162.1, 178.6; EIMS: m/z 270/272 [M⁺], 143, 130, 129, 128, 127, 111, 101, 77, 75; Anal. Calcd. for C₁₁H₁₃ClN₂O₂S (270.74): C, 48.80; H, 4.10; N, 10.35. Found: C, 48.61; H, 3.98; N, 11.63.

5-[1-(3,4-Dichlorophenoxy)propyl]-1,3,4-oxadiazole-2-thione (6g). Yield: 78%; m.p. 96-98°C; IR (KBr, cm⁻¹): νmax 3121, 3072, 2965, 2934, 1588, 1503, 1277; ¹H-NMR (300 MHz, CDCl₃): δ 1.09 (3H, t, J = 7.5 Hz, CH₂CH₃), 1.27 (1H, s, SH), 2.06-2.24 (2H, m, CH₂CH₃), 5.08 (1H, t, J = 6.9 Hz, OCHCH₂CH₃), 6.84 (1H, dd, J = 9.0, 3.0 Hz, Ar-H), 7.10 (1H, d, J = 2.7 Hz, Ar-H), 7.35 (1H, d, J = 9.0 Hz, Ar-H); ¹³C-NMR (75 MHz, CDCl₃): δ 9.3, 26.2, 72.9, 115.4, 118.1, 126.1, 131.0, 133.0, 155.9, 161.6, 178.5; EIMS: m/z 304/306 [M⁺], 189, 164, 163, 162, 161, 147, 145, 142, 101, 75; Anal. Calcd. for C₁₁H₁₃Cl₂N₂O₂S (305.18): C, 43.29; H, 3.30; N, 9.18. Found: C, 43.05; H, 3.22; N, 8.94.

5-[1-(4-Methylphenoxy)propyl]-1,3,4-oxadiazole-2-thione (6h). Yield: 80%; m.p. 76-77 °C; IR (KBr, cm⁻¹): νmax 3086, 2970, 2933, 2753, 1613, 1506, 1466, 1265; ¹H-NMR (300 MHz, CDCl₃): δ 1.09 (3H, t, J = 7.5 Hz, CH₂CH₃), 2.03-2.22 (2H, m, Ar-H), 6.87 (2H, d, J = 8.4 Hz), 7.09 (2H, d, J = 8.1 Hz, Ar-H), 11.62 (1H, s, NH); ¹³C-NMR (75 MHz, CDCl₃): δ 9.6, 20.7, 26.6, 73.0, 116.1, 130.3, 132.4, 155.1,

3.5.2 General procedure for the coupling of benzothiazolyl chloroacetamide with 1,3,4-oxadiazole-2-thiones/thiols

The respective 1,3,4-oxadiazole-2-thione/thiol (6a-h) (0.952 mmol), benzothiazolyl chloroacetamide¹¹⁵ (1.047 mmol) and anhydrous potassium carbonate (3.808 mmol) were mixed in acetone (40 mL) with stirring. The resulting reddish solution was refluxed till completion of reaction (TLC). The reaction mixture was cooled to room temperature and filtered to remove K₂CO₃. The filtrate was concentrated on a rotary evaporator and added to cold water. The resulting solid was filtered, dissolved in acetone and boiled with decolorizing charcoal. The charcoal was filtered and the filtrate concentrated to afford pure benzothiazole derivative (7a-h).

N-(Benzothiazole-2-yl)-2-[5-(1-(4-bromophenoxy)ethyl)-1,3,4-oxadiazole-2-ylthio)acetamide (7a). Yield: 75%; m.p. 191-192°C; IR (KBr, cm⁻¹): νmax 3180, 3064, 2972, 2876, 1693, 1605, 1568, 1483, 1264; ¹H-NMR (300 MHz, DMSO-d₆): δ 1.65 (3H, d, J = 6.6 Hz, OCHMe), 4.43 (2H, s, SCH₂), 5.84 (1H, q, J = 6.6 Hz, OCHMe), 6.98 (2H, m, Ar-H), 7.32 (1H, d, J = 7.9, 0.9 Hz, Ar-H), 7.39-7.48 (3H, m, Ar-H), 7.77 (1H, d, J = 7.8 Hz, Ar-H), 7.99 (1H, d, J = 7.8 Hz, Ar-H), 12.78 (1H, s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 18.9, 36.1, 67.4, 113.9, 118.5, 121.2, 122.4, 124.2, 126.7, 132.9, 148.9, 156.3, 158.1, 164.4, 166.7, 166.8; EIMS: m/z 378/380 [M⁺], 363, 361, 205, 177, 173, 171, 150, 134, 120, 76; FABMS: m/z 491/493 [M+H]⁺; Anal. Calcd. for C₁₉H₁₅BrN₄O₃S₂ (491.38): C, 46.44; H, 3.08; N, 11.40. Found: C, 46.77; H, 3.04; N, 11.53.

N-(Benzothiazole-2-yl)-2-[5-(1-(4-chlorophenoxy)ethyl)-1,3,4-oxadiazole-2-ylthio)acetamide (7b). Yield: 83%; m.p. 179-180°C; IR (KBr, cm⁻¹): νmax 3176, 3063, 2979, 2925, 2873, 1693, 1608, 1571, 1513, 1479, 1267; ¹H-NMR (300 MHz, DMSO-d₆): δ 1.65 (3H, d, J = 6.6 Hz, OCHMe), 4.43 (2H, s, SCH₂), 5.84 (1H, d, J = 6.6 Hz, OCHCH₃), 7.03 (2H, m, Ar-H), 7.27-7.35 (3H, m, Ar-H), 7.45 (1H, d, J = 7.9, 0.6 Hz, Ar-H), 7.77 (1H, d, J = 8.1 Hz, Ar-H), 7.99 (1H, d, J = 7.8 Hz, Ar-H), 12.78 (1H,
s, NH); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 18.9, 36.1, 67.5, 118.1, 121.2, 122.3, 124.3, 126.1, 126.7, 129.9, 149.0, 155.8, 158.1, 164.4, 166.7, 166.8; EIMS: m/z 334, 332, 317, 205, 191, 177, 150, 149, 134, 127, 111, 91, 75; FABMS: m/z: 447/449 [M+H]$^+$; Anal. Calcd. for C$_{19}$H$_{13}$ClN$_4$O$_5$S$_2$ (446.63): C, 51.06; H, 3.38; N, 12.54. Found: C, 50.83; H, 3.42; N, 12.37.

$N$-(Benzothiazole-2-yl)-2-[5-(1-(3,4-dichlorophenoxy)ethyl)-1,3,4-oxadiazole-2-ylthio]acetamide (7c). Yield: 68%; m.p. 195-196°C; IR (KBr, cm$^{-1}$): $\nu_{\text{max}}$ 3179, 3067, 2972, 2928, 1687, 1602, 1564, 1478, 1265; $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.65 (3H, d, $J = 6.3$ Hz, OCHMe), 4.43 (2H, s, SCH$_2$), 5.93 (1H, q, $J = 6.3$ Hz, OCHMe), 7.03 (1H, dd, $J = 8.7$, 3.0 Hz, Ar-H), 7.29-7.51 (4H, m, Ar-H), 7.77 (1H, d, $J = 8.1$ Hz, Ar-H), 7.98 (1H, d, $J = 7.5$ Hz, Ar-H), 12.78 (1H, s, NH); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 18.7, 36.1, 67.7, 117.0, 118.3, 121.2, 122.3, 124.2, 124.3 126.7, 131.6, 132.2, 149.1, 156.4, 158.1, 164.5, 166.2, 166.5; EIMS: m/z 480 [M$^+$], 379, 377, 281, 207, 193, 191, 134, 125, 103, 89; FABMS: m/z 481/483 [M+H]$^+$; Anal. Calcd. for C$_{19}$H$_{21}$ClN$_4$O$_5$S$_2$ (481.38): C, 52.22; H, 4.84; N, 12.82. Found: C, 47.55; H, 2.72; N, 11.56.

$N$-(Benzothiazole-2-yl)-2-[5-(1-(4-methylphenoxy)ethyl)-1,3,4-oxadiazole-2-ylthio]acetamide (7d). Yield: 74%; m.p. 205-206°C; IR (cm$^{-1}$): $\nu_{\text{max}}$ 3180, 3063, 2973, 2874, 1693, 1605, 1569, 1483, 1263; $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.63 (3H, d, $J = 6.6$ Hz, OCHMe), 2.18 (3H, s, Ar-Me), 4.43 (2H, s, SCH$_2$), 5.74 (1H, d, $J = 6.6$ Hz, OCHMe), 6.87 (2H, d, $J = 8.7$ Hz, Ar-H), 7.35 (2H, d, $J = 8.7$ Hz, Ar-H), 7.32 (1H, dd, $J = 7.9$, 0.9 Hz, Ar-H), 7.45 (1H, dd, $J = 8.2$, 1.2 Hz, Ar-H), 7.77 (1H, d, $J = 8.1$ Hz, Ar-H), 7.99 (1H, d, $J = 7.8$ Hz, Ar-H), 12.77 (1H, s, NH); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 19.1, 20.2, 36.1, 67.3, 116.3, 121.2, 122.3, 124.2, 126.7, 130.4, 131.3, 149.0, 154.9, 158.1, 164.2, 166.7, 167.3; EIMS: m/z 312, 297, 221, 205, 191, 177, 149, 134, 107, 91, 77, 65; FABMS: m/z 427 [M+H]$^+$; Anal. Calcd. for C$_{20}$H$_{18}$N$_4$O$_5$S$_2$ (426.51): C, 56.32; H, 4.25; N, 13.14. Found: C, 55.93; H, 4.19; N, 13.19.

$N$-(Benzothiazole-2-yl)-2-[5-(1-(4-bromophenoxy)propyl)-1,3,4-oxadiazole-2-ylthio]acetamide (7e). Yield: 71%; m.p. 178-180°C; IR (KBr, cm$^{-1}$): $\nu_{\text{max}}$ 3180, 3064,
2972, 2876, 1693, 1605, 1568, 1483, 1264; \(^1\)H-NMR (300 MHz, DMSO-d6): \(\delta\) 0.92 (3H, t, \(J = 7.4\) Hz, CH\(_2\)CH\(_3\)), 1.92-2.07 (2H, m, CH\(_2\)CH\(_3\)), 4.42 (2H, s, SCH\(_2\)), 5.64 (1H, t, \(J = 6.6\) Hz, OCH\(_2\)CH\(_3\)), 6.97 (2H, m, Ar-H), 7.32 (1H, adt, \(J = 7.3, 0.6\) Hz, Ar-H), 7.39-7.47 (3H, m, Ar-H), 7.77 (1H, d, \(J = 8.1\) Hz, Ar-H), 7.99 (1H, d, \(J = 7.8\) Hz, Ar-H), 12.78 (1H, s, NH); \(^{13}\)C-NMR (75 MHz, DMSO-d6): \(\delta\) 9.5, 26.4, 36.1, 72.2, 113.9, 118.5, 121.2, 122.3, 124.2, 126.7, 132.8, 149.0, 156.7, 158.0, 164.4, 166.2, 166.7; EIMS: m/z 392, 390, 363, 361, 219, 191, 177, 173, 171, 157, 155, 150, 149, 134, 119, 105; FABMS: m/z 505/507 [M+H]\(^+\); Anal. Calcd. for C\(_{20}\)H\(_{17}\)BrN\(_4\)O\(_3\)S\(_2\) (505.41): C, 47.53; H, 3.39; N, 11.09. Found: C, 47.31; H, 3.25; N, 10.89.

\(N\)-(Benzothiazole-2-yl)-2-[5-(1-(4-chlorophenoxy)propyl]-1,3,4-oxadiazole-2-ylthio]acetamide (7f). Yield: 61%; m.p. 186-188°C; IR (KBr, cm\(^{-1}\)): \(\nu\) max 3178, 3063, 2975, 2925, 2869, 1693, 1606, 1570, 1513, 1482, 1266; \(^1\)H-NMR (300 MHz, DMSO-d6): \(\delta\) 0.92 (3H, t, \(J = 7.30\) Hz, CH\(_2\)CH\(_3\)), 1.97-2.07 (2H, m, CH\(_2\)CH\(_3\)), 4.43 (2H, s, SCH\(_2\)), 5.64 (1H, t, \(J = 6.75\) Hz, OCH\(_2\)CH\(_3\)), 7.02 (2H, m, Ar-H), 7.26-7.35 (3H, m, Ar-H), 7.24 (1H, adt, \(J = 8.2, 1.2\) Hz, Ar-H), 7.77 (1H, d, \(J = 7.8\) Hz, Ar-H), 7.99 (1H, d, \(J = 7.8\) Hz, Ar-H), 12.78 (1H, s, NH); \(^{13}\)C-NMR (75 MHz, DMSO-d6): \(\delta\) 9.5, 26.4, 36.1, 72.3, 118.1, 121.2, 122.3, 124.3, 126.2, 126.7, 129.9, 149.0, 156.2, 158.1, 164.4, 166.2, 166.7; EIMS: m/z 4160, 317, 219, 191, 177, 169, 150, 149, 134, 127, 111, 99; FABMS: m/z 461/463 [M+H]\(^+\); Anal. Calcd. for C\(_{20}\)H\(_{17}\)ClN\(_4\)O\(_3\)S\(_2\) (460.96): C, 52.11; H, 3.72; N, 12.15. Found: C, 51.92; H, 3.85; N, 12.23.

\(N\)-(Benzothiazole-2-yl)-2-[5-(1-(3,4-dichlorophenoxy)propyl]-1,3,4-oxadiazole-2-ylthio]acetamide (7g). Yield: 56%; m.p. 197-198°C; IR (KBr, cm\(^{-1}\)): \(\nu\) max 3177, 3064, 2669, 2932, 2874, 1690, 1603, 1568, 1478, 1265; \(^1\)H-NMR (300 MHz, DMSO-d6): \(\delta\) 1.03 (3H, t, \(J = 7.4\) Hz, CH\(_2\)CH\(_3\)), 2.10-2.20 (2H, m, CH\(_2\)CH\(_3\)), 4.63 (2H, s, SCH\(_2\)), 5.82 (1H, t, \(J = 6.8\) Hz, OCH\(_2\)CH\(_3\)), 7.15 (1H, dd, \(J = 9.0, 3.0\) Hz, Ar-H-6), 7.36 (1H, adt, \(J = 8.0, 1.2\) Hz, Ar-H), 7.44 (1H, d, \(J = 3.0\) Hz, Ar-H-2), 7.49 (1H, adt, \(J = 7.3, 1.2\) Hz, Ar-H), 7.55 (1H, d, \(J = 9.0\) Hz, Ar-H-5), 7.79 (1H, d, \(J = 7.5\) Hz, Ar-H), 12.78 (1H, s, NH); \(^{13}\)C-NMR (75 MHz, DMSO-d6): \(\delta\) 18.7, 28.7, 35.9, 72.7, 116.7, 118.2, 121.7, 123.9, 124.42, 126.3, 131.3, 132.2, 149.0, 157.0, 161.7, 164.5, 166.5, 166.9; EIMS: m/z 353, 351, 235, 191, 177, 149, 134, 109; FABMS: m/z 495/497 [M+H]\(^+\); Anal. Calcd. for C\(_{20}\)H\(_{16}\)Cl\(_2\)N\(_4\)O\(_3\)S\(_2\) (495.40): C, 48.49; H, 4.84; N, 3.26. Found: C, 48.31; H, 3.15; N, 11.31.
N-(Benzothiazole-2-yl)-2-[5-(1-(4-methylphenoxy)propyl]-1,3,4-oxadiazole-2-ylthio]acetamide (7h). Yield: 75%; m.p. 178-180°C; IR (KBr, cm⁻¹): νmax 3180, 3065, 2971, 2929, 2871, 1696, 1606, 1569, 1510, 1483, 1264; ¹H-NMR (300 MHz, DMSO-d₆): δ 0.92 (3H, t, J = 7.5 Hz, CH₂CH₃), 1.91-2.11 (2H, m, CH₂CH₃), 2.17 (3H, s, Ar-Me), 4.42 (2H, s, SCH₂), 5.53 (1H, t, J = 6.6 Hz, OCHCH₂CH₃), 6.87 (2H, d, J = 8.4 Hz, Ar-H), 7.03 (2H, d, J = 8.4 Hz, Ar-H), 7.31 (1H, at, J = 7.5 Hz, Ar-H), 7.44 (1H, at, J = 7.5 Hz, Ar-H), 7.77 (1H, d, J = 8.1 Hz, Ar-H), 7.98 (1H, d, J = 7.8 Hz, Ar-H), 12.78 (1H, s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 9.6, 20.5, 26.5, 36.2, 72.3, 116.2, 121.1, 122.2, 124.2, 126.7, 130.4, 131.3, 149.6, 155.3, 158.3, 164.3, 166.6, 166.8; EIMS: m/z 326, 311, 297, 235, 219, 191, 177, 150, 121, 107, 91, 77, 65; FABMS: m/z 441 [M+H]⁺; Anal. Calcd. for C₂₁H₂₀N₄O₅S₂ (440.54): C, 57.25; H, 4.58; N, 12.72. Found: C, 57.05; H, 4.64; N, 12.76.

3.6 General procedure for the Synthesis of 3-alkyl-4-amino-1H-1,2,4-triazole-5-thiones (8a-i)

A solution of potassium hydroxide (0.04 moles) and the respective hydrazide (0.04 moles) in methanol (100 mL) was treated with carbon disulfide (0.04 moles). The mixture was stirred for 12-16 hours at room temperature. Dry diethyl ether (200 mL) was added and the precipitated solid was filtered, washed with ether and vacuum dried at 78°C in drying pestle. The potassium salts of substituted dithiocarbazinic acids were obtained in nearly quantitative yield and used as such in the next step.

A suspension of the potassium salt of substituted dithiocarbazinic acid (0.02 moles), hydrazine hydrate (0.04 moles) and 2.0 mL of water was refluxed with stirring for 0.5-1.5 hour. The color of the reaction mixture changed to green with the evolution of hydrogen sulfide and a homogeneous solution was formed. When the evolution of hydrogen sulfide ceased (lead acetate test), the reaction mixture was diluted with cold water (100 mL) and acidified with concentrated hydrochloric acid resulting in the precipitation of a solid product. The product was filtered, washed with cold water and recrystallized from aqueous ethanol.
4-Amino-3-[1-(4-methylbenzenesulfonamido)ethyl]-1H,4H-1,2,4-triazole-5-thione (8a). Yield: 75%; m.p. 210-212°C; IR (KBr, cm\(^{-1}\)): \(\nu_{\text{max}}\) 3387, 3289, 3041, 2925, 1597, 1317, 1155; \(^1\)H-NMR (500 MHz, CD\(_3\)OD): \(\delta\) 1.45 (3H, d, \(J = 7.1\) Hz, CHCH\(_2\)), 2.36 (3H, s, Me-ArSO\(_2\)), 4.66 (1H, q, \(J = 7.3\) Hz, CHCH\(_3\)), 7.25 (2H, d, \(J = 8.1\) Hz, Ar-H), 7.59 (2H, d, \(J = 8.2\) Hz, Ar-H); \(^13\)C-NMR (75 MHz, Acetone-\(d_6\)): \(\delta\) 19.3, 20.6, 45.4, 126.7, 129.2, 137.7, 143.5, 151.5, 167.9; EIMS: m/z 313 [M\(^+\)], 249, 171, 155, 142, 116, 91. **Anal.** Calcd. for C\(_{11}\)H\(_{15}\)N\(_3\)O\(_2\)S\(_2\) (313.07): C, 42.16; H, 4.82; N, 22.35; S, 20.46. Found: C, 41.61; H, 4.73; N, 22.13; S, 19.76.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-methylpropyl]-1H,4H-1,2,4-triazole-5-thione (8b). Yield: 71%; m.p. 162-164°C; IR (KBr, cm\(^{-1}\)): \(\nu_{\text{max}}\) 3415, 3267, 3041, 2956, 1553, 1369, 1153; \(^1\)H-NMR (300 MHz, CD\(_3\)OD): \(\delta\) 0.82 (3H, d, \(J = 6.6\) Hz, CHCH\(_3\)), 1.05 (3H, d, \(J = 6.6\) Hz, CHCH\(_3\)), 2.14 (1H, m, CH(CH\(_3\))\(_2\)), 2.39 (3H, s, Me-ArSO\(_2\)), 4.25 (1H, d, \(J = 8.4\) Hz, CHNH), 7.23 (2H, d, \(J = 8.1\) Hz, Ar-H), 7.56 (2H, d, \(J = 8.1\) Hz, Ar-H); \(^13\)C-NMR (75 MHz, CD\(_3\)OD): \(\delta\) 17.9, 18.1, 20.3, 31.7, 55.0, 126.4, 129.0, 137.0, 143.7, 150.9, 167.0; EIMS: m/z 341 [M\(^+\)], 298, 171, 155, 116, 91, 65. **Anal.** Calcd. for C\(_{13}\)H\(_{19}\)N\(_3\)O\(_2\)S\(_2\) (341.09): C, 45.73; H, 5.61; N, 20.51; S, 18.78. Found: C, 45.66; H, 5.68; N, 20.60; S, 18.90.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-3-methylbutyl]-1H,4H-1,2,4-triazole-5-thione (8c). Yield: 67%; m.p. 178-180°C; IR (KBr, cm\(^{-1}\)): \(\nu_{\text{max}}\) 3345, 3279, 3057, 2965, 1567, 1372, 1158; \(^1\)H-NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 0.72 (3H, d, \(J = 6.1\) Hz, CHCH\(_3\)), 0.79 (3H, d, \(J = 6.1\) Hz, CHCH\(_3\)), 1.47-1.54 (3H, m, CHCH\(_2\)), 2.32 (2H, s, Me-ArSO\(_2\)), 4.46 (1H, dd, \(J = 8.0, 7.5\) Hz, CHNH), 5.30 (2H, br s, NH\(_2\)), 7.25 (2H, d, \(J = 8.0\) Hz, Ar-H), 7.54 (2H, d, \(J = 8.1\) Hz, Ar-H), 8.18 (1H, s, NHSO\(_2\)), 13.32 (1H, s, NH); \(^13\)C-NMR (75 MHz, DMSO-\(d_6\)): \(\delta\) 21.0, 21.1, 22.5, 23.9, 41.8, 46.6, 126.5, 129.1, 137.5, 142.7, 151.7, 166.3; EIMS: m/z 355 [M\(^+\)], 298, 184, 171, 155, 91, 57; **Anal.** Calcd. for C\(_{14}\)H\(_{21}\)N\(_3\)O\(_2\)S\(_2\) (355.11): C, 47.30; H, 5.95; N, 19.70; S, 18.04. Found: C, 46.97; H, 6.01; N, 19.53; S, 17.67.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-methylbutyl]-1H,4H-1,2,4-triazole-5-thione (8d). Yield: 59%; m.p. 144-146°C; IR (KBr, cm\(^{-1}\)): \(\nu_{\text{max}}\) 3341, 3299, 3194, 2963, 1548, 1383, 1159; \(^1\)H-NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 0.69 (3H,
dd, J = 8.0, 6.9, CH₂CH₃, 0.71 (3H, d, J = 7.4 Hz, CHCH₃), 0.98 (1H, m), 1.42 (1H, m), 1.51 (1H, m), 2.35 (3H, s, Me-ArSO₂), 3.39 (1H, at, J = 8.7 Hz, CHN), 5.25 (2H, br s, NH₂), 7.31 (2H, d, J = 8.1 Hz, Ar-H), 7.51 (2H, d, J = 8.1 Hz, Ar-H), 8.96 (1H, br s, NHSO₂), 12.68 (1H, s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 14.9, 15.6, 21.1, 24.6, 36.6, 59.1, 126.9, 129.0, 138.5, 142.3, 151.7, 169.3; EIMS: m/z 240, 184, 155, 91, 65, 57; Anal. Calcd. for C₁₅H₂₁N₃O₅S₂ (355.11): C, 47.30; H, 5.95; N, 19.70; S, 18.04. Found: C, 47.23; H, 5.87; N, 19.49; S, 17.86.

4-Amino-3-[1-(4-methylbenzenesulfonylamido)-2-hydroxyethyl]-1H,4H-1,2,4-triazole-5-thione (8e). Yield: 52%; m.p. 208°C; IR (KBr, cm⁻¹): v max 3426, 3262, 3037, 2930, 1560, 1327, 1161; ¹H-NMR (500 MHz, DMSO-d₆): δ 2.33 (3H, s, Me-ArSO₂), 3.54 (1H, dd, J = 10.1, 6.4 Hz), 3.62 (1H, dd, J = 10.0, 7.5 Hz), 4.47 (1H, dd, J = 14.8, 7.2 Hz, CH⁺H(OH)), 5.25 (2H, s, NH₂), 7.27 (2H, d, J = 8.0 Hz, Ar-H), 7.59 (2H, d, J = 8.1 Hz, Ar-H), 8.1 (1H, br s, NHSO₂), 13.40 (1H, s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 21.1, 50.5, 61.6, 126.5, 129.1, 137.5, 142.7, 149.9, 166.2; EIMS: m/z 329 [M⁺], 298, 171, 155, 144, 128, 91, 69, 65; Anal. Calcd. for C₁₅H₁₅N₃O₅S₂ (329.06): C, 40.11; H, 4.59; N, 21.26; S, 19.47. Found: C, 39.40; H, 4.43; N, 21.13; S, 19.83.

