Synthesis of some pyridine substituted α,β unsaturated carbonyl compounds as potential bacterial fatty acid biosynthesis enoyl ACP reductase inhibitors

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MS10043

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Certificate of Examination

This is to certify that the dissertation titled "*Synthesis of some pyridine substituted α,β unsaturated carbonyl compounds as potential bacterial fatty acid biosynthesis enoyl ACP reductase inhibitors*" submitted by Mr. Ashish Kumar (Reg. No. MS10043) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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Date: 24/04/2015

DECLARATION

The work presented in this dissertation has been carried out by me under the guidance of Dr. Sugumar Venkataramani at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Ashish Kumar

(Candidate)

Dated: 24/04/2015

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Sugumar Venkataramani
(Supervisor)

Acknowledgement

Throughout the course of this project, I was accompanied and supported by many people. I would express my gratitude for all of them.

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Ashish Kumar

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List of Abbreviations

FAB	:	Fatty acid Biosynthesis
DNA	:	Deoxyribosenucleic acid
AMR	:	Anti-microbial resistance
CoA	:	Coenzyme A
ACP	:	Acyl carrier protein
ENR	:	Enoyl-acyl carrier protein reductase
NADH	:	Nicotinamide adenine dinucleotide
KatG	:	Mycobacterium tuberculosis catalase-peroxidase
Pd	:	Palladium
EDC.HCl	:	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HOBt	:	Hydroxybenzotriazole
DCC	:	N,N'-Dicyclohexylcarbodiimide
CDI	:	Carbonyldiimidazole
K^{t}OBu	:	Potassium *tert*-Butoxide
MeOH	:	Methanol
EtOH	:	Ethanol
DCM	:	Dichloromethane
DMAP	:	Dimethylaminopyridine
TMSCl	:	Trimrthylchlorosilane

$SOCl_2$	:	Thionyl chloride
TEA	:	Triethylamine
PPh_3	:	Triphenylphosphine
$Pd(OAc)_2$	:	Palladium acetate
DMF	:	Dimethylformamide
THF	:	Tetrahydrofuran
ToTP	:	Tri-*ortho*-tolylphosphine
K_2CO_3	:	Potassium carbonate
Cs_2CO_3	:	Cesium carbonate
KOH	:	Potassium hydroxide
LiOH	:	Lithium hydroxide
Na_2SO_4	:	Sodium sulphate
DIPEA	:	N,N-Diisopropylethylamine
R.T.	:	Room temperature
Equiv.	:	Equivalence
TLC	:	Thin Layer Chromatography
NMR	:	Nuclear Magnetic Resonance Spectroscopy
FT-IR	:	Fourier Transform - Infrared Spectroscopy

CONTENTS

ABSTRACT

Antibiotics are an important tool for humanity to counter the threat posed by the microbial diseases. With the significant developments in chemistry and medicine, scientists have been able to develop several classes of drug molecules that can cater to our needs. However bacteria being an evolving organism has been able to develop its own defense mechanisms and thus attaining the resistance against drug molecules. The threat of Antimicrobial Resistance (AMR) is so acute that even the World Health Organization (WHO) has recognized this fact and urged scientists to come up with new class of drugs which once again could give us an upper hand against our oldest foe. One such emerging class of drug molecules is the "Bacteria fatty acid biosynthesis (FAB) inhibitors", which specifically target the fatty acid biosynthetic pathway in the bacterial cell. The limited resistance in bacteria, sequential conservation of the FAB cycle in several species, and rather underexplored pathway are the key features making them an attractive domain for further exploration. In our work here, we present the synthesis of pyridine based α,β-unsaturated carbonyl compounds which are modelled around certain known drug molecules (still under clinical trials) in this category and thus, could potentially serve the purpose of FAB inhibitors.

CHAPTER 1

Introduction

1.1 Antibiotics

An *Antibiotic* is a term generally referring to "a compound which is capable of inhibiting the growth and selectively killing the pathogenic microorganisms". Antibiotics have been the greatest triumphs of scientific medicine that save millions of lives throughout the world. The source of the antibiotic can be another microorganism, semisynthetic or fully synthetic. The first antibacterial agent used was Sulfanilamide in 1930s. Its success prompted the exploration in the new domain and was soon followed up by Penicillin. The discovery of Penicillin is credited to Sir Alexander Fleming. The pioneering efforts of Florey and Chain then laid the foundation of wide scale production and medical administration of antibiotics. [1] From then on many new drug molecules began to unfold such as Streptomycin, vancomycin, Erythromycin, etc and the diseases like tuberculosis became less threatening. Science and medicine finally had unlocked the secret to save millions. Such was the success of antibiotics that the scientific and medicinal community ceased to develop many new drugs. However, bacteria being an evolving organism, slowly and steady started developing resistance to the drugs. The signs of resistance were visible for sulfanilamide and penicillin way before 1950s, but by the end of 1990s resistant strains of bacteria began to show up for every known antibiotic. The innovation gap seen in the field of antibiotic research from 1970s-2000s again brought back the old enemy and now with even more strong armory at its disposal. [2] Thus, again began the quest to develop new antibiotics that could help counter the looming threat.

The antibiotics can be classified into different categories (Fig.1). An antibiotic can work by killing the bacteria (bactericidal) or by inhibiting its multiplication (bacteriostatic). Each antibiotic has its own range of bacterial species upon which it can be effective. Based on the spectrum of activity, they can be narrow spectrum antibiotics which target only a few species, or the broad spectrum antibiotics which effective against a large number of bacterial species. Antibiotics can work within a bacterial cell in different ways. For example, beta lactams like penicillin inhibit cell wall synthesis. Quinolones and rifampin

target the Nucleic acids and aminoglycosides target protein synthesis. Certain class of antibiotics such as sulfa drugs can also interfere with a critical cell metabolism and thus kills or inhibit its growth. The emergence of resistance strains of bacteria is the chief reason for development of new drug molecules. When a bacteria becomes resistant to certain class of drug, it becomes important to target other metabolisms within the cell to curtail its advantage of resistance.

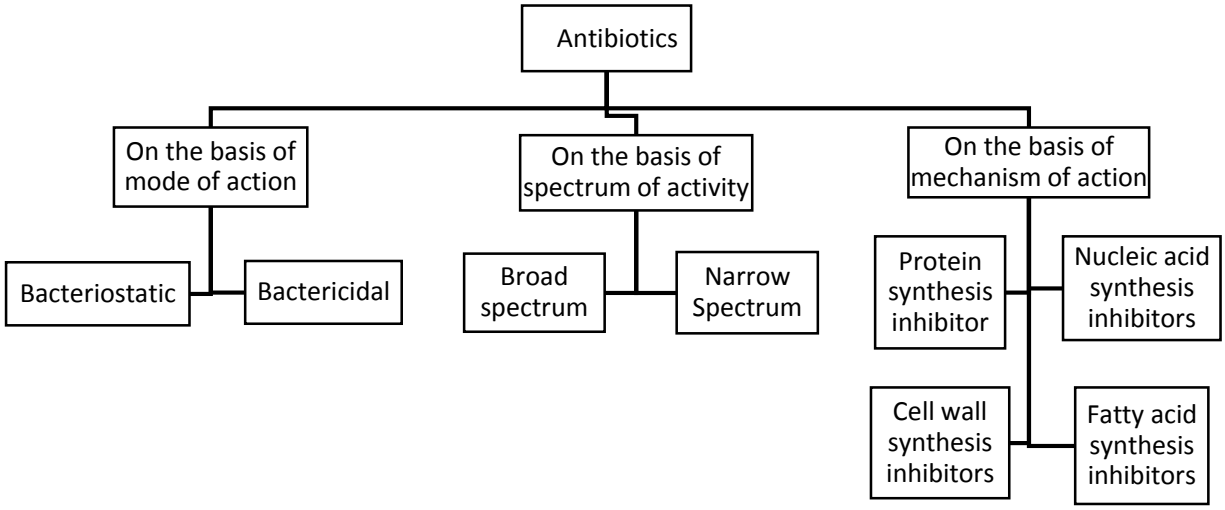


Fig.1: Classification of antibiotics

1.2 Antibiotic Resistance:

Emergence of the resistance in the bacteria is a result of the evolutionary process. If the concentration of drug required to inhibit or kill the microorganism is greater than the normal use, then the microorganism is considered to be resistant to that particular drug. Bacteria may have an intrinsic resistance to antibiotic. However, in most of the cases the resistance is genetically acquired. A random mutation can generate a resistant cell. Mostly the bacteria owe their antibiotic resistance to the proteins encoded by plasmids that they harbor. Plasmids are small, circular, extra-chromosomal pieces of DNA that can be transferred from one bacterium to another. These plasmids often contain genes that encode proteins that confer resistance to a wide array of antibiotics. [3] The common changes seen in a resistant bacterial cell are:

