Chapter- 4A

Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation
Chapter- 4A

Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation

4A.1. Introduction

Benzopyrans or chromans have great importance in medicinal chemistry due to their broad spectrum of biological activities such as antioxidants, diabetes, cardiovascular, multidrug resistance reversal, antifertility, anti-HIV agent, ischemia, etc.\(^1\),\(^2\) On the other hand prenylated aromatic compounds (Fig. 1 & Fig. 2) exhibit anti-microbial, pro-apoptotic, and anti-proliferative activities against a number of cell lines.\(^3\)\(^-\)\(^8\) The rich variety of prenylated molecules, containing chroman and chromene unit\(^9\) are generally characterized by low cellular toxicity and good membrane permeability, which make them ideal drug template in medicinal chemistry. In addition to this the chroman unit is abundant in natural products, and possess a broad array of biological activities viz antimicrobial, antiviral, mutagenicity, antiproliferative, sex pheromone, antitumor, and central nervous system activity.\(^10\) The fused dimethylpyran system, which are naturally synthesized by the condensation of a phenol with an active isoprene unit (2-methy-1,3-butadiene) are ‘privileged substructure’ in numerous bioactive compounds such as the antifungal benzopyran methylripariochromene A (I),\(^11\) the antibacterial pyranoflavanone 5-methyllupinifoliol (II),\(^12\) and the antitumor acridone alkaloid acronycin III (Fig. 1).\(^13\)

![Figure 1. Biologically active compounds containing chromene unit](image)

Very recently 3,3-dimethyl-3\(H\)-benzofuro[3,2,f][1]-benzopyran (IV), or its dihydro analogue (V), were synthesized and evaluated for their antitubercular activities (Fig. 2).\(^14\)
Chapter 4A  Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation

Figure 2. Benzopyran derivative (IV), or its dihydro analogue (V) as antimycobacterial agent

On the other hand five membered heterocyclic compounds including pyrrolidine, pyrrole, imidazolidine, imidazole, pyrazole, oxazole, isoxazoline etc. have occupied an important place in organic, bioorganic and medicinal chemistry. These compounds are of great importance among various classes of organic compounds due to their synthetic utilities and wide range of biological activities such as anti-cancer, anti-microbial, anti-fungal, anti-viral and anti-diabetic. The five member N heterocyclic compounds, pyrrolidine derivatives are common structural features in many natural products and properly designed biologically active molecules (Fig. 3). In addition, pyrrolidines are also important building blocks in organic syntheses, which can be used for pharmaceutical purposes and as ligands of transition metal catalysts.

Figure 3. Biologically active synthetic and natural products containing pyrrolidine unit
Pyrrolidine unit is also present in the neuroprotective agent kaitocephalin,\textsuperscript{22} the influenza drug A-315675,\textsuperscript{23} and the antitumor antibiotic bioxalomycin \(\beta_1\),\textsuperscript{24} and several other biologically active alkaloids,\textsuperscript{25} excitatory amino acid inhibitors\textsuperscript{26} and angiotensin converting enzyme (ACE) inhibitors (Fig. 3).\textsuperscript{27} In 2006 Bogatcheva et al.\textsuperscript{28} reported a library of new diamine scaffolds with their antimycobacterial activity and found that the pyrrolidine containing scaffold is an important hit to develop new antimycobacterial agent (Fig. 4). Perumal et al.\textsuperscript{29} reported the antimycobacterial activity of spiro pyridopyrrolizines and pyrrolidines derivatives against H37Rv strain.

![Figure 4. Diamine scaffold](image)

Moreover, a family of compounds consisting of both the benzopyran and pyrrolidine rings such as hexahydrochromeno[4,3-b]pyrrolidine or benzopyrano[3,4-c]pyrrolidine skeleton was known to be antagonists of the muscular nicotin receptor (Fig. 5),\textsuperscript{30} selective dopamine D3 receptor.\textsuperscript{31} Similar structure motifs are common in various natural compounds, such as martinelline and sceletium alkaloid A-4\textsuperscript{32} (Fig. 5).

![Figure 5. Biologically important compounds containing benzopyrano [3,4-c]pyrrolidine or similar structure motifs](image)

### 4A.2. Earlier methods of synthesis of chroman ring

A number of methods have been reported in literature involving different synthetic strategies and a few of them are discussed below.
4A.2.1. By Grignard reaction

The arylbutanone derivative on treatment with methyl magnesium iodide gives the alcohol, which on acid catalysed cyclization affords 2,2-dimethylchroman (Fig. 6).³³

![Diagram of Grignard reaction](image)

Similarly the Grignard reaction of methyl magnesium iodide with β-orthomethoxyphenylethylmethylketone produces alcohol intermediate which on demethylation with hydrobromic acid gives 2,2-dimethylchroman (Fig. 7).³⁴

![Diagram of Grignard reaction](image)

4A.2.2 By the reduction of chromenones

In the presence of diborane as reducing agent³⁵ chroman-4-ones were transformed into corresponding chroman, e.g. 6,7-dimethoxy-2,2-dimethylchroman-4one on reduction with diborane gave 86% yield of chroman (Fig. 8).

![Diagram of diborane reduction](image)

4A.2.3 By reaction of phenol and hydroxycoumarin with prenylated compounds
The 2,2-dimethylchromans were synthesized by the reaction of 3,3-dimethylallyl alcohol with different substituted phenols in the presence of acid catalyst. The reaction through isoprenylated intermediates (Fig. 9)\textsuperscript{36} which subsequently undergo intramolecular cyclization resulting in the benzopyran nucleus.

Wolform \textit{et al.}, reported the reaction of 3,3-dimethylallyl bromide with phenol in the presence of zincchloride in order to get dimethylchromans (Fig. 10).\textsuperscript{37}

An elegant method has been reported by Ahluwalia \textit{et al.},\textsuperscript{38a} for the preparation of 2,2-dimethylchroman, by reaction of phenol and isoprene in presence of orthophosphoric acid. Similarly orthophosphoric acid catalyst condensation of phenol derivative with 2-methylbut-3-en-2-ol give the 2,2-dimethylchroman in very good yields\textsuperscript{38b} (Fig. 11).
Further a method for the synthesis of the linear pyranocoumarins from 7-hydroxycoumarin was developed by Ahluwalia et al. In this reaction the cyclization of preformed 8-iodo-7-(1',1'-dimethylprop-2-ynloxy)-coumarin derivatives by heating in N,N-dimethylaniline afforded linear pyranocoumarins (Fig. 12).

4A.2.4. By novel domino reaction

Meng et al., reported a novel domino reaction catalyzed by triphenylphosphine for synthesis of the highly functionalized chroman derivatives. A γ-CH₃ of allenoate undergoes cyclization to form chroman derivative as shown in figure 13.

4A.2.5. By Mitsunobu reaction

A general two-step synthesis of the 2-substituted chroman ring system based on an intermolecular Mitsunobu reaction between 2-bromophenol and chiral halopropanols, followed by cyclization to the chroman has been reported by Hodgetts (Fig. 14).
4A.3 Earlier methods of synthesis of chromene

4A.3.1 By one-pot tandem reactions

Xie et al.,\textsuperscript{42} reported the asymmetric synthesis of functionalized 2-amino-2-chromene derivatives with high enantioselectivities via one-pot tandem reactions. The reaction of unsaturated ketones with malononitrile catalyzed by 9-amino-9-deoxyepiquinine in combination with (R)-1,1'-binaphth-2,2'-diyl hydrogen phosphate (Fig 15) gave the chromane derivatives A and B as shown below.

![Catalyst](image)

\begin{center}
\textbf{Figure 15.}
\end{center}

4A.3.2. By Using Ring-Closing Olefin Metathesis

Chang et al.,\textsuperscript{43} reported a practical and highly efficient procedure for the preparation of a diverse chromene derivatives using ring-closing metathesis (RCM) as shown below. This methodology is emerging as a new tool in synthetic organic chemistry (Fig. 16).

![Catalyst](image)

\begin{center}
\textbf{Figure 16.}
\end{center}
Adler et al.\textsuperscript{44} reported a novel and direct method to synthesis a variably substituted 2,2-dimethyl-2H-chromenes regioselectively under microwave irradiation in CDCl\textsubscript{3}. This protocol offers a mild reaction environment (neutral, no added catalyst) and displays tolerance toward acid- and base-sensitive protecting groups (Fig. 17).

**Figure 17.**

\textbf{4A.3.3. By Baylis-Hillman methodology}

Kaye et al.\textsuperscript{45} reported the chemoselective synthesis of the chromene derivatives from activated alkenes such as vinyl ketones, sulphones or sulphonates, in which the activating group (R3) effectively inhibits cyclization via the acyl substitution pathway (Fig. 18). Thus, a salicyaldehyde derivative on reaction with activated alkene in the presence of DABCO, results in a Baylis-Hillman products, which further undergoes cyclization and dehydration to give the chromene derivatives.

**Figure 18.**

\textbf{4A.4. Earlier methods of synthesis of pyrrolidine ring}

The synthesis of pyrrolidine ring commonly involves a ring closure process\textsuperscript{46-48} and intermolecular cyclization involving 3+2 cycloaddition.\textsuperscript{49,50} Among these, few of the general methods used for the preparation of pyrrolidines are described as below.

\textbf{4A.4.1. Synthesis of pyrrolidine ring by cyclization of alkenes}

\textbf{4A.4.1.1 Acid (H\textsuperscript{+}) catalyzed cyclization}

Schlummer and co-workers\textsuperscript{51} have carried out cyclization of the N-substituted
aminoalkenes in the presence of triflic or sulfuric acid at 100 °C, to afford respective pyrrolidine in excellent yield (Fig. 19).

![Figure 19.]

**4A.4.1.2. Palladium-catalyzed cyclization**

Wolfe et al.⁵² have reported the stereoselective synthesis of N-acyl- and N-Boc-protected pyrrolidines via palladium catalyzed reactions of γ-(N-acylamino) alkenes and γ-(N-Boc-amino) alkenes with aryl bromides. Generally high level of diastereoselectivity was reported in this reaction (Fig. 20).

![Figure 20.]

**4A.4.1.3. Ruthenium catalyzed cyclization**

Ruthenium catalysed⁵³ cyclization of α,ω-dienes is an efficient method for the construction of pyrrolidine ring. The reaction of N,N-diallyl-p-toluenesulfonamide with one equivalent of trimethyl(vinylxoy)silane in presence of a Ru-catalyst (5 mol%) at ambient temperature led to the formation of two products in 65% and 21% yield respectively (Fig. 21).

![Figure 21.]
Fustero et al.\textsuperscript{54} have developed the first cross metathesis for the synthesis of 2,5-substituted pyrrolidines, catalyzed by a Hoveyda-Grubbs second generation catalyst Cl\textsubscript{2}(IMes)Ru=CH(o-i-PrOC\textsubscript{6}H\textsubscript{4})/BF\textsubscript{3}.OEt\textsubscript{2}. Thus a vinyl ketone on reaction with N-protected alkene derivative in the presence of Grubbs catalyst results into pyrrolidine derivative (Fig. 22).

\begin{equation}
\text{O} \quad + \quad \text{R}_1 \text{R}_2 \text{R}_3 \quad \text{NHCbz} \quad \xrightarrow{[\text{Ru}]\text{Lewis acid}} \quad \text{Solvent/\textDelta or microwave} \quad \text{R}_1 \text{R}_2 \text{R}_3 \quad \text{N} \quad \text{Cbz}
\end{equation}

**Figure 22.**

**4A.4.1.4. Ionic iodine atom transfer cyclization**

\textit{N}-Tosyl pyrrolidine derivatives can effectively be synthesized from \(\gamma\)-iodoolefins and chloramine-T (CT) in acetonitrile at room temperature. The cyclization proceeds with high stereoselectivity via a cyclic iodonium intermediate\textsuperscript{55} (Fig. 23).

\begin{equation}
\text{R}_1 \text{R}_2 \text{I} \quad + \quad \text{Cl} \quad \text{N} \quad \text{S} \quad \text{O} \quad \text{O} \quad \text{MeCN, rt, 48 h} \quad \xrightarrow{\text{MeCN, rt, 48 h}} \quad \text{Ts R}_1 \text{R}_2 \text{I}
\end{equation}

**Figure 23.**

**4A.4.1.5. Osmium catalysed cyclization**

The osmium-catalyzed\textsuperscript{56} oxidative cyclization of 1,4-dienes derived from amino alcohol initiators is an effective method for the synthesis of pyrrolidines. The cyclization of enantiopure \textit{syn}- and \textit{anti}-amino alcohols resulted enantiopure \textit{cis}- and \textit{trans}-2,5-disubstituted pyrrolidines, respectively (Fig 24).

\begin{equation}
\text{OH} \quad \text{NHTs} \quad \xrightarrow{\text{catalytic Os (IV)}} \quad \text{HO}_2^- \quad \text{anti aminoalcohol}
\end{equation}

\textit{trans} pyrrolidine, >95 % ee

**Figure 24.**
4A.4.1.6. Gold catalysed cyclization

A mild and effective hydroamination of unactivated olefins catalysed by Au[P(tBu)$_2$(O-biphenyl)Cl] and AgOTf complex in 1:1 has been reported to offer substituted pyrrolidine derivative in good yields and enantioselectively.\(^5^7\) The hydroamination of alkenes in presence of Au[P(tBu)$_2$(O-biphenyl)Cl] and AgOTf complex in 1:1 afford pyrrolidine or piperidine derivatives. The treatment of an N-4-pentenyl or N-5-hexenyl urea with a catalytic 1:1 mixture of a gold(I) N,N-diaryl imidazol-2-ylidine complex and AgOTf gave the corresponding nitrogen heterocycle\(^5^8\) (Fig. 25).