4-Amino-3-[1-(4-methylbenzenesulfonylamido)-2-phenylethyl]-1H,4H-1,2,4-triazole-5-thione (8f). Yield: 62%; m.p. 200-202°C; IR (KBr, cm⁻¹): v max 3412, 3278, 3034, 2928, 1578, 1340, 1158; ¹H-NMR (300 MHz, Acetone-d₆): δ 2.25 (3H, s, Me-ArSO₂), 3.09 (1H, m, CH⁺HPh), 4.49 (1H, m, CH⁺HPh), 4.64 (1H, m, CHN₃H), 4.81 (2H, br s, NH₂), 6.97 (1H, br s, NHSO₂), 7.03-7.10 (3H, m, Ar-H), 7.16 (2H, ad, J = 7.8 Hz, Ar-H), 7.42 (2H, d, J = 8.1 Hz, Ar-H), 7.49 (d, 2H, J = 8.1 Hz, Ar-H); ¹³C-NMR (75 MHz, Acetone-d₆): δ 21.5, 32.3, 51.8, 127.3, 127.8, 128.1, 129.4, 130.3, 130.5, 137.1, 144.2, 149.3, 168.3; EIMS: m/z 389 [M⁺], 298, 274, 155, 91, 77, 65; Anal. Calcd. for C₁₇H₁₉N₅O₅S₂ (389.09): C, 52.43; H, 4.92; N, 17.99; S, 17.05. Found: C, 52.23; H, 5.08; N, 17.69; S, 17.19.

4-Amino-3-[1-(4-methylbenzenesulfonylamido)-3-methylthiopropyl]-1H,4H-1,2,4-triazole-5-thione (8g). Yield: 69%; m.p. 176-178°C; IR (KBr, cm⁻¹): v max 3320, 3253, 3045, 2928, 1567, 1383, 1163; ¹H-NMR (500 MHz, CD₃OD): δ 2.02 (3H, s,
4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-(1H-imidazol-4-yl)ethyl]-1H,4H-1,2,4-triazole-5-thione (8h). Yield: 73%; m.p. 238°C; IR (KBr, cm⁻¹): νmax 3343, 3231, 3044, 2927, 1561, 1319, 1157; ¹H-NMR (500 MHz, DMSO-d₆) δ 2.32 (3H, s, Me-ArSO₂), 2.87 (1H, dd, J = 14.4, 7.3 Hz, CHH Imidazole), 3.23 (1H, dd, J = 14.4, 8.0 Hz, CHHImidazole), 4.68 (1H, dd, J = 6.8, 6.6 Hz, CHNH), 5.25 (2H, br s, NH₂), 6.68 (1H, s, Imidazole-H), 7.23 (2H, d, J = 8.0 Hz, Ar-H), 7.43 (1H, s, Imidazole-H), 7.49 (2H, d, J = 8.0 Hz, Ar-H), 8.25 (1H, br s, NHSO₂), 11.72 (1H, br s, Imidazole-NH), 13.30 (1H, br s, NH); ¹³C-NMR (75 MHz, Acetone-d₆): δ 21.5, 32.4, 50.7, 127.7, 128.0, 130.1, 135.7, 136.1, 138.3, 144.3, 151.4, 168.1; EIMS: m/z 246, 171, 155, 107, 91, 89, 81, 65; Anal. Calcd. for C₁₄H₁₇N₇O₂S₂ (379.09): C, 44.31; H, 4.52; N, 25.84; S, 16.90. Found: C, 44.41; H, 4.71; N, 26.19; S, 16.53.

4-Amino-3-[1-(4-methylbenzenesulfonamido)-2-1H-indolylethyl]-1H,4H-1,2,4-triazole-5-thione (8i). Yield: 56%; m.p. 248-250°C; IR (KBr, cm⁻¹): νmax 3434, 3281, 3051, 2930, 1580, 1315, 1159; ¹H-NMR (500 MHz, CD₂OD): δ 2.31 (3H, s, Me-ArSO₂), 3.18-3.27 (2H, m, CH₂-Indole), 4.84 (1H, dd, J = 7.6, 7.5 Hz, CHNH), 6.90-6.96 (2H, m, Indole-H), 7.04 (1H, s, NH), 7.06 (1H, d, J = 8.2 Hz, Indole-H), 7.25 (1H, d, J = 7.3 Hz, Indole-H), 7.32 (2H, d, J = 8.0 Hz, Ar-H), 7.43 (2H, d, J = 8.0 Hz, Ar-H); ¹³C-NMR (75 MHz, DMSO-d₆): δ 21.5, 29.50, 49.9, 109.3, 111.8, 118.4, 118.8, 121.3, 124.6, 126.5, 127.2, 129.4, 136.5, 137.7, 142.8, 152.2, 166.96; EIMS: m/z 273, 155, 131, 130, 91, 77, 65; Anal. Calcd. for C₁₉H₂₀N₆O₂S₂ (428.11): C, 53.25; H, 4.70; N, 19.63; S, 14.97. Found: C, 52.97; H, 4.57; N, 19.43; S, 15.03.
3.7 General procedure for the synthesis of thiosemicarbazides (10a-m).

To a solution of carboxylic acid hydrazide (5.90 mmol) in absolute MeOH (30 mL) was added a solution of the respective isothiocyanate (5.9 mmol) in absolute MeOH (20 mL) with stirring and the mixture was heated under reflux for 2-3 h. After cooling, the resulting solid was filtered and recrystallized from EtOH/water to give pure thiosemicarbazides (10a-m).

1-[(2-Hydroxy-2-phenyl)acetyl]-4-(4-bromophenyl)thiosemicarbazides (10a).
Yield: 85%; m.p. 56°C; IR (ATR, cm⁻¹): ν_max 3485, 3227, 3119, 2952, 1690, 1642, 1590, 1511, 1484, 1170; ¹H-NMR (300 Hz, DMSO-d₆): δ 5.12 (1H, s, CHO), 6.11 (1H, br s, OH), 7.33-7.48 (9H, m, Ar-H), 9.36 (1H, br s, NH), 9.80 (1H, br s, NH), 10.29 (1H, s, NH); EIMS: m/z 217, 215, 213, 173, 171, 148, 134, 107, 79, 77.

1-[(2-Hydroxy-2-phenyl)acetyl]-4-(4-chlorophenyl)thiosemicarbazide (10b).
Yield: 81%; m.p. 162-164°C; IR (ATR, cm⁻¹): ν_max 3479, 3227, 3125, 2946, 1689, 1643, 1591, 1510, 1488, 1170; ¹H-NMR (300 Hz, DMSO-d₆): δ 5.12 (1H, d, J = 2.4 Hz, CHO), 6.12 (1H, br s, OH), 7.26-7.50 (9H, m, Ar-H), 9.39 (1H, br s, NH), 9.81 (1H, br s, NH), 10.31 (1H, s, NH); EIMS: m/z 208, 171, 170, 169, 148, 114, 107, 79.

1-[(2-Hydroxy-2-phenyl)acetyl]-4-(4-fluorophenyl)thiosemicarbazide (10c).
Yield: 87%; m.p. 178-180°C; IR (ATR, cm⁻¹): ν_max 3424, 3228, 3119, 2952, 1679, 1610, 1548, 1505, 1448, 1414, 1366, 1170; ¹H-NMR (300 Hz, DMSO-d₆): δ 5.11 (1H, d, J = 4.2 Hz, CHO), 6.10 (1H, br s, OH), 7.16-7.50 (9H, m, Ar-H), 9.37 (1H, br s, NH), 9.73 (1H, br s, NH), 10.29 (1H, s, NH); EIMS: m/z 301, 212, 208, 194, 111, 110, 107, 105, 95, 79.

1-[(2-Hydroxy-2-phenyl)acetyl]-4-(4-methylphenyl)thiosemicarbazide (10d).
Yield: 87%; m.p. 109-111°C; IR (ATR, cm⁻¹): ν_max 3479, 3244, 3130, 3033, 2952, 1692, 1644, 1594, 1505, 1449, 1414, 1366, 1170; ¹H-NMR (300 Hz, DMSO-d₆): δ 5.12 (1H, d, J = 2.4 Hz, CHO), 6.12 (1H, br s, OH), 7.15 (2H, d, J = 8.1 Hz, Ar-H),
7.25-7.37 (5H, m, Ar-H), 7.48 (2H, d, J = 8.1 Hz, Ar-H), 9.24 (1H, br s, NH), 9.65 (1H, br s, NH), 10.28 (1H, s, NH); EIMS: m/z 297, 220, 208, 163, 148, 149, 121, 107, 91, 79, 77, 65.

1-[(2-Hydroxy-2-phenyl)acetyl]-4-(4-nitropheno|)thiosemicarbazide (10e). Yield: 79%; m.p. 174-175°C; IR (ATR, cm$^{-1}$): $\nu_{\text{max}}$ 3427, 3217, 3083, 2944, 1678, 1643, 1598, 1552, 1504, 1494, 1172; $^1$H-NMR (300 Hz, DMSO-d$_6$): $\delta$ 5.13 (1H, s, CHOCH), 6.18 (1H, br s, OH), 7.27-7.38 (3H, m, Ar-H), 7.49 (2H, d, J = 7.2 Hz, Ar-H), 7.87 (2H, d, J = 9.3 Hz, Ar-H), 8.23 (2H, dd, J = 9.0, 2.4 Hz, Ar-H), 9.69 (1H, br s, NH), 10.08 (1H, br s, NH), 10.38 (1H, s, NH); EIMS: m/z 208, 180, 148, 150, 134, 122, 107, 90, 79, 77.

1-[3-Methyl-2-(4-methylphenylsulfonamido)butanoyl]-4-(4-chlorophenyl)thiosemicarbazide (10f). Yield: 95%; m.p. 212-214°C; IR (cm$^{-1}$): $\nu_{\text{max}}$ 3461, 3379, 3273, 1649, 1335, 1265, 1157; $^1$H-NMR (300 MHz, Acetone-d$_6$): $\delta$ 0.85 (3H, d, J = 6.7 Hz, CHCH$_3$), 0.86 (3H, d, J = 6.8 Hz, CHCH$_3$), 2.05 (1H, m, CH$_2$(CH$_3$)$_2$), 2.40 (3H, s, Me-ArSO$_2$), 3.47 (1H, dd, J = 6.5, 6.4 Hz, CHNH), 7.00 (1H, d, J = 5.5 Hz, NHSO$_2$), 7.35 (2H, d, J = 8.8 Hz, Ar-H), 7.41 (2H, d, J = 8.2 Hz, Ar-H), 7.79 (2H, d, J = 8.8 Hz, Ar-H), 7.81 (2H, d, J = 8.2 Hz, Ar-H), 8.95 (1H, br s, NH), 9.11 (1H, br s, NH), 9.76 (1H, br s, NH); EIMS: m/z 327, 226, 171, 170, 169, 155, 127, 111, 91, 65.

1-[4-Methyl-2-(4-methylphenylsulfonamido)pentanoyl]-4-(4-chlorophenyl)thiosemicarbazide (10g). Yield: 89%; m.p. 159-161°C; IR (KBr, cm$^{-1}$): $\nu_{\text{max}}$ 3469, 3358, 3267, 1657, 1333, 1248, 1161; $^1$H-NMR (500 MHz, CD$_3$OD): $\delta$ 0.54 (3H, d, J = 5.7 Hz, CHCH$_3$), 0.81 (3H, d, J = 6.1 Hz, CHCH$_3$), 1.45-1.51 (3H, m, CH$_2$CH (CH$_3$)$_2$), 2.41 (3H, s, Me-ArSO$_2$), 3.59 (1H, br s, NH), 7.31 (2H, d, J = 8.1 Hz, Ar-H), 7.38 (2H, d, J = 8.1 Hz, Ar-H), 7.65 (2H, d, J = 8.6 Hz, Ar-H), 7.76 (2H, d, J = 8.2 Hz, Ar-H), 8.91 (1H, br s, NH), 9.18 (1H, br s, NH), 9.87 (1H, br s, NH); EIMS: m/z 228, 171, 170, 169, 155, 127, 111, 91, 88, 65.

1-[3-Methyl-2-(4-methylphenylsulfonamido)pentanoyl]-4-(4-chlorophenyl)thiosemicarbazide (10h). Yield: 86%; m.p. 84-87°C; IR (KBr, cm$^{-1}$): $\nu_{\text{max}}$ 3465, 3387, 3271, 1663, 1338, 1239, 1155; $^1$H-NMR (300 MHz, Acetone-d$_6$): $\delta$ 0.67 (3H, t, J =
7.8 Hz, CH$_2$CH$_3$), 0.84 (3H, d, J = 6.7 Hz, CHCH$_2$), 1.14 (1H, m, CHHCH$_3$), 1.48 (1H, m, CHHCH$_3$), 1.75 (1H, m, CH$_2$CHNH), 2.40 (3H, s, Me-ArSO$_2$), 3.72 (1H, dd, J = 9.9, 6.6 Hz, CHNH), 6.68 (1H, d, J = 9.8 Hz, NHSO$_2$), 7.34 (2H, d, J = 8.6 Hz, Ar-H), 7.36 (2H, d, J = 8.6 Hz, Ar-H), 7.68 (2H, d, J = 8.2 Hz, Ar-H), 7.81 (2H, d, J = 8.2 Hz, Ar-H), 8.96 (1H, s, NH), 9.14 (1H, s, NH), 9.78 (1H, s, NH); EIMS: m/z 240, 171, 170, 169, 155, 127, 111, 99, 91, 65.

1-[3-Hydroxy-2-(4-methylbenzenesulfonamido)butanoyl]-4-(4-chlorophenyl)thiosemicarbazide (10i). Yield: 82 %; m.p. 192-194 °C; IR (KBr, cm$^{-1}$): $\nu_{\text{max}}$ 3397-3263, 1653, 1341, 1243, 1157; $^1$H-NMR (500 MHz, CD$_3$OD): $\delta$ 1.03 (3H, d, J = 6.3 Hz, CHCH$_3$), 2.40 (3H, s, Me-ArSO$_2$), 3.64 (1H, d, J = 4.5 Hz, CHNH), 4.03 (1H, m, CH$_3$CHOH), 7.30 (2H, d, J = 8.8 Hz, Ar-H), 7.36 (2H, d, J = 8.1 Hz, Ar-H), 7.58 (2H, d, J = 8.7 Hz, Ar-H), 7.77 (2H, d, J = 8.2 Hz, Ar-H), 8.89 (1H, s, NH), 9.04 (1H, s, NH), 9.63 (1H, s, NH); EIMS: m/z 228, 171, 170, 169, 155, 127, 111, 91, 88, 65.

1-[4-Methylthio-2-(4-methylbenzenesulfonamido)butanoyl]-4-(4-chlorophenyl)thiosemicarbazide (10j). Yield: 91 %; m.p. 200-201 °C; IR (KBr, cm$^{-1}$): $\nu_{\text{max}}$ 3463, 3351, 3253, 1661, 1337, 1236, 1156; $^1$H-NMR (500 MHz, CD$_3$OD): $\delta$ 1.83 (1H, m, CHHSMe), 1.90 (3H, s, SCH$_2$), 1.95 (1H, m, CHFSMe), 2.28 (1H, m), 2.39-2.46 (4H, m), 3.83 (1H, dd, J = 7.4, 5.5 Hz, CHNH), 7.31 (2H, d, J = 8.7 Hz, Ar-H), 7.37 (2H, d, J = 8.0 Hz, Ar-H), 7.62 (2H, d, J = 8.7 Hz, Ar-H), 7.72 (1H, d, J = 7.6 Hz, NH), 7.77 (2H, d, J = 8.0 Hz, Ar-H), 8.93 (1H, s, NH), 9.07 (1H, s, NH), 9.69 (1H s, NH); EIMS: m/z 258, 171, 170, 169, 155, 127, 111, 99, 91, 75, 65, 61, 57.

1-[3-Mercapto-2-(4-methylbenzenesulfonamido)propanoyl]-4-(4-chlorophenyl)thiosemicarbazide (10k). Yield: 71 %; m.p. 117-118 °C; IR (KBr, cm$^{-1}$): $\nu_{\text{max}}$ 3463, 3376, 3251, 2553, 1654, 1339, 1244, 1160; $^1$H-NMR (300 MHz, Acetone-$d_6$): $\delta$ 2.39 (3H, s, Me-ArSO$_2$), 3.63-3.71 (2H, m, SCH$_2$), 4.29 (1H, d, J = 6.4 Hz, CHNH), 4.32 (1H, br s, SH), 7.0 (1H, br s, NHSO$_2$), 7.31 (2H, d, J = 8.6 Hz, Ar-H), 7.37 (2H, d, J = 8.2 Hz, Ar-H), 7.55 (2H, d, J = 8.6 Hz, Ar-H), 7.58 (2H, d, J = 8.2 Hz, Ar-H), 8.96 (1H, s, NH), 9.13 (1H, s, NH), 9.64 (1H s, NH); EIMS: m/z 171, 155, 107, 91, 65.
1-[3-Hydroxy-2-(4-methylbenzenesulfonamido)propanoyl]-4-(4-chlorophenyl)thiosemicarbazide (10). Yield: 73%; m.p. 170-172 °C; IR (KBr, cm⁻¹): νmax 3387-3235, 1665, 1337, 1155, 1246; ¹H-NMR (500 MHz, CD₂OD): δ 2.41 (3H, s, Me-ArSO₂), 3.58 (1H, dd, J = 10.5, 6.7 Hz), 3.70 (1H, dd, J = 10.5, 5.5 Hz), 3.79 (1H, dd, J = 6.2, 5.9 Hz), 5.31 (1H, br s, OH), 7.30 (2H, d, J = 8.7 Hz, Ar-H), 7.37 (2H, d, J = 8.1 Hz, Ar-H), 7.56 (2H, d, J = 8.7 Hz, Ar-H), 7.77 (2H, d, J = 8.1 Hz, Ar-H), 8.87 (1H, s, NH), 9.13 (1H, s, NH), 9.81 (1H, s, NH); EIMS: m/z 240, 171, 170, 169, 155, 127, 111, 99, 91, 75, 65.

1-[3-(1H-Imidazol-5-yl)-2-(4-methylphenylsulfonamido)propanoyl]-4-(4-chlorophenyl)thiosemicarbazide (10m). Yield: 85%; m.p. 176-178 °C; IR (KBr, cm⁻¹) νmax 3421, 3361, 3297, 1658, 1336, 1244, 1156; ¹H-NMR (500 MHz, CD₂OD): δ 2.39 (3H, s, Me-ArSO₂), 3.13-3.21 (m, 2H, CH₂CH), 4.85 (1H, dd, J = 9.0, 6.0 Hz, CHNH), 7.25 (1H, s, NH), 7.29 (2H, d, J = 8.1 Hz, Ar-H), 7.37 (2H, d, J = 6.9 Hz, Ar-H), 7.43 (2H, d, J = 6.9 Hz, Ar-H), 7.61 (2H, d, J = 8.2 Hz, Ar-H), 8.43 (1H, s, NH), 8.97 (1H, s, NH), 9.18 (1H, s, NH), 9.73 (1H, s, NH); EIMS: m/z 200, 171, 170, 127, 111, 91, 75.

3.8 Synthesis of 1,3,4-oxadiazoles and 1,2,4-triazoles

3.8.1 General procedure for the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles (11a-e)

The respective thiosemicarbazide (1.0 eq) was dissolved in methanol (10 mL) and mercuric acetate [Hg(OAc)₂] (1.1 eq) added. The reaction mixture was refluxed for 2-3 hours while completion of the reaction was monitored by TLC. The reaction mixture was filtered and concentrated in vacuo. The resulting solid was recrystallized from aqueous ethanol.

2-(Hydroxybenzyl)-5-(4-bromophenyl)amino-1,3,4-oxadiazole (11a). Yield: 79%; m.p. 160-162 °C; IR (ATR, cm⁻¹): νmax 3425, 3061, 2926, 1581, 1493, 1486; ¹H-NMR (300 Hz, CD₂OD): δ 4.57 (1H, s, CHOH), 5.90 (1H, s, CHO), 7.51-7.31 (9H, m, Ar-H); ¹³C-NMR (75 MHz, Acetone-d₆): δ 67.1, 113.8, 119.0, 126.3, 128.1, 128.4,
131.9, 138.2, 139.2, 160.1, 161.1; **EIMS**: m/z 345 [M⁺], 239, 196, 155, 133, 131, 107, 106, 105, 77.

2-(Hydroxybenzyl)-5-(4-chlorophenyl)amino-1,3,4-oxadiazole (11b). Yield: 76%; m.p. 166-168 °C; **IR** (ATR, cm⁻¹): νmax 3451, 3069, 3031, 2942, 1608, 1576, 1517, 1488; **¹H-NMR** (300 Hz, CD3OD): δ 4.56 (1H, s, CHOH), 5.89 (1H, s, OH), 7.51-7.26 (9H, m, Ar-H); **¹³C-NMR** (75 MHz, Acetone-d₆): δ 67.1, 118.6, 126.3, 128.1, 128.2, 128.4, 128.9, 137.8, 139.5, 160.1, 161.1; **EIMS**: m/z 301 [M⁺], 195, 107, 106, 77, 69.

2-(Hydroxybenzyl)-5-(4-fluorophenyl)amino-1,3,4-oxadiazole (11c). Yield: 79%; m.p. 175-177°C; **IR** (ATR, cm⁻¹): νmax 3443, 3063, 3026, 2926, 1603, 1571, 1512, 1486, 1253; **¹H-NMR** (300 Hz, DMSO-d₆): δ 4.73 (1H, d, J = 5.3 Hz, CHOH), 5.01 (1H, s, CHO), 7.10-7.49 (9H, m, Ar-H), 9.62 (1H, s, NH); **¹³C-NMR** (75 MHz, DMSO-d₆): δ 72.8, 115.9 (d, J = 21.8 Hz), 120.6 (d, J = 7.5 Hz), 127.1, 128.1, 128.6, 132.1, 140.7, 158.1 (d, J = 237.0 Hz), 159.3, 161.7; **EIMS**: m/z 285 [M⁺], 272, 224, 194, 175, 149, 148, 136, 110, 105, 77.

2-(Hydroxybenzyl)-5-(4-methylphenyl)amino-1,3,4-oxadiazole (11d). Yield: 81%; m.p. 172-173 °C; **IR** (ATR, cm⁻¹): νmax 3426, 3081, 3062, 2941, 1606, 1571,1508, 1489; **¹H-NMR** (300 Hz, CD3OD): δ 2.37 (3H, s, Ar-Me), 5.59 (1H, s, CHO), 6.88 (2H, d, J = 8.3 Hz, Ar-H), 7.07 (2H, d, J = 7.6, 5.5 Hz), 7.17-7.22 (5H, m, Ar-H); **¹³C-NMR** (75 MHz, Acetone-d₆): δ 19.8, 67.1, 117.1, 126.3, 128.3, 128.3, 129.4, 131.3, 136.4, 139.7, 160.5, 160.7; **EIMS**: m/z 283 [M⁺], 281, 176, 148, 133, 132, 107, 106, 105, 91, 65.

2-(Hydroxybenzyl)-5-(4-nitrophenyl)amino-1,3,4-oxadiazole (11e). Yield: 66%; m.p. 179-180°C; **IR** (ATR, cm⁻¹): νmax 3260, 3105, 2930, 1627, 1595, 1557, 1515, 1508, 1306; **¹H-NMR** (300 MHz, DMSO-d₆): δ 5.96 (1H, d, J = 4.8 Hz, CHO), 6.73 (1H, d, J = 4.8 Hz, CHO), 7.33-7.50 (5H, m, Ar-H), 7.73 (2H, d, J = 9.0 Hz, Ar-H), 8.26 (2H, d, J = 9.0 Hz, Ar-H), 11.40 (1H, s, NH); **¹³C-NMR** (75 MHz, DMSO-d₆): δ 66.6, 117.2, 126.0, 126.8, 128.6, 128.9, 139.9, 141.6, 145.2, 159.7, 162.1; **EIMS**: m/z 312 [M⁺], 206, 165, 133, 119, 107, 79, 77.
3.8.2 General procedure for the synthesis of triazoles (12a-e, 13a-g)

The respective thiosemicarbazide (10a-l; 1.10 mmol) was added portion wise to a solution of NaOH (5%, 30 mL) and the reaction mixture was heated under reflux for 4 h. After cooling, the reaction mixture was filtered and the filtrate was acidified with 6 N HCl to pH 2-3. The precipitated solid was filtered, washed thoroughly with water and recrystallized from EtOH/water.

3-(Hydroxybenzyl)-4-(4-bromophenyl)-2H-1,2,4-triazole-3-thione (12a). Yield: 64%; m.p. 188-190 °C; IR (ATR, cm⁻¹): νmax 3128, 3027, 2937, 1578, 1497, 1488; ¹H-NMR (300 Hz, CD₂OD): δ 5.68 (1H, s, CHOH), 6.92 (2H, d, J = 8.6 Hz, Ar-H), 7.08 (2H, dd, J = 7.4, 5.4 Hz, Ar-H), 7.51 (2H, d, J = 8.6 Hz, Ar-H), 7.27-7.19 (3H, m, Ar-H); ¹³C-NMR (75 MHz, Acetone-δ₆): δ 67.8, 124.4, 126.5, 128.0, 128.3, 131.2, 133.4, 134.8, 139.2, 155.3, 171.5; EIMS: m/z 359/361 [M⁺], 256, 255, 228, 213, 155, 132, 131, 107, 106, 105.

3-(Hydroxybenzyl)-4-(4-chlorophenyl)-2H-1,2,4-triazole-3-thione (12b). Yield: 67%; m.p. 178-180 °C; IR (ATR, cm⁻¹) νmax 3125, 3022, 2934, 1557, 1489, 1485; ¹H-NMR (300 Hz, CD₂OD): δ 5.68 (1H, s, CHOH), 6.99 (2H, d, J = 8.7 Hz, Ar-H), 7.08 (2H, dd, J = 7.4, 5.2 Hz, Ar-H), 7.24-7.19 (5H, m, Ar-H); ¹³C-NMR (75 MHz, Acetone-δ₆): δ 67.5, 126.4, 127.8, 128.0, 128.9, 130.7, 133.1, 134.5, 139.0, 153.5, 170.4; EIMS: m/z 315/317 [M⁺], 245, 210, 183, 169, 132, 107, 106, 105, 99.