1. Change in Porin size/shape: Restricting the entry of antibiotics. Literature reports have indicated the loss or functional change of porins in a large number of organisms, such as *E.coli*. [4,5,6]
2. Efflux Pumps: To throw out the antibiotic molecules immediately. Efflux was first described as a mechanism of resistance to tetracycline in *Escherichia coli.* [7]
3. Structurally modifying the target site. Example: Modification of DNA Gyrase (enzyme essential for DNA Replication) by mutation can render the fluoroquniolones ineffective.
4. Producing enzymes to destroy antibiotic molecules. Example: Production of β-lactamases to destroy the β-lactam rings.

Due to the ability of bacteria to overcome the drug, there is always a need to develop new drug molecules that can target new sites or metabolisms in the bacteria. One such class of antibiotics that have developed is "Fatty acid biosynthesis inhibitors".

1.3 Fatty acid Biosynthesis:

Fatty acid synthesis is the creation of fatty acids from acetyl-CoA and malonyl-CoA precursors through action of enzymes called fatty acid synthases (Fig.2). It is an important metabolic reaction as fatty acids are necessary for building and reproducing cell membranes. The important steps involved in this cycle are:

1. Condensation of malonyl-CoA and acetyl-CoA by β-ketoacyl-ACP synthase.
2. Reduction of carbonyl to hydroxyl by β-ketoacyl-ACP reductase.
3. Dehydration of alcohol to alkene by β-hydroxyacyl-ACP dehydrase.
4. Reduction of alkene to alkane by enoyl-ACP reductase.

Fatty acid synthesis is divided into two classes: type I and type II. In type I, the active sites that catalyze the cycle are present on a single protein or may be divided between two interacting proteins. However, for type II, the active sites of each enzymatic cycle are distinctly distributed onto single protein.

The bacterial species, predominantly, show the presence of FAB-II and hence this becomes an attractive targets for antibiotics. Also, the protein sequence and arrangement of active sites are different for the humans and bacteria. Thus, selectively, the bacterial cell can be targeted without affecting the mammalian fatty acid synthesis. [8, 9]

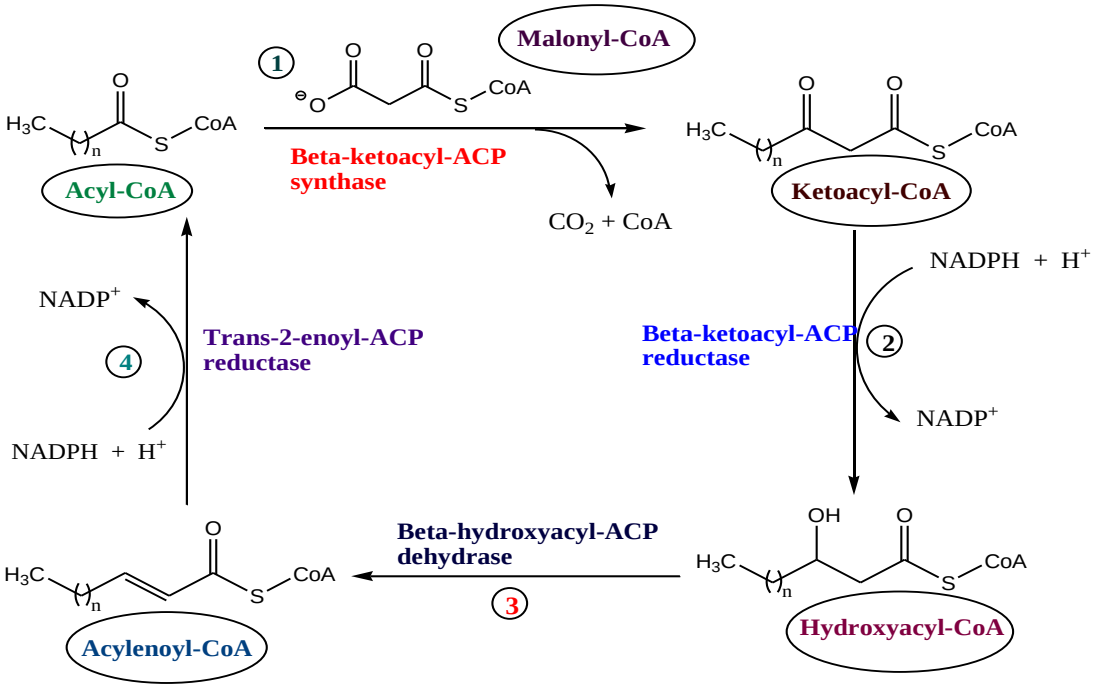


Fig.2: Fatty acid biosynthesis

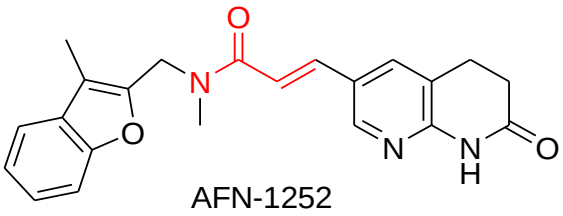
1.4 Fatty acid Biosynthesis inhibitors:

An inhibitor interferes in the normal functioning and alters the action of enzymes/proteins by site specific binding. One such class of inhibitors specifically target the fatty acid biosynthesis process in the bacteria and hence serve the purpose of antibiotic. These are known as "Fatty acid biosynthesis inhibitors" and the common examples include *Triclosan* and *Isoniazid*. Triclosan is a broad spectrum antibiotic that acts by inhibiting the enoyl-acyl carrier protein reductase (ENR) enzyme. This enzyme is coded to the FAB I gene in the bacteria. This increases the binding affinity of enzyme to NAD^+ (Nicotinamide adenine dinucleotide) and forms ENR-NAD^+ complex, which is stable and no longer participates in the fatty acid synthesis. [9] Triclosan, on the other hand, gets activated by a bacterial enzyme KatG, and then binds to NADH cofactor to form a complex. The complex then further binds to lnhA or enoyl-acyl carrier protein reductase and blocks the sites to which the substrate is supposed to attach. Henceforth, inhibiting the fatty acid synthesis. [10]

Resistance has been observed to these drugs for a few species of streptococci and therefore there is an urgent need to develop new drugs.