![Figure 25.](image)

4A.4.1.7. Copper catalyzed cyclization

Chemler et al\(^5^9\) have reported a diastereoselective copper-promoted intramolecular aminooxygenation of various alkene substrates to give different in good yields and diastereoselectivity. The synthetic utility of the method was demonstrated by a short and efficient formal synthesis of (+)-monomorine (Fig. 26).

![Figure 26.](image)
4A.4.2. Synthesis of pyrrolidine ring by cyclization of allenes

An asymmetric intramolecular hydroamination of allenes to give vinyl pyrrolidine and piperidine under the influence of phosphine gold(I)-bis-p-nitrobenzoate complexes as catalyst has been reported by Toste et al.\textsuperscript{60} as shown below (Fig 27).

![Catalyst: P(3,5-xylyl)\textsubscript{2}AuPnB, P(3,5-xylyl)\textsubscript{2}AuPnB, OPnB: p-nitrobenzoate](image)

Widenhoefer et al.\textsuperscript{61} have also reported the Au(I)-catalyzed intramolecular hydroamination of N-allenyl carbamates as an effective method to prepare different cyclic amines as shown above (Fig. 27).

4A.4.3. Via MCR of aldehydes, amines and 1,1-cyclopropanediesters

Kerr et al.\textsuperscript{62} have prepared pyrrolidine derivatives by three component reaction of aldehyde, amines and 1,1-cyclopropanediesters. The reaction involves an \textit{in situ} generation of aldimines, followed by the reaction of 1,1-cyclopropanediesters in presence of a Lewis acid Yb(OTf)\textsubscript{3}. The major diastereomer formed has substituents at the C-2 and C-5 substituents in \textit{cis} geometry (Fig 28).

![Figure 28.](image)
4A.4.4. Via aza-payne rearrangement

The aza-payne rearrangement\(^{63}\) of 2,3-aziridin-1-ols under basic reaction conditions favours the formation of epoxy amines which on subsequent nucleophilic attack of the epoxide by the ylide results in a bis-anion. The latter upon 5-exo-tet ring-closure yields respective pyrrolidine derivatives (Fig. 29).

4A.4.5. Synthesis of pyrrolidine by double-Michael reaction

Sriramurthy et al.\(^{64}\) reported a stereoselective synthesis of pyrrolidines via aminoacid-derived pronucleophiles as Michael donors and electron deficient acetylenes as Michael acceptors in presence of bisphosphine-catalyst (Fig. 30).

4A.4.6. By [3 +2] annulation method

Polyfunctionalized pyrrolidines were synthesized by stereoselective [3+2]-annulation of 1,3-bis(silyl)propenes and N-Ts-R-amino aldehydes followed by desilylation and stereospecific Tamao-Fleming oxidation as shown below (Fig. 31).\(^{65,66}\)

![Figure 29.](image-url)

![Figure 30.](image-url)

![Figure 31.](image-url)
4A.4.7. By alkenylative cyclization

Zhao et al.\textsuperscript{67} reported a divalent palladium-catalyzed intramolecular enyne coupling reactions initiated by acetoxypalladation of alkynes. The reaction involves the acetoxypalladation of the alkyne, followed by insertion of the alkene. The protonolysis of the carbon-palladium bond in the presence of bidentate nitrogen ligands is a key step during the Pd(II) catalytic cycle (Fig. 32). The method is useful to construct a variety of synthetically important pyrrolidines.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure32.png}
\caption{Figure 32.}
\end{figure}

4A.4.8. By iodo-aldol cyclization

The N-Ts linked enoate aldehydes and ketones undergo intramolecular iodo-aldol cyclization to give N-tosylated pyrrolidine (Fig. 33). The reactions occur in good yields and are highly selective for the \textit{trans}-products.\textsuperscript{68}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure33.png}
\caption{Figure 33.}
\end{figure}

4A.4.9. By reduction of substituted pyrroles

The heterogeneous catalytic hydrogenation of highly substituted pyrroles in the presence of 5\% Rh/Al\textsubscript{2}O\textsubscript{3} affords fully reduced functionalized pyrrolidines (Fig 34). The reaction resulted in excellent diastereoselectivity.\textsuperscript{69}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure34.png}
\caption{Figure 34.}
\end{figure}
4A.4.10. By CP*Ir complex-catalyzed cyclization

The Cp*Ir complex catalyzed N-heterocyclization of primary amines with alkane diols resulted into five membered cyclic amines in good to excellent yields along with water as a by product of the reaction\(^7\) (Fig. 35).

\[
R_1\text{NH}_2 + R_2\text{OH} \xrightarrow{\text{cat. [Cp*IrCl}_2/\text{NaHCO}_3, \text{toluene/90-110 °C}}} R_1\text{N}R_2
\]

Figure 35.

4A.4.11. By N-heterocyclization of primary amines and hydrazines with dihalides

A simple and efficient microwave irradiated synthesis of nitrogen-containing heterocycles from alkyl dihalides (ditosylates) and primary amines via cyclocondensation in an alkaline aqueous medium was reported by Ju\(^7\) (Fig. 36).

\[
R_1\text{NH}_2 + X\text{X}_n \xrightarrow{\text{aq. K}_2\text{CO}_3, \text{MW}} R_1\text{N}\text{X}_n
\]

Figure 36.

4A.4.12. Organocatalytic approach to enantioselective synthesis of pyrrolidine

An enantioselective organocatalytic, one-pot synthesis of pyrrolidine, core structures was realized starting from readily available glycine esters by combination with several different organocatalytic reactions (Fig. 37).\(^7\)

\[
\text{Hantzsch ester} \quad \text{CF}_3\text{COOH, EtOH/H}_2\text{O} \quad 60 \text{ °C, 24 h}
\]

Figure 37.
4A.4.13. By 1,3-dipolar cycloaddition of azomethine ylides

Galley et. Al., reported highly regioselective synthesis of pyrrolidines by using azomethine ylides and chiral unsaturated ketones in the presence of DBU/AgOAc as catalyst (Fig. 38).

\[
\begin{align*}
\text{Figure 38.}
\end{align*}
\]

Recently in our group J. Pandey et al. reported a diastereoselective synthesis of tetrasubstituted glycosyl pyrrolidines by 1,3-dipolar cycloaddition of azomethine ylides and glycosyl olefins. 1,3-Dipolar cycloaddition of \textit{in situ} generated azomethine ylides in presence of LDA with glycosyl \textit{E}-olefins led to diastereoselective formation of C-glycosylated proline esters in very good yields (Fig. 39).

\[
\begin{align*}
\text{Figure 39.}
\end{align*}
\]

4A.5 Basis of Work

Hybrid molecules with two or more than two pharmacophore is gaining moment now a days in drug development. As mentioned above both chroman and pyrrolidine derivatives possess a wide range of biological activities. The antitubercular activity in both the scaffolds prompted us to synthesize chromanylated and chromenylated pyrrolidines and evaluate them antitubercular activity.
4A.6. Present work

The present work describes the hybridization of two antitubercular pharmacophore (pyrrolidine and chroman) to get novel molecules and their bio­evaluations\textsuperscript{76,77} against \textit{M. tuberculosis} H37Ra and \textit{M. tuberculosis} H37Rv. The compounds have been synthesized by [3+2] cycloaddition of chroman derived nitrostyrene, nitrochromene with benzylidene ethyl glycinites.

4A.7. Synthesis of tetrasubstituted pyrrolidines

The compounds synthesized in this section are-

- Ethyl (2\textit{R},3\textit{R},4\textit{S},5\textit{S}) 3-(2,2-dimethylchroman-6-yl)-4-nitro-5-phenylpyrrolidine-2-carboxylate (88),
- Ethyl (2\textit{R},3\textit{R},4\textit{S},5\textit{S})-3-(2,2-dimethylchroman-6-yl)-4-nitro-5-(4-methoxyphenyl) pyrrolidine-2-carboxylate (89),
- Ethyl(2\textit{R},3\textit{R},4\textit{S},5\textit{S})-3-(2,2-dimethylchroman-6-yl)-4-nitro-5-(3-nitrophenyl) pyrrolidine-2-carboxylate (90),
- Ethyl (\textit{I}S,3\textit{S},3\textit{a}R,9\textit{b}S)-3,4-bis(4-methoxyphenyl)-3\textit{a}-nitro-1,2,3,3\textit{a},4,9\textit{b}-hexahydrochromeno [3,4-c]pyrrole-1-carboxylate (91),
- Ethyl (\textit{I}S,3\textit{S},3\textit{a}R,9\textit{b}S)-4-(4-methoxyphenyl)-3\textit{a}-nitro-3-(4-nitrophenyl)-1,2,3,3\textit{a},4,9\textit{b}-hexahydrochromeno[3,4-c]pyrrole-1-carboxylate (92).

The synthetic strategy begins with readily available 4-hydroxybenzaldehyde (79). The latter was reacted with 2-methyl-1,3-butadiene (isoprene) in the presence of H\textsubscript{3}PO\textsubscript{4} to give 2,2-dimethylchroman-6-carboxaldehyde (80).\textsuperscript{78} The reaction of compound 80 with nitromethane in the presence of ammonium/acetic acetic acid at 80 °C afforded (\textit{E})-2,2-dimethyl-6-(2-nitrovinyl)chroman (81) (Scheme 1).\textsuperscript{79}

\begin{center}
\includegraphics[width=\textwidth]{Scheme_1}
\end{center}

The structure of compound 81 was established on the basis of its spectroscopic data and analysis. FABMS of the compound showed a peak at m/z 234 [M+H]\textsuperscript{+} corresponding to its molecular formula C\textsubscript{13}H\textsubscript{15}NO\textsubscript{3}. In \textsuperscript{1}H NMR spectrum both the olefinic protons were observed as doublet at \(\delta\) 7.92 (1H, \(J = 13.48\) Hz) and \(\delta\) 7.49 (1H, \(J = 13.48\) Hz) while the
two aromatic protons were appeared as multiplet in the range of $\delta$ 7.29-7.25. Another aromatic proton was seen as doublet at $\delta$ 6.80 with coupling constant 8.3 Hz. The four methylene protons were visible as two distinct triplets at $\delta$ 2.82 (2H, $J = 6.4$ Hz) and $\delta$ 1.86 (2H, $J = 6.4$ Hz). The six protons of the gem dimethyl group of pyran ring appeared as singlet at $\delta$ 1.35. In $^{13}$C NMR spectrum the olefinic carbons were observed at $\delta$ 139.4 and $\delta$ 135.0 while the aromatic carbons appeared at $\delta$ 158.4, 131.7, 129.0, 122.1, 122.0 and 118.9. The two methylene carbons were seen at $\delta$ 32.8 and $\delta$ 22.6. The two methyl carbons were observed at $\delta$ 27.3.