3-(Hydroxybenzyl)-4-(4-fluorophenyl)-2H-1,2,4-triazole-3-thione (12c). Yield: 63%; m.p. 82-85 °C; IR (ATR, cm⁻¹): νmax 3453, 3069, 3026, 2930, 1599, 1562, 1508, 1487, 1216; ¹H-NMR (300 Hz, DMSO-δ₆): δ 5.59 (1H, d, J = 5.4 Hz, CHOH), 6.37 (1H, d, J = 5.4 Hz, CHOH), 7.13-7.31 (9H, m, Ar-H), 13.91 (1H, s, NH); ¹³C-NMR (75 MHz, DMSO-δ₆): δ 66.9, 116.3 (d, J = 23.5 Hz), 126.8, 128.1, 128.3, 130.4 (d, J = 3 Hz), 131.4 (d, J = 9.8 Hz), 139.7, 154.0, 162.5 (d, J = 244.5 Hz), 169.2; EIMS: m/z 301/303 [M⁺], 300, 284, 272, 224, 194, 167, 153, 136, 122, 109, 105, 77.

3-(Hydroxybenzyl)-4-(4-methylphenyl)-2H-1,2,4-triazole-3-thione (12d). Yield: 63%; m.p. 82-85 °C; IR (ATR, cm⁻¹): νmax 3435, 3076, 3034, 2935, 1609, 1567, 1506,
3-(Hydroxybenzyl)-4-(4-nitrophenyl)-2H-1,2,4-triazole-3-thione (12e). Yield: 64%; m.p. 122-123°C; IR (KBr, cm⁻¹) 3429, 3125, 3022, 2934, 1557, 1508, 1489, 1485, 1337; ¹H-NMR (300 MHz, DMSO-d₆): δ 5.93 (1H, d, J = 4.8 Hz, CHO), 6.76 (1H, d, J = 4.8 Hz, CHOH), 7.33-7.50 (5H, m, Ar-H), 7.83 (2H, d, J = 9.0 Hz, Ar-H), 8.32 (2H, d, J = 9.0 Hz, Ar-H), 12.87 (1H, s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 67.6, 116.4, 126.1, 126.7, 128.9, 129.1, 139.3, 141.9, 146.2, 162.7, 171.1; EIMS: m/z 328 [M⁺], 195, 180, 122, 107, 77.

4-(4-Chlorophenyl)-5-[2-methyl-1-(4-methylbenzenesulfonamido)propyl]-2H-1,2,4-triazole-3-thione (13a). Yield: 85%; m.p. 100-102 °C; [α]D²⁵ + 47° (c 1.2, acetone); IR (KBr, cm⁻¹): v_max 3476, 3277, 1335, 1235, 1156; ¹H-NMR (500 MHz, CD₃OD): δ 0.78 (3H, d, J = 6.7 Hz, CHCH₃), 0.81 (3H, d, J = 6.7 Hz, CHCH₃), 1.94 (1H, m, CH(CH₃)₂), 2.39 (3H, s, Ar-Me), 3.98 (1H, dd, J = 8.4, 7.1 Hz, CH(NH), 6.99 (1H, d, J = 8.4 Hz, NHSO₂), 7.29 (2H, d, J = 8.8 Hz, Ar-H), 7.35 (2H, d, J = 8.1 Hz, Ar-H), 7.62 (2H, d, J = 8.8 Hz, Ar-H), 7.63 (2H, d, J = 8.1 Hz, Ar-H), 12.62 (1H, br s, NH); ¹³C-NMR (75 MHz, Acetone-d₆): δ 17.4, 20.9, 32.1, 55.2, 119.2, 126.7, 129.8, 130.0, 130.6, 132.5, 135.2, 137.9, 143.0, 151.7, 169.5; EIMS: m/z 436 [M⁺], 393, 281, 254, 226, 155, 91, 65; Anal. Calcd. for C₁₉H₂₁ClN₄O₂S₂ (436.98): C, 52.22; H, 4.84; N, 12.82. Found: C, 52.16; H, 4.79; N, 12.61.

4-(4-Chlorophenyl)-5-[3-methyl-1-(4-methylbenzenesulfonamido)butyl]-2H-1,2,4-triazole-3-thione (13b). Yield: 83%; m.p. 190-192 °C; [α]D²⁵ + 27° (c 0.66, CHCl₃); IR (KBr, cm⁻¹): v_max 3478, 3417, 1313, 1235, 1161; ¹H-NMR (500 MHz, CD₃OD): δ 0.46 (3H, d, J = 5.9 Hz, CHCH₃), 0.72 (3H, d, J = 6.0 Hz, CHCH₃), 1.50-1.64 (3H, m, CHCH₃), 2.39 (3H, s, Ar-Me), 4.12 (1H, dd, J = 8.4, 4.3 Hz, CH(NH), 7.10 (1H, br s, NH), 7.35 (2H, d, J = 8.2 Hz, Ar-H), 7.36 (2H, d, J = 8.5 Hz, Ar-H), 7.60 (2H, d, J = 8.2 Hz, Ar-H), 7.63 (2H, d, J = 8.5 Hz, Ar-H), 12.61 (1H, br s, NH);
$^{13}$C-NMR (75 MHz, Acetone-$d_6$): δ 20.4, 21.5, 22.7, 24.2, 43.6, 47.9, 126.8, 129.7, 129.8, 130.4, 130.8, 136.9, 137.0, 144.2, 154.9, 168.7; EI-MS: m/z 450 [M$^+$], 393, 295, 241, 239, 155, 91, 69, 65; Anal. Calcd. for C$_{20}$H$_{22}$ClN$_4$O$_2$S$_2$ (451.01): C, 53.26; H, 5.14; N, 12.42. Found: C, 53.13; H, 5.13; N, 12.36.

4-(4-Chlorophenyl)-5-[2-methyl-1-(4-methylbenzenesulfonylamido)butyl]-2H-1,2,4-triazole-3-thione (13c). Yield: 83%; m.p. 122-124 °C; [α]$_D^{25}$ + 42° (c 1.01, CHCl$_3$); IR (KBr, cm$^{-1}$): ν$_{max}$ 3473, 3217, 1336, 1239, 1158; $^1$H-NMR (500 MHz, CD$_3$OD): δ 0.64 (3H, t, J = 7.4 Hz, CH$_2$CH$_2$), 0.76 (3H, d, J = 6.8 Hz, CHCH$_3$), 0.84 (1H, m, CHHCH$_3$), 1.47 (1H, m, CHHCH$_3$), 1.74 (1H, m, CH$_2$CHCH$_3$), 2.39 (3H, s, Ar-Me), 3.98 (1H, d, J = 6.9 Hz, CHNH), 6.99 (1H, br s, NHSO$_2$), 7.29 (2H, d, J = 8.6 Hz, Ar-H), 7.36 (2H, d, J = 8.2 Hz, Ar-H), 7.62 (2H, d, J = 8.6 Hz, Ar-H), 7.64 (2H, d, J = 8.2 Hz, Ar-H), 12.62 (1H, br s, NH); $^{13}$C-NMR (75 MHz, Acetone-$d_6$): δ 14.8, 20.5, 24.2, 37.7, 51.1, 60.5, 119.0, 127.1, 128.6, 128.9, 129.3, 138.2, 143.1 144.2, 154.8, 171.2; EI-MS: m/z 450 [M$^+$], 393, 239, 155, 111, 91, 65; Anal. Calcd. for C$_{20}$H$_{22}$ClN$_4$O$_2$S$_2$ (451.01): C, 53.26; H, 5.14; N, 12.42. Found C, 53.31; H, 5.08; N, 12.31.

4-(4-Chlorophenyl)-5-(1-(4-methylbenzenesulfonylamido)-2-hydroxypropyl)-2H-1,2,4-triazole-3-thione (13d). Yield: 72%; m.p. 121-122 °C; [α]$_D^{25}$ + 54° (c, 1.1, CHCl$_3$); IR (KBr, cm$^{-1}$): ν$_{max}$ 3418, 3365, 3271, 1339, 1241, 1158; $^1$H-NMR (500 MHz, DMSO-$d_6$): δ 0.98 (3H, d, J = 6.2 Hz, CHOCHCH$_3$), 2.36 (3H, s, Ar-Me), 3.66 (1H, m, CHOHH$_3$), 3.80 (1H, dd, J = 6.2, 6.1 Hz, CHNH), 4.97 (1H, d, J = 5.6 Hz, OH), 6.74 (2H, d, J = 8.1 Hz, Ar-H), 7.17 (1H, br s, NHSO$_2$), 7.31 (2H, d, J = 8.0 Hz, Ar-H), 7.53 (2H, d, J = 8.1 Hz, Ar-H), 7.61 (2H, d, J = 8.0 Hz), 13.8 (1H, s, NH); $^{13}$C-NMR (75 MHz, CD$_3$OD): δ 17.6, 20.1, 54.4, 67.7, 126.9, 129.3, 129.4, 130.3, 132.0, 135.6, 137.2, 143.8, 150.1, 168.3; EI-MS: m/z 356, 355, 171, 155, 152, 111, 91, 65; Anal. Calcd. for C$_{18}$H$_{19}$ClN$_4$O$_2$S$_2$ (438.95): C, 49.25; H, 4.36; N, 12.76. Found: C, 49.19, H, 4.31; N, 12.65.

4-(4-Chlorophenyl)-5-[1-(4-methylbenzenesulfonylamido)-3-methylthiopropyl]-2H-1,2,4-triazole-3-thione (13e). Yield: 79%; m.p. 105-107 °C; [α]$_D^{25}$ + 38° (c 0.90, CHCl$_3$); IR (KBr, cm$^{-1}$): ν$_{max}$ 3417, 3254, 1320, 1221, 1157; $^1$H-NMR (500 MHz,
Acetone-$d_6$: δ 1.74 (3H, s, CH$_2$S), 1.90 (2H, m, SCH$_2$CH$_2$), 2.20 (2H, m, SCH$_2$CH$_2$), 2.33 (3H, s, Ar-Me), 4.12 (1H, dd, J = 13.7, 7.0 Hz, CHNH), 7.30 (2H, d, J = 8.8 Hz, Ar-H), 7.31 (2H, d, J = 8.2 Hz, Ar-H), 7.47 (2H, d, J = 8.2 Hz, Ar-H), 7.61 (2H, d, J = 8.8 Hz, Ar-H), 8.34 (1H, d, J = 7.0 Hz, NH$_2$SO$_2$), 13.87 (1H, s, NH); $^{13}$C-NMR (75 MHz, Acetone-$d_6$): δ 15.0, 21.6, 29.7, 33.1, 48.0, 127.0, 119.2, 129.6, 129.8, 130.4, 130.9, 136.8, 144.2, 153.7, 168.7; EIMS: m/z 468 [M$^+$], 313, 265, 239, 238, 210, 155, 111, 91, 75, 65, 61, 59; Anal. Calcd. for C$_{19}$H$_{21}$ClN$_4$O$_2$S$_3$ (469.04): C, 48.65; H, 4.51; N, 11.94. Found: C, 48.59; H, 4.43; N, 11.86.

4-(4-Chlorophenyl)-5-[1-(4-methylbenzenesulfonamido)-2-mercaptoethyl]-2H-1,2,4-triazole-3-thione (13f). Yield: 63%; m.p. 141-143 °C; [α]$_D^{25}$ + 22° (c 0.53, CHCl$_3$); IR (KBr, cm$^{-1}$): $\nu$$_{max}$ 3287, 3243, 2558, 1335, 1243, 1158; $^1$H-NMR (300 MHz, Acetone-$d_6$): δ 2.41 (3H, s, Ar-Me), 3.74-3.83 (2H, m, CH$_2$SH), 4.19 (1H, at, J = 6.4 Hz, CHNH), 4.38 (1H, br s, SH), 7.07 (1H, br s, NH$_2$SO$_2$), 7.31 (2H, d, J = 8.6 Hz, Ar-H), 7.33 (2H, d, J = 8.0 Hz, Ar-H), 7.56 (2H, d, J = 8.6 Hz, Ar-H) 7.57 (2H, d, J = 8.0 Hz, Ar-H), 12.62 (1H, br s, NH); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): δ 18.9, 21.8, 56.5, 116.5, 120.3, 125.9, 128.8, 129.4, 132.6, 138.7, 146.7, 152.3, 170.4; EIMS: m/z 215, 170, 155, 138, 126, 111, 99, 91, 75, 73, 63; Anal. Calcd. for C$_{17}$H$_{17}$ClN$_4$O$_2$S$_3$ (440.99): C, 46.30; H, 3.89; N, 12.70. Found: C, 46.25; H, 3.79; N, 12.63.

4-(4-Chlorophenyl)-5-[1-(4-methylbenzenesulfonamido)-2-hydroxyethyl]-2H-1,2,4-triazole-3-thione (13g). Yield: 69%; m.p. 115-118 °C; [α]$_D^{25}$ + 63° (c 1.51, CHCl$_3$); IR (KBr, cm$^{-1}$): $\nu$$_{max}$ 3413, 3387, 3235, 1337, 1246, 1155; $^1$H-NMR (300 MHz, DMSO-$d_6$): δ 2.36 (3H, s, Ar-Me), 3.46 (1H, dd, J = 10.1, 6.5 Hz, CHHOH), 3.57 (1H, dd, J = 10.1, 8.7 Hz, CHHOH), 3.83 (dd, 1H, J = 8.4, 6.3 Hz, CHNH), 5.22 (br s, 1H, OH), 7.22 (2H, d, J = 8.7 Hz, Ar-H), 7.31 (2H, d, J = 8.1 Hz, Ar-H), 7.47 (2H, d, J = 8.7 Hz, Ar-H), 7.63 (2H, d, J = 8.1 Hz, Ar-H), 8.32 (1H, br s, NH$_2$SO$_2$), 13.85 (1H, br s, NH); $^{13}$C-NMR (75 MHz, Acetone-$d_6$): δ 21.5, 51.1, 62.9, 127.0, 129.8, 130.0, 130.9, 132.4, 134.7, 137.5, 143.5, 151.2, 168.0; EIMS: m/z 393 [M$^+$], 209, 171, 170, 169, 155, 152, 111, 91, 65; Anal. Calcd. for C$_{17}$H$_{17}$ClN$_4$O$_3$S$_2$ (424.92): C, 48.05; H, 4.03; N, 13.19. Found: C, 47.96; H, 4.12; N, 13.09.
3.9  General procedure for the synthesis of thiazoles (14a-e,h).

The respective thiosemicarbazide (1.10 mmol) was added portion wise to conc. H$_2$SO$_4$ (20 mL) at 0 °C with continuous stirring. The reaction mixture was stirred for 4 hr at 23 °C and then allowed to stand overnight. The mixture was neutralized with dil. NaOH to afford a precipitate which was filtered, washed with excess of water and recrystallized from EtOH/water to afford 1,3,4-thiazole derivatives (14a-e,h).

2-(4-Chlorophenyl)amino-5-[2-methyl-1-(4-methylbenzenesulfonamido)propyl]-1,3,4-thiazole (14a). Yield: 82%; m.p. 148-150 °C; $[\alpha]_D^{25} + 35^o$ (c 0.84, CHCl$_3$);
IR (KBr, cm$^{-1}$): $\nu_{\text{max}}$ 3276, 3223, 1328, 1155; $^1$H-NMR (500 MHz, Acetone-$d_6$): $\delta$ 0.83, 0.99 (6H, 2 x d, $J = 6.7$ Hz, CHMe$_2$), 2.07 (1H, m, CHMe$_2$), 2.35 (3H, s, Me-ArSO$_2$), 4.44 (1H, dd, $J = 8.5$, 7.8 Hz, NHCHCHMe$_2$), 7.07 (2H, d, $J = 8.1$ Hz, Ar-H), 7.23 (2H, d, $J = 8.1$ Hz, Ar-H), 7.34 (2H, d, $J = 8.8$ Hz, Ar-H), 7.64 (2H, d, $J = 8.8$ Hz, Ar-H), 9.41 (1H, br s, NH); $^{13}$C-NMR (75 MHz, Acetone-$d_6$): $\delta$ 19.3, 21.3, 33.3, 59.9, 119.2, 125.6, 127.2, 129.3, 129.7, 140.0, 142.5, 142.8, 162.1, 164.8; EIMS: m/z 393, 329, 184, 169, 155, 152, 111, 99, 91, 65; Anal. Calcd. for C$_{19}$H$_{21}$ClN$_4$O$_2$S$_2$ (436.98): C, 52.22; H, 4.84; N, 12.82. Found: C, 52.29; H, 4.82; N, 12.77.

2-(4-Chlorophenyl)amino-5-[1-(4-methylbenzenesulfonamido)-3-methylbutyl]-1,3,4-thiazole (14b). Yield: 79%; m.p. 208-210 °C; $[\alpha]_D^{25} + 37^o$ (c 0.88, CHCl$_3$);
IR (KBr, cm$^{-1}$): $\nu_{\text{max}}$ 3315, 3227, 1325, 1159; $^1$H-NMR (500 MHz, CD$_2$OD): $\delta$ 0.78, 0.85 (6H, 2 x d, $J = 6.5$ Hz, CH(CH$_3$)$_2$), 1.62 (1H, m, CH(CH$_3$)$_2$), 1.69 (2H, dd, $J = 7.5$, 7.0 Hz, CHCH$_2$CH), 2.28 (3H, s, Ar-Me), 4.71 (1H, dd, $J = 7.8$, 4.5 Hz, CHNH), 7.17 (1H, br s, NHSO$_2$), 7.27 (2H, d, $J = 8.1$ Hz, Ar-H), 7.35 (2H, d, $J = 8.9$ Hz, Ar-H), 7.64 (2H, d, $J = 8.1$ Hz, Ar-H), 7.65 (2H, d, $J = 8.9$ Hz, Ar-H), 9.51 (1H, br s, NH); $^{13}$C-NMR (75 MHz, Acetone-$d_6$): $\delta$ 20.5, 21.2, 21.7, 24.2, 44.7, 52.3, 119.0, 126.3, 127.1, 128.8, 129.4, 138.4, 139.8, 143.0, 162.9, 164.9; EIMS: m/z 450 [M$^+$], 393, 294, 241, 239, 226, 184, 171, 155, 152, 111, 99, 91, 65; Anal. Calcd. for C$_{20}$H$_{22}$ClN$_4$O$_2$S$_2$ (451.01): C, 53.26; H, 5.14; N, 12.42. Found: C, 53.37; H, 5.06; N, 12.37.
2-(4-Chlorophenyl)amino-5-[1-(4-methylbenzenesulfonamido)-2-methylbutyl]-1,3,4-thiadiazole (14c). Yield: 76%; m.p. 74-76 °C; [α]D25 + 72° (c 1.7, CHCl3); IR (KBr, cm⁻¹): νmax 3287, 3197, 1332, 1156; ¹H-NMR (500 MHz, Acetone-d₆): δ 0.80-0.86 (6H, m, CH₃CH₂CH₂CH₃), 1.17 (1H, m, CHHCH₃), 1.47 (1H, m, CHHCH₃), 1.72 (1H, m, CH₂CH₂CH₃), 2.39 (3H, s, Ar-Me), 3.72 (1H, dd, J = 9.8, 6.7 Hz, CHNH), 6.74 (1H, d, J = 9.8 Hz, NHSO₂), 7.36 (2H, d, J = 8.5 Hz, Ar-H), 7.59 (2H, d, J = 8.1 Hz, Ar-H), 7.64 (2H, d, J = 8.5 Hz, Ar-H), 7.68 (2H, d, J = 8.1 Hz), 10.13 (1H, br s, NH); ¹³C-NMR (75 MHz, Acetone-d₆): δ 14.8, 20.5, 24.7, 37.7, 51.1, 60.4, 119.0, 127.1, 128.6, 128.9, 129.3, 129.4, 138.2, 143.1, 162.9, 164.9; EIMS m/z 450 [M⁺], 393, 240, 184, 169, 155, 152, 111, 99, 91, 65; Anal. Calcd. for C₂₀H₂₃ClN₄O₂S₂ (451.01): C, 53.26; H, 5.14; N, 12.42. Found: C, 53.33; H, 5.18; N, 12.33.

2-(4-Chlorophenyl)amino-5-[1-(4-methylbenzenesulfonamido)-2-hydroxypropyl]-1,3,4-thiadiazole (14d). Yield: 67%; m.p. 180-182 °C; [α]D25 + 28° (c 0.66, CHCl₃); IR (KBr, cm⁻¹): νmax 3487, 3361, 3279, 1341, 1157; ¹H-NMR (500 MHz, Acetone-d₆): δ 1.10 (3H, d, J = 6.3 Hz, CHCH₃), 2.33 (3H, s, Ar-Me), 3.65 (1H, br s, OH), 4.14 (1H, m, CH₂CH₃OH), 4.61 (1H, dd, J = 7.9, 3.6 Hz, CHNH), 6.93 (1H, d, J = 6.9 Hz, NHSO₂), 7.32 (2H, d, J = 8.0, Ar-H), 7.37 (2H, d, J = 8.6 Hz, Ar-H), 7.53 (2H, d, J = 8.9 Hz, Ar-H), 7.74 (2H, d, J = 8.2 Hz, Ar-H), 9.87 (1H, br s, NH); ¹³C-NMR (75 MHz, Acetone-d₆): δ 16.8, 21.0, 58.2, 75.0, 120.2, 125.6, 127.4, 129.2, 129.5, 137.4, 138.3, 143.0, 160.1, 166.4; EIMS: m/z 356, 355, 171, 155, 152, 111, 91, 65; Anal. Calcd. for C₁₉H₁₉ClN₄O₂S₂ (438.95): C, 49.25; H, 4.36; N, 12.76. Found: C, 49.33; H, 4.30; N, 12.81.

2-(4-Chlorophenyl)amino-5-[1-(4-methylbenzenesulfonamido)-3-methylthiopropyl]-1,3,4-thiadiazole (14e). Yield: 73%; m.p. 174-176 °C; [α]D25 + 74° (c 1.78, CHCl₃); IR (KBr, cm⁻¹): νmax 3324, 3221, 1335, 1154; ¹H-NMR (500 MHz, Acetone-d₆): δ 1.95 (3H, s, SCH₂), 2.18 (2H, m, SCH₂CH₂), 2.33 (3H, s, Ar-Me), 2.44 (2H, m, SCH₂CH₂), 4.87 (1H, dd, J = 14.4, 7.9 Hz, CHNH), 7.20 (1H, br s, NHSO₂), 7.32 (2H, d, J = 8.1 Hz, Ar-H), 7.35 (2H, d, J = 8.8 Hz, Ar-H), 7.65 (2H, d, J = 8.8 Hz, Ar-H), 7.69 (2H, d, J = 8.1 Hz, Ar-H); ¹³C-NMR (75 MHz, Acetone-d₆): δ 14.8, 21.4, 29.6, 34.5, 52.5, 119.2, 125.7, 127.1, 129.4, 129.9, 138.4, 139.9, 143.2, 162.7, 165.0; EIMS: m/z 313, 265, 239, 155, 152, 111, 91, 75, 61, 65; Anal. Calcd. for
C_{19}H_{21}ClN_{4}O_{3}S_{3} (469.04): C, 48.65; H, 4.51; N, 11.94. Found: C, 48.57; H, 4.57; N, 11.87.

2-(4-Chlorophenyl)amino-5-[2-(1H-imidazole-4-yl)-1-(4-methylbenzenesulfonylamido)ethyl]-1,3,4-thiadiazole (14h). Yield: 77%; m.p. 247-248 °C (dec.); [α]_{D}^{25} + 68° (c 1.62, CHCl_{3}); IR (KBr, cm\(^{-1}\)): \(v_{\text{max}}\) 3406, 3368, 3297, 1336, 1156; \(^{1}\text{H-NMR}\) (500 MHz, CD_{3}OD): δ 2.31 (3H, s, Ar-Me), 3.30-3.33 (2H, m, CH_{2}CHNH), 4.97 (1H, dd, \(J = 9.0, 6.0\) Hz, CHNH), 7.15 (1H, s, CH-Imidazole), 7.23 (2H, d, \(J = 8.1\) Hz, Ar-H), 7.29 (2H, d, \(J = 6.9\) Hz, Ar-H), 7.48 (2H, d, \(J = 6.9\) Hz, Ar-H), 7.56 (2H, d, \(J = 8.2\) Hz, Ar-H), 8.40 (1H, s, CH-Imidazole); \(^{13}\text{C-NMR}\) (75 MHz, CD_{3}OD): δ 21.4, 31.8, 53.4, 117.7, 119.2, 125.7, 126.8, 129.4, 129.8, 130.9, 134.9, 138.1, 139.9, 143.1, 161.9, 165.1; EIIMS m/z 246, 171, 155, 125, 123, 107, 91, 85, 69, 65; Anal. Calcd. for C_{20}H_{19}ClN_{6}O_{3}S_{2} (474.99): C, 50.57; H, 4.03; N, 17.69. Found: C, 50.53; H, 4.06; N, 17.55.

3.10 General procedure for one pot two step synthesis of 1,3,4-oxadiazoles (15a-d')

Aryloxyalkanoic acid hydrazide (5a-h, 1.0 eq) was dissolved in methanol (30mL) and arylisothiocyanate in methanol (10mL) was added slowly on stirring. The resulting reaction mixture was refluxed and consumption of isothiocyanate (2-3 hr) was monitored by TLC. Rrequired quantity of mercuric acetate was added and continued the reflux. After 4-5 hours TLC indicated the formation of one major product. The reaction mixture was cooled to room temperature, filtered and concentrated in vacuo. The resulting solid was recrystallized from aqueous ethanol.