1.5 Aim of Our Research:

The focus of our research lies on the fatty acid biosynthesis inhibitors. We aim at developing the molecules modelled on a known promising molecule "AFN-1252" (Fig.3) developed by Affinium Pharmaceuticals, Inc. (Toronto, Canada). [11] This molecule is essentially an α,β-unsaturated amide and is in its PHASE-III stage of clinical trials. Other than this, no drug molecule of this category is commercially available which gives us an opportunity to come up with alternate molecules modelled around it. Another set of molecules developed by Glaxo-Smith Kline also categorize as FAB I inhibitors. These molecules are indole based α,β-unsaturated amides (Fig.4) and tend to occupy the region of the active site where the enoyl substrate is expected to bind. [12]



AFN-1252

Fig.3

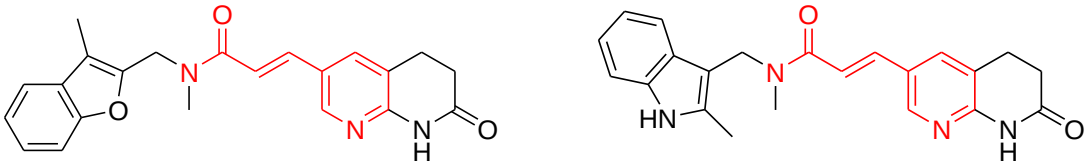


Fig.4: Molecules screened by Glaxo-Smith Kline as FAB inhibitors

Fig.5 shows the library of our target compounds. These compounds are expected to target the final reduction process in the FAB-II cycle by inhibiting the enoyl-ACP reductase. These retain the core moiety of α,β-unsaturated amides. The present class of known molecules of such kind, are the ones with aliphatic amides. However in our work, we have tried to develop the cyclic amides in the form of prolines, pyrrolidines, piperidines and amino thiaoles. Some thiazole ring containing compounds have been found to show the therapeutic abilities, therefore making it an attractive target for us to accommodate in our work. [13] Since the aminopyridine based enoyl-ACP Reductase inhibitors have already

been screened by Glaxo-Smith Kline with promising results, we also tried to incorporate the same moiety in our work. [14]

To understand the effect of additional substituent at the olefinic carbon in the activity, we included the Knoevenagel condensation products of various active methylene compounds with pyridine carboxyldehyde. Besides this strategy also provides additional advantages in further derivatization and heterocycles formation.

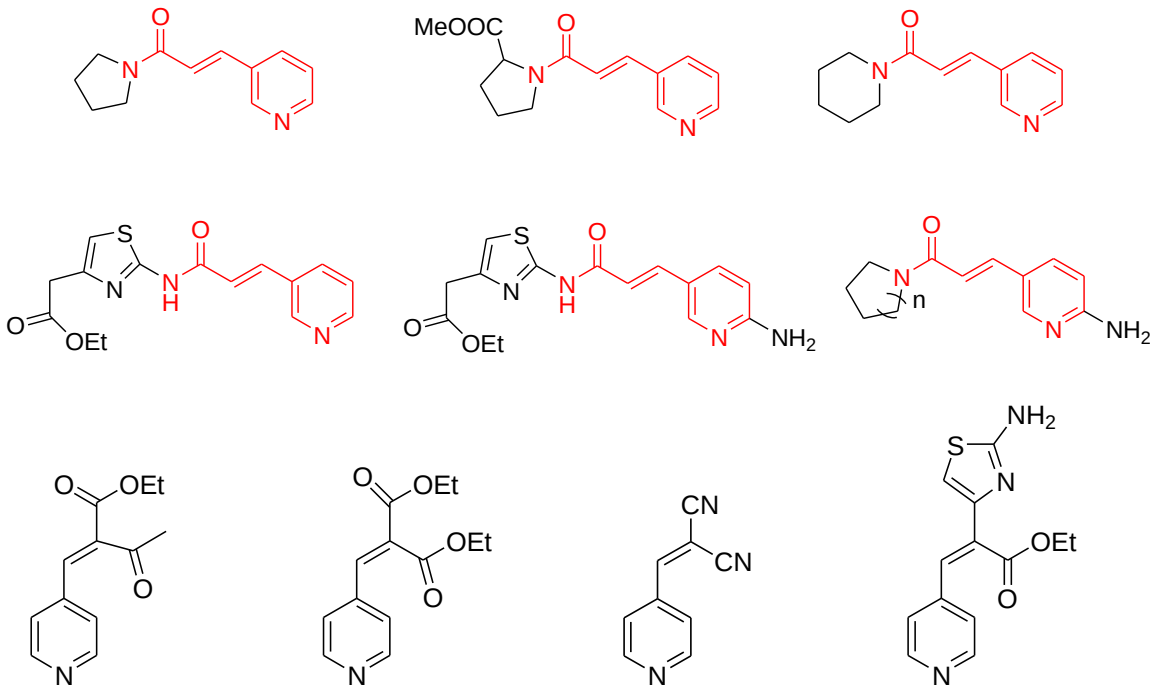


Fig.5: Target molecules of our study

1.6 Retrosynthetic routes adopted:

In order to achieve the targeted molecules, the retrosynthetic route as shown in Fig.6 was adopted. Two different routes have been considered. In the first startegy, the acrylic amides were prepared using the cyclic amines (proline, piperidine and pyrrolidine) and acrylic acid. For proline derivatives, the carboxylic group was esterified prior to the Heck coupling. (Route 1, Fig.6)

A Heck reaction was performed for the bromopyridines and ethyl acrylate. The resultant product was then used to form the amides using proline, pyrrolidine, piperidine and

aminothiazole. (Route 2, Fig. 6)

A Knoevenagel Condensation was performed for the pyridine carboxyldehyde and active methylene compounds, such as malononitrile, ethylacetoacetate and diethylmalonate to prepare the α,β-unsaturated carbonyl derivatives which was then planned for further derivatization. (Fig.6)