However, 2-(4-methoxyphenyl)-3-nitro-2H-chromene (84) was prepared by modification of the earlier reported method by neat reaction of 2-aryl-nitroethylene (83) with salicylaldehyde in the presence of DBU (Scheme 2).

![Scheme 2.](image)

The structure of compound 84 was established by its spectroscopic data. FABMS of the compound showed a peak at m/z 284 [M+H]$^+$ corresponding to its molecular formula C$_{16}$H$_{13}$N$_{14}$O$_4$. In $^1$H NMR spectrum the H-2 and H-4 protons of the chromene ring appeared as singlet at $\delta$ 6.51 and $\delta$ 8.03 respectively while four aromatic protons were visible as multiplet in the range of $\delta$ 7.34-7.25. The triplet of one aromatic proton was observed as doublet at $\delta$ 7.02 with $J = 7.44$ Hz while rests of the three aromatic protons appeared as multiplet in the range of $\delta$ 6.85-6.79. The methoxy protons were observed as singlet at $\delta$ 3.75. In $^{13}$C NMR spectrum the C-2 carbon of chromene ring was observed at $\delta$ 75.5 while methoxy carbon was seen at $\delta$ 55.4. The rest of the aromatic carbons and chromene ring carbons were visible in the range of $\delta$ 112.2-159.0.

Syntheses of the required dipole intermediate benzylidine ethyl glycinates (87a-87d) were carried out by reacting ethyl glycinate hydrochloride (86) with different aromatic aldehydes (85a-85d) in the presence of triethylamine/anhy. MgSO$_4$ in CH$_2$Cl$_2$ (Scheme 3, Table 1).
Table 1. Synthesis of benzylidine ethyl glycinates (87a-87d)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>R₁</th>
<th>R₂</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>87a</td>
</tr>
<tr>
<td>2</td>
<td>OCH₃</td>
<td>H</td>
<td>87b</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>NO₂</td>
<td>87c</td>
</tr>
<tr>
<td>3</td>
<td>NO₂</td>
<td>H</td>
<td>87d</td>
</tr>
</tbody>
</table>

The substituted benzylidine ethyl glycinates (87a-87d) were used as such without purification for the subsequent (3+2) cycloaddition reaction with (E)-2,2-dimethyl-6-(2-nitrovinyl)chroman 81 (Scheme 3). The azomethine ylide of benzylidine ethyl glycinates 87a was generated in situ by reacting it with DBU/LiBr in acetonitrile at room temperature. Reaction of azomethine ylide of 87a with (E)-2,2-dimethyl-6-(2-nitrovinyl)chroman (81) in the presence of triethylamine, anhy. MgSO₄ at rt afforded the desired product ethyl-3-(2,2-dimethylchroman-6-yl)-4-nitro-5-phenylpyrrolidine-2-carboxylate (88), along with small amount of other isomer as observed on TLC (Scheme 4). The major product was isolated by column chromatography and was characterized on the basis of its spectroscopic data. The stereochemistry in compound 88 was speculated on the basis of literature precedents and the mechanism (Fig. 39). It has earlier been reported by Galley et al that in such cycloaddition reaction involving a chiral dienophile and azomethine ylide the relative orientation of substituents in major isomer at C2/C3 and C3/C4 is anti and at C4/C5 is syn. Furthermore stereochemistry is attained via regiospecific endo cycloaddition of the W-shaped dipole to the E configured dipolarophiles in the ester series.
**Figure 39.** Reaction mechanism and Houk’s model of the transition state for the formation of major diastereomer.

Formation of the above major diastereomer 10 can be explained by Houk’s model or Houk’s inside alkoxy effect. The transition state for the formation of major diastereomer (88) involves attack of the W-shaped ylide with the E configured olefin oriented away from the most bulky chromanyl moiety. The ethoxy substituent occupies the stereoelectronically favoured inside position and the small H atom the crowded outside region while the attack of ylide from other side of olefin, the less stable transition structure would be possible because of unfavourable steric interactions (Figure 38).

**Scheme 4.**

The structure of compound 88 was established by its spectroscopic data. FABMS of the compound showed a peak at m/z 425 [M+H]+ corresponding to its molecular formula C$_{24}$H$_{28}$N$_2$O$_5$. In $^1$H NMR spectrum the aromatic protons were observed as two distinct doublets and one multiplets at $\delta$ 6.75 (d, 1H, $J = 8.2$ Hz), 6.96 (d, 2H, $J = 9.2$ Hz), 7.25-7.43 (m, 5H) respectively. The one proton of pyrrolidine ring was observed as doublet at around $\delta$ 5.17 (d, $J = 2.6$ Hz) while the rest of the three protons of pyrrolidine ring were observed as two separate multiplet in the range of $\delta$ 4.67 (m, 1H) and $\delta$ 3.98-4.06 (m, 2H) respectively. The multiplet of two protons corresponding to OCH$_2$ of ethyl ester groups were observed at $\delta$ 4.24-4.32. The four methylene protons of benzopyran ring were
observed as two distinct triplet at δ 2.73 (t, 2H, J = 7.7 Hz) and at δ 1.84 (t, J = 7.6 Hz, 2H). The nine methyl protons of benzopyran ring and ethyl ester group appeared as multiplet in the range of δ 1.34-0.82. In 13C NMR spectrum the carbonyl carbon of ester group was observed at δ 171.8 while all the aromatic carbons were observed in the range of δ 118.44-154.31. The pyrrolidine ring carbons were seen at δ 68.04, 80.18 (C), and 97.58. The three methylene carbons were observed at δ 61.93 (OCH₂), δ 48.12 (CH₂), δ 33.07 (CH₂) while the three methyl carbons were observed at δ 55.4 (OCH₃), 14.68, 13.92 (CH₃). Similarly, reaction of the nitrostyrene 81 with the benzylidene ethyl glycinate 87b and 87c separately as above led to the formation of the desired products 89 and 90 respectively in good yields (Scheme 5). The structure of the compounds 89 and 90 were established on the basis of their spectroscopic data. The FABMS of compounds 89 and 90 displayed [M+H]+ peaks at 455, 470 corresponding to their molecular formulae C24H27O7N3, C25H30O6N2 respectively. The 1H NMR, 13C NMR and IR spectral data were similar to that for compound 88 described above. The detail one available in experimental section.

\[ \text{EtO}_2 \text{N} = \text{CH}_3, \text{R} = \text{H} \]

**Scheme 5.**

In another set of the reaction 3-nitrochromene derivative (84) reacted with benzylidene ethylglycinate 87a and 87b separately under above reaction condition to give ethyl-4-(4-methoxyphenyl)-3a-nitro-3-(4-methoxyphenyl)-1,2,3,3a,4,9b-hexahydrochromeno[3,4-c]pyrrole-1-carboxylate (91) and ethyl-4-(4-methoxyphenyl)-3a-nitro-3-(4-methoxyphenyl)-1,2,3,3a,4,9b-hexahydrochromeno[3,4-c]pyrrole-1-carboxylate 92 respectively (Scheme 6).
The structure of compound 92 was established by its spectroscopic data and analysis. FABMS of the compound showed a peak at m/z 520 [M+H]^+ corresponding to its molecular formula C_{28}H_{28}N_{2}O_{7}. In $^1$H NMR spectrum the aromatic protons were observed as three doublets at δ 6.60 (d, 2H, J = 8.6 Hz), δ 6.76 (d, 1H, J = 7.9 Hz), 8.19 (d, 2H, J = 8.6 Hz); and two triplets δ 6.96-7.19 (m, 5H), δ 7.46-7.55 (m, 2H). The three protons of pyrrolidine ring were observed at δ 4.06 (d, 1H, J = 3.7 Hz), δ 4.98 (s, 1H) and δ 5.41 (s, 1H) while the methylene proton (OCH$_2$) appeared as quartet at δ 4.35 (J = 7.08 Hz). The methoxy protons were observed as singlet at δ 3.70, the only exchangeable proton NH was seen as multiplet at δ 2.91 while the methyl protons of the ethoxy group were observed as triplet at δ 1.50 (J = 7.1 Hz). In $^{13}$C NMR spectrum the carbonyl carbon of ester group was observed at δ 172.68 while the all aromatic carbons were observed in the range of δ 114.4-160.4. The pyrrolidine ring carbons were appeared at δ 68.95, 75.58 and 45.34. The methylene carbon (OCH$_2$) was observed at δ 62.69 while two methoxy carbons and one methyl carbon observed at δ 55.54 (OCH$_3$), 55.55(OCH$_3$) and 14.76 (CH$_3$) respectively.

4A.8. Biological activity

All synthesized compounds were evaluated against *M. tuberculosis* H37 Ra and *M. tuberculosis* H37 Rv strains following earlier protocol.$^{74,75}$

As evident from table 2, among all of the tested compounds, compounds 88, 90 and 92 were found to be active at MIC 12.5 µg/ml against *M. tuberculosis* H37Rv while compounds 89 and 91 were not active at 12.5 µg/ml concentration. Further none of them active against *M. tuberculosis* H37Ra as MIC value were >12.5 µg/ml. Although with this limited number of compounds, no definite structure activity relationship could be established, yet the activity against virulent strain of mycobacterium reveals that compounds are specific to virulent strain of mycobacterium.
Table 2. Antitubercular activity of synthesized compounds (88-92) against *M. tuberculosis*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>MIC (µg/ml) against <em>M. tuberculosis</em></th>
<th>MIC (µg/ml) against <em>M. tuberculosis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H37Rv</td>
<td>H37Ra</td>
</tr>
<tr>
<td>1</td>
<td>88</td>
<td>12.5</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
<td>&gt;12.5</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>12.5</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>4</td>
<td>91</td>
<td>&gt;12.5</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>5</td>
<td>92</td>
<td>12.5</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>6</td>
<td>Isoniazid</td>
<td>0.75</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Ethambutol</td>
<td>3.25</td>
<td>-</td>
</tr>
</tbody>
</table>

*ND = Not done, MIC = Minimum inhibitory concentration*

**4A.9. Experimental Section**

Commercially available reagent grade chemicals were used as received. All reaction was followed by TLC on E. Merck Kieselgel 60 F<sub>254</sub>, with detection by UV light, spraying a 20% KMnO<sub>4</sub> aq solution. Column chromatography was performed on silica gel (60-120 mesh & 100-200 mesh, E. Merck). IR spectra were recorded as thin films or in KBr soln with a Perkin Elmer Spectrum RX-1 (4000-450 cm<sup>-1</sup>) spectrophotometer. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Brucker DRX -300 or 200 in CDCl<sub>3</sub> or CDCl<sub>3</sub> + CCl<sub>4</sub>. Chemical shift values are reported in ppm relative to TMS (tetramethylsilane) as internal reference, unless otherwise stated; s (singlet), d (doublet), t (triplet), m (multiplet), dd (double doublet); *J* in hertz. ESI mass spectra were performed using Quattro II (Micromass). Elemental analyses were performed on a Perkin-Elmer 2400 II elemental analyzer.

**4A.9.1. Preparation of 2,2-di-methyl chroman-6-carbaldehyde (80)**

To a solution of 4-hydroxybenzaldehyde (1 g, 8.19 mmol), orthophosphoric acid (0.94 mL, 16.39 mmol) in petroleum ether (10 mL), isoprene (1.64 mL, 16.39 mmol) was added slowly at 0 °C. The reaction mixture was stirred at room temperature for 16 h. After completion of reaction (TLC), the reaction mixture was pour into beaker containing crushed ice and sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried over sodium sulphate then concentrate under reduced pressure. Purification of the residue by silica gel chromatography (EtOAc/n-hexane, 1:3) provided 2,2-di-methyl chroman-6-carbaldehyde 2 as liquid, 0.8g, 51.3 %. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ 1.35
Chapter 4A  Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation

(s, 6H, CH₃x2), 1.81-1.85 (m, 2H, CH₂), 2.82 (t, 2H, J = 6.7 Hz, OCH₂), 6.85 (d, 1H, J = 9.0 Hz, ArH), 7.58-7.61(m, 2H, ArH), 9.80 (s, 1H, CHO).