2-(4-Bromophenyl)amino-5-[1-(4-bromophenoxy)ethyl]-1,3,4-oxadiazole (15a): Yield: 71%; m.p. 152-153°C (dec.); IR (ATR, cm\(^{-1}\)): \(v_{\text{max}}\) 3247, 3062, 2936, 1620, 1593, 1571, 1486; \(^{1}\text{H-NMR}\) (300 MHz, Acetone-\(d_{6}\)): δ 1.75 (3H, d, \(J = 6.6\) Hz, OCHCH_{3}), 5.69 (1H, q, \(J = 6.6\) Hz, OCHCH_{3}), 7.05 (2H, m, Ar-H), 7.45 (2H, m, Ar-H), 7.51 (2H, m, Ar-H), 7.63 (2H, m, Ar-H), 9.74 (1H, s, NH); \(^{13}\text{C-NMR}\) (75 MHz, Acetone-\(d_{6}\)): δ 18.2, 67.4, 113.6, 114.0, 118.1, 119.0, 131.9, 132.4, 138.0, 156.6,
2-(4-Bromophenyl)amino-5-[1-(4-methylphenoxy)ethyl]-1,3,4-oxadiazole (15b):
Yield: 74%; m.p. 164-165°C; IR (ATR, cm⁻¹): v_max 3250, 3067, 1609, 1587, 1569, 1488; H-NMR (300 MHz, DMSO-d₆): δ 1.66 (3H, d, J = 6.3 Hz, OCHCH₃), 2.21 (3H, s, MeArO), 5.68 (1H, q, J = 6.3 Hz, OCHCH₃), 6.93 (2H, m, Ar-H), 7.07 (2H, m, Ar-H), 7.50 (4H, m, Ar-H), 10.75 (1H, s, NH); C-NMR (75 MHz, DMSO-d₆): δ 19.2, 20.5, 67.2, 113.9, 116.3, 119.4, 130.4, 131.1, 132.3, 138.3, 155.0, 159.5, 160.3; EIMS: m/z 373/375 [M⁺], 269, 268, 267, 266, 200, 198, 172, 170, 157, 155, 107, 91, 69.

2-(4-Fluorophenyl)amino-5-[1-(4-bromophenoxy)ethyl]-1,3,4-oxadiazole (15c):
Yield: 68%; m.p. 148-149°C; IR (ATR, cm⁻¹): v_max 3255, 3063, 2940, 1613, 1584, 1570, 1487; H-NMR (300 MHz, Acetone-d₆): δ 1.80 (3H, d, J = 6.6 Hz, OCHCH₃), 5.50 (1H, q, J = 6.6 Hz, OCHCH₃), 6.92 (2H, m, Ar-H), 7.05 (2H, m, Ar-H), 7.34-7.40 (4H, m, Ar-H), 8.45 (1H, br s, NH); C-NMR (75 MHz, Acetone-d₆): δ 19.1, 67.5, 114.5, 116.1 (d, J_C,F = 23.2 Hz), 117.7, 119.4 (d, J_C,F = 7.5 Hz), 132.5, 133.5 (d, J_C,F = 2.3 Hz), 156.1, 158.9 (d, J_C,F = 240.7 Hz), 159.2, 160.8; EIMS: m/z 377/379 [M⁺], 206, 173, 171, 157, 155, 138, 136, 110, 95, 76, 69.

2-(4-Fluorophenyl)amino-5-[1-(4-chlorophenoxy)ethyl]-1,3,4-oxadiazole (15d):
Yield: 79%; m.p. 134-136°C; IR (ATR, cm⁻¹): v_max 3251, 3059, 2947, 1623, 1581, 1574, 1483; H-NMR (300 MHz, DMSO-d₆): δ 1.68 (3H, d, J = 6.3 Hz, OCHCH₃), 5.76 (1H, q, J = 6.3 Hz, OCHCH₃), 7.08 (2H, d, J = 8.7 Hz, Ar-H), 7.17 (2H, at, J = 9.0 Hz, Ar-H), 7.34 (2H, d, J = 9.0 Hz, Ar-H), 7.53 (2H, m, Ar-H), 10.60 (1H, br s, NH); C-NMR (75 MHz, DMSO-d₆): δ 19.0, 67.6, 116.1 (d, J_C,F = 22.5 Hz), 118.1, 119.1 (d, J_C,F = 8.3 Hz), 126.0, 129.8, 135.4 (d, J_C,F = 1.5 Hz), 156.0, 157.8 (d, J_C,F = 237.0 Hz), 158.9, 160.6; EIMS: m/z 333 [M⁺], 206, 155, 138, 128, 126, 110, 95, 69.

2-(4-Fluorophenyl)amino-5-[1-(3,4-chlorophenoxy)ethyl]-1,3,4-oxadiazole (15e):
Yield: 81%; m.p. 152-153°C; IR (ATR, cm⁻¹): v_max 3247, 3067, 2987, 1626, 1583, 1570, 1489; H-NMR (300 MHz, DMSO-d₆): δ 1.68 (3H, d, J = 6.3 Hz, OCHCH₃),
5.86 (1H, q, J = 6.3 Hz, OCH\textsubscript{3}), 7.08 (1H, dd, J = 9.0, 3.0 Hz, 3,4-Cl\textsubscript{2}Ar-H-6), 7.18 (2H, m, Ar-H), 7.41 (1H, d, J = 3.0 Hz, 3,4-Cl\textsubscript{2}Ar-H-2), 7.51-7.56 (3H, m, Ar-H), 10.63 (1H, s, NH); \textsuperscript{13}C-NMR (75 MHz, DMSO-d\textsubscript{6}): δ 18.7, 67.7, 115.1 (d, J\textsubscript{CF} = 22.5 Hz), 117.1, 118.3, 119.1 (d, J\textsubscript{CF} = 7.5 Hz), 124.2, 131.6, 132.2, 135.3 (d, J\textsubscript{CF} = 2.3 Hz), 156.6, 158.0 (d, J\textsubscript{CF} = 236.3 Hz), 158.6, 160.7; \textbf{EIMS}: m/z 206, 191, 162/164, 138, 125, 110, 95, 69.

2-(4-Fluorophenyl)amino-5-[1-(4-methylphenoxy)ethyl]-1,3,4-oxadiazole (15f)
Yield: 83%; m.p. 144-146°C; IR (ATR, cm\textsuperscript{-1}): ν\textsubscript{max} 3253, 3073, 2942, 1632, 1586, 1492; \textsuperscript{1}H-NMR (300 MHz, DMSO-d\textsubscript{6}): δ 1.73 (3H, d, J = 6.3 Hz, OCH\textsubscript{CH\textsubscript{3}}), 2.24 (3H, s, Me-ArO), 5.59 (1H, q, J = 6.3 Hz, OCH\textsubscript{CH\textsubscript{3}}), 6.94 (2H, m, Ar-H), 7.08-7.17 (4H, m, Ar-H), 7.65-7.72 (2H, m, Ar-H), 9.47 (1H, s, NH); \textsuperscript{13}C-NMR (75 MHz, Acetone-d\textsubscript{6}): δ 18.3, 19.6, 67.3, 115.1 (d, J\textsubscript{CF} = 35.3 Hz), 116.0, 118.8 (d, J\textsubscript{CF} = 7.5 Hz), 129.9, 131.0, 135.1 (d, J\textsubscript{CF} = 3 Hz), 155.3, 158.1 (d, J\textsubscript{CF} = 237.8 Hz), 159.4, 160.4; \textbf{EIMS}: m/z 313 [M\textsuperscript{+}], 206, 176, 138, 110, 77, 69.

2-(4-Chlorophenyl)amino-5-[1-(4-bromophenoxy)ethyl]-1,3,4-oxadiazole (15g)
Yield: 81%; m.p. 152-153°C; IR (ATR, cm\textsuperscript{-1}) ν\textsubscript{max} 3231, 3050, 2944, 1623, 1596, 1573, 1486; \textsuperscript{1}H-NMR (300 MHz, Acetone-d\textsubscript{6}): δ 1.76 (3H, d, J = 6.6 Hz, OCH\textsubscript{CH\textsubscript{3}}), 5.69 (1H, q, J = 6.6 Hz, OCH\textsubscript{CH\textsubscript{3}}), 7.05 (2H, m, Ar-H), 7.37 (2H, m, Ar-H), 7.46 (2H, m, Ar-H), 7.68 (2H, m, Ar-H), 9.85 (1H, s, NH); \textsuperscript{13}C-NMR (75 MHz, Acetone-d\textsubscript{6}): δ 19.1, 67.3, 114.5, 119.0, 119.6, 129.9, 133.3, 138.4, 157.5, 159.9, 161.1, 161.2; \textbf{EIMS}: m/z 393/395 [M\textsuperscript{+}], 224, 222, 199, 174, 173, 172, 171, 128, 126, 157, 155, 113, 111, 99, 76, 69.

2-(4-Chlorophenyl)amino-5-[1-(4-chlorophenoxy)ethyl]-1,3,4-oxadiazole (15h)
Yield: 77%; m.p. 165-166°C; IR (ATR, cm\textsuperscript{-1}) ν\textsubscript{max} 3261, 3061, 2954, 1633, 1594, 1579, 1488; \textsuperscript{1}H-NMR (300 MHz, DMSO-d\textsubscript{6}): δ 1.68 (3H, d, J = 6.3 Hz, OCH\textsubscript{CH\textsubscript{3}}), 5.78 (1H, q, J = 6.3 Hz, OCH\textsubscript{CH\textsubscript{3}}), 7.08 (2H, m, Ar-H), 7.33-7.40 (4H, m, Ar-H), 7.54 (2H, m, Ar-H), 10.78 (1H, br s, NH); \textsuperscript{13}C-NMR (75 MHz, Acetone-d\textsubscript{6}): δ 18.2, 67.4, 118.1, 119.0, 126.0, 129.4, 129.9, 137.9, 156.0, 159.1, 160.4; \textbf{EIMS}: m/z 349/351 [M\textsuperscript{+}], 224, 222, 129, 127, 126, 113, 111, 99, 75, 69.
2-(4-Chlorophenyl)amino-5-[1-(3,4-dichlorophenoxy)ethyl]-1,3,4-oxadiazole (15i): Yield: 73%; m.p. 147-148°C; IR (ATR, cm⁻¹): νmax 3246, 1620, 1598, 1573, 1494, 1470; ¹H-NMR (300 MHz, Acetone-d₆): δ 1.78 (3H, d, J = 6.6 Hz, OCHCH₃), 5.76 (1H, q, J = 6.6 Hz, OCHCH₃), 7.09 (1H, dd, J = 9.0, 2.7 Hz, 3,4-Cl₂Ar-H-6), 7.33 (1H, d, J = 2.7 Hz, 3,4-Cl₂Ar-H-2), 7.38 (2H, m, Ar-H), 7.49 (1H, d, J = 9.0 Hz, 3,4-Cl₂Ar-H-5), 7.68 (2H, m, Ar-H), 9.35 (1H, br s, NH); ¹³C-NMR (75 MHz, Acetone-d₆) δ 18.0, 67.8, 116.4, 118.0, 118.7, 124.4, 126.6, 129.0, 131.0, 132.3, 137.6, 156.7, 158.7, 160.3; EIMS: m/z 201, 169, 153, 144, 125, 111, 75.

2-(4-Chlorophenyl)amino-5-[1-(4-methylphenoxy)ethyl]-1,3,4-oxadiazole (15j): Yield: 80%; m.p. 157-158°C; IR (ATR, cm⁻¹): νmax 3232, 3054, 2944, 1623, 1597, 1588, 1487; ¹H-NMR (300 MHz, Acetone-d₆): δ 1.73 (3H, d, J = 6.6 Hz, OCHCH₃), 2.24 (3H, s, Me-ArO), 5.60 (1H, q, J = 6.6 Hz, OCHCH₃), 6.95 (2H, m, Ar-H), 7.08 (2H, m, Ar-H), 7.35 (2H, m, Ar-H), 7.68 (2H, m, Ar-H), 9.68 (1H, br s, NH); ¹³C-NMR (75 MHz, Acetone-d₆) δ 18.4, 19.6, 67.3, 116.0, 118.6, 126.4, 128.9, 129.9, 131.0, 137.6, 155.2, 159.5, 160.1; EIMS: m/z 329 [M⁺], 281, 222, 207, 154, 126, 108, 91, 69.

2-(4-Nitrophenyl)amino-5-[1-(3,4-dichlorophenoxy)ethyl]-1,3,4-oxadiazole (15k): Yield: 75%; m.p. 118-119°C (dec); IR (ATR, cm⁻¹): νmax 3334, 3097, 2941, 1633, 1592, 1579, 1523, 1488, 1327; ¹H-NMR (300 MHz, DMSO-d₆): δ 1.70 (3H, d, J = 6.3 Hz, OCHCH₃), 5.91 (1H, q, J = 6.3 Hz, OCHCH₃), 7.09 (1H, dd, J = 9.0 Hz, J = 2.7 Hz, 3,4-Cl₂Ar-H-6), 7.41 (1H, d, J = 2.7 Hz, 3,4-Cl₂Ar-H-2), 7.55 (1H, d, J = 9.0 Hz, 3,4-Cl₂Ar-H-5), 7.73 (2H, d, J = 9.0 Hz, 4-NO₂Ar-H), 8.25 (2H, J = 9.0 Hz, 4-NO₂Ar-H), 11.49 (1H, s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 18.5, 67.7, 117.1, 117.3, 118.0, 118.3, 124.2, 125.9, 131.6, 132.2, 145.1, 156.5, 159.5, 169.0; EIMS: m/z 233, 165, 163, 161, 137, 133, 122, 99, 63.

2-(4-Nitrophenyl)amino-5-[1-(4-methylphenoxy)ethyl]-1,3,4-oxadiazole (15l): Yield: 73%; m.p. 182-184°C; IR (ATR, cm⁻¹): νmax 3310, 3108, 2929, 1623, 1595, 1586, 1506, 1486, 1330; ¹H-NMR (300 MHz, DMSO-d₆): δ 1.68 (3H, d, J = 6.6 Hz, OCHCH₃), 2.21 (3H, s, Me-ArO), 5.72 (1H, q, J = 6.6 Hz, OCHCH₃), 6.94 (2H, d, J = 8.7 Hz, MeAr-H), 7.09 (2H, J = 8.7 Hz, MeAr-H), 7.72 (2H, m, NO₂Ar-H), 8.25
(2H, m, NO₂Ar-H), 11.44 (1H, s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 19.2, 20.5, 67.2, 116.3, 117.2, 125.9, 130.4, 131.2, 141.6, 145.1, 155.0, 159.8, 160.2; EIMS: m/z 233, 207, 149, 107, 95, 69.

2-(4-Methylphenyl)amino-5-[1-(4-bromophenoxy)ethyl]-1,3,4-oxadiazole (15m):
Yield: 76%; m.p. 160-161°C; IR (ATR, cm⁻¹): ν max 3237, 3062, 2937, 1617, 1594, 1585, 1488; ¹H-NMR (300 MHz, DMSO-d₆): δ 1.67 (3H, d, J = 6.3 Hz, OCHCH₃), 2.24 (3H, s, Me-ArNH), 5.76 (1H, q, J = 6.3 Hz, OCHCH₃), 7.03 (2H, m, BrAr-H), 7.13 (2H, d, J = 8.4 Hz, MeAr-H), 7.40 (2H, d, J = 8.4 Hz, MeAr-H), 7.47 (2H, m, BrAr-H), 10.47 (1H, s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 19.0, 20.7, 67.3, 113.8, 117.4, 118.6, 129.9, 131.3, 132.8, 136.4, 156.5, 158.7, 160.7; EIMS: m/z 373 [M⁺], 202, 174, 172, 157, 155, 134, 106, 91, 79, 81, 69, 65.

2-(4-Methylphenyl)amino-5-[1-(4-chlorophenoxy)ethyl]-1,3,4-oxadiazole (15n):
Yield: 76%; m.p. 152-153°C; IR (cm⁻¹): ν max 3266, 3042, 2946, 1621, 1608, 1569, 1488; ¹H-NMR (300 MHz, Acetone-d₆): δ 1.75 (3H, d, J = 6.6 Hz, OCHCH₃), 2.28 (3H, s, Me-ArNH), 5.66 (1H, q, J = 6.6 Hz, OCHCH₃), 7.1 (2H, m, BrAr-H), 7.16 (2H, d, J = 8.7 Hz, MeAr-H), 7.32 (2H, m, BrAr-H), 7.53 (2H, d, J = 8.7 Hz, MeAr-H), 9.35 (1H, br s, NH); ¹³C-NMR (75 MHz, Acetone-d₆): δ 18.2, 19.8, 67.5, 117.2, 117.6, 126.2, 129.4, 129.5, 131.5, 136.2, 156.2, 158.7, 160.6; EIMS: m/z 329 [M⁺], 202, 134, 129, 127, 106, 91, 69.

2-(4-Methylphenyl)amino-5-[1-(3,4-dichlorophenoxy)ethyl]-1,3,4-oxadiazole (15o):
Yield: 79%; m.p. 146-147°C; IR (ATR, cm⁻¹): ν max 3256, 3067, 2934, 1576, 1603, 1488; ¹H-NMR (300 MHz, DMSO-d₆): δ 1.68 (3H, d, J = 6.3 Hz, OCHCH₃), 2.24 (3H, s, Me-ArNH), 5.86 (1H, q, J = 6.3 Hz, OCHCH₃), 7.08 (1H, dd, J = 9.0, 3.0 Hz, 3,4-Cl₂Ar-H-6), 7.13 (1H, d, J = 9.0 Hz, 3,4-Cl₂Ar-H-5), 7.40 (2H, J = 8.7 Hz, MeAr-H), 7.42 (1H, d, J = 3.0 Hz, 3,4-Cl₂Ar-H-2), 7.56 (2H, d, J = 8.7 Hz, MeAr-H), 10.48 (1H, br s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 18.7, 20.8, 67.7, 117.1, 117.5, 118.3, 124.1, 129.9, 131.3, 131.6, 132.2, 136.4, 156.6, 158.4, 160.7; EIMS: m/z 215, 202, 191, 164, 163, 162, 161, 134, 106, 99, 77, 69.
2-(4-Methylphenyl)amino-5-[1-(4-methylphenoxy)ethyl]-1,3,4-oxadiazole (15p): Yield: 76%; m.p. 120-121°C; IR (ATR, cm⁻¹): vₘₐₓ 3263, 3071, 2927, 1608, 1587, 1486; ^1H-NMR (300 MHz, DMSO-d₆): δ 1.66 (3H, d, J = 6.3 Hz, OCH₃), 2.22 (3H, s, Me-Ar), 2.24 (3H, s, Me-Ar), 5.66 (1H, t, J = 6.3 Hz, OCHCH₃), 6.93 (2H, m, Ar-H), 7.08-7.14 (4H, m, Ar-H), 7.41 (2H, m, Ar-H), 10.42 (1H, br s, NH); ^13C-NMR (75 MHz, DMSO-d₆) δ 19.2, 20.6, 20.8, 67.3, 116.3, 117.5, 129.9, 130.4, 131.1, 131.2, 136.5, 155.0, 159.2, 160.7; EIMS: m/z 309 [M⁺], 202, 145, 134, 132, 107, 91, 69, 65.

2-(4-Bromophenyl)amino-5-[1-(3,4-dichlorophenoxy)ethyl]-1,3,4-oxadiazole (15q): Yield: 81%; m.p. 139-141°C; IR (cm⁻¹): vₘₐₓ 3245, 3067, 2970, 1609, 1593, 1578; ^1H-NMR (300 MHz, DMSO-d₆): δ 1.68 (3H, d, J = 6.6 Hz, OCHCH₃), 5.87 (1H, q, J = 6.6 Hz, OCHCH₃), 7.08 (1H, dd, J = 9.0, 3.0 Hz, 3,4-Cl₂Ar-H-5), 7.42 (1H, d, J = 3.0 Hz, 3,4-Cl₂Ar-H-2), 7.49-7.57 (5H, m, Ar-H, 3,4-Cl₂Ar-H-5), 10.79 (1H, s, NH); ^13C-NMR (75 MHz, DMSO-d₆): δ 18.8, 67.7, 113.9, 117.1, 118.3, 119.4, 124.2, 131.6, 132.1, 132.3, 138.3, 156.6, 158.8, 160.4; EIMS: m/z 268, 266, 255, 257, 209, 207, 200, 198, 172, 170, 157, 155, 133, 130, 95, 91, 75, 69.

2-(4-Bromophenyl)amino-5-[1-(4-bromophenoxy)propyl]-1,3,4-oxadiazole (15r). Yield: 73%; m.p. 145-146°C; IR (ATR, cm⁻¹): vₘₐₓ 3255, 3057, 2973, 1615, 1590, 1568; ^1H-NMR (300 MHz, Acetone-d₆): δ 1.06 (3H, at, J = 6.6 Hz, OCHCH₂CH₃), 2.08 (2H, m, OCHCH₂CH₃), 5.45 (1H, t, J = 6.6 Hz, OCHCH₂CH₃), 7.05 (2H, m, Ar-H), 7.45 (2H, m, Ar-H), 7.51 (2H, m, Ar-H), 7.63 (2H, m, Ar-H), 9.74 (1H, s, NH); ^13C-NMR (75 MHz, Acetone-d₆): δ 8.8, 26.3, 72.6, 113.6, 114.0, 118.0, 119.0, 131.9, 132.4, 138.0, 157.0, 158.3, 160.1; EIMS: m/z 451/453 [M⁺], 283, 282, 281, 280, 240, 238, 173, 172, 171, 170, 157, 155, 83, 76, 69.

2-(4-Bromophenyl)amino-5-[1-(4-bromophenoxy)propyl]-1,3,4-oxadiazole (15s). Yield: 71%; m.p. 113-115°C; IR (ATR, cm⁻¹): vₘₐₓ 3273, 3066, 2977, 1618, 1587, 1565; ^1H-NMR (300 MHz, DMSO-d₆): δ 0.99 (3H, t, J = 7.5 Hz, OCHCH₂CH₃), 1.97-2.05 (2H, m, OCHCH₂CH₃), 5.46 (1H, t, J = 6.9 Hz, OCHCH₂CH₃), 6.92 (2H, m, Ar-H), 7.08 (2H, d, J = 8.4 Hz, MeAr-H), 7.47-7.53 (4H, m, Ar-H), 10.78 (1H, s, NH); ^13C-NMR (75 MHz, DMSO-d₆): δ 9.8, 20.5, 26.6, 72.3, 113.8, 115.8, 119.4,
2-(4-Nitrophenyl)amino-5-[1-(3,4-dichlorophenoxy)propyl]-1,3,4-oxadiazole (15t). Yield: 70%; m.p. 184-185°C; IR (ATR, cm⁻¹): νmax 3323, 3109, 2934, 1618, 159, 1588, 1513, 1485, 1333; ¹H-NMR (300 MHz, DMSO-d₆): δ 1.07 (3H, t, J = 7.5 Hz, CH₂CH₃), 2.03-2.13 (2H, m, OCHCH₂CH₃), 5.71 (1H, t, J = 6.6 Hz, OCHCH₂), 7.09 (1H, dd, J = 9.0, 3.0 Hz, 3,4-Cl₂Ar-H-6), 7.41 (1H, d, J = 3.0 Hz, 3,4-Cl₂Ar-H-2), 7.54 (1H, d, J = 9.0Hz, 3,4-Cl₂Ar-H-5), 7.73 (2H, m, Ar-H), 8.26 (2H, m, Ar-H), 11.45 (1H, br s, NH); ¹³C-NMR (75 MHz, DMSO-d₆) δ 9.6, 26.3, 72.6, 117.1, 117.4, 118.3, 124.3, 125.9, 131.6, 132.2, 141.7, 156.9, 158.8, 160-3; EIMS: m/z 408/410 [M⁺], 247, 165, 163, 161, 137, 122, 83, 76, 75, 69.

2-(4-Bromophenyl)amino-5-[1-(4-chlorophenoxy)propyl]-1,3,4-oxadiazole (15u). Yield: 76%; m.p. 135-137°C; IR (ATR, cm⁻¹): νmax 3308, 3136, 2972, 1596, 1552, 1487; ¹H-NMR (300 MHz, Acetone-d₆): δ 1.07 (3H, t, J = 7.5 Hz, CH₂CH₃), 2.10-2.21 (2H, m, OCHCH₂CH₃), 5.44 (1H, t, J = 6.6 Hz, OCHCH₂), 7.09 (2H, m, Ar-H), 7.31 (2H, m, Ar-H), 7.52 (2H, m, Ar-H), 7.62 (2H, m, Ar-H), 9.61 (1H, br s, NH); ¹³C-NMR (75 MHz, Acetone-d₆): δ 8.8, 26.4, 72.7, 114.0, 117.6, 119.0, 126.2, 129.4, 131.9, 138.0, 156.6, 158.4, 160.2; EIMS: m/z 407/409 [M⁺], 283, 282, 281, 280, 238, 236, 173, 171, 157, 155, 130, 129, 128, 127, 113, 111, 99, 83, 75, 69.

2-(4-Chlorophenyl)amino-5-[1-(3,4-dichlorophenoxy)propyl]-1,3,4-oxadiazole (15v). Yield: 80%; m.p. 148-150°C; IR (ATR, cm⁻¹): νmax 3247, 3050, 2978, 2872, 1587, 1575, 1506, 1493; ¹H-NMR (300 MHz, DMSO-d₆): δ 0.99 (3H, t, J = 7.5 Hz, CH₂CH₃), 1.98-2.14 (2H, m, OCHCH₂CH₃), 5.67 (1H, t, J = 6.9 Hz, OCHCH₂CH₃), 6.82 (1H, d, J = 8.4 Hz, 3,4-Cl₂Ar-H-5), 7.08 (1H, dd, J = 9.0, 3.0 Hz, 3,4-Cl₂Ar-H-6), 7.37-7.41 (3H, m, Ar-H, 3,4-Cl₂Ar-H-2), 7.54 (2H, m, Ar-H), 10.78 (1H, br s, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ 9.6, 26.3, 72.6, 117.1, 118.3, 119.0, 123.8, 126.0, 129.4, 131.6, 132.1, 141.7, 156.9, 158.0, 160.4; EIMS: m/z 397/399 [M⁺], 205, 203, 196, 194, 163, 161, 154, 152, 138, 136, 128, 126, 113, 111, 83, 69.