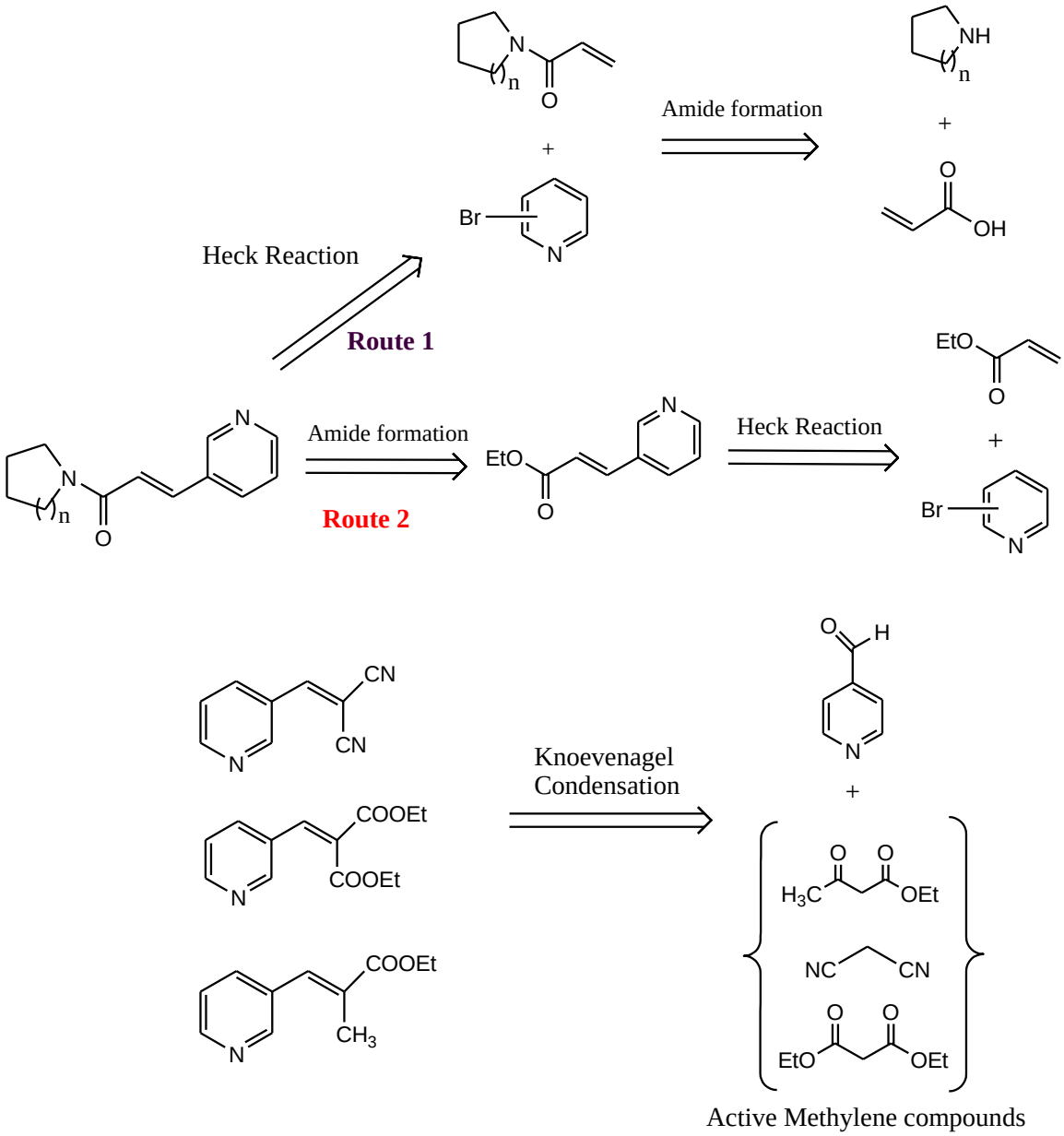


Fig.6: Retrosynthetic routes adopted for the synthesis of targeted molecules

Since Heck coupling, Knoevenagel condensation and acid-amine coupling are the most important reactions in the current context, a brief introduction to such reactions are given below.

1.6.1 Heck Reaction:

Richard F Heck developed an important coupling reaction between aryl halides and olefins using a palladium catalyst. In 1972, he made an important modification to his earlier reported reaction by generating the organopalladium complex from an organohalide and Pd(0) by an oxidative addition. [15] The mechanism of Heck reaction is depicted in Fig. 7.

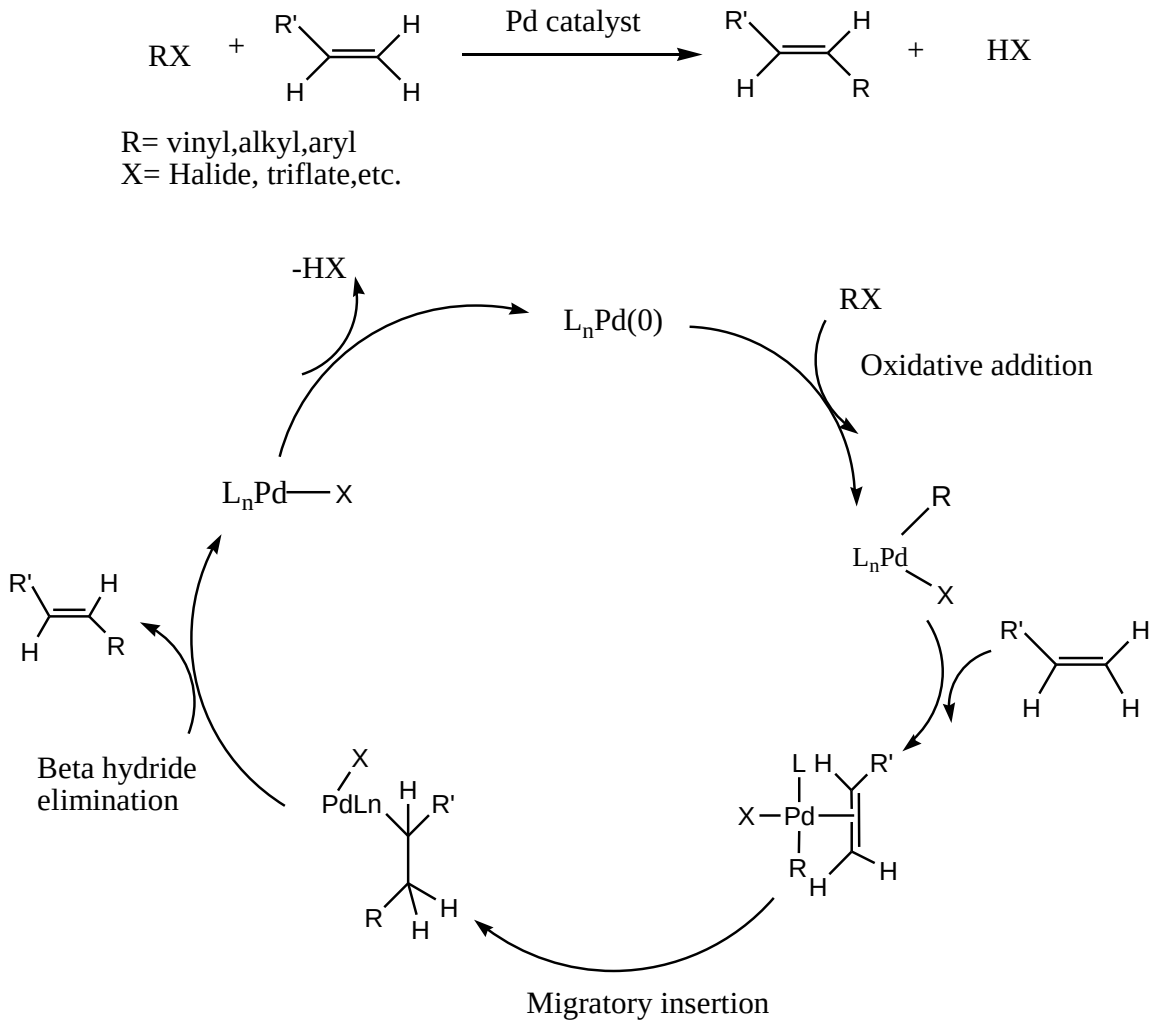


Fig. 7: Mechanism of Heck reaction

1.6.2 Amide Formation:

There are many synthetic routes available for carrying out the amide coupling. The most common methods involve the use of acid chlorides (Schotten-Baumann Reaction) [16]. The acryloyl chloride can prepared using the standard thionyl chloride procedure. [17] This acid chloride can then be coupled with the amine. Amides can also be generated using the acid amine coupling reactions involving coupling agents like EDC.HCl, HOBt, DCC, etc. The use of the coupling agents generates an activated ester which then can be easily coupled to amine. [18] Esters can also be converted to amides in strong alkali and basic conditions. Other methods required for such conversion make use of tin and boron compounds. [19, 20] Use of K^{t}OBu to get the necessary strong basic condition has also been reported in literature. [21]

1.6.3 Knoevenagel Condensation:

Named after the German chemist, Emil Knoevenagel, it is the condensation of active methylene compounds with aldehydes or ketones to afford α,β-unsaturated compounds. It usually involves an amine base as an organocatalyst. [22] The reaction initiates with the deprotonation of active methylene compound by the base to generate an enolate. Simultaneously the base also forms an iminium ion with the aldehyde which then undergoes an attack from the enolate and following a rearrangement, results in the olefin compound. (Fig. 9) Literature reports also suggest the phosphane catalyzed Knoevenagel condensation for α-cyanoacrylates and α-cynoacrylonitriles. [23] The mechanism reported for PPh_3 catalyzed reaction is shown in Fig.8