4A.9.2. Preparation of (Z)-2,2-dimethyl-6-(2-nitrovinyl)chroman (81)

To a stirred solution of 2,2-dimethylchroman-6-carbaldehyde 80 (1g, 5.26 mmol), nitromethane (5 mL, as reagent and solvent), ammonium acetate (0.41g, 5.26 mmol) and acetic acid (1mL) was heated at 80 °C for 4h. After completion of reaction (TLC), the reaction mixture was poured onto ice, filtered and the filtrate was concentrated under reduced pressure and extracted with ethyl acetate. The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure to get compound 81 as syrup. Yield 0.8 g (65.23 %). FT-IR (KBr, cm⁻¹) 1657, 1256; ¹H NMR (200MHz, CDCl₃) δ 1.35 (s, 6H, C(CH₃)₂), 1.86 (t, 2H, J = 6.4 Hz, CH₂), 2.82 (t, 2H, J = 6.4 Hz, CH₂), 6.80 (d, 1H, J = 8.3 Hz, ArH), 7.25-7.29 (m, 2H, ArH), 7.42 (d, 1H, J = 13.4 Hz, CH=), 7.85 (d, 1H, J = 13.4 Hz, CH=). ¹³C NMR (50 MHz, CDCl₃) δ 22.6 (CH₂), 27.3 (CH₃), 32.8 (CH₂), 118.9 (ArCH), 122.1 (ArC), 129.0 (ArCH), 131.7, 135.0, 139.4 (ArCH), 158.4 (ArC); ESMS (m/z): 234 [M+ H]⁺.

4A.9.3. Preparation of 2-(4-methoxyphenyl)-3-nitro-2H-chromene (84)

To a stirred solution of (E)-1-methoxy-4-(2-nitrovinyl)benzene 83 (1g, 5.58 mmol), salicyaldehyde (0.88g, 6.13 mmol), 0.242 mL of DBU (30 mol %) was added at room temperature. After completion of reaction (TLC), the reaction mixture was extracted with ethyl acetate. The organic layer was dried (Na₂SO₄) and concentrated under reduced pressure to get compound 84 as yellow solid. Yield 1.3 g (82.2 %). FT-IR (KBr, cm⁻¹) 1657, 1256; ¹H NMR (200MHz, CDCl₃) δ 3.75 (s, 3H, OCH₃), 6.51 (s, 1H, H-2), 6.79-6.85 (m, 3H, ArH), 6.98-7.02 (m, 1H, ArH), 7.25-7.34 (m, 4H, ArH), 8.03 (s, 1H, H-4), ESMS (m/z): 234 [M+ H]⁺.

4A.9.4. Preparation of benzylidene ethyl glycinates 87a-87d

To the stirred solution of ethyl glycinate hydrochloride salt (2 eq.) and anhydrous MgSO₄ in dry CH₂Cl₂, aromatic aldehyde (1 eq.) was added and reaction mixture was further stirred for 3-4 h at ambient temperature. After the completion of the reaction, the reaction mixture was filtered and concentrated under reduced pressure to get the desired
benzylidene ethyl glycinates (87a-87d). The freshly prepared benzylidene ethyl glycinates (87a-87d) were used as such without purification.

**4A.9.2. General procedure for preparation of compound (88-92)**

The solution of DBU (10 mol%) and LiBr (10 mol%) in anhydrous THF (solvent) was stirred magnetically at 0 °C under an inert (N₂) atmosphere for about 10 min., [(4-metroxybenzylidene)-amino]-acetic acid ethyl ester (1 mol) in anhyd. THF was slowly added drop wise to the above stirring reaction mixture at 0°C and stirring continued for further 30 min. After complete addition the reaction mixture was brought to room temperature and solution of compound 2 (1 mol) in anhyd. THF was slowly added drop wise. The reaction mixture was stirred at room temperature for further 1 hrs. After completion of the reaction (TLC), the reaction mixture was extracted with ethyl acetate (2 x 70 mL), the organic layer was dried (anh. Na₂SO₄) and evaporated under reduced pressure to get a crude product. The latter was chromatographed (SiO₂ column, 60-120 mesh) using a gradient of hexane: ethyl acetate (6:4) to give the desired compound 3 as liquid.

**4A.9.2.2. Ethyl (2R,3R,4S,5S) 3-(2,2-dimethylchroman-6-yl)-4-nitro-5-phenylpyrrolidine-2-carboxylate (88)**

It was obtained by the reaction of compound 81 (0.5 g, 2.14 mmol), [(benzylidene)-amino]-acetic acid ethyl ester (0.41g, 2.14 mmol) and DBU-LiBr (1:1, 10 mol%) as liquid, 0.40 g, in 43.9 % yield. FT-IR (KBr, cm⁻¹) 3401, 1728, 1603; ¹H NMR (200MHz, CDCl₃) δ 0.82-1.34 (m, 9H, OCH₂CH₃ & C(CH₃)₂), 1.84 (t, J =7.6 Hz, 2H, CH₂), 2.73 (t, 2H, J = 7.7 Hz, CH₂), 3.98-4.06 (m, 2H), 4.24-4.32 (m, 2H, OCH₂), 4.67 (m, 1H, CH), 5.17 (d, 1H, J = 2.6 Hz, CH), 6.75 (d, 1H, J = 8.2 Hz, ArH), 6.96 (d, 2H, J = 9.2 Hz, ArH), 7.25-7.43 (m, 5H, ArH); ¹³C NMR (50 MHz, CDCl₃) δ 13.92, 14.68 (CH₃), 22.9 (CH₂), 27.3, (CH₃), 33.07 (CH₂), 48.12 (CH₂), 61.93 (OCH₂), 6804 (CH), 80.18 (C), 97.58 (CH), 118.44 (ArCH), 121.80 (ArC), 126.58 (ArCH), 128.44, 128.11 (ArC), 128.80, 129.09, 129.20 (ArCH), 130.01, 135.08, 139.09 (ArC), 154.31 (ArC), 171.8 (COOEt); ESMS (m/z): 425 [M+ H]+; Elemental analysis for C₂₄H₂₈N₂O₅: C, 67.91; H, 6.65; N, 6.60 %; Found: C, 67.89; H, 6.69; N, 6.58.
4A.9.2.1. Ethyl (2R,3R,4S,5S)-3-(2,2-dimethylchroman-6-yl)-4-nitro-5-(4-methoxyphenyl)pyrrolidine-2-carboxylate (89)

It was obtained by the reaction of compound 81 (0.5 g, 2.14 mmol), [(4-methoxybenzylidene)-amino]-acetic acid ethyl ester (0.47g, 2.14 mmol) and DBU-LiBr (1:1, 10 mol%) as liquid, 0.65 g, in 66.6 % yield. FT-IR (KBr, cm⁻¹) 1657, 1730, 3402; ¹H NMR (200MHz, CDCl₃) δ 0.87 (t, 3H, J = 7.1 Hz, OCH₂CH₃), 1.22-1.33 (m, 6H, C(CH₃)₂), 1.79 (m, 2H, CH₂), 2.16 (bs, 1H, NH), 2.75 (t, 2H, J = 6.7 Hz, CH₂), 3.75-3.82 (m, 5H, OCH₃ & CH₂), 4.27-4.32 (m, 2H, OCH₂), 4.65 (d, 1H, J = 8.5 Hz, CH), 5.11 (m, 1H, CH), 6.70 (d, 1H, J = 8.2 Hz, ArH), 6.89-6.96 (m, 4H, ArH), 7.48 (d, 2H, J = 8.7 Hz); ¹³C NMR (50 MHz, CDCl₃) δ 14.68 (CH₃), 22.8 (CH₂), 27.3, 27.2 (CH₃), 33.14 (CH₂), 55.4 (OCH₃), 61.28 (OCH₂), 64.36, 67.53 (CH), 95.30 (C), 114.50, 114.80 (ArC), 117.98 (ArCH), 121.13 (ArC), 127.20 (ArCH), 128.31 (ArC), 128.53, 129.30 (ArCH), 129.96 (ArC), 154.31, 160.42 (ArC), 172.11 (COOEt); ESMS (m/z): 455 [M+ H⁺]; Elemental analysis for C₂₅H₃₀N₂O₆: C, 66.06; H, 6.65; N, 6.16; O, 21.12 %; Found: C, 66.10; H, 6.69; N, 6.13 %.

4A.9.2.3. Ethyl(2R,3R,4S,5S)-3-(2,2-dimethylchroman-6-yl)-4-nitro-5-(3-nitrophenyl) pyrrolidine-2-carboxylate (90)

It was obtained by the reaction of compound 81 (0.5 g, 2.14 mmol), [(3-nitrobenzylidene)-amino]-acetic acid ethyl ester (0511g, 2.14 mmol) and DBU-LiBr (1:1, 10 mol%) as liquid, 0.42 g, in 42.2 % yield. FT-IR (KBr, cm⁻¹) 3422, 1727, 1609; ¹H NMR (200MHz, CDCl₃) δ 1.26-1.34 (m, 9H, OCH₂CH₃ & C(CH₃)₂), 1.77 (t, J = 6.6 Hz, 2H, CH₂), 2.74 (t, 2H, J = 6.6 Hz, CH₂), 4.09-4.11 (m, 2H), 4.25-4.32 (m, 2H, OCH₂), 4.99 (d, 1H, CH), 5.24 (d, 1H, J = 3.7 Hz, CH), 6.74 (d, 1H, J = 8.1 Hz, ArH), 6.96 (m, 2H, ArH), 7.54-7.58 (d, 1H, J = 7.9 Hz, ArH), 7.70 (d, 1H, ArH), 8.16 (d, 1H, J = 8.17Hz, ArH), 8.28 (s, 1H, ArH); ¹³C NMR (50 MHz, CDCl₃) δ 14.61 (CH₃), 22.92 (CH₂), 27.30, 27.34 (CH₃), 33.03 (CH₂), 62.02 (OCH₂), 66.42, 67.33, 74.84 (CH), 96.76 (CH), 118.53 (ArCH), 121.94 (ArC), 122.67, 124.00, 126.55 (ArCH), 130.07, 132.78 (ArCH), 138.02, 148.70, 154.44 (ArC), 172.11 (COOEt); ESMS (m/z): 470 [M+ H⁺]; Elemental analysis for C₂₅H₂₇N₃O₇: C, 61.40; H, 5.80; N, 8.95; O, 23.85 %; Found: C, 61.35; H, 5.76; N, 8.90 %.
Chapter 4A  Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation

4A.9.2.4. Ethyl (1S,3S,3aR,9bS)-3,4-bis(4-methoxyphenyl)-3a-nitro-1,2,3,3a,4,9b-hexahydrochromeno[3,4-c]pyrrole-1-carboxylate (91)

It was obtained by the reaction of compound 84 (0.5 g, 1.76 mmol), [-(4-methoxybenzylidene)-amino]-acetic acid ethyl ester (0.39 g, 1.76 mmol) and DBU-LiBr (1:1, 10 mol%) as white solid, m. p. 125 °C, 0.54 g, in 60.8 % yield. FT-IR (KBr, cm⁻¹) 3431, 1729, 1596; ¹H NMR (200MHz, CDCl₃) δ 1.29 (t, 3H, J = 7.2 Hz, CH₃), 3.72-3.79 (m, 7H, OCH₃×2 & CH), 4.22-4.32 (q, 2H, J = 7.14 Hz, OCH₂CH₃), 5.38-5.41 (m, 2H, CH₂), 6.01 (s, 1H, CH), 6-67-7.12 (m, 11H, ArH), 8.16 (d, J = 8.0 Hz, ArH); ESMS (m/z): 505 [M+ H⁺]; Elemental analysis for C₂₅H₂₅N₂O₇: C, 66.66; H, 5.59; N, 5.55 % found: C, 66.63; H, 5.63; N, 5.52 %.