2-(4-Chlorophenyl)amino-5-(4-methylphenoxy)propyl]-1,3,4-oxadiazole (15w). Yield: 84%; m.p. 129-130°C; IR (ATR, cm⁻¹): νmax 3246, 3063, 2975, 2877, 1608,
1599, 1540, 1493; $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.06 (3H, t, $J = 7.5$ Hz, CH$_2$(CH$_3$)$_2$), 2.11-2.20 (2H, m, OCHCH$_2$CH$_3$), 2.23 (3H, s, Me-ArO), 5.36 (1H, t, $J = 6.9$ Hz, OCHCH$_2$CH$_3$), 6.94 (2H, m, Ar-H), 7.08 (2H, m, Ar-H), 7.38 (2H, m, Ar-H), 7.69 (2H, m, Ar-H), 9.61 (1H, br s, NH); $^{13}$C-NMR (75 MHz, Acetone-$d_6$): $\delta$ 8.9, 19.6, 26.5, 72.6, 115.9, 118.6, 126.5, 128.9, 129.9, 131.0, 137.6, 155.6, 158.8, 160.1; EIMS: m/z 343/345 [M$^{+}$], 238, 236, 191, 152, 128, 126, 107, 91, 83, 65.

2-(4-Fluorophenyl)amino-5-[1-(4bromophenoxy)propyl]-1,3,4-oxadiazole (15x)
Yield: 78%; m.p. 145-146°C; IR (ATR, cm$^{-1}$): $\nu_{max}$ 3303, 3056, 2973, 2873, 1605, 1507, 1485; $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.99 (3H, d, $J = 7.5$ Hz, CH$_2$CH$_3$), 1.95-2.16 (2H, m, OCHCH$_2$CH$_3$), 5.56 (1H, t, $J = 6.9$ Hz, OCHCH$_2$CH$_3$), 7.02 (2H, m, Ar-H), 7.18 (2H, m, Ar-H), 7.44-7.556 (4H, m, Ar-H), 10.69 (1H, s, NH); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 9.7, 26.4, 72.3, 113.8, 115.1 (d, $J_{C,F} = 36.0$ Hz), 118.6, 119.0 (d, $J_{C,F} = 7.5$ Hz), 132.8, 135.3 (d, $J_{C,F} = 2.3$ Hz), 156.8, 157.8 (d, $J_{C,F} = 237.0$ Hz), 158.1, 160.6; EIMS: m/z 391/393 [M$^{+}$], 220, 178, 173, 171, 157, 155, 138, 136, 110, 95, 77.

2-(4-Fluorophenyl)amino-5-[1-(3,4-dichlorophenoxy)propyl]-1,3,4-oxadiazole (15y)
Yield: 76%; m.p. 140-141°C; IR (ATR, cm$^{-1}$): $\nu_{max}$ 3321, 3047, 2963, 2880, 1608, 1512, 1488; $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.99 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_3$), 2.01-2.12 (2H, m, OCHCH$_2$CH$_3$), 5.66 (1H, t, $J = 6.6$ Hz, OCHCH$_2$), 7.08 (1H, dd, $J = 9.0, 3.0$ Hz, 3,4-Cl$_2$Ar-H-6), 7.17 (2H, m, Ar-H), 7.40 (1H, d, $J = 3.0$ Hz, 3,4-Cl$_2$Ar-H-2), 7.51-7.56 (3H, m, Ar-H, 3,4-Cl$_2$Ar-H-5), 10.61 (1H, br s, NH); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): $\delta$ 9.6, 26.3, 72.6, 116.1 (d, $J_{C,F} = 22.5$ Hz), 117.1, 118.3, 119.1 (d, $J_{C,F} = 8.3$ Hz), 124.2, 131.6, 132.2, 135.3 (d, $J_{C,F} = 2.3$ Hz), 157.0, 157.8 (d, $J_{C,F} = 237.0$ Hz), 157.9, 160.7; EIMS: m/z 220, 164, 163, 162, 161, 138, 136, 110, 95, 83, 75.

2-(4-Fluorophenyl)amino-5-[1-(4-methylphenoxy)propyl]-1,3,4-oxadiazole (15z)
Yield: 74%; m.p. 132-134°C; IR (ATR, cm$^{-1}$): $\nu_{max}$ 3311, 2973, 1624, 1575, 1558, 1540, 1489; $^1$H-NMR (300 MHz, DMSO-$d_6$): $\delta$ 0.99 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_3$), 1.95-2.10 (2H, m, OCHCH$_2$CH$_3$), 2.21 (3H, s, Me-ArO), 5.45 (1H, t, $J = 6.9$ Hz, OCHCH$_2$), 6.93 (2H, m, Ar-H), 7.08 (2H, m, Ar-H), 7.17 (2H, m, Ar-H), 7.53 (2H, m,
Ar-H), 10.61 (1H, br s, NH); $^{13}$C-NMR (75 MHz, Acetone-$d_6$): δ 9.8, 20.5, 26.6, 72.3, 116.1 (d, $J_{CF} = 20.3$ Hz), 118.9 (d, $J_{CF} = 7.5$ Hz), 135.4 (d, $J_{CF} = 2.3$ Hz), 128.9, 129.9, 131.0, 137.6, 155.4, 157.8 (d, $J_{CF} = 237.0$), 158.6, 160.5 (C-2); EIMS: m/z 327 [M$^+$], 298, 220, 207, 178, 149, 138, 127, 83, 65.

2-(4-Nitrophenyl)amino-5-[1-(4-bromophenoxy)propyl]-1,3,4-oxadiazole (15a*). Yield: 71%; m.p. 181-182°C; IR (ATR, cm$^{-1}$): ν$_{max}$ 3258, 3062, 2945, 1625, 1581, 1557, 1524, 1486, 1327; $^1$H-NMR (300 MHz, Acetone-$d_6$): δ 1.08 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_3$), 2.10-2.23 (2H, m, OCHCH$_2$CH$_3$), 5.50 (1H, t, $J = 6.6$ Hz, OCHCH$_2$), 7.06 (2H, m, Ar-H), 7.46 (2H, m, Ar-H), 7.88 (2H, m, Ar-H), 8.28 (2H, m, Ar-H), 10.25 (1H, br s, NH); $^{13}$C-NMR (75 MHz, Acetone-$d_6$): δ 8.8, 26.4, 72.6, 113.7, 117.0, 118.0, 125.2, 132.4, 142.1, 144.5, 157.0, 159.0, 159.7; EIMS: m/z 281, 246, 207, 109, 91, 69.

2-(4-Nitrophenyl)amino-5-(4-methylphenoxy)propyl]-1,3,4-oxadiazole (15b*). Yield: 69%; m.p. 170-172°C; IR (ATR, cm$^{-1}$): ν$_{max}$ 3257, 3043, 2935, 1626, 1594, 1575, 1513, 1489, 1345; $^1$H-NMR (300 MHz, DMSO-$d_6$): δ 1.01 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_3$), 2.00-2.14 (2H, m, OCHCH$_2$CH$_3$), 2.21 (3H, s, Me-ArO), 5.50 (1H, t, $J = 6.9$ Hz, OCHCH$_2$), 6.93 (2H, m, Ar-H), 7.07 (2H, m, Ar-H), 7.72 (2H, m, Ar-H), 8.25 (2H, m, Ar-H), 11.41 (1H, br s, NH); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): δ 8.9, 20.5, 26.6, 72.4, 116.3, 117.3, 125.9, 130.4, 131.2, 141.7, 145.1, 155.4, 159.6, 159.9; EIMS: m/z 354 [M$^+$], 207, 190, 163, 149, 137, 107, 91.

2-(4-Methylphenyl)amino-5-[1-(4-bromophenoxy)propyl]-1,3,4-oxadiazole (15c*). Yield: 79%; m.p. 123-124°C; IR (ATR, cm$^{-1}$): ν$_{max}$ 3263, 1622, 1608, 1569, 1485; $^1$H-NMR (300 MHz, DMSO-$d_6$): δ 0.98 (3H, t, $J = 7.5$ Hz, CH$_2$CH$_3$), 2.01-2.08 (2H, m, OCHCH$_2$CH$_3$), 2.23 (3H, s, Me-ArNH), 5.54 (1H, t, $J = 6.9$ Hz, OCHCH$_2$), 7.02 (2H, d, $J = 9.0$ Hz, BrAr-H), 7.12 (2H, d, $J = 8.1$ Hz, MeAr-H), 7.40 (2H, d, $J = 8.1$ Hz, MeAr-H), 7.46 (2H, d, $J = 9.0$ Hz, BrAr-H), 10.46 (1H, br s, NH); $^{13}$C-NMR (300 MHz, Acetone-$d_6$): δ 9.7, 20.8, 26.4, 72.3, 113.8, 117.4, 118.6, 129.9, 131.3, 132.8, 136.4, 156.8, 158.0, 160.7; EIMS: m/z 217, 216, 174, 157, 155, 154, 134, 132, 106, 91, 77, 65.
2-(4-Methylphenyl)amino-5-[1-(3,4-dichlorophenoxy)propyl]-1,3,4-oxadiazole (15d’). Yield: 75%; m.p. 121-122°C IR (ATR, cm⁻¹): νₘₐₓ 3268, 3049, 2921, 2860, 1615, 1586, 1506, 1463; \(^1\)H-NMR (300 MHz, CDCl₃): δ 1.09 (3H, t, J = 7.5 Hz, CH₂CH₃), 1.96-2.26 (2H, m, OCHCH₂CH₂), 2.33 (3H, s, Me-ArNH), 5.23 (1H, t, J = 7.5 Hz, OCHCH₂), 6.91 (1H, dd, J = 9.0, 2.7 Hz, 3,4-Cl₂Ar-Hs), 7.14-7.17 (3H, m, Ar-H), 7.25-7.33 (3H, m, Ar-H), 8.35 (1H, br s, NH); \(^1\)C-NMR (75 MHz, CDCl₃): δ 9.6, 20.8, 26.7, 73.1, 115.3, 117.9, 118.1, 125.4, 129.6, 129.9, 130.9, 133.1, 134.8, 156.4, 158.1, 160.1; EIMS: m/z 377/379 [M⁺], 216, 162, 161, 134, 132, 106, 91, 65.
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Synthesis

of

Pseudodisaccharides
Chapter 1

INTRODUCTION
Introduction

Carbohydrates play an important part in biological functions. They are found in nature in the form of monosaccharides, disaccharides, oligosaccharides and polysaccharides. Two sugar units may be coupled together by formal dehydration resulting in the formation of a disaccharide through an α- or β-glycosidic bond. A glycosidic bond is formed between a glycosyl donor (sugar) and a glycosyl acceptor that may be a molecule of sugar or any aglycon1 (Figure 1).

![Figure 1: Formation of a glycosidic bond](image)

The glycosidic bond is one of the most stable bonds in natural polymers. It is hundred times more stable than phosphodiester bond in DNA² and has an estimated half life of approximately five million years under neutral conditions. Although glycosidic bond is very stable, nature has produced a class of enzymes called glycoside hydrolases which catalyze the hydrolysis of the glycosidic bond by a factor of $10^{17}$ thus hydrolysing it in a very short time³. Two widely accepted mechanisms for glycoside hydrolysis by 1) retaining hydrolases, and 2) inverting hydrolases, are well documented⁴ (Figure 2). Glycosidic hydrolysis proceeds either via oxycarbenium ion formation (Figure 2, a) or a direct double displacement in an $S_N2$ fashion (Figure 2, b).

(a)

(b)
The naturally occurring glycosidases have numerous applications in textile, food and pulp processing, enzyme replacement therapy and as catalyst in oligosaccharide synthesis. The increased hydrolysis of glycosidic bond results into various bacterial and viral infections and some glycosidase inhibitors are already in use or under investigation as therapeutic agents.

The enzymes used to inhibit the hydrolysis of the glycosidic bond are called glycosidase inhibitors. Glycosidase inhibitors mimic the charge in the transition state of the enzymatic hydrolysis. The most common method to mimic this charge is the introduction of the nitrogen atom. The diseases such as diabetes, cancer, HIV, influenza, and various bacterial and viral infections may also be cured by designing specific enzyme inhibitors. Glycosidase inhibitors are seen as targets for future drug development.

Hydrolytically stable sugars are important in the inhibition of enzymes, as these induce specificity for an enzyme and increase binding affinity. Pseudodisaccharides/neodisaccharides are important examples of such potential enzyme inhibitors. The term pseudodisaccharide includes disaccharide-like compounds (Figure 3. I) in which one or both of the monosaccharides are replaced with e.g., carbasugars or inositols. This term is also used to describe non-glycosidically linked disaccharides where two anomeric centres are not involved in the inter-glycosidic linkage (Figure 3. II, III, IV). The later structures are also called neodisaccharides as these are non natural disaccharides that consist of two sugars rather than pseudosugars.
**Figure 3:** a) Disaccharide: acetal-linked (I); b) Pseudodisaccharides: ether-linked (II); amine-linked (III), thioether-linked (IV).

Chemical modification of carbohydrates may give more stable modified structures; glycomimetics. Glycomimetics are molecules that resemble a carbohydrate but they have been modified in some way. The concept of stereochemical mimicry is used in glycomimetics, by modification of a monosaccharide, either substitution with another sugar or a non sugar residue. In glycomimetics, it is proposed that one of the sugars binds in its natural orientation to a carbohydrate binding site in an enzyme or lectin, while the other sugar residue assumes an unnatural orientation. Thus a glycoside mimic is recognized but not hydrolyzed by the glycosidases. Nilsson *et al* has shown that some mannoses can mimic galactose in binding to galectins, the proposed binding mode being supported by molecular modelling. Jenkins *et al* has reported that *N*-substituted 3-aminoaltrose derivatives (V) can act as glucosidase inhibitors, and has proposed the aminosugar mimics the protonated exocyclic oxygen (VI) in the natural substrate.
Valienamine (VII)\textsuperscript{12} and acarbose (VIII)\textsuperscript{13} are important examples of enzyme inhibitors. Valienamine inhibits intestinal \(\alpha\)-glucosidase and sucrase, while acarbose is a well known inhibitor of \(\alpha\)-glucosidase, dextran sucrase, alpha-amylase, glucoamylase and cyclomaltodextrin glucanotransferase.

The synthesis of non natural amine linked disaccharides is not very common in literature, although, \(C_2\) symmetric (6–6) \(N\)-linked neodisaccharides are well documented\textsuperscript{14}. Almost 30 years ago, first example of a sec–sec \(N\)-linked compound appeared in the literature\textsuperscript{15}. Recently, a few reports of unsymmetrically substituted amine pseudodisaccharides appeared. Kroutil \textit{et al} used a method based on aziridine opening by amines for the synthesis of diamino compounds\textsuperscript{16}. Thiem \textit{et al} produced \(pri–sec\) linked pseudodisaccharides by reductive amination\textsuperscript{17}. Thioglycosides (IV, IX) have also been reported as potential enzyme inhibitors or enzyme inhibiting scaffolds\textsuperscript{18a,b}. The selenodisaccharides (X) also inhibit the glycosidases\textsuperscript{19}.

The diversity in nature has been the main source of inspiration towards the synthesis of new carbohydrate structures. Since the isolation of Coyolosa - a hypoglycaemic agent reported to have (6-6) tail-to-tail ether linked disaccharides (XI) structure\textsuperscript{20}, several articles on the synthesis of such a linkage have been published. Either linked disaccharides are not unknown in nature. A \textit{pri–sec} glucose(4-5)ribose ether linked disaccharide (XII) in an exotoxin from \textit{Bacillus Thuringiensis} was isolated\textsuperscript{21} and latter synthesized\textsuperscript{22} by Sorm \textit{et al}.
Sec-sec ether linkage is more difficult to synthesize than pri-sec or pri-pri ether linkage\textsuperscript{24}, it requires an \textit{S}_N\textit{2} reaction at a secondary carbon whereas \textit{S}_N\textit{1} reaction is highly disfavoured\textsuperscript{23}. Reports on the synthesis of pri-pri and pri-sec ether linked disaccharides have also been found in literature\textsuperscript{23}. Kovac \textit{et al} has observed sec-sec ether linked disaccharides as by products\textsuperscript{35} in his tin acetal \textit{\beta}-mannoside-forming alkylations. Paulsen \textit{et al} have also reported the synthesis of various sec-secs ether linked disaccharides\textsuperscript{26}.

In recent years, the synthesis of non-glycosidic pseudodisaccharides has got a great attention. The synthesis of thioether-linked pseudodisaccharides (XIII)\textsuperscript{27} as well as selenoglycosides (XIV)\textsuperscript{28} have recently been reported along with amine-bridged pseudodisaccharides (XV, XVI)\textsuperscript{17}. 
Figure 4: Some di- and trisaccharide mimetics

The role of glycosidase inhibitors in the recognition of important biological processes is gaining importance. The amine and ether bridged disaccharides may be designed to mimic the charge in the transition state of the glycosidase catalyzed hydrolysis of the glycosidic bond. The amine linkage may be stable under the hydrolytic conditions as it will result into the formation of ammonium ion. The ether linkage is also relatively stable under the acidic hydrolysis. The proposed structures may be protonated by the carboxylic acid residue in the enzyme active site but not be hydrolyzed due to the formation of either ammonium or oxonium ions, respectively, in amine or ether linked structures.
Synthetic plan

The glycosidase inhibitors are the main focus for the future drug development. Keeping in view the prime importance of glycosidase inhibitors the present project was designed to synthesize the molecules which may mimic the charge in the transition state of the enzyme hydrolysis. The synthetic plan consists of synthesis of amine and ether linked pseudodisaccharides.

The amine-linked pseudodisaccharides may be synthesized using two approaches. The first approach involves the stereospecific epoxide ring opening and the second one is based on the Mitsunobu chemistry. The pri-sec amine linkage may be established using epoxide ring opening approach and the pri-pri linkage may be established by Mitsunobu reaction.

![Chemical structures](image)

3,6-amine-linkage  2,6-amine-linkage  6,6-amine-linkage

The ether-linked disaccharides may be synthesized by the direct $S_N$2 substitution on 1,2:5,6-di-O-isopropylidene-$\alpha$-D-allofuranose-3-triflate using different secondary alcohols as nucleophiles under basic conditions.

![Chemical structure](image)

sec-sec ether-linkage
Chapter 2

Results

and

Discussion
Results and Discussion

2.1 Synthesis of amine and ether-linked pseudodisaccharides

In order to reach the target amine and ether-linked structures, first of all important precursors *i.e.*, sugar alcohols, amines, sulfonamides and epoxides were synthesized by a multistep sequence (Schemes 1-4).

The primary alcohol (4) (Scheme 1) was synthesized following an already published procedure. Methyl-α-D-glucopyranoside (1) was selectively protected at the primary position taking the advantage of the steric bulk associated with the trityl group, the secondary hydroxyls remained unaffected. The trityl protected tetrol (2) was benzyl protected (3) at the remaining hydroxyl groups with benzyl bromide and the trityl protection selectively removed under acidic conditions to afford the primary alcohol derivative (4). Methyl-2,3,4-tri-O-benzyl-6-azido-α-D-glucopyranoside (5) was synthesized from alcohol (4) via mesitylation, followed by the reaction with sodium azide. The azide (5) was selectively reduced to amine (6) (the *in situ* poisoning of the catalyst prevented the cleavage of the benzyl ethers) using 10% palladium on activated charcoal. The amine (6) was converted to its triflyl (7) and nosyl amide (8) by the reaction with triflic anhydride and 2-nitrobenzenesulfonyl chloride, respectively (Scheme 1).

![Chemical reaction diagram](image)

**Scheme 1**: From methyl-α-D-glucopyranoside to protected 6-sulfonamide
In a similar fashion alcohol (9)\textsuperscript{31} (scheme 2) was converted to azide (10) followed by reduction to amine (11)\textsuperscript{32}.

![Scheme 2: From methyl-2,4-6-tri-O-benzyl-α-D-mannopyranoside to C-6 amine](image)

The C-3 glucose amine (16) was synthesized from 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (12) (scheme 3). 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (12)\textsuperscript{33} was oxidized to its corresponding C-3 ketone (13) by pyridinium dichromate in the presence of 4Å molecular sieves. The C-3 ketone (13) was reduced to the corresponding C-3 alcohol-1,2:5,6-di-O-isopropylidene-α-D-allofuranose (14)\textsuperscript{34}; sodium borohydride (NaBH₄) reduction yielded excellent stereoselectivity. The C-3 allose alcohol (14) was converted to C-3 glucose azide (15), by an S\textsubscript{N}2 reaction on allose triflate, and was subsequently reduced to the corresponding C-3 amine (16)\textsuperscript{34} with glucose configuration, by the same method adopted for C-6 amines (6,11).

![Scheme 3: Synthesis of glucose-3-amine](image)

The alcohol (22)\textsuperscript{35} (scheme 4) was synthesized starting from methyl-α-D-
mannopyranoside (17) following a series of reactions. Monobenzylidene derivative, methyl-4,6-di-O-benzylidene-α-D-mannopyranoside (18) was synthesized by the reaction of benzaldehyde dimethylacetal and methyl-α-D-mannopyranoside\(^{36}\). Methyl-4,6-di-O-benzylidene-α-D-mannopyranoside (18) was converted to methyl-3-O-benzyl-4,6-di-O-benzylidene-α-D-mannopyranoside (19)\(^{36}\) and methyl-2-O-benzyl-4,6-di-O-benzylidene-α-D-mannopyranoside (20)\(^{36}\) using Garegg’s phase transfer conditions\(^{37}\) in 20 and 62% yields, respectively. The monobenzylated alcohol (20) was oxidized to the corresponding C-3 ketone (21) by pyridinium dichromate in the presence of 4Å molecular sieves. The C-3 ketone (21) was subjected to reduction\(^{38}\). Two products methyl-2-O-benzyl-4,6-di-O-benzylidene-α-D-altropyranoside (22) and methyl-2-O-benzyl-4,6-di-O-benzylidene-α-D-mannopyranoside (20) were isolated in 52 and 20% yields, respectively.

![Scheme 4: Synthesis of secondary alcohols starting from mannose](image)

2.1.1 Synthesis of amine-linked pseudodisaccharides

For the creation of amine-linkage, two different approaches were employed. The first approach was the stereospecific epoxide ring opening, leading to trans diaxial disaccharides while the second approach involved the Mitsunobu coupling reaction.

2.1.1.1 Epoxide opening approach

The epoxide opening reaction was selected because of its predictable stereochemical outcome. The epoxides (23) and (24) with manno and allo configuration,
respectively, were synthesized by already published methods\(^{39}\).

![Structural formulas of compounds 23 and 24](image)

Reaction of *manno* epoxide (23) with C-6 amines (6 and 11) in the presence of LiClO\(_4\), in refluxing acetonitrile gave the *alto* configured *N*-linked pseudodisaccharides with (3-6) connectivity (25,26). In a similar manner, the reaction of *allo* epoxide (24) led to the formation of (2-6) *N*-linked pseudodisaccharides (27,28). The *trans* dixial ring opening\(^{40}\) of conformationally locked epoxides led to excellent regioselectivity in both cases.

\[
\begin{align*}
23 + 6 & \xrightarrow{(i)} 25 \\
23 + 11 & \xrightarrow{(i)} 26 \\
24 + 6 & \xrightarrow{(i)} 27 \\
24 + 11 & \xrightarrow{(i)} 28
\end{align*}
\]

(25: 84h, 79% \(84\text{h}, 79\%\), 17% epoxide recovered)

Reagents and conditions: (i) Reactions carried out with epoxide 23 or 24 (1 eq), amine 6 or 11 (1.1 eq), LiClO\(_4\) (2 eq) in refluxing acetonitrile

**Scheme 5: Epoxide opening reaction**
The structures of the disaccharides (25-28) were established on the basis of NMR and high resolution mass spectrometry. The assignment of the rings (i.e., glucopyranoside or mannopyranoside) was made on the basis of the coupling constants of anomeric protons with H-2. The anomeric proton in glucose has axial-equatorial coupling constant larger than the corresponding equatorial-equatorial coupling in mannose. The 2-6 or 3-6 connectivity was established on the basis of the shielding caused by nitrogen to the proton and carbon at position 2 or 3, respectively. The exact assignments of protons and carbons were made by the two dimensional NMR spectroscopy. The calculated values of HRMS were found in good agreement with the experimental results.

2.1.1.2 Mitsunobu approach

This approach was used to prepare pri-pri N-linked pseudodisaccharides (scheme 6), both $C_2$-symmetric and unsymmetric products were obtained. The sulphonamide (7,8) was reacted with the primary alcohol (4,9) under standard Mitsunobu$^{41}$ conditions (DIAD or DEAD and TPP in THF). It was found that cooling to 0°C was necessary as attempting the reaction between alcohol (4) and sulphonamide (7) failed to give the Mitsunobu product at room temperature.

The coupling was confirmed by NMR and high resolution mass spectrometry (HRMS). The disappearance of NH signal indicated that the coupling reaction has taken place. The coupling of two glucose units was easy to confirm in case of 2-nitrobenzene sulphonamide (nosylamide). The integration of protons along with the HRMS confirmed the coupling. The sugar protons exhibited double integration when compared to the integration of the aromatic protons belonging to nosyl moiety. Where as, in case of trifluoromethane sulphonamide the disappearance of the signal for NH proton indicated the formation of the disaccharide, while the HRMS exhibited molecular ion peak corresponding to molecular weight of the dimerized product. The coupling of glucose and mannose was confirmed by the NMR spectral data. The ring assignment was made on the basis of $^1\text{H}$-$^1\text{H}$ coupling constants as done in epoxide ring opening products. The calculated HRMS values for disaccharides were found in very good agreement with the experimentally observed values.
Reagents and conditions: (i) DIAD (2+2 eq), Ph$_3$P (2+2 eq), THF, 0 °C→RT, 7 h; (ii) DIAD (2+0.5 eq), Ph$_3$P (2+0.5 eq), THF, 0 °C→RT, 26 h; (iii) DIAD (3 eq), Ph$_3$P (3 eq), THF, 0 °C→RT, 3 h; (iv) DEAD (2+2 eq), Ph$_3$P (2+2 eq), THF, 0 °C→RT, 48 h.