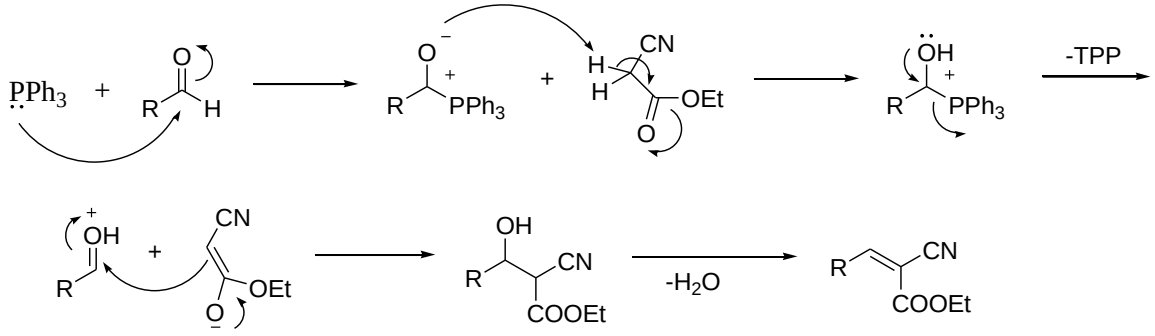


Fig. 8: Plausible mechanism reported for PPh_3 catalyzed Knoevenagel Condensation

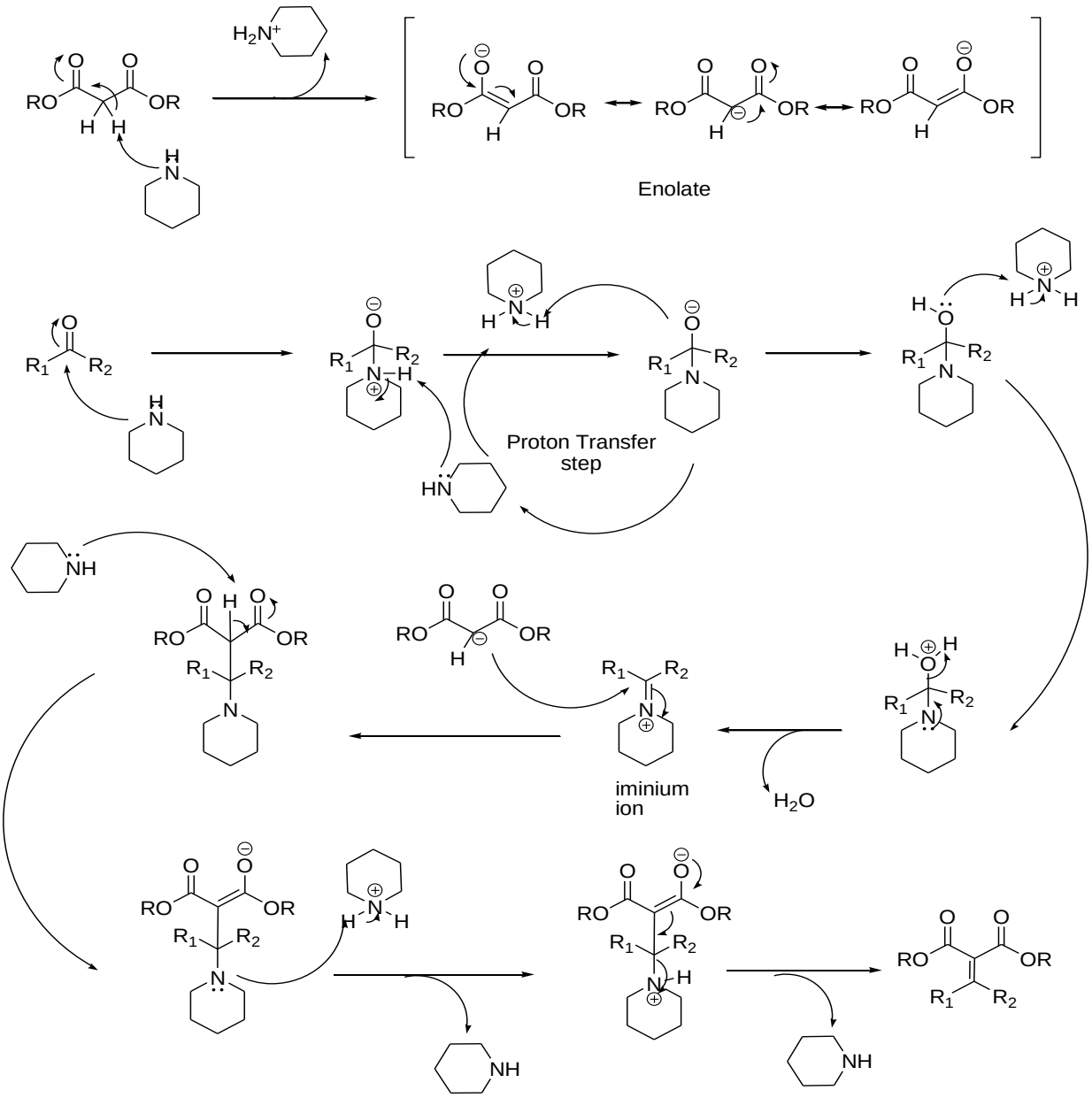


Fig. 9: Mechanism of Knoevenagel Condensation

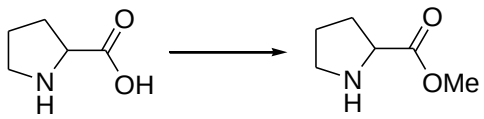
CHAPTER 2

RESULT AND DISCUSSION

Following the route 1 in our retrosynthetic route, the first step was to prepare the amides of the various cyclic amines (proline, pyrrolidine and Piperidine). In case of proline it was necessary to carry out the protection of the acid group prior to the next step.

1) Protection of carboxylic acid group in proline (Preparation of L-proline methyl ester):

The preparation of L-proline methyl ester **(1)** was carried out using the various synthetic routes reported in literature involving trimethylchlorosilane (TMSCl) and thionyl chloride. (Scheme 1). [24, 25]



1

Yield = 60 %

Conditions: $SOCl_2$/TMSCl (1.5 equiv.), 80 ^oC Reflux, 12h

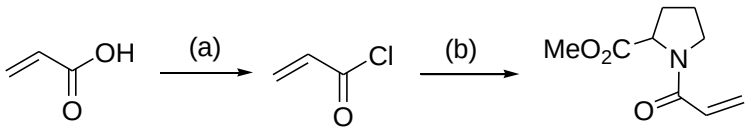
Scheme 1

The reaction with TMSCl gave product in very poor yield. One probable reason that we suspect was that the TMSCl contained moisture. The presence of moisture can significantly affect the yield in such reactions. In case of $SOCl_2$ the yield improved significantly. Also it is necessary to neutralize the excess of $SOCl_2$ once the reaction is over, as the hydrochloride salt is formed initially. The $SOCl_2$ used was not distilled, therefore limiting the yield to 60%. Also, MeOH was not dried. The yields improved in bulk reactions.

2) Preparation of L-proline acryl amide:

1 was used to prepare L-proline acryl amide based on the literature report. (Scheme 2). [26] In order to prepare acryloyl chloride from acrylic acid, $SOCl_2$ was used along with catalytic

amount of benzoquinone (to inhibit polymerization of acrylic acid). The acid chloride was then reacted to the *L*-proline methyl ester to yield the desired product **(2)**. Again, the $SOCl_2$ used was not distilled and thus limited the yield. The solvents used in the reaction were dried before.



2

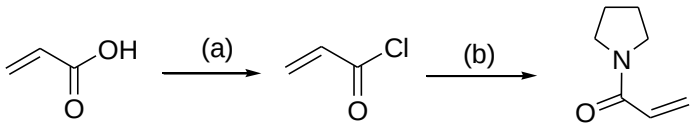
yield = 60%

Conditions: (a) $SOCl_2$ (1.05 equiv.), benzoquinone (catalytic), DCM, R.T., 1hr (b) L-proline methyl ester (1 equiv.), TEA (3 equiv.), DMAP (catalytic), DCM, R.T., 1hr.