4A.9.2.5. Ethyl (1S,3S,3aR,9bS)-4-(4-methoxyphenyl)-3a-nitro-3-(4-nitrophenyl)-1,2,3,3a,4,9b-hexahydrochromeno[3,4-c]pyrrole-1-carboxylate (92)

It was obtained by the reaction of compound 84 (0.5 g, 1.76 mmol), [-(4-nitrobenzylidene)-amino]-acetic acid ethyl ester (0.41 g, 1.76 mmol) and DBU-LiBr (1:1, 10 mol%) as white solid, m. p. 179-180 °C, 0.49 g, in 54.5 % yield. FT-IR (KBr, cm⁻¹) 3385, 1740, 1602, 1545; ¹H NMR (200MHz, CDCl₃) δ 1.50 (t, 3H, J = 7.1 Hz, CH₃), 2.91 (m, 1H, NH), 3.70 (s, 3H, OCH₃), 4.06 (d, 1H, J = 3.7 Hz, CH), 4.35 (q, 2H, J = 7.08 Hz, OCH₂CH₃), 4.98 (s, 1H, CH), 5.41 (s, 1H, CH), 6.60 (d, 2H, J = 8.6 Hz, ArH), 6.76 (d, 1H, J = 7.9 Hz, ArH), 6.96-7.19 (m, 5H, ArH), 7.46-7.55 (m, 2H, ArH), 8.19 (d, 2H, J = 8.6 Hz, ArH); ¹³C NMR (50 MHz, CDCl₃) δ 14.76 (CH₃), 45.34, 55.55, 55.54 (OCH₃), 62.69 (OCH₂), 68.95, 75.58 (CH), 114.40, 118.77 (ArCH), 123.82, 124.42, 128.65, 129.22, 129.56, 129.98 (ArCH), 142.42, 149.03, 150.30, 160.44 (ArC), 172.68 (COOEt); ESMS (m/z): 520 [M+ H⁺]; Elemental analysis for C₂₅H₂₃N₃O₇: C, 62.42; H, 4.85; N, 8.09 found: C, 62.38; H, 4.80; N, 8.00.

4A.10. Bio-evaluation methods

4A.10.1. Activity against M. tuberculosis H₃⁷Ra strain

All of the synthesized compounds were evaluated for their efficacy against M. tuberculosis H₃⁷Ra at active concentration ranging from 50 µg/mL to MIC using two-fold dilutions in the initial screen. Log phase culture of M. tuberculosis H₃⁷Ra is diluted...
Chapter- 4A

Synthesis of chromanyl pyrroldine as hybrid molecules and their antitubercular evaluation

so as to give final OD550 nm of 0.05 in Sauton’s medium. In 96-well white plates 190 mL of culture is dispensed in each well. A dimethyl sulfoxide (DMSO) solution of test compounds is dispensed into 96-well plates so as to make final test concentration of 25 mg/mL (5 mg test compound is dispensed into 10 mL of DMSO). Then the plate is incubated at 37 °C/5% CO₂ for 5 days. On 5th day 15 mL Alamar Blue solution is added to each well of the plate. The plate is again incubated overnight at 37 °C/5% CO₂ incubator. The fluorescence is read on BMG polar star with excitation frequency at 544 nm and emission frequency at 590 nm. The compounds, which were found to be active (>90% inhibition as compared with control) at this concentration are then tested at 6 serial dilutions starting from 50 to 1.56 µg/mL.

4A.10.2. Activity against M. tuberculosis H37Rv strain

Drug susceptibility and determination of MIC of the test compounds/drugs against M. tuberculosis H37Rv were performed by agar microdilution method where two-fold dilutions of each test compound were added into 7H10 agar supplemented with OADC and organism. A culture of M. tuberculosis H37Rv growing on L–J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of 1 mg/mL concentration of extracts/compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 Middle Brook’s medium (containing 1.7 mL medium and 0.2 mL OADC supplement) at different concentrations of compound keeping the volume constant i.e. 0.1 mL. Medium was allowed to cool by keeping the tubes in slanting position. These tubes were then incubated at 37 °C for 24 h followed by streaking of M. tuberculosis H37Rv (5 \( \cdot 10^4 \) bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound.
4A.11. References


Chapter 4A

Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation


34. (a) Claisen, L.; Tictze, E. Ber., 1926, 59, 2344; (b) John, W.; Gunther, P. Ber., 1941, 74, 879.


56. Timothy J. Donohoe, Peter J. Lindsay-Scott, Jeremy S. Parker, Cedric K. A. Callens, Received January 8, 2010 organic letter Org. Lett., Vol. xx, No. x, XXXX
Chapter 4A  Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation


4.12. Copies of Mass, $^1$H NMR, $^{13}$C NMR spectra of selected compounds
Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation

ESMS of compound 89

\[
\text{MSD Channel} \quad 724.4958 \quad \text{RT:} \quad 0.40-0.59 \quad \text{AV:} \quad 4.1 \quad \text{SB:} \quad 2.00 \quad \text{NL:} \quad 3.99E-01
\]

\[
\text{m/z:} \quad 455.2, 455.4, 408.4, 409.4, 276.3, 354.4
\]

\[\text{1H NMR of compound 89}\]
Chapter 4A  Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation

$^{13}$C NMR of compound 89

ESMS of compound 90
Chapter 4A

Synthesis of chromanyl pyrrolidine as hybrid molecules and their antitubercular evaluation

$^1$H NMR of compound 90

$^{13}$C NMR of compound 90
Chapter-4B

Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents
Chapter-4B

Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

4B.1. Introduction

Five membered heterocyclic compounds such as oxazole\(^1\), oxazoline\(^2\), isoxazole\(^3\), dihydroisoxazole or isoxazoline\(^4\)-\(^{14}\) have occupied an important place among various classes of heterocyclic organic compounds due to their synthetic utility and wide range of biological activities.\(^1\)-\(^{14}\) The dihydroisoxazole is representative of the active pharmacophore in several biologically important molecules (Fig. 1) such as antifungal and antibacterial\(^4\), antitubercular\(^5\), siderophore analogs\(^6\), antidepressant\(^7\), \(\beta\)-galactosidase inhibitor\(^8\), aminoacyl synthetase inhibitor.\(^9\) The isoxazolines on the other hand are versatile intermediates for the synthesis of a variety of bioactive compounds.\(^10\),\(^11\) Owing to the labile nature of the N–O bond under mild reducing conditions, dihydroisoxazoles provide an easy access to a variety of fascinating 1,3-difunctional aminoalcohols\(^12\),\(^13\) which themselves are known for their antitubercular activities.

![Mycobactin analogue](image)

![\(\beta\)-Galactosidase inhibitor](image)

![Antitubercular](image)

![Antidepressant](image)

\textbf{Figure 1:} Representative biologically active molecules containing oxazoline and isoxazoline ring system

As discussed in chapter 1 several isoxazoline linked nitrofurans series (Chapter 1, Fig. 41) are potent antitubercular displaying \textit{in vitro} and \textit{in vivo} activities.
Chapter-4B Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

Linezolid, the parent molecule was the first commercially available oxazolidinone, showing activity against Gram-positive bacteria\textsuperscript{14} including multidrug- resistant bacteria by binding to 50S subunit of bacterial ribosome and thus blocking the synthesis of bacterial proteins.\textsuperscript{15} Further, several mycobacterial mycobactins contain this isooxazoline unit but the significance of the isooxazoline in mycobactins is still debated. Despite of unique ability to treat the bacterial infection, resistance against linezolid was reported in early 2001.\textsuperscript{16}

**Figure 2**: Diagram showing the lead optimization or generation by modifying structure of linezolid the parent hit by bioisosteric replacement of oxazolidinone ring to isooxazoline ring

To counteract the resistance problems several modifications in linezolid is reported recently by different research groups via simplifying the molecular diversity, isosteric replacement of oxazolidinone by isooxazoline ring and incorporating other pharmacophores (Fig. 2)

4B.2. Earlier methods of synthesis of isooxazoline ring

The synthesis of isooxazoline ring commonly involves a (3+2) cycloaddition reaction. Few of the general methods used for the preparation of isoxazoline are described as below.
4B.2.1. By phenylisocyanate mediated cyclization reaction

Nitroalkene which was prepared from racemic anhydride in a few steps, undergoes in situ nitrile oxide formation in the presence of PhNCO later on intramolecular cyclization to afford the racemic trans-hydrindane (Fig 3).\(^\text{17}\)

![Figure 3.](image)

4B.2.2. By sodium hypochlorite mediated cyclization reaction

The diastereomeric mixture of isoxazolines was achieved by the cycloaddition reaction of aldoxime with alkene in the presence of NaClO. In the synthesis of butenolides, (-)-mintlactone and (+)-isomintlactone, NaClO mediated cycloaddition is a key step (Fig. 4).\(^\text{18}\)

![Figure 4.](image)

Lygo \textit{et al} reported the construction of isoxazoline intermediate by using sodium hypochlorite and triethylamine in cyclization reaction of oxime and alkene. The reaction of an alkenyl oxime derived from D-ribose was conducted by exposing it to sodium hypochlorite, thereby affording the required oxazolidine (Fig. 5).\(^\text{19}\)

![Figure 5.](image)
4B.2.3. By N-chlorosuccinimide and triethylamine mediated cyclization reaction

Ray and co-workers have synthesized a pyrimidoazepinone derivative via intramolecular cycloaddition reaction of an oxime which generate oximoyl chloride with NCS to generate the corresponding oximoyl chloride, subsequent addition of base effected the generation of the nitrile oxide and its cycloaddition with the terminal olefin (Fig. 6).20,21

Figure 6.

In our group a diastereoselective synthesis of glycosylisoxazoline reported22 by using sugar derivative oxime and different alkenes in presence of NCS and DBU at 0°C-rt (Fig. 7). These compounds displayed antimicrobacterial activity.

Figure 7.
4B.2.4. By metal-catalysed reactions

4B.2.4.1. By Mg(II) cation catalysed reaction

Faita et al. have reported,\textsuperscript{23} Mg(II) cation catalysed 1,3-cycloadditions of supported Evans' chiral auxiliary with nitrile oxides to get a series of dihydroisoxazoles (Fig. 8).

\[
\text{Ms} - \equiv \text{N} - \overline{\text{O}} \quad \text{Mg(CIO}_4\text{)}_2
\]

\textbf{Figure 8.}

Jedlovska et al. have demonstrated\textsuperscript{27} the synthesis of 4-acyl-substituted C5/C6 anti isoxazolines by using MgX\textsubscript{2} (X = Br or I) in 1,3-dipolar cycloadditions of mesityl nitrile oxide to chiral unsaturated enones (Fig. 9).

\[
\text{R} = \text{Me}, \quad \text{X} = \text{Br} \text{ or I} \quad \text{MgX}_2 (\text{X} = \text{Br or I})
\]

\textbf{Figure 9.}

4B.2.4.2. By dialkylzinc catalysed reaction

Inomata et al.\textsuperscript{25,26} reported the synthesis of chiral isoxazoline by employing a dialkylzinc derivative as a catalyst of the nitrile oxide cycloaddition to allylic alcohols in the presence of diisopropyl (R,R)-tartrate [(R,R)-DIPT]. The nitrile oxide, generated in situ by direct oxidation of the corresponding aldoxime, coordinated to zinc metal of a zinc-bridging intermediate before giving, via cycloaddition, the corresponding chiral isoxazoline and catalytic amounts of (R,R)-DIPT (0.2 equiv) were sufficient to provide high enantioselectivities (Fig. 10).
Chapter-4B  Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

![Chemical Structure](image)

Figure 10.

4B.2.4.3. By Zr(IV) alkoxides catalysed reaction

Application of Zr(IV) alkoxides in a nitrile oxide cycloaddition for the synthesis of isoxazoline has been reported by Porco et al.27 The nitrile oxide was generated in situ from the corresponding ethyl chloro-oximinoacetate and diisopropylethylamine, which reacted with a chiral exocyclic vinyl epoxide in the presence of Zr(Ot-Bu)₄ to afforded the corresponding spiroisoxazoline as diastereomers mixture (Fig 11).

![Chemical Structure](image)

Figure 11.

4B.3. Basis of work

Dihydroisoxazoles or isoxazoline are interesting heteroaromatic structures due to their broad biological properties. Because of interesting antitubercular activity in dihydroisoxazoles derivatives and piperazine analogue of phenylisoxazoline IV (Fig. 2), it was thought to synthesized few novel but simple dihydroisoxazoline and see their antitubercular activity.