Scheme 6: Mitsunobu coupling reaction

The attempts to establish *pri-sec* or *sec-sec* linkage using Mitsunobu reaction and *sec-sec* linkage using epoxide opening reaction were unsuccessfull. Reaction between triflamide (7) and C-3 alcohol (14) [DIAD (2+1 eq), PPh$_3$ (2+1 eq), 24h], did not result into any detectable amount of the product and only starting materials were recovered. This result was probably an outcome of steric bulk associated with the secondary alcohol. The reaction was repeated in the presence of methanol but still no \(N\)-linked pseudodisaccharide was detectable, instead only \(N\)-methylated product 33 (99% yield) was isolated.
Similarly, reactions between amine (16) and epoxides (23) and (24) were also unsuccessful and only unreacted starting materials were seen on TLC. It seems that again steric crowding is mainly responsible for the failure of the sec-sec N-linked pseudodisaccharide formation.

2.1.1.3 Deprotection of disaccharides

All disaccharides (25-32) were successfully deprotected (scheme 7) in one step (i.e., removal of benzyl ethers, benzylidene acetics and sulfonamide protection) using dissolving metal\textsuperscript{42} conditions to give the free pseudodisaccharides as their bis-methyl glycosides (34-39) (Scheme 3). Sodium metal was first added to the liquid ammonia followed by slow addition of the sugar solution in THF. Due to low solubility of protected disaccharides at −78 °C in ammonia/THF mixtures, the alternate procedure in which ammonia was condensed into a THF solution of disaccharide and then sodium added was not found successful. In the later procedure, some starting material was often recovered from the reaction mixture along with the product but no partially deprotected species were observed in any of the reactions.

The fully protected altrose derivatives were found to be in \textsuperscript{4}C\textsubscript{1} chair predominantly, as judged by the $^3J$ coupling constants in $^1$H-NMR [e.g., for a (3–6) N-linked compound 25: $J_{1,2} < 1$ Hz; $J_{2,3} = 1.4$ Hz; $J_{3,4} = 4.1$ Hz; $J_{4,5} = 9.8$ Hz; and for a (2–6) N-linked compound 27: $J_{1,2} < 1$ Hz; $J_{2,3} = 2.8$ Hz; $J_{3,4} = 2.8$ Hz; $J_{4,5} = 9.6$ Hz]. When the conformationally rigid benzylidene protection is removed, however, both the 3-substituted and the 2-substituted altrose residues lose this conformational rigidity in solution, either adopting a single or multiple conformations, as indicated by the $^1$H-NMR coupling constants [e.g., for a (3–6) N-linked compound 35: $J_{1,2} = 3.3$ Hz; $J_{2,3} = 5.7$ Hz; $J_{3,4} = 4.7$ Hz and $J_{4,5} = 7.8$ Hz; and for a (2–6) N-linked compound 37: $J_{1,2} = 4.6$ Hz; $J_{2,3} = 8.2$ Hz and $J_{3,4} = 3.6$ Hz].

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Scheme 7: Deprotection of disaccharides: (i) Na, NH₃ (g), THF, -78 °C
2.1.2 Synthesis of sec-sec ether-linked pseudodisaccharides

In an attempt towards the synthesis of (3–6) amine-linked disaccharide; the reaction of sulfonamide (8), triflate (39) and sodium hydride in DMF (Scheme 8) resulted in the formation of a new product. The sulfonamide (8) was recovered completely unreacted. The $^1$H-NMR spectrum of the resulting product exhibited eight singlets between 1.3-1.6 ppm integrating for twenty four protons i.e., eight methyl protons consistent with two bis-isopropylidene protected sugar moieties; doublets at 5.79 and 5.87 ppm for two anomic protons; a doublet at 4.70 ppm was assigned to H-2 ($J_{1,2} = 3.5$ Hz), with no coupling to H-3, consistent with a gluco configuration. The H-2 of the second ring was observed as a triplet ($J = 4.2$ Hz) at 4.64 ppm as expected for an allo configuration. The mass spectrum revealed a peak at $m/z$ 525, consistent with M+Na$^+$ for M=502. This data is consistent with a gluco-allo (3-3) ether-linked structure (40), formed in 48% yield based on dimerization of the triflate (39).

The triflate (39) was treated with sodium hydride and DMF (Scheme 8) in the absence of sulphonamide. Once again, gluco-allo (3-3) ether linked product (40) was formed as the major product (52%), but this time a second product was also isolated (8%). The $^1$H and $^{13}$C-NMR spectra of this compound exhibited signals consistent with only the diisopropylidene protected monosaccharide, but its mass spectrum showed a peak at $m/z$ 525, consistent with M+Na$^+$ for M=502. In $^1$H-NMR, H-2 appeared as a doublet at 4.65 ppm ($J_{1,2} = 3.6$ Hz) consistent with the gluco configuration. Based on the spectral information, the compound was assigned the $C_2$-symmetric gluco-gluco (3-3) ether-linked dimeric structure (41).

Scheme 8: Formation of sec-sec ether linkage: (i) 39, NaH, DMF, RT; 4, 48%; (ii) NaH, DMF, RT; 40, 52%; 41, 8%; (iii) NaOH, DMF, RT; 40, 51%; 41, 14%.
The formation of the products (40) and (41) can be explained on the basis of partial hydrolysis of the triflate by the presence of trace hydroxide. The hydroxide attack on the triflate sulphur may give the allo alcoholate, which then attacks another molecule of triflate (39) in an SN2 fashion and inversion results in dimeric unsymmetrical (3-3) ether-linked gluco-allo disaccharide (40). In an alternative reaction, the hydroxide may attack C-3 of triflate (39) and SN2 substitution may result into alcohol with gluco configuration, which in turn deprotonates to alcoholate and attacks another triflate to give C2-symmetric gluco-gluco (3-3) ether-linked disaccharide (41), formed as a result of double inversion. In order to suppress the reaction of hydroxide with triflate, the reaction was carried out under completely anhydrous conditions, but the formation of both products was always observed. The reaction was also carried out with sodium hydroxide rather than sodium hydride. Once again, both 40 (51%) and 41 (14%) were formed although the reaction took a longer time.

Thus, various secondary carbohydrate alcohols 10, 12, 19, 20, 22 and 42 were treated with allo triflate (39) and NaH in DMF, and ether-linked disaccharides 40, 41, 43-46 were formed as the major products in all cases, although small amounts of the unsymmetrical 40 and symmetrical 41 dimers were often seen, especially when large excess of triflate (39) was used (see Table ).
**Table:** Etherification reactions; all reactions carried out in DMF. \(^a\) isolated yields based on alcohols. \(^b\) it is possible that small quantities of 40 and 41 derived solely from triflate 39 artificially inflate the yields in these cases. For entry 1, 0.28 eq triflate 39 was recovered. For entry 2, 0.05 eq triflate 39 was recovered, along with disaccharide 41 accounting for 0.33 eq triflate 39.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Alcohol (1 eq)</th>
<th>Eq Triflate 39</th>
<th>Product</th>
<th>Yield (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Image" /></td>
<td>1.5</td>
<td><img src="image2.png" alt="Image" /></td>
<td>89% (^b)</td>
</tr>
<tr>
<td>2</td>
<td><img src="image3.png" alt="Image" /></td>
<td>1.5</td>
<td><img src="image4.png" alt="Image" /></td>
<td>63% (^b)</td>
</tr>
<tr>
<td>3</td>
<td><img src="image5.png" alt="Image" /></td>
<td>3.3</td>
<td><img src="image6.png" alt="Image" /></td>
<td>78%</td>
</tr>
<tr>
<td>4</td>
<td><img src="image7.png" alt="Image" /></td>
<td>2.5</td>
<td><img src="image8.png" alt="Image" /></td>
<td>95%</td>
</tr>
<tr>
<td>5</td>
<td><img src="image9.png" alt="Image" /></td>
<td>2.0</td>
<td><img src="image10.png" alt="Image" /></td>
<td>64%</td>
</tr>
<tr>
<td>6</td>
<td><img src="image11.png" alt="Image" /></td>
<td>2.0</td>
<td><img src="image12.png" alt="Image" /></td>
<td>34%</td>
</tr>
</tbody>
</table>
$^1$H-NMR coupling constants indicated that the furanose rings (labelled II in 40, 43-46) all had the gluco configuration. The configuration of the ether-linked disaccharides derived from the alcohols 19, 20, 22, 42, suggests that the reaction proceeds through a straightforward $S_N2$ mechanism, involving inversive of configuration at the C-3 of alloste triflate (39).

The attempted recrystallization on the ether-linked pseudodisaccharides resulted in the formation of needle like crystals of gluco-allo (40) disaccharide suitable for recording X-ray diffractions. This resolved structure of 3-O-(1,2:5,6-di-O-isopropylidene-3-deoxy-α-D-allofuranos-3-yl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (40) in an unambiguous manner.

**Figure 5:** X-ray structure of 40 as thermal ellipsoids drawn at 50% probability and showing crystallographic atom numbering scheme. **Selected dihedral angles (°):** C3$_{II}$-O3$_{II}$-C3$_{II'}$-C4$_{II}$, $-166.2$; C3$_{II'}$-O3$_{II'}$-C3$_{II'}$-C2$_{II}$, $+78.3$; C3$_{II}$-O3$_{II}$-C3$_{II}$-C2$_{II}$, $-99.7$; C3$_{II}$-O3$_{II}$-C3$_{II}$-C4$_{II}$, $+149.7$. **Bond angle (°):** C3$_{II}$-O3$_{II}$-C3$_{II}$, $+114.3$. **Bond lengths (Å):** O3$_{II}$-C3$_{II}$, 1.412; O3$_{II}$C3$_{II}$, 1.434.
Conclusions

Two new approaches for the synthesis of amine-linked neodisaccharides, i.e., sulfonamide-based Mitsunobu chemistry and opening of epoxides by carbohydrate amines were investigated. These two approaches were successfully utilized in the formation of some pri-pri and pri-sec N-linked pseudodisaccharides.

It was also discovered that allo triflate (39) provides a good electrophilic centre for S_N2 reactions with secondary alkoxide nucleophiles, including furanoses, axial and equatorial pyranose hydroxyls and a carbasugar, under standard alkylation conditions. This has been successfully applied to the synthesis of various sec-sec carbohydrate ether-linked disaccharides. However, attempts to synthesize sec-sec amine-linkage by epoxide opening and pri-sec or sec-sec amine-linkage by Mitsunobu reaction were unsuccessful. It was observed that probably the steric factors are responsible for this.
Chapter 3

EXPERIMENTAL
Experimental

3.1 Materials and Methods

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance II 400 or 500 MHz spectrometers; multiplicities are quoted as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), apparent triplet (at) or apparent triplet of doublets (atd). Multiplicities assigned using DEPT spectra. Signals were assigned using COSY and HSQC experiments. All chemical shifts are quoted on the δ-scale in parts per million (ppm). Residual solvent signals were used as an internal reference. Low- and high-resolution electrospray (ES) mass spectra were recorded using a Bruker Microtof instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm; concentrations are given in g/100 mL. Thin layer chromatography (TLC) was carried out on Merck Kieselgel sheets, pre-coated with 60F_254 silica. Plates were visualised using UV light and developed using 10% sulfuric acid, or an ammonium molybdate (10% w/v) and cerium (IV) sulfate (2% w/v) solution in 10% sulfuric acid. Flash column chromatography was carried out on silica gel (35–70 micron, Grace). DMF was distilled or bought anhydrous from Aldrich, THF and DCM were dried before use. Reactions performed under an atmosphere of nitrogen were maintained by an inflated balloon.

3.2 Synthesis of methyl 6-O-trityl-α-D-glucopyranoside (2)

Triphenylmethylchloride (53.74 g, 193.3 mmol) and 4-dimethylaminopyridine (DMAP) (750 mg) were added to a stirred solution of methyl α-D-glucopyranoside (1) (25 g, 128.8 mmol) in pyridine (200 mL) and the reaction mixture stirred at 80°C. After two and a half hour, TLC (ethyl acetate/methanol, 9:1) indicated complete conversion of the starting material (R_f = 0.1) to a single product (R_f = 0.5). The reaction mixture was diluted with distilled water (5 mL) and concentrated in vacuo. The resulting syrup was dissolved in dichloromethane (800 mL) and washed with a saturated ammonium chloride solution (2 x 200 mL). The organic phase was dried (MgSO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (pentane/ethyl acetate - DCM) to give pure product (31g,
55%). $^1$H-NMR (400 MHz, CDCl$_3$): δ 2.11 (1H, d, $J = 9.4$ Hz, OH), 2.53 (1H, d, $J =$ 2.7 Hz, OH), 2.65 (1H, d, $J_{OH,4} =$ 2.2 Hz), 3.43 (3H, s, OCH$_3$), 3.38-3.43 (3H, m, H-5, H-6, H-6'), 3.50-3.56 (2H, m, H-2, H-3), 3.67 (1H, m, H-4), 4.77 (1H, d, $J_{1,2} =$ 3.8, H-1), 7.22-7.47 (15H, m, Ar-H).

3.3 Synthesis of methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (4)

Benzyl bromide (5.1 ml, 42.7 mmol) was added to a stirred solution of the triol (2) (6.2 g, 14.2 mmol) in dimethyl formamide (40 mL). The reaction mixture was cooled to 0°C and sodium hydride (2.1 g, 88.2 mmol) was added portion-wise. The reaction mixture turned into a brownish solution. The mixture was allowed to warm to room temperature. After 20 hr, TLC (pentane/ethyl acetate, 6:1) indicated complete conversion of starting material into the product ($R_f = 0.4$). The reaction mixture was quenched with methanol (15 mL) and most of the solvent evaporated in vacuo. The mixture was diluted with DCM (250 mL) and washed with distilled water (2 x 50 mL). The organic phase was dried (MgSO$_4$), filtered and concentrated in vacuo. The resulting brownish oil was purified by dry flash chromatography (pentane - pentane/ethyl acetate, 6:1) to afford the trity ether (3).

Trityl ether (3) (10.0 g) was stirred in ethanol:acetic acid mixture (1:1, 100 mL) at 80°C. After 41 hr, TLC (pentane/ethyl acetate, 4:1) revealed the presence of starting material then 1 M HCl was added (10 mL). After 4 hr, TLC indicated complete conversion into the product ($R_f = 0.3$). The reaction mixture was diluted with water and extracted with ethyl acetate (2 x 500 mL). The combined organic phases dried (MgSO$_4$) and concentrated in vacuo. The resulting residue was purified by flash column chromatography (toluene/ethyl acetate, 8:2) to give the deprotected alcohol (53 % over two steps). $^1$H-NMR (400 MHz, CDCl$_3$): δ 1.61 (1H, dd, $J = 5.3$ Hz, $J =$ 7.2 Hz, OH), 3.36 (3H, s, OCH$_3$), 3.49 (1H, dd, $J_{1,2} =$ 3.6 Hz, $J_{2,3} =$ 9.7 Hz, H-2), 3.52 (1H, dd, $J =$ 9.6 Hz, $J =$ 9.0 Hz, H-4), 3.62-3.69 (3H, m, H-5, H-6, H-6'), 4.00 (1H, at, $J =$ 9.2 Hz, H-3), 4.56 (1H, d, H-1), 4.63, 4.88 (2H, ABq, $J = 11.0$ Hz, Ph-CH$_2$), 4.66, 4.80 (2H, ABq, $J = 12.2$ Hz, Ph-CH$_2$), 4.83, 4.99 (2H, ABq, $J_{AB} =$ 10.9 Hz, Ph-CH$_2$), 7.25-7.37 (15H, m, Ar-H).
3.4 Synthesis of 1,2:5,6-di-\(O\)-isopropylidene-3-oxo-\(\alpha\)-D-glucofuranose (13)

2,3:5,6-Di-\(O\)-isopropylidene glucofuranose (12) (5.0 g, 19.08 mmol) was dissolved in dichloromethane (150 mL) under nitrogen. Molecular sieves (4Å) (21.0 g) and pyridinium dichromate (PDC) (35.9 g, 95.4 mmol) were added and the mixture stirred at room temperature overnight. After 20 hr, TLC (pentane/ethyl acetate, 3:1) indicated conversion of the reactant (\(R_f = 0.23\)) into the product (\(R_f = 0.18\)). The reaction mixture was passed through a column (dichloromethane/ether, 25:1) to remove the black material from the product (4.3 g, 89%). \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.33, 1.43, 1.45 (3 x s, 12H, 2 x C(CH\(_3\))\(_2\)), 4.03-4.01 (2H, m, H-5, H-6), 4.33-4.39 (3H, m, H-2, H-4, H-6’), 6.13 (1H, d, \(J_{1,2} = 4.5\) Hz, H-1).

3.5 Synthesis of 1,2:5,6-di-\(O\)-isopropylidene-\(\alpha\)-D-allofuranose (14)

1,2:5,6-Di-\(O\)-isopropylidene-3-oxoglucofuranose (4.3 g, 16.6 mmol) was dissolved in tetrahydrofuran (THF) (115 mL) and methanol (15 mL) with stirring under an atmosphere of nitrogen and cooled to -15 °C. Sodium borohydride (NaBH\(_4\)) (1.68 g, 44.21 mmol) was added and stirring continued. After 3 h, TLC (pentane/ethyl acetate, 3:1) indicated conversion into one major product (\(R_f = 0.1\)). The reaction mixture was added to a mixture of ammonium chloride (NH\(_4\)Cl) and ice (100 mL), extracted with ethyl acetate (2 x 100 mL), dried (Na\(_2\)SO\(_4\)) and concentrated in vacuo. The crude product (3.8 g, 87%) was pure. \(^1\)H-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.37, 1.38, 1.46, 1.58 (12H, 4 x s, 4 x CH\(_3\)), 1.24 (1H, d, \(J_{OH,3} = 6.0\) Hz, OH), 3.81 (1H, dd, \(J_{5,6} = 4.7\) Hz, \(J_{5,6'} = 8.5\) Hz, H-6), 4.10-3.99 (3H, m, H-4, H-5, H-6’), 4.31 (1H, dat, \(J = 4.7\) Hz, \(J_{6,5} = 5.2\) Hz, H-2), 5.81 (1H, d, H-1).

3.6 General procedure for the synthesis of methyl 6-azido-2,3,4-tri-\(O\)-benzyl-6-deoxy-\(\alpha\)-D-gluco/mannopyranoside (5, 10)

Methyl 2,3,4-tri-\(O\)-benzyl-\(\alpha\)-D-gluco/mannopyranoside (1.0 eq) was dissolved in dichloromethane (15 mL) under nitrogen and cooled to 0°C. Methanesulfonyl chloride (MsCl) (1.1 eq) and triethyl amine (TEA) (1.5 eq) were added. The reaction
mixture turned into a thick suspension. After two and a half hour, TLC (pet. ether/ethyl acetate, 1:1) indicated complete conversion of the starting material. The reaction mixture was poured into ammonium chloride and ice (100 mL), extracted with ethyl acetate (2 x 75 mL), dried (MgSO₄) and concentrated in vacuo. The crude product was pure enough to be used in the next step.

Crude methansulfonyl derivative was dissolved in dimethyl formamide (DMF) (80 mL) under nitrogen, and sodium azide (NaN₃) (3.0 eq) was added. The reaction mixture was stirred at 60 °C overnight. After 16 h, TLC (pet. ether/ethyl acetate, 3:1) indicated complete conversion of the starting material into one major product. The reaction mixture was concentrated on a rotary to remove most of the solvent, diluted with dichloromethane (DCM) (200 mL) and washed with brine (2 x 50 mL). The organic phase was dried (MgSO₄) and concentrated in vacuo. The residue was purified by flash column chromatography (pet. ether/ethyl acetate, 10:1) to give the 6-azido sugar.

**Methyl 6-azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranoside (5).** Yield: 92 % over two steps; Rf: 0.6 (pet. ether/ethyl acetate, 3:1); $^1$H-NMR (400 MHz, CDCl₃): δ 3.33 (1H, dd, J₅,₆ = 5.7, J₆,₆' = 3.1 Hz, H-6), 3.40 (3H, s, OCH₃), 3.39-3.46 (2H, m, H-4, H-6'), 3.54 (1H, dd, J₄,₂ = 3.6, J₂,₃ = 9.6 Hz, H-2), 3.78 (1H, ddd, J₅,₆ = 2.4 Hz, J₄,₅ = 8.1 Hz, H-5), 3.99 (1H, at, J = 9.2 Hz, H-3), 4.58, 4.90 (2H, ABq, $J_{AB} = 11.0$ Hz, Ph-CH₂), 4.62 (1H, d, H-1), 4.67, 4.80 (2H, ABq, $J = 11.7$ Hz, Ph-CH₂), 4.82, 5.00 (2H, ABq, $J_{AB} = 10.9$ Hz, Ph-CH₂), 7.24-7.49 (15H, m, Ar-H).

**Methyl 6-azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-mannopyranoside (10).** Yield: 75% (over two steps); Rf 0.9 (pentane/ethyl acetate; 3:1); $^1$H-NMR (400 MHz, CDCl₃): δ 3.34 (3H, s, OCH₃), 3.41 (1H, dd, J₅,₆ = 6.4 Hz, J₆,₆' = 12.9 Hz, H-6), 3.46 (1H, dd, J₅,₆' = 2.4 Hz, H-6'), 3.74 (1H, m, H-5), 3.79-3.87 (3H, m, H-2, H-3, H-4), 4.60, 4.95 (2H, ABq, $J_{AB} = 11.0$ Hz, Ph-CH₂), 4.61 (2H, s, Ph-CH₂), 4.70, 4.76 (2H, ABq, $J_{AB} = 12.5$ Hz, Ph-CH₂), 4.73 (1H, s, H-1), 7.26-7.39 (15H, m, Ar-H).
3.7 General procedure for the synthesis of methyl 6-amino-2,3,4-tri-O-benzyl-α-D-gluco/mannopyranoside (6, 11)

Methyl 6-azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco/mannopyranoside (1.0 eq) was dissolved in ethyl acetate (5 mL) and required amount of 10 % Pd on charcoal (5% by weight) was added. Evacuated the air from the reaction flask and flowed in argon to create the inert atmosphere. Passed hydrogen (H₂) and again evacuated the flask, repeated this four times and then left stirring over night in hydrogen (H₂) atmosphere. After 16 h, TLC (DCM/MeOH, 15:1) showed the formation of one major product (Rf 0.3). The mixture was filtered through celite and concentrated in vacuo. The crude product was purified by flash column chromatography (DCM/MeOH, 15:1).

Methyl 6-amino-2,3,4-tri-O-benzyl-α-D-glucopyranoside (6). Yield: 90 %; 1H-NMR (400 MHz, CDCl₃): δ 2.01 (2H, br s, NH₂), 2.73 (1H, br s, H-6), 3.00 (1H, ad, J = 13.2, H-6'), 3.34 (1H, at, J = 9.7 Hz, H-4), 3.38 (3H, s, OCH₃), 3.50 (1H, dd, J₁₂ = 9.6 Hz, J₂₃ = 3.6 Hz, H-2), 3.59 (1H, ddd, J = 2.7, 6.7 Hz, J₄₅ = 9.5 Hz, H-5), 4.00 (1H, at, J = 9.2 Hz, H-3), 4.57 (1H, d, H-1), 4.62, 4.88 (2H, ABq, JAB = 11.1 Hz, Ph-CH₂), 4.67, 4.79 (2H, ABq, JAB = 12.1 Hz, Ph-CH₂), 4.82, 5.00 (2H, ABq, JAB = 10.9 Hz), 7.26-7.38 (15H, m, Ar-H).

Methyl 6-amino-2,3,4-tri-O-benzyl-α-D-mannopyranoside (11). Yield 87 %; 1H-NMR (400 MHz, CDCl₃): δ 1.85 (2H, br s, NH₂), 2.84 (1H, dd, J₅₆ = 7.0 Hz, J₆₆' = 13.2 Hz, H-6), 3.05 (1H, dd, J₅₆ = 2.3 Hz, H-6'), 3.31 (3H, s, OCH₃), 3.52 (1H, ddd, J₄₅ = 9.7 Hz, H-5), 3.77 (1H, at, J = 9.6 Hz, H-4), 3.80 (1H, m, H-2), 3.89 (1H, dd, J₂₃ = 3.0 Hz, J₃₄ = 9.3 Hz, H-3), 4.62 (2H, s, Ph-CH₂), 4.63, 4.94 (2H, ABq, JAB = 11.0 Hz, Ph-CH₂), 4.70 (1H, d J₁₂ = 1.8 Hz, H-1), 4.71, 4.79 (2H, ABq, JAB = 12.3 Hz, Ph-CH₂), 4.82, 5.00 (2H, ABq, JAB = 10.9 Hz), 7.26-7.38 (15H, m, Ar-H).

3.8 Synthesis of methyl 6-amino-2,3,4-tri-O-benzyl-6-deoxy-6-N-(trifluoromethanesulfonyl)-α-D-glucopyranoside(7)

Methyl 6-amino-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranoside (340 mg, 0.73 mmol) was dissolved in dichloromethane (3 mL), triethylamine (0.19 mL, 1.47
mmol) was added under an atmosphere of nitrogen and the reaction mixture was cooled with stirring below 0°C (sodium chloride + ice). Triflic anhydride (121 µL, 0.73 mmol) dissolved in dichloromethane (2 mL) was added slowly over 50 min. After 6 h, TLC (DCM/MeOH; 50:1) revealed the formation of one major product (Rf 0.4). The reaction mixture was poured into ice water (100 mL) and extracted with dichloromethane (3 x 75 mL). The combined organic layers were dried (MgSO4) and concentrated in vacuo. The crude product was purified by flash column chromatography (DCM/MeOH; 60:1) to afford the pure sulfonyl derivative (280 mg, 64 %); [α]D25 +51.6 (c, 1.0 in CHCl3); 1H-NMR (400 MHz, CDCl3): δ 3.30 (1H, dd, J5,6 = 6.1 Hz, J6,6' = 13.2 Hz, H-6), 3.36 (1H, dd, J3,4 = 8.8 Hz, J4,5 = 9.8 Hz, H-4), 3.37 (3H, s, OCH3), 3.45 (1H, m, H-6'), 3.46 (1H, dd, J1,2 = 3.5 Hz, J2,2' = 9.7 Hz, H-2), 3.72 (1H, ddd, J3,6' = 3.5 Hz, H-5), 4.02 (1H, dd, H-3), 4.52 (1H, d, H-1), 4.65, 4.92 (2H, ABq, JAB = 10.8 Hz, Ph-CH2), 4.66, 4.81 (2H, ABq, JAB = 12.1 Hz, Ph-CH2), 4.83, 5.02 (2H, ABq, JAB = 10.8 Hz, Ph-CH2), 5.03 (1H, m, NH), 7.28-7.39 (15H, m, Ar-H); 13C-NMR (400 MHz, CDCl3): δ 44.8 (t, C-6), 55.5 (q, OCH3), 68.9 (d, C-5), 73.6, 75.0, 75.8 (3 x t, 3 x Ph-CH2) 77.6 (d, C-4), 79.8 (d, C-2), 81.7 (d, C-3), 98.2 (d, C-1), 127.9, 128.1, 128.2, 128.3, 128.5, 128.6, 128.7, 128.7 (8 x d, 9 x Ar-CH) 137.7, 138.0, 138.5 (3 x s, 3 x Ar-C); m/z (ES+) 618 (M+Na+, 100%); HRMS: Caled. for C29H32F3NO7SNa (MNa+) 618.1744; Found 618.1738.