Scheme 2

3) Preparation of pyrrolidine acryl amide:

The same reaction of amide formation was carried out with pyrrolidine as well to give pyrrolidine acryl amide **(3)** (Scheme 3). Reaction was also carried out using TMSCl as trimethylsiloxy group would act as a good leaving group upon the attack of amine nucleophile. However, the presence of moisture in TMSCl hampered the reaction. (Table 3) In case of thionyl chloride too, the yields were lower. The use on undistilled thionyl chloride does not convert the acrylic acid completely to the acid chloride and hence lowers the yield in subsequent steps.



3

yield = 35 %

Conditions: (a) $SOCl_2$/TMSCl, benzoquinone (catalytic), DCM, R.T. (b) pyrrolidine, TEA, DMAP (catalytic), DCM, R.T.

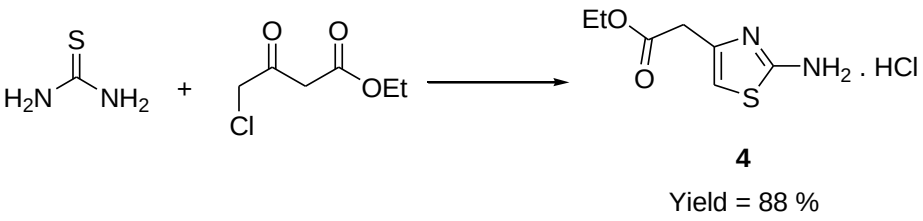
Scheme 3

Table 1:

Entry	Reagent	Pyrrolidine	TEA	Temp.	Time	Yield
1.	TMSCl (1.5 equiv.)	1.0 equiv.	-	R.T	3h	<5 %
2.	TMSCl (2 equiv.)	5.0 equiv.	-	R.T	3h	<5 %
3.	$SOCl_2$ (1.05 equiv.)	1.0 equiv.	1.05 equiv.	0 ^oC addition and then R.T	6h	10 %
4.	$SOCl_2$ (1.05 equiv.)	1.1 equiv.	3.3 equiv.	0 ^oC addition and then R.T	6h	35 %

4) Substrate scope for amines:

In order to prepare some different amine substrate, we carried out the synthesis for **(4)**. The reaction was successfully carried out between thiourea and ethyl-4-chloroacetoacetate (Scheme 9) with excellent yield. Since, thiazole ring containing compounds have already been known to show therapeutic abilities, we also tried to incorporate them into our targets. [13]



4

Yield = 88 %

Conditions: Ethanol, 80 ^oC Reflux, 6h

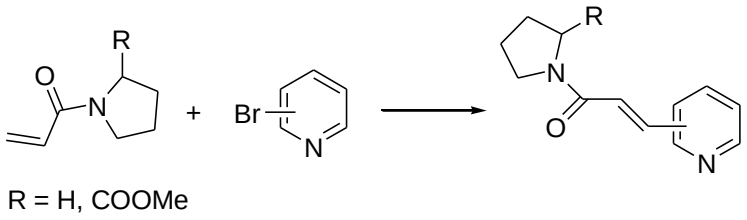
Scheme 4

5) Preparation of heck coupled products:

a) Heck reaction between acrylamide and bromopyridine:

Following the general protocols available in the literature reports, the heck reactions were carried out between pyrrolidine acryl amide/L-proline acrylamide and bromopyidines.

$Pd(OAc)_2$, PPh_3 as ligand and TEA as base were used. The reaction yielded product only in the case of 3-bromopyridine (Table 4). However, NMR showed the presence of impurities along with the product. The polarity of the amides and coupled products was found to be similar and therefore the purification via column chromatography was difficult.

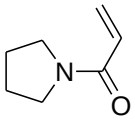
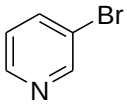
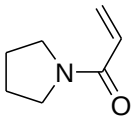
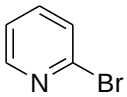
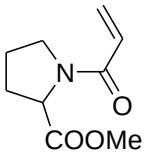
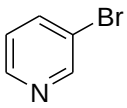
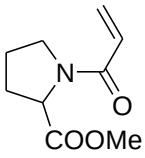
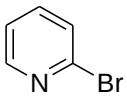


R = H, COOMe

Conditions: $Pd(OAc)_2$ (11 mol %), PPh_3 (30 mol %), TEA, DMF

Scheme 5(a)

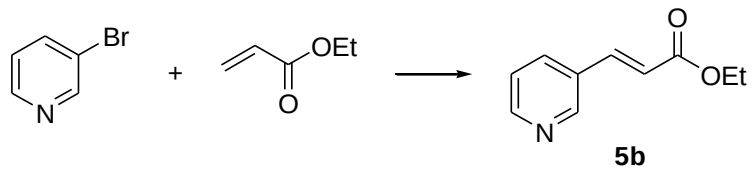
Table 2(a):

Entry	Acryl amide (1 eq.)	bromopyridine (2 eq.)	TEA	Time	Temp	Status
1.			1.3 equiv.	24h	130 °C	Crude product *
2.			1.0 equiv.	18h	130 °C	No product
3.			1.0 equiv.	24h	130 °C	Crude product *
4.			1.1 equiv.	24h	130 °C	No product

Since the yields of these amides after Heck coupling were low, we adopted an alternative strategy, viz route 2.

(b) Heck reaction between 3-bromopyridine and ethyl acrylate:

The heck coupling of 3-brompyridine and ethyl acrylate (Scheme 5(b)) gave positive results in good purity. The reaction was optimized for Diisopropylethylamine as base and ToTP as ligand. (Table 5, entry 5). When PPh_3 was being used as a ligand, then it was getting oxidized to form triphenylphosphine oxide ($O=PPh_3$) and thus hindering the targeted product formation. ToTP showed no such side product formation and gave maximum conversion.

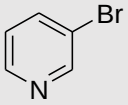


5b

Conditions: $Pd(OAc)_2$ (5 mol %), Ligand (30 mol %), Base (2 equiv.), DMF, 130 ^oC, 24h

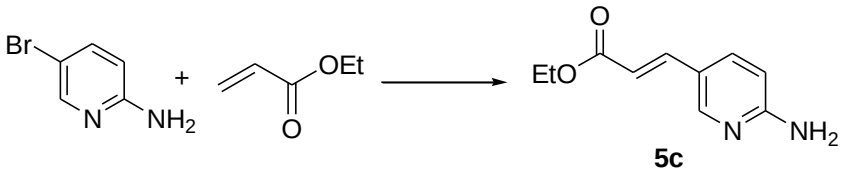
Scheme 5(b)

Table 2(b):

Entry no.		Ethyl acrylate	Base	Ligand	Yield
1.	1 equiv.	1.5 equiv.	K_2CO_3	PPh_3	23 %
2.	1 equiv.	1.5 equiv.	Cs_2CO_3	PPh_3	33 %
3.	1 equiv.	1.5 equiv.	TEA	PPh_3	16 %
4.	1 equiv.	1.5 equiv.	DIPEA	ToTP	17 %
5.	1 equiv.	1.5 equiv.	DIPEA	ToTP	88 %

(c) Heck reaction between 2-amino-5-bromopyridine and ethyl acrylate:

Following the already optimized conditions for 3-bromopyridine and ethyl acrylate, heck coupling was tried for the 2-amino-5-bromopyridne and ethyl acrylate with $Pd(OAc)_2$ as a catalyst, ToTP as a ligand and diisopropylethylamine as a base (Scheme 5(c)). The reaction was highly successful and gave pure product **5c** in high yields.