4B.4. Present work

The present work describes the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles and their bioevaluation against M. tuberculosis H37Ra and M. tuberculosis H37Rv. The compounds have been synthesized by [3+2] cycloaddition of aromatic aldoximes with alkenes.
4B.5. The synthesis of 5-benzyl-3-phenyl dihydroisoxazoles

The compounds synthesized in this section are-
5-((4-Chlorophenoxy)methyl)-3-phenyl-4,5-dihydroisoxazole (98), 4-((3-phenyl-4,5-dihydroisoxazol-5-yl) methoxy)benzonitrile (99), 4-chloro-2-((3-phenyl-4,5-dihydroisoxazol-5-yl) methyl)phenol (100), 4-hydroxy-3-((3-phenyl-4,5-dihydroisoxazol-5-yl) methyl)benzonitrile (101), 4-chloro-3-methyl-2-((3-phenyl-4,5-dihydroisoxazol-5-yl)methyl)phenol (102), 4-hydroxy-3-((3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)benzonitrile (103), 4-chloro-2-((3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)phenol (104), 4-chloro-2-((3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)3-methyl phenol (105), 4-chloro-2-((3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-3-methyl phenol (106), 3-((3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-hydroxybenzonitrile (107), 4-hydroxy-3-((3-(3-nitrophenyl)-4,5-dihydroisoxazol-5-yl)methyl)benzonitrile (108), 5-chloro-4-methyl-2-((3-(3-nitrophenyl)-4,5-dihydroisoxazol-5-yl) methyl)phenol (109).

The synthetic strategy begins with aromatic aldoximes (94a-94d) which were prepared by the reaction of hydroxylamine hydrochloride and different aromatic aldehydes (93a-93d) in ethanol in the presence of NN-dimethylaminopyridine (DMAP). Their (94a-94d) physical constants and spectral data matched literature values. 28

The O-allylated substrate (96a-96c) were prepared by the reaction of phenol (95a-95c) with allylbromide in presence of base K₂CO₃, and TBAB as phase transfer catalyst and subsequent heating at 120-130 °C (Clasein rearrangement), resulted into corresponding C-allylphenols (97a-97c). 29 The spectroscopic data of O-allylated substrate (96a-96c) and C-allylphenols (97a-97c) were full agreement with their literature reported values. 29,30
Chapter-4B  Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

Scheme 2.

In the first attempt of the reaction, hetero Diels- Alder reaction of (E)-benzaldehyde oxime 94a with 1-(allyloxy)-4-chlorobenzene 96a in acetonitrile as organic solvent was carried out at ambient temperature in the presence of N-chlorosuccinimide (NCS) and triethylamine as a base resulted into 5-((4-chlorophenoxy)methyl)-3-phenyl-4,5-dihydroisoxazole (98) in 69% yield (scheme 3).

Scheme 3.

The structure of compound 98 was established on the basis of spectroscopic data. FABMS spectrum showed a peak at m/z 288 [M+H]+ corresponding to its molecular formula C_{16}H_{14}O_{2}NCl. The IR absorptions at ν_{max} = 1589 cm\(^{-1}\) indicated the existence of the C=N group. The \(^1\)H NMR spectrum of compound 98 showed the H-5 proton as a multiplet in the range of δ 5.14-5.05 while two methylene protons of OCH\(_2\) were observed as multiplet in the range of δ 4.17-4.02. The H-4 protons were seen as multiplet in the range of δ 3.59-3.33. The aromatic proton were visible as two multiplet in the range of δ 7.71-7.67 (m, 2H), δ 7.43-7.40 (m, 3H), and two doublets at δ 7.22 (2H), δ 6.83 ppm (2H) with coupling constant, \(J = 8.8\) Hz. In the \(^13\)C NMR spectrum of compound 98, methylene (OCH\(_2\)) carbon was observed at δ 67.6 while the CH and CH\(_2\) carbons of dihydroisoxazole were visible at δ 77.3 and δ 36.2 respectively. The quaternary carbon of C=N appeared at δ 155.8 and aromatic carbon at their usual chemical shift. Similarly reaction of 94a with 96b under above reaction condition afforded 5-((4-
cyanophenoxy)methyl)-3-phenyl-4,5-dihydroisoxazole 99. The structure of compound 7 was established on the basis of their spectroscopic data.

In order to see the effect of C-allylated phenol on biological activity, study was extended with the reaction of (E)-benzaldehyde oxime (94a) and 2-allyl-4-chlorophenol (97a) under above reaction condition to get compound 4-chloro-2-((3-phenyl-4,5-dihydroisoxazol-5-yl)methyl)phenol 100 (Scheme 4).

\[
\begin{align*}
\text{94a} & \quad + \quad \text{97a} \\
& \quad \text{(i) NCS/Acetonitrile/rt/3-4 h} \\
& \quad \text{(ii) Et}3\text{N/0 °C-rt/8-11 h} \\
& \quad \text{100}
\end{align*}
\]

Scheme 4.

The structure of compound 100 was established on the basis of its spectroscopic data. FABMS spectrum showed a peak at m/z 288 [M+H]+ corresponding to its molecular formula C_{16}H_{14}O_{2}Cl. The IR spectrum of compound 100 showed an absorption at \( \nu_{\text{max}} = 1607 \text{ cm}^{-1} \) and 3386 cm\(^{-1}\) due to the dihydroisoxazole C=N and OH bonds respectively. In the \(^1\text{H NMR}\) spectrum of compound 100 showed a multiplet of dihydroisoxazole ring H-4a & CH\(_2\) in the range of \( \delta 3.17-2.91 \) (m, 3H\(_\text{a}\)) while dihydroisoxazole H-4b proton appeared as double doublet at \( \delta 3.40 \) (\( J_1 = 16.6 \text{ Hz, } J_2 = 10.2 \text{ Hz, } 1H \)). The H-5 of dihydroisoxazole ring was observed as multiplet in the range of \( \delta 5.06-4.97 \). All aromatic protons were seen as multiplets in the range \( \delta 7.62-7.58 \) (m, 2H\(_\text{a}\)), \( \delta 7.38-7.35 \) (m, 3H\(_\text{a}\)), \( \delta 7.10-7.03 \) (m, 2H\(_\text{a}\)), \( \delta 6.86-6.80 \) (m, 1H\(_\text{a}\)). The \(^{13}\text{C NMR}\) spectrum of compound 100 methylene (CH\(_2\)) carbon signal observed at \( \delta 35.0 \) while CH and CH\(_2\) dihydroisoxazole carbons were appeared at \( \delta 80.4 \) and \( \delta 37.9 \) respectively. The quaternary carbon of C=N appeared at \( \delta 156.4 \) and aromatic carbons were observed at \( \delta 152.6, 127.3, 123.9, 123.5 \) (ArC), 129.7, 129.1, 127.7, 127.4, 125.5, 117.4 (ArCH).

To investigate the scope of different substrates in this cycloaddition, the above aromatic aldoximes (94a-94d) were reacted with another C-allyl substrate (97a-97c) to get the desired dihydroisoxazole derivatives (101-109) (Scheme 5, Table 1).
Chapter-4B  Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

\[
\text{Ar} = \text{N} \quad \text{OH} \\
(94a-94d) \quad (97a-97c) \\
\rightarrow \quad \text{OH} \\
(101-109)
\]

(i) NCS / Acetonitrile / rt / 3-4 h  
(ii) Et\textsubscript{3}N / 0 °C / rt / 8-11 h

Scheme 5.

All the above compounds 101-109 were characterized on the basis of their spectroscopic data. ESMS of the compounds 101-109 were displayed their respective [M+H]+ peaks at 279, 302, 309, 318, 332, 322, 313, 324, 347 corresponding to their molecular formulae C\textsubscript{17}H\textsubscript{14}N\textsubscript{2}O\textsubscript{2}, C\textsubscript{17}H\textsubscript{16}ClN\textsubscript{2}O\textsubscript{3}, C\textsubscript{18}H\textsubscript{16}N\textsubscript{2}O\textsubscript{3}, C\textsubscript{17}H\textsubscript{16}ClNO\textsubscript{3}, C\textsubscript{18}H\textsubscript{18}ClNO\textsubscript{3}, C\textsubscript{16}H\textsubscript{13}ClNO\textsubscript{2}, C\textsubscript{17}H\textsubscript{13}ClN\textsubscript{2}O\textsubscript{2}, C\textsubscript{17}H\textsubscript{13}N\textsubscript{3}O\textsubscript{4}, C\textsubscript{17}H\textsubscript{15}ClN\textsubscript{2}O\textsubscript{4}. The IR spectrum of above compounds showed an absorption at around \(\nu_{\text{max}} = 1607 \text{ cm}^{-1}\) and 3386 cm\(^{-1}\) due to the dihydroisoxazole C=N and OH bonds respectively.

Table 1. Synthesis of 5-benzyl-3-phenyl dihydroisoxazole derivatives (101-109)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Aromatic aldoxime</th>
<th>C-allyl substrate</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94a</td>
<td>97b</td>
<td><img src="image1" alt="Product Image" /></td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>94a</td>
<td>97c</td>
<td><img src="image2" alt="Product Image" /></td>
<td>68</td>
</tr>
<tr>
<td>3</td>
<td>94b</td>
<td>97b</td>
<td><img src="image3" alt="Product Image" /></td>
<td>55</td>
</tr>
<tr>
<td>4</td>
<td>94b</td>
<td>97a</td>
<td><img src="image4" alt="Product Image" /></td>
<td>71</td>
</tr>
<tr>
<td>5</td>
<td>94b</td>
<td>97c</td>
<td><img src="image5" alt="Product Image" /></td>
<td>56</td>
</tr>
</tbody>
</table>
In the $^1$H NMR spectra of the above compounds, the aromatic protons appeared as multiplet or as doublet or double doublet at their usual chemical shift value. The other protons of the isoxazoline ring were appeared at their usual chemical shift and with usual pattern of multiplicities. In $^{13}$C NMR spectra, all the carbons were observed at their usual chemical shifts. (Experimental)

4B.6. Biological Activity

The synthesized compounds were evaluated against *M. tuberculosis* H37Ra$^{31}$ and *M. tuberculosis* H37Rv strains following earlier protocol.$^{32}$ Among all of the tested compounds, 4-chloro-3-methyl-2-((3-phenyl-4,5-dihydroisoxazol-5-yl) methyl)phenol (102) was found active at minimum inhibitory concentration (MIC) 3.12 µg/mL against *M. tuberculosis* H37Rv (Table 2). The compounds 100, 103 and 106 were active with MIC 12.5 µg/mL against *M. tuberculosis* H37Rv while rest of the compounds inactive at MIC 12.5 µg/mL. As evident from the table 2, the all of the screened compounds were more specific towards virulent strain of *M. tuberculosis*.

Although with this limited number of compounds, no definite structure activity relationship could be established, yet the observation made point out that these compounds are more specific towards virulent strain (*M. tuberculosis* H37Rv) than the avirulent strain (*M. tuberculosis* H37Ra).
Chapter-4B Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

Table 2. Antitubercular activity of 5-benzyl-3-phenyl dihydroisoxazole derivatives (98-109) against *M. Tuberculosis* H37Ra and *M. Tuberculosis* H37Rv

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (µg/ml) H37Ra</th>
<th>MIC (µg/ml) H37Rv</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
<td>&gt;50</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>99</td>
<td>&gt;50</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>100</td>
<td>&gt;50</td>
<td>12.5</td>
</tr>
<tr>
<td>101</td>
<td>&gt;50</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>102</td>
<td>&gt;50</td>
<td>3.12</td>
</tr>
<tr>
<td>103</td>
<td>&gt;50</td>
<td>12.5</td>
</tr>
<tr>
<td>104</td>
<td>&gt;50</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>105</td>
<td>&gt;50</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>106</td>
<td>&gt;50</td>
<td>12.5</td>
</tr>
<tr>
<td>107</td>
<td>&gt;50</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>108</td>
<td>&gt;50</td>
<td>&gt;12.5</td>
</tr>
<tr>
<td>109</td>
<td>&gt;50</td>
<td>&gt;12.5</td>
</tr>
</tbody>
</table>

MIC = Minimum inhibitory concentration, the lowest concentration of the compound which inhibits the growth of mycobacterium >90%; MIC of the drugs used as control, INH 0.65, rifampicin 0.75, and ethambutol 3.25 µg/mL against *M. tuberculosis* H37Rv.