3.9 Synthesis of methyl 6-amino-2,3,4-tri-O-benzyl-6-deoxy-N-(2-nitrobenzenesulfonyl)-α-D-glucopyranoside (8)

Methyl 6-amino-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranoside (766 mg, 1.65 mmol) was dissolved in dichloromethane (25 mL), and triethylamine (260 µL, 2.48 mmol), 2-nitrobenzenesulfonyl chloride (440mg, 1.98 mmol) and then 4-N,N-dimethylamino pyridine (DMAP) (20 mg, 0.16 mmol) were added under nitrogen atmosphere at room temperature. After 3 h, TLC (pet. ether/ethyl acetate; 2:1) indicated the formation of one major product (Rf 0.3). The reaction mixture was poured into ice water (100 mL) and extracted with dichloromethane (3x75 mL). The combined organic layers were dried (MgSO4) and concentrated in vacuo. The crude product was purified by flash column chromatography (pet. ether/ethyl acetate; 4:1-3:1-2:1) yielding pure sulfonyl derivative (1.04 g, 97 %). [α]D25 +61.4 (c, 1.0 in
CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ 3.18 (1H, adt, J = 6.0 Hz, J₉,₆' = 12.8 Hz, H-6), 3.26 (3H, s, OCH₃), 3.31 (1H, ddd, J₆,₅ = 3.0 Hz, J₅,₄NH = 6.0 Hz, H-6'), 3.38 (1H, dd, J₁,₄ = 9.0 Hz, J₄,₅ = 9.8 Hz, H-4), 3.41 (1H, dd, J₁,₂ = 3.6, J₂,₃ = 9.7 Hz, H-2), 3.66 (ddd, 1H, J₃,₅ = 5.7 Hz, H-5), 3.94 (1H, at, J = 9.2 Hz, H-3), 4.42 (1H, d, H-1), 4.62, 4.77 (2H, ABq, JAB = 12.1 Hz, Ph-CH₂), 4.63, 4.89 (2H, ABq, JAB = 10.9 Hz, Ph-CH₂), 4.81, 4.97 (2H, ABq, JAB = 10.9 Hz, Ph-CH₂), 5.49 (1H, at, J = 6.2 Hz, NH), 7.25-7.35 (15H, m, Ar-H), 7.65-7.70 (2H, m, Ar-H), 7.80 (1H, m, Ar-H), 8.04 (1H, m, Ar-H); ¹³C-NMR (100 MHz, CDCl₃): δ 44.4 (t, C-6), 55.5 (q, OCH₃), 69.1 (d, C-5), 73.6, 75.2, 75.9 (3 x t, 3 x Ph-CH₂), 78.0 (d, C-4), 79.9 (d, C-2), 81.8 (d, C-3), 98.2 (d, C-1), 125.4, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.6, 128.7, 131.3, 132.8, 133.6 (12 x d, 13 x Ar-CH), 133.8, 138.0, 138.1, 138.7, 148.2 (5 x s, 5 x Ar-C). m/z (ES⁺) 1319 (2M+Na⁺, 10), 687 (M+K⁺, 15), 671 (M+Na⁺, 100), 666 (M+NH₄⁺, 80%); HRMS: Calcd. for C₃₄H₄₀N₂O₅S (MNH₄⁺) 666.2480; Found 666.2458.

3.10 General procedure for the Mitsunobu reaction

Methyl 6-amino-2,3,4-tri-O-benzyl-6-deoxy-6-N-(substitutedsulfonyl)-α-D-glucopyranoside (1.0 eq) and methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranoside/mannopyranoside (1.0 eq) were dissolved in tetrahydrofuran (3 mL), under nitrogen with stirring. The reaction mixture was cooled to 0°C and triphenylphosphine (3.0 eq) was added. After 10 min diisopropyl azodicarboxylate (DIAD) (3.0 eq) was added slowly. The yellow solution turned into a milky suspension within 10 min, removed the ice bath after 30 min and continued stirring at room temperature. After 2 h, reaction mixture became a clear solution. After two and a half hour, TLC (pet. ether/ethyl acetate, 3.1) indicated formation of one major product. The reaction mixture was concentrated the crude product purified using flash column chromatography.

N,N-Bis(methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosid-6-yl) trifluoromethanesulphonamide (29). Reaction time: 7 hr; Rf: 0.9 (DCM/MeOH; 50:1); Flash column chromatography (DCM, DCM/MeOH, 50:1); Yield: 78%; [α]D²³ +51.1 (c, 1.0 in CHCl₃); ¹H-NMR δ (400 MHz, CDCl₃): δ 3.05 (2H, dd, J = 9.6, 9.0 Hz, H-4), 3.23-3.29 (8H, m, OCH₃, H-6), 3.43 (2H, dd, J₁₂ = 3.6 Hz, J₁₂₃ = 9.7, H-2), 3.84 (4H, br s, H-5, H-6'), 3.96 (2H, at, J = 9.2 Hz, H-3), 4.47 (2H, d, H-1), 4.55, 4.84 (4H,
**N,N-Bis(methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosid-6-yl) 2-nitrobenzenesulfonylamine (30).** Reaction time: 26 hr; Rf: 0.5 (toluene/ethyl acetate, 3.5:1); Flash column chromatography (toluene/ethyl acetate, 4:1); Yield: 78%; [α]D21 +85.9 (c, 1.0 in CHCl3); 1H-NMR (400 MHz, CDCl3): δ 3.12 (2H, at, J = 9.3 Hz, H-4), 3.22 (6H, s, OCH3), 3.41 (2H, dd, J1,2 = 3.6 Hz, J2,3 = 9.7 Hz, H-2), 3.51 (2H, dd, J5,6 = 9.6 Hz, J6,6' = 15.4 Hz, H-6), 3.66 (2H, at, J = 9.6 Hz, H-5), 3.88 (2H, at, J = 9.2 Hz, H-3), 4.02 (2H, ad, J = 15.1 Hz, H-6'), 4.38 (2H, d, H-1), 4.61, 4.76 (4H, ABq, JAB = 12.3 Hz, Ph-CH2), 4.62, 4.89 (4H, ABq, JAB = 10.9 Hz, Ph-CH2), 4.79, 4.97 (4H, ABq, JAB = 10.9 Hz, Ph-CH2), 7.25-7.37 (31H, m, Ar-H), 7.51-7.52 (2H, m, Ar-H), 7.86 (1H, m, Ar-H); 13C-NMR (100 MHz, CDCl3): δ 49.4 (t, C-6), 55.4 (q, OCH3), 70.9 (d, C-5), 73.3, 74.8, 75.8 (3 x s, 3 x Ph-CH2), 79.1 (d, C-4), 79.6 (d, C-2), 81.9 (d, C-3), 97.8 (d, C-1), 123.6, 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 128.8, 131.4, 132.4, 133.1, 133.9 (13 x d, 13 x Ar-CH), 134.5, 138.0, 138.2, 147.6 (5 x s, 5 x Ar-C); m/z (ES+) 1133 (M+K+, 55%), 1117 (M+Na+, 100%), 1112 (M+NH4+, 8%); HRMS: Calcd. for C62H66N2O14SNa (MNa+) 1117.412; Found 1117.4088.

**N-(Methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosid-6-yl) N-(methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-mannopyranosid-6-yl) trifluoromethanesulfonamide (31).** Reaction time: 3 hr Rf: 0.5 (pet. ether/ethyl acetate, 3:1) Flash column chromatography (pet. ether/ethyl acetate, 6:1); Yield: 87%; [α]D21 +67.8 (c, 1.0 in CHCl3); 1H-NMR (400 MHz, CDCl3): δ 3.10 (1H, dd, J = 9.0 Hz, 9.7 Hz, H-4), 3.19, 3.29 (6H, 2 x s, 2 x OCH3), 3.42 (1H, dd, J5,6 = 10.0 Hz, J6,6' = 15.2, H-6), 3.47 (1H, dd, J1,2 = 3.6 Hz, J2,3 = 9.7 Hz, H-2), 3.49-3.53 (2H, m, H-4, H-6), 3.75 (1H, dd, J1,2 = 1.9 Hz, J2,3 = 3.0 Hz, H-2'), 3.85 (1H, dd, J3,4 = 9.0 Hz, H-3), 3.79-3.95 (4H, br m, H-5, H-6', H-5', H-6'), 3.98 (1H, at, J = 9.2 Hz, H-3), 4.50 (1H, d, H-
\[ \text{N-(Methyl 2,3,4-tri-O-benzyl-6-deoxy-\(\alpha\)-D-glycospyranosid-6-yl)} \text{ N-(methyl 2,3,4-tri-O-benzyl-6-deoxy-\(\alpha\)-D-mannopyranos-6-yl) 2-nitrobenzenesulfonamide (32).} \]

Reaction time: 12 hr; \( R_{f} \) 0.3 (pet. ether/ethyl acetate, 3:1) Flash column chromatography (pet. ether/ethyl acetate, 6:1); Yield: 78 %; \([\alpha]_{D}^{23} +54.4 \) (c, 1.0 in CHCl\(_3\)); \( ^1\text{H-NMR} \) (400 MHz, CDCl\(_3\)): \( \delta \) 3.14, 3.26 (6H, 2 x s, 2 x OCH\(_3\)), 3.21 (1H, at, \( J = 9.7 \) Hz, H-4\(_{\text{II}}\)), 3.48 (1H, dd, \( J_{1,2} = 3.5, J_{2,3} = 9.7 \) Hz, H-2\(_{\text{II}}\)), 3.61-3.74 (7H, m, H-5\(_{\text{II}}, H-2_{\text{III}}, H-4_{\text{III}}, H-5_{\text{III}}, H-6_{\text{III}}, H-6'_{\text{III}}\)), 3.78 (1H, dd, \( J_{2,3} = 3.0, J_{3,4} = 8.6 \) Hz , H-3\(_{\text{II}}\)), 3.92 (1H, at, \( J = 9.2 \) Hz, H-3\(_{\text{II}}\)), 4.06 (2H, br at, \( J = 12.8 \) Hz, H-6\(_{\text{II}}, H-6'_{\text{II}}\)), 4.45 (1H, d, \( J_{1,2} = 1.8 \) Hz, H-1\(_{\text{II}}\)), 4.47 (1H, d, H-1\(_{\text{II}}\)), 4.60 (2H, s, Ph-CH\(_2\)), 4.63-4.67 (4H, m, 2 x Ph-CHH Ph-CH\(_2\)), 4.72, 4.78 (2H, ABq, \( J_{AB} = 12.1 \) Hz, Ph-CH\(_2\)), 4.81, 5.00 (2H, ABq, \( J_{AB} = 10.8 \) Hz, Ph-CH\(_2\)), 4.92, 5.95 (2H, ABq, \( J_{AB} = 11.0 \) Hz, Ph-CH\(_2\)); 7.30-7.41 (3H, m, Ar-H), 7.52-7.53 (2H, m, Ar-H), 7.93 (1H, m, Ar-H); \( ^{13}\text{C-NMR} \) (100 MHz, CDCl\(_3\)): \( \delta \) 49.3, 49.5 (2 x t, C-6\(_{\text{II}}, C-6_{\text{III}}\)), 54.8, 55.2 (2 x q, 2 x OCH\(_3\)), 70.1, 71.7 (2 x d, C-5\(_{\text{II}}, C-5_{\text{III}}\)), 72.0, 72.9, 73.3, 74.8, 75.7 (5 x t, 6 x Ph-CH\(_2\)), 74.7 (d, C-2\(_{\text{II}}\)), 76.0 (d, C-4\(_{\text{II}}\)), 79.4 (d, C-4\(_{\text{II}}\)), 79.7 (d, C-2\(_{\text{II}}\)), 80.1 (d, C-3\(_{\text{II}}\)), 81.9 (d, C-3\(_{\text{II}}\)), 97.6 (d, C-1\(_{\text{II}}\)), 98.8 (d, C-1\(_{\text{III}}\)), 123.6, 127.7, 127.7, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 131.4, 132.4, 132.9, 133.2 (18 x d, 22 x Ar-CH), 134.6, 138.1, 138.2, 138.3, 138.7, 147.6 (7 x s, 8 x Ar-C); \( m/z \) (ES\(^+\)) 1133 (M+K\(^{+}\), 10%), 1117 (M+Na\(^{+}\), 100%), 1112 (M+NH\(_4\)^{+}, 5%); HRMS: Calcd. for C\(_{62}\)H\(_{66}\)N\(_2\)O\(_{14}\)SNa (MNa\(^{+}\)) 1117.4127; Found 1117.4152.
3.11 General procedure for epoxide opening reactions

Methyl 6-amino-2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco/mannopyranoside (1.1 eq.), epoxide (1.0 eq.) and Lithium perchlorate (2.0 eq.) were dissolved in acetonitrile (3.0 mL) and reaction mixture stirred under reflux at 90 °C. TLC (DCM/MeOH; 15:1) (45-65 h) showed consumption of epoxide (Rf = 0.8 / 0.9) and formation of one major product. The reaction mixture was poured into water (50 mL) and extracted with ethyl acetate (2 x 75 mL). The combined organic phase dried (MgSO4) and concentrated in vacuo. The crude product was purified by flash column chromatography (DCM/MeOH; 50:1).

Methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco/puranosid-6-yl) (methyl 4,6-O-benzylidene-3-deoxy-α-D-altropyranosid-3-yl)amine (25). Reaction time: 45 hr; Rf: 0.3 (DCM/MeOH; 15:1); Yield 59%; [α]D24 = +58.28 (c, 1.0 in CHCl3); IR (neat): ν max 3428 (br, NH, OH); 1H-NMR (500 MHz, CDCl3): δ 2.07 (2H, br s, NH, OH), 2.88 (1H, dd, J5,6 = 5.0 Hz, J6,6′ = 13.0 Hz, H-6′), 3.13-3.16 (2H, m, H-6′, H-3′), 3.25, 3.31 (6H, 2 x s, 2 x OCH3), 3.49 (1H, dd, J1,2 = 3.5 Hz, J2,3 = 9.7 Hz, H-2′), 3.59 (1H, at, J = 9.3 Hz, H-4′), 3.72 (1H, ddd, J = 8.1, 2.7 Hz, H-5′), 3.77 (1H, at, J = 10.3 Hz, H-6′), 3.90 (1H, br s, H-2′), 3.97 (1H, at, J = 9.3 Hz, H-3′), 4.03 (1H, dd, J = 9.8 Hz, J = 4.1 Hz, H-4′), 4.15 (1H, adt, J5,6′ = 5.1 Hz, J = 10.0 Hz, H-5′), 4.27 (1H, dd, H-6′), 4.54 (1H, d, H-1′), 4.56 (1H, s, H-1′), 4.65, 4.77 (2H, ABq, JAB = 11.6 Hz, Ph-CH2), 4.69, 4.87 (2H, ABq, JAB = 11.7 Hz, Ph-CH2), 4.80, 4.96 (2H, ABq, JAB = 10.7 Hz, Ph-CH2), 5.56 (1H, s, Ph-CH2), 7.25-7.36 (18H, m, Ar-H), 7.44-7.46 (2H, m, Ar-H); 13C-NMR (125 MHz, CDCl3): δ 48.7 (t, C-6′), 55.2, 55.6 (2 x q, OCH3), 58.8 (d, C-5′), 58.8 (d, C-3′), 69.5 (t, C-6′), 69.9, (d, C-5′), 70.0 (d, C-2′), 73.5, 74.9, 75.9 (3 x t, 3 x Ph-CH2), 77.4 (d, C-4′), 79.1 (d, C-4′), 80.3 (d, C-2′), 82.3 (d, C-3′), 98.1 (d, C-1′), 102.1 (d, C-1′), 102.4 (d, Ph-CH), 126.3, 127.7, 127.9, 128.0, 128.1, 128.4, 128.5, 128.6, 129.2 (9 x d, 12 x Ar-CH), 137.7, 138.4, 138.8, 138.9 (4 x s, 4 x Ar-C); m/z (ES+) 728 (M+H+; 100%); HRMS: Calcd. for C42H39NO10 (MH+) 728.3429; Found 728.3430.

Methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosid-6-yl) (methyl 4,6-O-benzylidene-2-deoxy-α-D-altropyranosid-2-yl)amine (26). Reaction time: 65 hr; Rf:
Methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-mannopyranosid-6-yl) (methyl 4,6-O-benzylidene-3-deoxy-α-D-altropyranosid-3-yl)amine (27). Reaction time: 46 hr; Rf 0.4 (DCM/MeOH; 15:1); Yield 58%; [α]$_D^{22}$ + 32.34 (c 1.0 in CHCl$_3$); IR (neat): $\nu_{\text{max}}$ 3413 (br, NH, OH); 1H-NMR (400 MHz, CDCl$_3$): $\delta$ 2.38 (2H, br s, NH, OH), 3.02 (1H, dd, $J_{5,6}$ = 6.3 Hz, $J_{6,6'}$ = 12.7 Hz, H-6'i), 3.19, 3.32 (6H, 2 x s, 2 x OCH$_3$), 3.19-3.24 (2H, m, H-6'i, H-3'i), 3.71 (1H, ddd, $J_{4,5}$ = 9.1 Hz, $J_{5,6'}$ = 2.5 Hz, H-5'i), 3.76-3.81 (2H, m, H-2'i, H-6'i), 3.88 (1H, dd, $J_{2,3}$ = 3.0 Hz, $J_{3,4}$ = 9.3 Hz, H-3'i), 3.93-3.98 (2H, m, H-4'i, H-2'i), 4.05 (1H, dd, $J_{3,4}$ = 4.0 Hz, $J_{4,5}$ = 9.4 Hz, H-4'i), 4.24 (1H, adt, $J_{5,6'}$ = 5.2 Hz, $J_{3,4}$ = 9.8 Hz, H-5'i), 4.29 (1H, dd, $J_{6,6'}$ = 9.8 Hz, H-6'i), 4.57 (1H, s, H-1'i), 4.62 (2H, s, Ph-CH$_2$), 4.68 (1H, d, $J_{1,2}$ = 1.7 Hz, H-1'i), 4.69, 4.92 (2H, ABq, $J_{AB}$ = 11.0 Hz, Ph-CH$_2$), 4.70, 4.76 (2H, ABq, $J_{AB}$ = 12.4 Hz, Ph-CH$_2$), 5.57 (1H, s, Ph-CH), 7.26-7.40 (18H, m, Ar-H), 7.46-7.50 (2H, m, Ar-H); 13C-NMR (100 MHz, CDCl$_3$): $\delta$ 49.1 (t, C-6'i), 54.6, 55.5 (2 x q, 2 x OCH$_3$), 58.6 (d, C-3'i), 58.7 (d, C-5'i), 69.5 (t, C-6'i), 69.9 (d, C-2'i), 70.9 (d, C-5'i), 72.1, 72.6, 74.9 (3 x t, 3 x Ph-CH$_2$), 74.7 (d, C-2'i), 76.4 (d, C-4'i), 77.1 (d, C-4'i), 80.3 (d, C-3'i), 99.8 (d, C-1'i), 102.2 (d, C-1'i), 110.0 (d, C-2'i), 117.2, 127.5, 128.1, 128.2, 128.3, 128.5, 128.6, 128.6, 129.2 (12 x d, 12 x Ar-CH), 137.4, 138.3, 138.3, 138.7 (4 x s, 4 x Ar-C); m/z (ES$^+$) 728 (M+H$^+$, 100%); HRMS Calcd. for C$_{42}$H$_{50}$NO$_{10}$ (M+H$^+$) 728.3429; Found 728.3435.
Methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-mannopyranos-6-yl) (methyl 4,6-O-benzylidene-2-deoxy-α-D-altropyranos-2-yl)amine (28). Reaction time: 24 hr; Rf: 0.5 (DCM/MeOH; 15:1); Yield: 89%; [α]$_{D}^{23}$ + 54.4 (c, 1.0 in CHCl$_3$); IR (neat): $\nu_{\text{max}}$ 3496 (br, NH, OH), $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 2.93 (1H, dd, J$_{5,6}$ = 5.8 Hz, J$_{6,6'}$ = 12.1 Hz, H-6), 2.99 (1H, dd, J$_{5,6'}$ = 2.9 Hz, H-6), 3.07 (2H, m, H-2$_H$, NH/OH), 3.32, 3.41 (6H, 2 x s, 2 x OCH$_3$), 3.69 (1H, m, H-5$_H$), 3.76 (1H, at, J = 10.2 Hz, H-6$_H$), 3.81 (1H, dd, J$_{1,2}$ = 1.9 Hz, J$_{2,3}$ = 3.0 Hz, H-2$_H$), 3.85 (1H, dd, J$_{3,4}$ = 2.8 Hz, J$_{4,5}$ = 9.8 Hz, H-4$_H$), 3.90 (1H, dd, J$_{3,4}$ = 9.3 Hz, H-3$_H$), 3.98 (1H, at, J = 9.4 Hz, H-4$_H$), 4.12 (1H, m, H-3$_H$), 4.18 (1H, atd, J$_{5,6}$ = 5.0 Hz, J = 10.0 Hz, H-5$_H$), 4.31 (1H, dd, H-6$_H$), 4.64 (1H, d, H-1), 4.64 (2H, s, Ph-CH$_2$), 4.65, 4.99 (2H, ABq, J$_{AB}$ = 11.1 Hz, Ph-CH$_2$), 4.69 (1H, d, J$_{1,2}$ = 1.3 Hz, H-1$_H$), 4.71, 4.81 (2H, ABq, J$_{AB}$ = 12.3 Hz, Ph-CH$_2$), 5.55 (1H, s, Ph-CH$_3$), 7.26-7.40 (18H, m, Ar-H), 7.47-7.50 (2H, m, Ar-H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 49.1 (t, C-6), 55.0, 55.7 (2 x q, 2 x OCH$_3$), 58.7 (d, C-5), 61.0 (d, C-2), 68.4 (d, C-3), 69.3 (t, C-6), 71.4 (d, C-5), 72.3, 73.0, 75.2 (3 x t, 3 x Ph-CH$_3$), 74.8 (d, C-2), 76.1 (d, C-4), 77.4 (d, C-4), 80.4 (d, C-3), 99.3 (d, C-1), 101.8 (d, C-1), 102.3 (d, Ph-CH$_2$), 126.4, 127.7, 127.8, 127.8, 127.9, 128.3, 128.5, 128.6, 129.1 (9 x d, 12 x Ar-CH), 137.7, 138.4, 138.8, 138.9 (4 x s, 4 x Ar-C); m/z (ES$^+$) 766 (M+K$^+$, 10%), 750 (M+Na$^+$, 45%), 728 (M+H$^+$, 100%); HRMS: Calcd. for C$_{42}$H$_{69}$NO$_{10}$Na (MNa$^+$) 750.3249; Found 750.3280.

3.12 General Procedure for the deprotection of amine-linked disaccharides

Ammonia was condensed (15-20 mL) in a flask at -78 °C. Sodium metal (required quantity) was added, the solution turned deep blue immediately. Protected pseudodisaccharide in tetrahydrofuran (2 mL) was added, followed by 3-4 drops of methanol. The reaction mixture (deep blue solution) was stirred at this temperature for 4-5 h, under nitrogen atmosphere. Then solid ammonium chloride (400 mg) was
added to quench the reaction. Ammonia was evaporated at room temperature. The crude product was dissolved in CHCl₃/EtOH (1:1) and filtered through cotton wool, repeated this twice to get rid of most of the salt. The resulting crude product purified by flash column chromatography using chloroform, methanol, acetic acid and water solvent system as the eluent (CMAW; 60:30:5:3).

(Methyl 6-deoxy-α-D-glucospyranosid-6-yl) (methyl 3-deoxy-α-D-altropyranosid-3-yl) ammonium acetate (34) R₆ 0.3 (CMAW); Yield 80%; [α]D²² = 56.5 (c, 1.0 in H₂O); ¹H-NMR (400 MHz, D₂O): δ 2.08 (3H, s, C(O)CH₃), 3.45 (1H, dd, J = 8.8 Hz, J = 9.8 Hz, H-6), 3.50 (1H, m, H-4), 3.52, 3.55 (6H, 2 x s, 2 x OCH₃), 3.68 (1H, dd, J₁₂ = 3.6 Hz, J₂₂ = 9.8 Hz, H-2), 3.72-3.78 (3H, m, H-3, H-3, H-6'), 3.85-3.97 (3H, m, H-5, H-6, H-6'), 4.07 (1H, adt, J = 9.6, 3.0 Hz, H-5'), 4.22 (1H, dd, J₁₂ = 3.3 Hz, J₂₂ = 5.7 Hz, H-2), 4.34 (1H, dd, J = 4.7 Hz, J = 7.9 Hz, H-4), 4.87 (1H, d, H-1), 4.94 (1H, d, H-1); ¹³C-NMR (100 MHz, CDCl₃): δ 21.0 (q, C(O)CH₃), 47.0 (t, C-6), 55.0, 55.13 (2 x q, 2 x OCH₃), 58.7 (d, C-3), 59.6 (t, C-6), 59.8 (d, C-4), 63.7 (d, C-2), 65.9 (d, C-5), 69.9 (d, C-2), 70.3 (d, C-4), 70.8 (d, C-5), 71.5 (d, C-3), 98.7 (d, C-1), 99.3 (d, C-1), 177.8 (s, C(O)CH₃); m/z (ES⁺) 370 (M+H⁺, 100%); HRMS Calcd. for C₁₄H₂₈NO₁₀ 370.1708 (MH⁺). Found 370.1726.