5c

Yield = 83 %

Conditions: $Pd(OAc)_2$ (5 mol %), ToTP (30 mol %), DIPEA (2 equiv.), DMF, 130^oC, 24h

Scheme 5 (c)

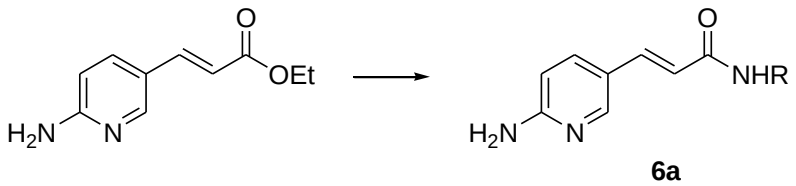
6) Amide formation from heck coupled products:

Once we developed the heck coupled product, the next step was to synthesize amides from them. In this regard, the Heck products of ethyl acrylate was treated with various amine nucleophiles for trans amidation.

a) Reaction between ethyl 3-(6-aminopyridin-3-yl)acrylate (5c) and amines:

5c was reacted with a number of amines (Scheme 6(a)). Primary amines were used in this case owing to their higher reactivity and less steric hindrance compared to the secondary amines. Only in the case of hydrazine, a polar product was formed, whereas in the other cases, there was no reaction. The reaction was also tested at 50 ^oC for 24h, but again it did not proceed.

A literature report suggested to use basic conditions to convert ester to amides directly.[27] So, we used K^tOBu as a base in the same reaction and heated at 80 ^0C. Again, we observed no reaction.



6a

Conditions: Amine (5 equiv), K^tOBu (1 equiv.), THF, 80 ^oC, 24h

Scheme 6(a)

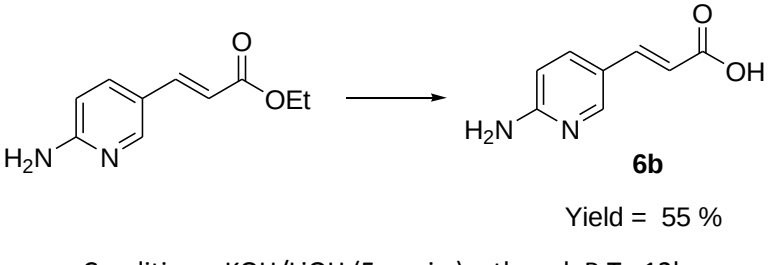
Table 3:

Entry	Amine	Status
1.	Hydrazine monohydrate	Highly Polar spot on TLC {Could not be isolated}
2.	Aniline	No Reaction
3.	4-Chloroaniline	No Reaction
4.	2-aminothiazole	No Reaction
5.	*p*-Anisidine	No Reaction
6.	*p*-Toluidine	No Reaction

Since we were not able to accomplish the direct conversion of ester to amides, we adopted the standard approach of first hydrolyzing the ester to acid and then follow it up with acid amine coupling

b) Hydrolysis of ethyl 3-(6-aminopyridin-3-yl)acrylate (5c) to its acid derivative:

Hydrolysis of an ester to acid is a well-known reaction. So, following the general protocols for ester hydrolysis, we carried out a reaction for hydrolysis of **5c** using base to obtain its acid derivative **6b**. (Scheme 6(b)).



6b

Yield = 55 %

Conditions: KOH/LiOH (5 equiv.), ethanol, R.T., 12h

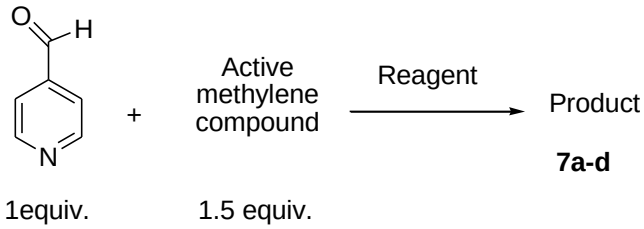
Scheme 6(b)

The yield of the product was low because of its partial solubility in ethanol. Minimum amount of ethanol-water had to be employed for this reaction in order to attain the product. The yields also improved on large scale reaction.

7) Knoevenagel Condensation:

As per our of our retrosynthetic route (Fig. 6), Knoevenagel Condensation reactions were carried out for various active methylene groups (like malononitirle, diethylmalonate, ethylacetoacetate, etc) and 4-Pyridine carboxyldehyde (Scheme 7).

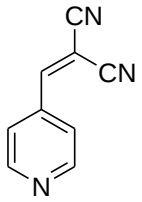
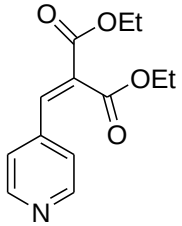
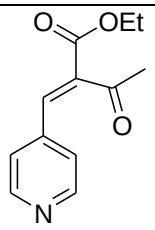
In case of Malononitrile, literature reports were followed and PPh_3 was employed as the necessary organocatalyst for carrying out the reaction. [23] In other cases, Piperidine was used.

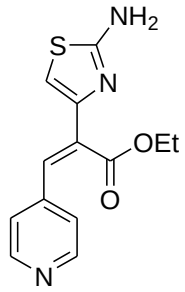


Conditions: DMAP (catalytic), Solvent, 70 – 80 ^oC

Scheme 7

Table 4:

Entry	Active methylene compound (2)	Product	Reagent	Solvent	Status*
1.	malononitrile		PPh_3 (0.2equiv.)	DMF	50 % conversion
2.	diethylmalonate		Piperidine (0.1 equiv.)	No solvent	80 % conversion
3.	ethylacetoacetate		Piperidine (0.1 equiv.)	No solvent	100 % conversion

4.	ethyl (2-amino-1,3-thiazol-4-yl)acetate		Piperidine (1.1 equiv.)	DMF	20 % conversion

*Conversions are reported on the basis of TLC monitoring.

When PPh_3 was used, it got oxidized to triphenylphosphine oxide and therefore could not catalyze the reaction properly. However, upon column isolation, multiple spots were observed lying very close to the product. Hence, the pure compound could not be isolated.

The route 1 of our retrosynthetic pathway where first the amides were synthesized and the coupled to Bromopyridine via Heck coupling, could not yield a pure product owing to the similar polarity of the impurities and the product. Therefore, route 2 was adopted where we carried out the heck reaction first between ethyl acrylate and Bromopyridine first. The conditions were optimized to obtain almost quantitative yield of the Heck product with ethyl acrylate. These were then followed up by hydrolysis of ester to obtain pure acid derivative. Thus, the results from the route 2 were encouraging.

Apart from that, Knoevenagel condensation reactions were also performed for 4-pyridine carboxyldehyde and various active methylene compounds. The aim was to create a set of molecules with different substitutions on the oleifinic carbon and to study their effects on antimicrobial activity. However, pure products could not be isolated mostly because of the incomplete conversion of the starting compounds.

CHAPTER 3

CONCLUSIONS & OUTLOOK

Pyridine substituted α,β-unsaturated amides are now being seen as promising molecules in the medicinal chemistry. Their role in the inhibition of the fatty acid biosynthesis in bacteria can well be exploited in form of antibiotics. Many molecules are already available in literature reports and some of them have even made it into the trial phases. Since there has been a huge innovation gap in development of the new antibiotics, the threat of antibiotic resistance has tormented the world for decades now. These new molecules could potentially be the next class of antibiotics and serve the purpose. Therefore we designed Pyridine substituted α,β-unsaturated carbonyl compounds as our target molecules to inhibit the FAB pathway in bacteria.

Two alternative synthetic routes, namely amide formation followed by Heck coupling and Heck coupling followed by amide formation were considered to achieve the target molecules. Due to the separation issues during chromatographic purification, the former route was not successful. Therefore, the alternative approach was followed, where the bromopyridine compounds were coupled with ethyl acrylate and then hydrolyzed to yield pure acid. The results were much more promising and encouraging in these approaches. The conditions were optimized to obtain almost quantitative yield of the Heck product with ethyl acrylate.

In order to understand the effect of substituents at the olefinic carbon in antibacterial activity, Knoevenagel condensation reaction was exploited using pyridine-4-carboxaldehyde with various active methylene compounds.