Further, it is also evident that the active compounds with MIC ≤ 12.5 possess a benzyl substituent at the 5-position of isooxazoline with electron withdrawing substituent at the 4-position. Any alteration of the positions of these substitutents led to decrease in antitubercular potential.

4B.7. Experimental Section

4B.7.1. Chemistry

Commercially available reagent grade chemicals were used as received. All reaction was followed by TLC on E. Merck Kieselgel 60 F254, with detection by UV light, spraying a 20% KMnO4 aq solution. Column chromatography was performed on silica gel (60-120 mesh & 100-200 mesh, E. Merck). IR spectra were recorded as thin films or on KBr pellets with a Perkin Elmer Spectrum RX-1 (4000-450 cm⁻¹) spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Brucker DRX -300 in CDCl₃ and DMSO. Chemical shift values are reported in ppm relative to TMS (tetramethylsilane) as internal reference, unless otherwise stated; s (singlet), d (doublet), t (triplet), m (multiplet); J in
hertz. FAB mass spectra were performed using a mass Spectrometer Jeol SX-102 and ESI mass spectra were performed using Quattro II (Micromass). Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer.

**4B.7.1.1. Typical procedure for the synthesis of 5-((4-chlorophenoxy)methyl)-3-phenyl-4, 5-dihydroisoxazole (98)**

To a solution of benzaldoxime 94a (1g, 8.26 mmol) in acetonitrile (10 mL) was added N-chlorosuccinimide (1.5g, 8.26 mmol) slowly at 0°C. The reaction mixture was stirred for 4 h at room temperature. Reaction progress i.e., formation of benzenecarboximidoyl chloride was observed by TLC. To a reaction mixture containing benzenecarboximidoyl chloride at 0°C was added triethylamine (0.22 mL, 25 mol %) followed by addition of alkene 96a (1.39 g, 8.26 mmol). The reaction mixture was stirred for 11 h, and extracts with ethyl acetate. The organic extracts was dried over sodium sulphate and concentrated under reduced pressure. The product was purified by column chromatography on silica gel (60-120 mesh) using ethyl acetate: hexane (4:6) as eluent to give compound 98 as solid, mp 153-154 °C, in 69 % yield. ESIMS m/z = 288 [M+H]+, IR νmax cm⁻¹ 3071 (aromatic CH stretching), 1589 (C=N stretching); ¹H NMR (200 MHz, CDCl₃) δppm = 7.71-7.67 (m, 2H, ArH), 7.43-7.40 (m, 3H, ArH), 7.22 (d, J = 8.8 Hz, 2H, ArH), 6.83 (d, J = 8.8 Hz, 2H, ArH), 5.14-5.05 (m, 1H, dihydroisoxazole ring CH), 4.17-4.02 (m, 2H, OCH₂), 3.59-3.33 (m, 2H, dihydroisoxazole ring CH₂). ¹³C NMR (50 MHz, CDCl₃) δppm = 155.8 (C=N), 155.1, 127.4, 125.0 (ArC), 129.0, 128.1, 128.0, 125.5, 114.0 (ArCH), 77.3 (dihydroisoxazole CH), 67.6 (OCH₂), 36.2 (dihydroisoxazole ring CH₂); Elemental analysis for C₁₆H₁₄ClNO₂: C, 66.79; H, 4.90; Cl, 12.32; N, 4.87; Found: C, 66.70; H, 4.93; Cl, 12.30; N, 4.85.

**4B.7.1.2. 4-((3-Phenyl-4,5-dihydroisoxazol-5-yl) methoxy)benzonitrile (99)**

It was obtained by the reaction of benzaldoxime 94a (2g, 16.5 mmol), NCS (1.8g, 16.5 mmol), alkene 96b (2.6g, 16.5 mmol) and triethylamine (25 mol%), as solid, mp 161-162°C, ESIMS m/z = 279 [M+H]+, IR νmax cm⁻¹ 2291 (CN stretching), 1603 (C=N stretching); ¹H NMR (200 MHz, CDCl₃) δppm = 7.69-7.55 (m, 4H, ArH), 7.42-7.40 (m, 3H, ArH), 7.22 (d, J = 8.8 Hz, 2H, ArH), 6.94 (d, J = 8.8 Hz, 2H, ArH), 5.12-5.09 (m, 1H, dihydroisoxazole ring CH), 4.24-4.09 (m, 2H, OCH₂), 3.61-3.32 (m, 2H, dihydroisoxazole ring CH₂). ¹³C NMR (50 MHz, CDCl₃) δppm = 161.6 (C=N), 167.2,
Chapter-4B  
Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

134.8, 101.2 (ArC), 139.4, 135.3, 134.1, 132.1, 121.1 (ArCH), 109.1 (CN) 83.9 (dihydroisoxazole CH), 74.7 (OCH₂), 42.2 (dihydroisoxazole ring CH₂); Elemental analysis for C₁₇H₁₄N₂O₂: C, 73.37; H, 5.07; N, 10.07; Found: C, 73.33; H, 5.00; N, 10.00.

4B.7.1.3. 4-Chloro-2-((3-phenyl-4,5-dihydroisoxazol-5-yl) methyl)phenol (100)

It was obtained by the reaction of benzaldoxime 94a (1g, 8.26 mmol), NCS (1.1g, 8.26 mmol), alkene 97a (1.5g, 8.9 mmol) and triethylamine (25 mol %), as solid, mp 139-140 °C, ESIMS m/z = 288 [M+H]+, IR νmax cm⁻¹ 3386 (OH stretching), 1607 (C=N stretching); ¹H NMR (200 MHz, CDCl₃) δppm = 7.62-7.58 (m, 2H, ArH), 7.38-7.35 (m, 3H, ArH), 7.10-7.03 (m, 2H, ArH), 6.86-6.80 (m, 1H, ArH), 5.06-4.97 (m, 1H, dihydroisoxazole ring CH), 3.40 (dd, J₁ = 16.6 Hz, J₂ = 10.2 Hz, 1H, dihydroisoxazole CH₃H₉b), 3.17-2.91 (m, 3H, dihydroisoxazole ring CH₃H₉b & CH₂). ¹³C NMR (50 MHz, CDCl₃) δppm = 156.4 (C=N), 152.6, 127.3, 123.9, 123.5 (ArC), 129.7, 129.1, 127.7, 127.4, 125.5, 117.4 (ArCH), 80.4 (dihydroisoxazole CH), 37.9 (dihydroisoxazole ring CH₂), 35.0 (benzyl CH₂); Elemental analysis for C₁₆H₁₄ClN₂O₂: C, 66.79; H, 4.90; Cl, 12.32; N, 4.87; Found: C, 66.70; H, 4.84; Cl, 12.28; N, 4.82.

4B.7.1.4. 4-Hydroxy-3-((3-phenyl-4,5-dihydroisoxazol-5-yl) methyl)benzonitrile (101)

It was obtained by the reaction of benzaldoxime 94a (1g, 8.26 mmol), NCS (1.1g, 8.26 mmol), alkene 97b (1.3g, 8.26 mmol) and triethylamine (25 mol%), as solid, mp 159-160 °C, ESIMS m/z = 279 [M+H]+, IR νmax cm⁻¹ 2220 (CN stretching), 1603 (C=N stretching); ¹H NMR (200 MHz, CDCl₃) δppm = 7.86 (s, 1H, ArH), 7.64-7.59 (m, 2H, ArH), 7.46-7.38 (m, 4H, ArH), 6.95 (d, J = 8.2 Hz, 1H, ArH), 5.10-5.06 (m, 1H, dihydroisoxazole ring CH), 3.49 (dd, J₁ = 16.7 Hz, J₂ = 10.3 Hz, 1H, dihydroisoxazole CH₃H₉b), 3.18-3.04 (m, 3H, dihydroisoxazole ring CH₃H₉b & CH₂). ¹³C NMR (50 MHz, CDCl₃) δppm = 162.0 (C=N), 164.9, 135.2, 130.8, 124.7, 101.6 (ArC), 140.9, 137.9, 135.5, 134.1, 132.1, 121.6 (ArCH), 108.4 (CN), 85.5 (dihydroisoxazole CH), 45.0 (benzyllic CH₂), 40.8 (dihydroisoxazole ring CH₂); Elemental analysis for C₁₇H₁₄N₂O₂: C, 73.37; H, 5.07; N, 10.07; Found: C, 73.30; H, 5.13; N, 10.02.
4B.7.1.5. 4-Chloro-3-methyl-2-((3-phenyl-4,5-dihydroisoxazol-5-yl)methyl)phenol (102)

It was obtained by the reaction of benzaldoxime 94a (5g, 41.3 mmol), NCS (5.52g, 41.3 mmol), alkene 97c (7.5g, 41.3 mmol) and triethylamine (25 mol%), as solid, mp 159-160 °C, ESIMS m/z = 302 [M+H]^+, IR \( \nu_{\text{max}} \) cm\(^{-1}\) 3407 (OH stretching), 1604 (C=N stretching); \(^1\)H NMR (200 MHz, CDCl\(_3\)) \( \delta_{ppm} = 7.65-7.60 \) (m, 2H, ArH), 7.38-7.30 (m, 3H, ArH), 7.00 (d, \( J = 8.6 \) Hz, 2H, ArH), 6.60 (d, \( J = 8.6 \) Hz, 2H, ArH), 5.04-4.95 (m, 1H, dihydroisoxazole ring CH), 3.28-3.21 (m, 2H, OCH\(_2\)), 3.11-3.06 (m, 2H, dihydroisoxazole ring CH\(_2\)), 2.37 (s, 3H, CH\(_3\)). \(^{13}\)C NMR (50 MHz, CDCl\(_3\)) \( \delta_{ppm} = 161.9 \) (C=N), 158.3, 140.2, 132.1, 129.7, 128.3 (ArC), 134.4, 133.7, 132.9, 130.9, 117.9 (ArCH), 85.1 (dihydroisoxazole CH), 43.7 (dihydroisoxazole ring CH\(_2\)), 36.3 (benzyl CH\(_2\)), 21.1 (CH\(_3\)); Elemental analysis for C\(_{11}\)H\(_{16}\)ClN\(_2\)O\(_2\): C, 67.66; H, 5.34; Cl, 11.75; N, 4.64; found C, 67.65; H, 5.39; Cl, 11.70; N, 4.63.

4B.7.1.6. 4-Hydroxy-3-((3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl) benzonitrile (103)

It was obtained by the reaction of 4-methoxybenzaldoxime 94b (1.4g, 9.9 mmol), NCS (1.32g, 9.9 mmol), alkene 97b (1.6g, 9.9 mmol) and triethylamine (25 mol%), as solid, mp 199-200°C, ESIMS m/z = 309 [M+H]^+, IR \( \nu_{\text{max}} \) cm\(^{-1}\) 2216 (CN stretching), 1607 (C=N); \(^1\)H NMR (200 MHz, CDCl\(_3\)) \( \delta_{ppm} = 9.36 \) (s, 1H, ArOH), 7.60-7.53 (m, 3H, ArH), 7.40 (dd, 1H, \( J_1 = 2.05 \) Hz, \( J_2 = 8.35 \) Hz, ArH), 6.99-6.88 (m, 3H, ArH), 5.10-5.01 (m, 1H, dihydroisoxazole ring CH), 3.83 (s, 3H, OCH\(_3\)), 3.32 (dd, \( J_1 = 16.6 \) Hz, \( J_2 = 10.1 \) Hz, 1H, dihydroisoxazole CH\(_{aH_b}\)), 3.17-2.98 (m, 3H, dihydroisoxazole ring CH\(_{aH_b}\) & CH\(_2\)). \(^{13}\)C NMR (50 MHz, CDCl\(_3\)) \( \delta_{ppm} = 160.1 \) (C=N), 160.9, 156.3, 125.7, 122.3, 101.3 (ArC), 135.5, 132.7, 128.4, 116.1, 114.5 (ArCH), 119.9 (CN), 79.4 (dihydroisoxazole CH), 55.6 (OCH\(_3\)), 39.6 (benzyl CH\(_2\)), 34.9 (dihydroisoxazole ring CH\(_2\)); Elemental analysis for C\(_{18}\)H\(_{16}\)N\(_2\)O\(_2\): C, 70.12; H, 5.23; N, 9.09; Found: C, 70.09; H, 5.29; N, 9.00.