(Methyl 6-deoxy-α-D-mannopyranosid-6-yl) (methyl 3-deoxy-α-D-altropyranosid-3-yl) ammonium acetate (35). R₆ 0.3 (CMAW); Yield 74%; [α]D²² = 60.0 (c, 1.0 in H₂O); ¹H-NMR (500 MHz, D₂O): δ 2.08 (3H, s, C(O)CH₃), 3.62 (1H, m, H-6), 3.60, 3.64 (2 x s, 2 x OCH₃), 3.76 (1H, at, J = 9.7 Hz, H-4), 3.79 - 3.84 (2H, m, H-6', H-3), 3.92-2.98 (2H, m, H-3, H-6), 4.02-4.12 (4H, m, H-2, H-5, H-5, H-6'), 4.29 (1H, dd, J = 3.5 Hz, J = 5.8 Hz, H-2), 4.41 (1H, dd, J = 7.8, 4.7 Hz, H-4), 4.94 (1H, H-1), 4.97 (1H, H-1'); ¹³C-NMR (125 MHz, CDCl₃): δ 21.0 (q, C(O)CH₃), 45.6 (t, C-6), 53.5, 53.7 (2 x q, 2 x OCH₃), 57.4 (d, C-3), 58.4 (t, C-6), 58.9 (d, C-4), 62.8 (d, C-2), 66.3, 69.5 (2 x d, C-5, C-5), 67.6 (d, C-4), 67.6 (d, C-2), 67.8 (d, C-3), 98.3 (d, C-1), 99.2 (2 x d, C-1), 179.0 (s, C(O)CH₃); m/z (ES⁺) 370 (M+H⁺, 100%); HRMS: Calcd. for C₁₄H₂₈NO₁₀ 370.1708 (MH⁺). Found 370.1702.

(Methyl 6-deoxy-α-D-glucospyranosid-6-yl) (methyl 2-deoxy-α-D-altropyranosid-2-yl) ammonium acetate (36). R₆ 0.2 (CMAW); Yield 88%; [α]D²² = 46.7 (c, 1.0
in H$_2$O), $^1$H-NMR (500 MHz, D$_2$O): δ 2.08 (3H, s, C(O)CH$_3$), 3.39-3.46 (2H, m, H-4$_i$, H-6$_i$), 3.55 (1H, m, H-2$_{II}$), 3.56, 3.58 (6H, 2 x s, 2 x OCH$_3$), 3.69 (1H, dd, J$_{1,2} = 3.7$ Hz, J$_{2,3} = 9.8$ Hz, H-2$_i$), 3.70 (1H, m, H-6$_{II}$), 3.77 (1H, at, J = 9.7 Hz, H-5$_{II}$), 3.90-3.92 (2H, m, H-6$_{II}$, H-6$_{I}$, H-6$_{I}$), 4.01-4.09 (3H, m, H-3$_{II}$, H-4$_{II}$, H-5$_{II}$), 4.26 (1H, dd, J = 8.2, 3.6 Hz, H-3$_{II}$), 4.95 (1H, d, H-1), 5.07 (1H, d, J$_{1,2} = 4.6$ Hz, H-1$_{II}$). $^{13}$C-NMR (125 MHz, D$_2$O): δ 21.0 (q, C(O)CH$_3$), 46.4 (t, C-6$_i$), 54.6, 54.7 (2 x q, 2 x OCH$_3$), 58.2 (d, C-2$_{II}$), 59.3 (t, C-6$_{II}$), 64.5, 66.2, 72.5 (3 x d, C-3$_{II}$, C-4$_{II}$, C-5$_{II}$), 65.0 (d, C-3$_{II}$), 69.6 (d, C-2$_i$), 70.3 (d, C-4$_i$), 71.3 (d, C-5$_i$), 96.1 (d, C-1$_{II}$), 98.3 (d, C-1$_i$); m/z (ES$^+$) 370 (M+H$^+$, 100%); HRMS: Calcd. for C$_{14}$H$_{28}$NO$_{10}$ 370.1708 (M$^+$); Found 370.1726.

(Methyl 6-deoxy-α-D-mannopyranosid-6-yl) (methyl 2-deoxy-α-D-altropyranosid-2-yl)ammonium acetate (37). R$_f$: 0.2 (CMAW); Yield 72%; [α]$^{22}_D + 42.2$ (c, 1.0 in H$_2$O), $^1$H-NMR (500 MHz, D$_2$O): δ 2.08 (3H, s, C(O)CH$_3$), 3.46 (1H, dd, J$_{5,6} = 5.6$ Hz, J$_{6,6'} = 13.2$ Hz, H-6$_i$), 3.53, 3.54 (6H, 2 x s, 2 x OCH$_3$), 3.55 (1H, dd, J$_{1,2} = 4.5$ Hz, J$_{2,3} = 9.8$ Hz, H-2$_{II}$), 3.67 (1H, at, J = 9.7 Hz, H-4$_i$), 3.70 (1H, dd, J$_{5,6'} = 3.0$ Hz, H-6$_{I}$), 3.86 (1H, dd, J$_{2,3} = 3.5$ Hz, J$_{3,4} = 9.6$ Hz, H-3$_i$), 3.88-3.94 (2H, m, H-6$_{II}$, H-6$_{II}$), 3.97 (1H, adt, J = 9.2 Hz, H-5$_i$), 4.03-4.08 (3H, m, H-2$_{II}$, H-4$_{II}$, H-5$_{II}$), 4.24 (1H, dd, J = 8.1, 3.8 Hz, H-3$_{II}$), 4.90 (1H, d, J$_{1,2} = 1.6$ Hz, H-1), 5.06 (1H, d, H-1$_{II}$), $^{13}$C-NMR (125 MHz, D$_2$O): δ 21.0 (q, C(O)CH$_3$), 46.6 (t, C-6$_i$), 54.5, 54.8 (2 x q, 2 x OCH$_3$), 58.6 (d, C-2$_{II}$), 59.6 (t, C-6$_{II}$), 64.7 (d, C-4$_{II}$), 65.3 (d, C-3$_{II}$), 67.3 (d, C-5$_i$), 68.7 (d, C-2$_i$), 69.0 (d, C-3$_i$), 72.5 (d, C-5$_i$), 96.4 (d, C-1$_{II}$), 100.2 (d, C-1$_i$), 178.4 (s, C(O)CH$_3$); m/z (ES$^+$) 370 (M+H$^+$, 100%); HRMS: Calcd. for C$_{14}$H$_{28}$NO$_{10}$ 370.1708 (M$^+$); Found 370.1723.

N,N-Bis(methyl 6-deoxy-α-D-glucopyranosid-6-yl)ammonium acetate (38). R$_f$: <0.1 (CMAW); Yield 62%; [α]$^{22}_D + 50.0$ (c, 1.0 in H$_2$O), $^1$H-NMR (500 MHz, D$_2$O): δ 2.07 (3H, s, C(O)CH$_3$), 3.38-3.47 (6H, m, H-4, H-6), 3.56 (6H, s, OCH$_3$), 3.69-3.73 (4H, m, H-2, H-6'), 3.79 (2H, at, J = 9.7 Hz, H-3), 4.07 (2H, adt, J = 9.9, 2.2 Hz, H-5), 4.97 (2H, d, J$_{1,2} = 3.5$ Hz, H-1), $^{13}$C-NMR (125 MHz, D$_2$O): δ 21.0 (q, C(O)CH$_3$), 47.0 (t, C-6), 53.9 (q, OCH$_3$), 65.1 (d, C-5), 69.3 (d, C-2), 69.9 (d, C-4), 70.9 (d, C-3), 97.8 (d, C-1); m/z (ES$^+$) 370 (M+H$^+$, 100%); HRMS: Calcd. for C$_{14}$H$_{28}$NO$_{10}$ 370.1708 (M$^+$); Found 370.1715.
(Methyl 6-deoxy-α-D-glucopyranosid-6-yl) (methyl 6-deoxy-α-D-mannopyranosid-6-yl) ammonium acetate (39). Rf: <0.1 (CMAW); Yield 86%; [α]D = 55.9 (c, 1.0 in H2O); 1H-NMR (500 MHz, D2O): δ 2.07 (3H, s, C(O)CH3), 3.43 (1H, at, J = 9.9 Hz, H-6), 3.45 (1H, at, J = 9.8 Hz, H-6), 3.49 (1H, at, J = 9.3 Hz, H-4\(\beta\)), 3.59, 3.60 (6H, 2 x s, 2 x OCH3), 3.60-3.77 (4H, m, H-2, H-4\(\beta\), H-6\(\beta\), H-6\(\alpha\)), 3.82 (1H, at, J = 9.7 Hz, H-3\(\alpha\)), 3.93 (1H, dd, J2,3 = 3.3 Hz, J3,4 = 9.6 Hz, H-3\(\beta\)), 4.05-4.11 (2H, m, H-5\(\beta\), H-5\(\alpha\)), 4.12 (1H, dd, J1,2 = 1.7 Hz, H-2\(\beta\)), 4.96 (1H, d, H-1\(\beta\)), 5.01 (1H, d, H-1\(\alpha\)); 13C-NMR (125 MHz, D2O): δ 21.0 (q, C(O)CH3), 46.5, 46.6 (2 x t, C-6\(\beta\), C-6\(\alpha\)), 53.1, 53.4 (2 x q, 2 x OCH3), 55.2 (d, C-4\(\beta\)), 64.8, 65.5 (2 x d, C-5\(\beta\), C-5\(\alpha\)), 66.2 (d, C-4\(\alpha\)), 67.5 (d, C-2\(\alpha\)), 67.8 (d, C-3\(\alpha\)), 68.7 (d, C-2\(\beta\)), 69.5 (d, C-4\(\alpha\)), 70.4 (d, C-3\(\beta\)), 97.3 (d, C-1\(\alpha\)), 98.9 (d, C-1\(\beta\)), 179.3 (s, C(O)CH3); m/z (ES+) 370 (M+H\(^+\), 100%); HRMS: Calcd. for C14H28NO10 370.1708 (MH\(^+\)); Found 370.1690.

3.13 General Procedure for the synthesis of ether-linked disaccharides

1,2:5,6-Di-O-isopropylidene-3-O-trifluoromethanesulfonyl-α-D-allofuranose (2.0 eq) and sugar alcohol (1.0 eq) were dissolved in dimethyl formamide (2.0 mL) at room temperature under nitrogen. Sodium hydride (2.0 eq) was added and the mixture stirred at room temperature. The reaction mixture turned brown within 10 min. After one and a half hour, TLC (pet. ether/ethanol; 3:1) revealed the formation of one major product. The reaction mixture was poured into brine (50 mL), extracted with ether (2 x 75 mL), dried (MgSO4) and concentrated in vacuo. The crude product was purified by flash column chromatography (pentane/ethyl acetate, 3:5:1).

3-O-(1,2:5,6-Di-O-isopropylidene-3-deoxy-α-D-allofuranos-3-yl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (40). Rf 0.3 (pentane/ethyl acetate; 3:1); [α]D = 50.1 (c, 1.0 in CHCl3); 1H-NMR (400 MHz, CDCl3): δ 1.32, 1.33, 1.35, 1.41, 1.44, 1.49, 1.54 (24H, 7 x s, 4 x C(CH3)2), 3.89 (1H, dd, J2,3 = 4.7 Hz, J3,4 = 8.4 Hz, H-3\(\beta\)), 3.92-4.11 (7H, m, H-4\(\beta\), H-6\(\beta\), H-6\(\alpha\), H-3\(\beta\), H-4\(\alpha\), H-6\(\alpha\), H-6\(\alpha\)), 4.28 (1H, at, J = 4.0 Hz, J = 6.8 Hz, H-5\(\beta\)), 4.37 (1H, at, J = 7.5, 5.9 Hz, H-5\(\alpha\)), 4.64 (1H, at, J = 4.2 Hz, H-2\(\alpha\)), 4.70 (1H, d, J1,2 = 3.5 Hz, H-2\(\beta\)), 5.79 (1H, d, J1,2 = 3.9 Hz, H-1\(\beta\)), 5.87 (1H, d, H-1\(\alpha\)). 13C-NMR (100 MHz, CDCl3): δ 25.4, 25.4, 26.4, 26.4, 26.8, 27.0, 27.0, 27.0
Bis(1,2:5,6-di-O-isopropylidene-3-deoxy-α-D-glucofuranos-3-yl) ether (41). R<sub>f</sub> 0.6 (pentane/ethyl acetate; 3:1); [α]<sub>D</sub>+24° -49.7 (c, 1.0 in CHCl<sub>3</sub>);<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 1.29, 1.32, 1.40, 1.48 (24H, 4 x s, 8 x CH<sub>3</sub>), 3.92 (2H, dd, J<sub>5,6</sub>= 5.6 Hz, J<sub>6,6′</sub>= 8.6 Hz, H-6), 4.04-4.09 (6H, m, H-3, H-4, H-6′), 4.15 (2H, atd, J<sub>4,5</sub>= 7.8 Hz, J = 5.8 Hz, H-5), 4.65 (2H, d, J<sub>1,2</sub>= 3.6 Hz, H-2), 5.81 (2H, d, H-1).<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>): δ 25.5, 26.3, 26.9, (3 x q, 4 x C(CH<sub>3</sub>)<sub>2</sub>), 67.8 (t, C-6), 72.4 (d, C-5), 81.1, 81.3 (2 x d, C-3, C-4), 82.3 (d, C-2), 105.7 (d, C-1), 109.1, 112.1 (2 x s, 2 x C(CH<sub>3</sub>)<sub>2</sub>); m/z (ES<sup>+</sup>) 525 (M+Na<sup>+</sup>, 100%). HRMS: Calcd. for C<sub>24</sub>H<sub>38</sub>O<sub>11</sub>Na (MNa<sup>+</sup>) 525.2306; Found 525.2339.

3-O-(Methyl 4,6-O-benzylidene-2-O-benzyl-3-deoxy-α-D-altropyranosid-3-yl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (43). R<sub>f</sub> 0.4 (pentane/ethyl acetate; 3:1); [α]<sub>D</sub>+24° +28.8 (c, 1.0 in CHCl<sub>3</sub>);<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ 1.23, 1.29, 1.40, 1.45 (12H, 4 x s, 4 x CH<sub>3</sub>), 3.37 (3H, s, OCH<sub>3</sub>), 3.58 (1H, d, J<sub>2,3</sub>= 2.6 Hz, H-2), 3.76 (1H, at, J = 10.1 Hz, H-6), 3.96-4.08 (4H, m, H-3, H-4, H-6′, H-6″), 4.15-4.24 (3H, m, H-3, H-4, H-5′, H-5″), 4.26 (1H, d, J<sub>1,2</sub>= 3.6 Hz, H-2″), 4.29 (1H, dd, J<sub>5,6</sub>= 5.2 Hz, H-6″), 4.50 (1H, aq J = 6.1 Hz, H-5″), 4.57, 4.72 (1H, AB<sub>q</sub>, J<sub>AB</sub>= 12.0 Hz, PhCH<sub>2</sub>), 4.68 (1H, s, H-1″), 5.54 (1H, s, PhCH), 5.80 (1H, d, H-1″), 7.26-7.41 (8H, m, Ar-H), 7.46-7.49 (2H, m, Ar-H).<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>): δ 25.2, 26.5, 26.7, 27.0 (4 x q, 2 x C(CH<sub>3</sub>)<sub>2</sub>), 55.2 (q, OCH<sub>3</sub>), 58.4 (d, C-5), 66.7 (t, C-6), 69.4 (t, C-6), 72.9 (t, PhCH<sub>2</sub>), 73.0 (d, C-5), 73.5, 76.3 (2 x d, C-3, C-4), 77.0 (d, C-2), 81.5, 82.5 (2 x d, C-3, C-4), 82.7 (d, C-2), 99.7 (d, C-1), 102.4 (d, PhCH), 105.3 (d, C-1), 108.7, 111.7 (2 x s, C(CH<sub>3</sub>)<sub>2</sub>), 126.3, 128.1, 128.3, 128.4, 128.7, 129.1 (6 x d, Ar-CH) 137.3, 137.8 (2 x s Ar-C); m/z (ES<sup>+</sup>) 637 (M+Na<sup>+</sup>, 100%); HRMS: Calcd. for C<sub>33</sub>H<sub>42</sub>O<sub>11</sub>Na (MNa<sup>+</sup>) 637.2619; Found 637.2626.

3-O-(Methyl 4,6-O-benzylidene-2-O-benzyl-3-deoxy-α-D-mannopyranosid-3-yl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (44). R<sub>f</sub> 0.6 (pentane/ethyl acetate;
3:1); [α]D$^{23}$ -4.9 (c, 1.0 in CHCl₃); $^1$H-NMR (400 MHz, CDCl₃): δ 1.17, 1.21, 1.40, 1.47 (12 H, 4 x s, 2 x C(CH₃)₂), 3.37 (3H, s, OCH₃), 3.77 (1H, dat, $J_{5,6'} = 4.6$ Hz, $J = 10.2$ Hz, H-3n), 3.85 (1H, dd, $J_{1,2} = 1.7$ Hz, $J_{2,3} = 3.2$ Hz, H-2n), 3.86 (1H, at, $J = 10.2$ Hz, H-6n), 3.96 (1H, dd, $J_{5,6} = 5.3$ Hz, $J_{6,6'} = 8.6$ Hz, H-6n), 3.98 (1H, dd, $J_{5,4} = 3.2$ Hz, $J_{4,5} = 10.1$ Hz, H-4n), 4.02 (1H, dd, $J_{3,4} = 8.8$ Hz, H-3n), 4.07 (1H, dd, $J_{5,6} = 6.0$ Hz, H-6n), 4.10 (1H, dd, $J_{4,5} = 10.1$ Hz, H-4n), 4.15 (1H, d, $J_{2,3} = 3.0$ Hz, H-3n), 4.23-4.28 (2H, m, H-5n), 6.61 (1H, d, $J_{1,2} = 1.6$ Hz, H-1n), 6.71, 4.83 (1H, ABq $J_{AB} = 12.0$ Hz, PhCH₂), 4.82 (1H, d, $J_{1,2} = 3.6$ Hz, H-2n), 5.62 (1H, s, PhCH), 5.75 (1H, d, H-1n), 7.25-7.40 (8H, m, Ar-H), 7.49-7.53 (2H, m, Ar-H). $^{13}$C-NMR (100 MHz, CDCl₃): δ 25.4, 26.0, 26.9, 27.0 (4 x q, 2 x C(CH₃)₂), 55.0 (q, OCH₃), 64.4 (d, C-5i), 68.0 (t, C-6n), 68.9 (t, C-6n), 72.4 (d, C-5n), 73.8 (t, PhCH₂), 77.3 (d, C-2n), 77.8 (d, C-4n), 78.2 (d, C-4i), 81.6 (d, C-3i), 83.5 (d, C-2n), 83.6 (d, C-3n), 100.7 (d, C-1i), 101.6 (d, PhCH), 105.4 (d, C-1n), 109.2, 111.7 (2 x s, 2 x C(CH₃)₂)), 125.9, 127.7, 127.9, 128.1, 128.3, 128.8 (6 x d, Ar-CH), 173.5, 138.2 (2 x s, Ar-C); m/z (ES<sup>+</sup>) 637 (M+Na<sup>+</sup>, 100%); HRMS: Calcd. for C₃₃H₄₂O₁₁Na (MNa<sup>+</sup>) 637.2619; Found 637.2588.

3-O-(Methyl 4,6-O-benzylidene-3-O-benzyl-2-deoxy-α-D-mannopyranosid-2-yl)-1,2:5,6 di-O-isopropylidene-α-D-glucofuranose (45). Rf: 0.5 (pentane/ethyl acetate; 3:1); [α]D$^{25}$ -19.0 (c, 1.0 in CHCl₃); $^1$H-NMR (400 MHz, CDCl₃): δ 1.17, 1.35, 1.42, 1.46 (12 H, 4 x s, 2 x C(CH₃)₂), 3.35 (3H, s, OCH₃), 3.77 (1H, dat, $J_{5,6'} = 4.1$ Hz, $J = 9.6$ Hz, H-5n), 3.82 (1H, at, $J = 9.9$ Hz, H-6n), 3.86 (1H, dd, $J_{1,2} = 1.5$ Hz, $J_{2,3} = 3.2$ Hz, H-2n), 3.91 (1H, dd, $J_{3,4} = 9.8$ Hz, H-3n), 3.97 (1H, dd, $J_{5,6} = 6.2$ Hz, $J_{6,6'} = 8.4$ Hz, H-6n), 4.03 (1H, at, $J = 9.3$ Hz, H-4n), 4.04 (1H, d, $J_{2,3} = 3.0$ Hz, H-3n), 4.09 (1H, dd, $J_{4,5} = 7.8$ Hz, H-4n), 4.15 (1H, dd, $J_{5,6} = 6.1$ Hz, H-6n), 4.23-4.31 (2H, m, H-6n), 4.70, 4.91 (1H, ABq $J_{AB} = 11.7$ Hz, PhCH₂), 4.78 (1H, d, $J_{1,2} = 1.5$ Hz, H-1n), 4.81 (1H, d, $J_{1,2} = 3.7$ Hz, H-2n), 5.62 (1H, s, PhCH), 5.83 (1H, d, H-1n), 7.24-7.39 (8H, m, Ar-H), 7.48-7.50 (2H, m, Ar-H). $^{13}$C-NMR (100 MHz, CDCl₃): δ 25.6, 26.3, 26.9, 27.0 (4 x q, 2 x C(CH₃)₂), 55.0 (q, OCH₃), 63.9 (d, C-5i), 67.8 (t, C-6n), 69.0 (t, C-6n), 72.9 (d, C-5n), 73.7 (t, PhCH₂), 75.7 (d, C-3i), 79.6 (d, C-4n), 80.2 (d, C-2n), 81.4 (d, C-4n), 83.5 (d, C-2n), 85.4 (C-3n), 100.6 (d, C-1i), 101.6 (d, PhCH), 105.6 (d, C-1n), 109.1, 111.7 (2 x s, 2 x C(CH₃)₂), 126.2, 127.7, 127.7, 128.3, 128.4, 129.0 (6 x d, Ar-CH), 137.6, 138.4 (2 x s, Ar-C); m/z (ES<sup>+</sup>) 637 (M+Na<sup>+</sup>, 100%); HRMS: Calcd. for C₃₃H₄₂O₁₁Na (MNa<sup>+</sup>) 637.2619; Found 637.2641.
3-O-(2,3,4,6-Tetra-0-benzyl-5a-carba-b-D-glucopyranosyl)-(1→3)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (46). Rf: 0.9 (pentane/ethyl acetate; 3:1); [a]D²⁴
+9.3 (c, 1.0 in CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ 1.16, 1.33, 1.41, 1.44 (12 H, 4 x s, 2 x C(CH₃)₂), 1.39 (1H, m, H-5a), 1.66 (1H, m, H-5β), 2.15 (1H, dat, J = 4.0 Hz, J₅₅₅₆ = 13.6 Hz, H-5a'), 3.37 (1H, at, J = 9.0 Hz, H-2α or H-3β), 3.43-3.54 (4H, m, H-1β, H-4α, H-6β, H-2α or H-3β), 3.57 (1H, dd, J₅₅₆ = 5.3 Hz, J₆₆₆ = 8.9 Hz, H-6α'), 3.95-3.98 (2H, m, H-3β, H-6β), 4.08 (1H, dd, J₅₅₆ = 6.2 Hz, J₆₆₆ = 8.4 Hz, H-6β'), 4.14 (1H, dd, J = 3.4 Hz, J = 3.2 Hz, J = 4.5, J = 7.0 Hz, H-4α), 4.29 (1H, qq, J = 6.4 Hz, H-5β), 4.45 (2H, s, PhCH₂), 4.51 (1H, d, J = 10.8 Hz, PhCHH'), 4.57 (1H, d, J = 1.2 Hz, J = 3.8 Hz, H-2α), 4.72 (1H, d, J = 11.2 Hz, PhCHH'), 4.84-4.92 (4H, m, PhCH₂, 2 x PhCHH'), 4.60 (1H, d, H-1β), 7.18-7.34 (20H, m, Ar-H); ¹³C-NMR (100 MHz, CDCl₃): δ 25.6, 26.0, 26.8, 26.9 (4 x q, 2 x C(CH₃)₂), 30.0 (t, C-5α), 39.0 (d, C-5), 67.3 (t, C-6β), 70.1 (t, C-6α), 72.9 (d, C-5β), 73.4, 75.4, 75.7, 75.9 (4 x t, 4 x PhCH₂), 79.9, 80.9, 81.0, 81.4, 82.9, 85.0, 86.6 (7 x d, C-1β, C-2α, C-3β, C-4β, C-4α, C-2β, C-3α, C-4α), 105.5 (d, C-5β), 108.9, 111.6 (2 x s, 2 x C(CH₃)₂), 127.4, 127.7, 127.7, 127.8, 127.9, 128.2, 128.5, 128.5, 128.6 (10 x d, Ar-CH), 138.5, 138.6, 138.8 (3 x s, 4 x Ar-C); m/z (ES⁺) 819 (M+K, 7%), 803 (M+Na+, 100%); HRMS: Calcd. for C₄₇H₆₅O₁₀Na (MNa⁺) 803.3766; Found 803.3741.
References

7. For the state of the art, see: Carbohydr. Res. 2007, 342, 1537–1982 (special issue on Glycomimetics).


28. Fournierre, V.; Cumpstey, I., unpublished.


List of Publications