4B.7.1.7. 4-Chloro-2-((3-(4-methoxyphenyl)-4,5-dihydroisoxazol-5-yl)methyl)phenol (104)

It was obtained by the reaction of 4-methoxybenzaldoxime 94b (1.4g, 6.6 mmol), NCS (0.88g, 6.6 mmol), alkene 97a (1.1g, 6.6 mmol) and triethylamine (25 mol%) as solid, mp151-152 °C, ESIMS m/z = 318 [M+H]^+, IR \( \nu_{\text{max}} \) cm\(^{-1}\) 3170 (OH stretching), 1607
4B.7.1.9. 4-Chloro-2-((3-(4-chlorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)3-methylphenol (106)

It was obtained by the reaction of 4-chlorobenzaldoxime 94c (1.5 g, 9.9 mmol), NCS (1.32g, 9.9 mmol), alkene 97a (1.6g, 9.9 mmol) and triethylamine (25 mol%) as white solid, mp148-149 °C, ESIMS m/z = 322 [M+H]+, IR νmax cm⁻¹ 3189 (OH stretching), 1598 (C=N); ¹H NMR (200 MHz, DMSO) δppm = 8.93 (s, 1H, ArOH), 6.74 (d, 2H, J = 8.02 Hz, ArH), 6.57 (d, 2H, J = 8.0 Hz, ArH), 6.30 (s, 1H, ArH), 6.17 (d, 1H, J = 8.1Hz, ArH), 6.90 (d, 1H, J = 8.1 Hz, ArH), 4.06 (m, 1H, dihydroisoxazole ring CH), 2.43-1.90
Chapter-4B Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

(m, 4H, dihydroisoxazole ring CH₂ & benzyl CH₂); ¹³C NMR (50 MHz, DMSO) 156.1 (C=N), 154.7, 128.8, 126.0, 122.6 (ArC), 134.8, 130.8, 129.1, 128.6, 127.6, 116.7 (ArCH), 80.4 (dihydroisoxazole CH), 39.2 (benzyl CH₂), 35.1 (dihydroisoxazole ring CH₂); Elemental analysis for C₁₆H₁₃Cl₂NO₇: C, 59.65; H, 4.07; Cl, 22.01; N, 4.35; Found: C, 59.60; H, 4.00; Cl, 22.00; N, 4.33.

4B.7.1.10. 3-((3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-hydroxybenzonitrile (107)

It was obtained by the reaction of 4-chlorobenzaldoxime 94c (1.5g, 9.6 mmol), NCS (1.28g, 9.6 mmol), alkene 97b (1.5g, 9.6 mmol) and triethylamine (25 mol%) as white solid, mp162-163 °C, ESIMS m/z = 313 [M+H]+, IR ν max cm⁻¹ 2220 (CN stretching), 1603 (C=N stretching); ¹H NMR (200 MHz, CDCl₃) δ ppm = 10.1 (s, 1H, ArOH), 7.58 (d, 2H, J = 8.5 Hz, ArH), 7.46-7.34 (m, 4H, ArH), 6.94 (d, 2H, J = 8.5 Hz, ArH), 5.09-5.02 (m, 1H, dihydroisoxazole ring CH), 3.32 (dd, J₁ = 16.6 Hz, J₂ = 10.2 Hz, 1H, dihydroisoxazole CH₆H₆b), 3.12-2.92 (m, 3H, dihydroisoxazole ring CH₂). ¹³C NMR (50 MHz, CDCl₃) δ ppm = 160.1 (C=N), 164.9, 148.3, 131.5, 125.4, 130.8, 129.1, 128.6, 127.6, 116.2 (ArCH), 80.5 (dihydroisoxazole CH), 39.0 (benzyl CH₂), 34.9 (dihydroisoxazole ring CH₂).

4B.7.1.11. 4-Hydroxy-3-((3-(3-nitrophenyl)-4,5-dihydroisoxazol-5-yl)methyl)-4-hydroxybenzonitrile (108)

It was obtained by the reaction of 3-nitrobenzaldoxime 94b (2.0g, 12.0 mmol), NCS (1.6g, 12.0 mmol), alkene 97b (1.5g, 12.0 mmol) and triethylamine (25 mol%) as solid, mp146-147 °C, ESIMS m/z = 324 [M+H]+, IR ν max cm⁻¹ 2220 (CN stretching), 1603 (C=N stretching); ¹H NMR (200 MHz, DMSO) δ = 9.90 (s, 1H, ArOH), 7.43 (s, 1H, ArH), 7.33 (d, 1H, J = 8.0 Hz, ArH), 7.14 (d, 1H, J = 7.2 Hz, ArH), 6.83-6.71 (m, 2H, ArH), 6.00 (d, J = 8.3 Hz, 1H, ArH), 6.02 (d, J = 8.3 Hz, 1H, ArH), 4.17-4.08 (m, 1H, dihydroisoxazole ring CH), 2.60 (dd, J₁ = 17.2 Hz, J₂ = 10.5 Hz, 1H, dihydroisoxazole CH₆H₆b), 2.32 (dd, J₁ = 17.2 Hz, J₂ = 7.2 Hz, 1H, dihydroisoxazole CH₆H₆b), 2.06 (m, 2H, dihydroisoxazole ring CH₂). ¹³C NMR (50 MHz, DMSO) 160.1 (C=N), 155.9, 148.3, 131.5, 125.4, 101.3 (ArC), 135.5, 133.0, 132.8, 130.8, 124.7, 121.1, 116.2 (ArCH), 119.9 (CN), 80.5 (dihydroisoxazole CH), 39.0 (benzyl CH₂), 34.9 (dihydroisoxazole ring CH₂).
Chapter 4B Application of 3+2 dipolar cycloaddition reaction in the synthesis of 3-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

CH₂); Elemental analysis for C₁₁H₁₃N₃O₄: C, 63.16; H, 4.05; N, 13.00; Found: C, 63.12; H, 4.10; N, 12.90.

4B.1.12. 5-Chloro-4-methyl-2-((3-(3-nitrophenyl)-4,5-dihydroisoxazole-5-yl)methyl)phenol (109)

It was obtained by the reaction of 3-nitrobenzaldoxime 94d (2.0g, 12.0 mmol), NCS (1.6g, 12.0 mmol), alkene 97c (2.19g, 12.0 mmol) and triethylamine (25 mol%) as solid, mp 150-151 °C, ESIMS m/z = 347 [M+H]+, IR νmax cm⁻¹ 2220 (CN stretching), 1603 (C=N stretching); ¹H NMR (200 MHz, CDCl₃) δ = 8.41-8.04 (m, 4H, ArOH & ArH), 7.63-7.55 (m, 1H, ArH), 7.09-7.05 (m, 1H, ArH), 6.73-6.67 (m, 1H, ArH), 5.12-5.04 (m, 1H, dihydroisoxazole ring CH), 3.32-3.02 (m, 4H, dihydroisoxazole ring CH₂ & benzyl CH₂), 2.38 (s, 3H, CH₃). ¹³C NMR (50 MHz, CDCl₃) 155.7 (C=N), 154.4, 148.8, 136.3, 132.2, 126.0, 124.7 (ArC), 132.5, 128.4, 123.9, 121.8, 118.5, 114.5 (ArCH), 82.3 (dihydroisoxazole CH), 39.1 (benzylic CH₂), 32.3 (dihydroisoxazole ring CH₂), 17.3 (CH₃); Elemental analysis for C₁₇H₁₅ClN₂0₄: C, 58.88; H, 4.36; Cl, 10.22; N, 8.08; Found C, 58.82; H, 4.33; Cl, 10.19; N, 8.04.

4B.8. Bio-evaluation methods

4B.8.1. Activity against M. tuberculosis H37Ra strain

All of the synthesized compounds were evaluated for their efficacy against M. tuberculosis H37Ra at active concentration ranging 50 µg/mL-1.56 µg/mL MIC using two-fold dilutions in the initial screen. Log phase culture of M. tuberculosis H37Ra is diluted so as to give final OD₅₅₀ nm of 0.05 in Sauton’s medium. In 96-well white plates 190 mL of culture is dispensed in each well. A dimethyl sulfoxide (DMSO) solution of test compounds is dispensed into 96- well plates so as to make final test concentration of 25 mg/mL (5 mg test compound is dispensed into 10 mL of DMSO). Then the plate is incubated at 37 °C/5% CO₂ for 5 days. On 5th day 15 mL Alamar Blue solution is added to each well of the plate. The plate is again incubated overnight at 37 °C/5% CO₂ incubator. The fluorescence is read on BMG polar star with excitation frequency at 544 nm and emission frequency at 590 nm. The compounds, which were found to be active (>90% inhibition as compared with control) at this concentration are then tested at 6 serial dilutions starting from 50 to 1.56 µg/mL.
4B.8.2. Activity against *M. tuberculosis* H37Rv strain

Drug susceptibility and determination of MIC of the test compounds/drugs against *M. tuberculosis* H37Rv were performed by agar microdilution method where two-fold dilutions of each test compound were added into 7H10 agar supplemented with OADC and organism. A culture of *M. tuberculosis* H37Rv growing on L–J medium was harvested in 0.85% saline with 0.05% Tween-80. A suspension of 1 mg/mL concentration of extracts/compounds was prepared in DMSO. This suspension was added to (in tubes) 7H10 Middle Brook’s medium (containing 1.7 mL medium and 0.2 mL OADC supplement) at different concentrations of compound keeping the volume constant i.e. 0.1 mL. Medium was allowed to cool by keeping the tubes in slanting position. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 \(10^4\) bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 30 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with H37Rv. The concentration at which complete inhibition of colonies occurred was taken as active concentration of test compound.
4.9. References

1. (a) Spinelli, O.A. Ital. Soc. Chem. 1999, 3, 301; (b) Conti, P. Dallanoce, C.;
Amici, M.D.; Micheli, C.D.; Klotz, K.N.; Bioorg. Med. Chem. 1998, 6, 401; (c)
1998, 8, 313; (e) Kang, Y.Y.; Shin, K.J.; Yoo, K.H.; Seo, K.J.; Hong, C.Y.; Lee,

2. 2. Wong, G. S. K.; Wu, W. 2-Oxazolines. In The Chemistry of Heterocyclic
Compounds; Palmer, D. C., Eds.; Oxazoles: Synthesis, Reactions and


Seest, E.P.; Thomas, R.C.; Toops, D.S.; Watt, W.; Wishka, D.G.; Ford, C.W.; Zurenko,
G.E.; Hamel, J.C.; Schaadt, R.D.; Stapert, D.; Yagi, B.H.; Adams, W.J.; Fries,
J.M.; Slatter, J.G.; Sams, J.P.; Oien, N.L.; Zaya, M.J.; Winkler, L.C.; Wynalda
M.A. J. Med. Chem. 2003, 46, 284; (b) Varshney, V.; Mishra, N. N.; Shukla, P.


Fernandez, J.; Martinez, S.; Nieto, C.; Pastor, J.; Bakker, M.H.; Biesmans, I.;

1999, 9, 277.


Med. Chem. Res. 1996, 6, 52; (b) Fuller, A. A.; Chen, B.; Minter, A. R.; Mapp, A.
Chapter-4B Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents


Chapter-4B  Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents


4.10 Copies of Mass, $^1$H NMR, $^{13}$C NMR spectra of selected compounds
Chapter-4B  Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

ESMS of compound 101

1H NMR spectra of compound 101
Chapter-4B  Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

$^{13}$C NMR spectra of compound 101

ESMS of compound 102
Chapter 4B: Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

$^1$H NMR spectra of compound 102

$^{13}$C NMR spectra of compound 102
Chapter-4B Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

ESMS of compound 104

$^1$H NMR of spectra compound 104
Chapter-4B  Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

13C NMR of spectra compound 104

ESMS of compound 106
Chapter 4B Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

\[ ^1\text{H NMR spectra of compound 106} \]

\[ \text{ESMS of compound 107} \]
Chapter 4B  Application of 3+2 dipolar cycloaddition reaction in the synthesis of 5-benzyl-3-phenyl dihydroisoxazoles as antitubercular agents

$^1$H NMR spectra of compound 107

$^{13}$C NMR spectra of compound 107