We already have developed the heck coupled acid derivative **(6b)**, which could be coupled with different amines to get our targeted molecules. For this, the simple acid-amine coupling pathways can be followed involving different coupling agents. The Knoevenagel Products could also be derivatized to form the necessary amides. Once the targeted molecules are developed, we would be able to do their biological studies.

The major limitation of this project is that we could not have enough target molecules synthesized and so the in vitro minimum inhibitory concentration (MIC) studies could not be studied.

Therefore, the biological studies and further derivatization of these system, in particular introduction of heterocyclic groups are planned to be carried out as future work. Also, we have planned to revisit the route 1 for synthesizing of our targets. The reaction conditions are to be modified and optimized to attain the pure products ready for the biological activity assignments.

CHAPTER 4

EXPERIMENTAL SECTION

4.1 GENERAL METHODS:

The solvents and reagents were purchased from commercial suppliers (Sigma Aldrich, Merck and HiMedia) and used without further purification. The reactions monitored by TLC was on run on silica gel plates (Merck) and visualized with UV light (254 nm and 360 nm), $KMnO_4$ and iodine stains. Column chromatography was performed on silica gel (60-120 mesh) purchased from HiMedia. The IR spectra were recorded on a Perkin – Elmer Spectrum two FT IR spectrometer FT-IR spectrometer. Mass spectra were recorded on a Waters SYNAPT G25 Spectrometer in a positive and negative Electron Spray Ionization mode. The ^{1}H NMR and ^{13}C NMR were recorded on an Avance-III, Bruker Biospin 400 MHz and 100 MHz respectively at room temperature. Chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane (δ 0.00). ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q). Splitting patterns that could not be interpreted or easily visualized were recorded as multiplet (m) or broad (br). Coupling constants are reported in Hertz (Hz).

4.2 SYNTHESIS:

1) General procedure for preparation of L-proline methyl ester:

L-proline (1 equiv.) was dissolved in methanol (10 mL) and thionyl chloride (1.5 equiv.) was then slowly added to it at 0 ^{o}C. The reaction mixture was then refluxed overnight at 80 ^{o}C. Reaction was monitored through TLC and upon completion, the solvent was evaporated under reduced pressure. Minimum amount of water was then added to it and the $NaHCO_3$ (1.5 equiv.) was added so as to quench the excess of $SOCl_2$ and HCl. The product was then isolated using acetonitrile and saturating the aqueous layer with NaCl. The solvent was then dried under reduced pressure to get a yellow oil as product.

L-Proline methyl ester (1): Yellow oil; Yield= 60 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.51 ppm (br s, 1 H), δ 3.85 ppm (br s, 3 H), δ 3.53-3.62 ppm (m, 2 H), δ 2.42-2.47 ppm (m,1H),

δ 2.07-2.21 ppm (m, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169 ppm, δ 59 ppm, δ 53 ppm, δ 45 ppm, δ 28 ppm, δ 23 ppm.

2) General procedure for preparation of amides:

Acrylic chloride was generated by adding $SOCl_2$ (1.3 equiv.) slowly to an ice cold solution of acrylic acid (1.0 equiv.) in 5mL DCM with a catalytic amount of benzoquinone. The reaction was run for about an hour. Simultaneously, a solution of amine (L-proline methyl ester/ Pyrrolidine (1.5 equiv.), TEA (3 equiv.) and DMAP (catalytic) was prepared in 5 mL DCM. To this, the acrylic chloride prepared was added slowly dropwise at R.T. The reaction is allowed to stir at R.T for about 6h and monitored through TLC. Once completed, to the reaction mixture, minimum amount (3-5 mL) of ice cold water is added. The product is extracted out using DCM (3x10 mL) and washed with HCl (3x10 mL), water (2x10 mL), NaOH (3x10 mL) and brine solution (10 mL). The collected organic extracts are dried over Na_2SO_4 and then dried under reduced pressure to yield brown colored viscous liquid as product.

L-Proline acrylamide (2): Brown viscous liquid; Yield= 60 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.25-6.40 ppm (m, 2 H), δ 5.73 ppm (dd, *J*=7.8, 4.3 Hz, 1 H), δ 4.56-4.58 ppm (m, 1 H), δ 3.6 ppm (s, 1 H), δ 3.55-3.60 (m, 2 H), δ 1.9-2.2 ppm (m, 4 H).

Pyrrolidine acyl amide (3): Dark Brown viscous liquid; Yield= 35 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.33-6.48 ppm (m, 2 H), δ 5.63-5.66 ppm (d, *J*=12 Hz, 1 H), δ 3.52 ppm (m, 4 H), δ 1.92 ppm (m, 4 H).

3) General procedure for Heck Reactions:

$Pd(OAC)_2$ (5-11 mol %) and Ligand {PPh_3/ ToTP} (20-30 mol %) were dissolved in 5 mL DMF with a constant purging of Argon into the solution for about 1hr. bromopyridine (1 equiv.), ethyl acrylate/ proline acryl amide/ pyrrolidine acrylamide and base (TEA/ K_2CO_3/ Diisopropylethylamine) were added to it in necessary amounts. The reaction mixture was then heated at 130 ^{o}C for about 24h with constant monitoring through TLC. Upon completion, the reaction mixture was filtered through Celite and the solvent was dried off under reduced pressure. The crude product was then purified by column chromatography. (In some cases acid-base workup was performed. After the drying of the solvent, minimum amount of water (3 mL) was added to the crude product. The pH was then adjusted to 3-5

and washed with ethyl acetate. The aqueous extracts were collected and $NaHCO_3$ was added to take pH at 7-8. The compound was extracted out using ethyl acetate and dried over Na_2SO_4. The solvents were dried under reduced pressure to yield product.)

(E)-ethyl 3-(pyridin-3-yl)acrylate (5b): brown oil, yield = 88 %, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 ppm (s, 1 H), δ 8.56 ppm (dd, *J*=4.9, 1.6 Hz, 1 H), δ 7.8 ppm (d, *J*= 8 Hz, 1 H), δ =7.63 ppm (d, J=*16.1* Hz, 1 H), δ 7.29 ppm (m, 1 H), δ 6.47 ppm (d, *J*=16.1 Hz, 1 H), δ 4.54 ppm (q, *J*=7 Hz, 2 H), δ 1.3 ppm (t, *J*=7.2 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166 ppm, δ 150 ppm, δ149 ppm, δ 140 ppm, δ 134 ppm, δ 123 ppm, δ 120 ppm, δ 60 ppm, δ 14 ppm; MS (ESI) *m/e* 178 $(M+H)^+$.

(E)-ethyl 3-(6-aminopyridin-3-yl)acrylate (5c): yellow solid, yield = 83 %, ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 ppm (d, *J*=2.1 Hz, 1 H), δ 7.69ppm (dd, *J*=8.8, 2.3 Hz, 1 H), δ 7.56ppm (d, *J*=16.1 Hz, 1 H), δ 6.53ppm (d, *J*=8.8 Hz, 1 H), δ 6.27ppm (d, *J*= 16.1 Hz, 1 H), δ 4.27ppm (q, *J*=7 Hz, 2 H), δ 1.34 ppm (t, *J*=7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167 ppm, δ159 ppm, 148 ppm, δ141 pm, δ 136 ppm, δ 120 ppm, δ 115 ppm, δ 109 ppm, δ 60 ppm, δ 14 ppm; MS (ESI) m/e 191 $(M-H)^+$.

4) General procedure for hydrolysis of ester to acid:

The heck coupled ester was dissolved in minimum amount of EtOH (5 ml) and a solution of LiOH/KOH in water (2-3 mL) was added at R.T. The reaction was stirred at R.T overnight. Upon completion, 12M HCl was added dropwise so that pH became 6. The precipitated solids were filtered and dried to afford the acid derivative in form of solid.

(E)-3-(6-aminopyridin-3-yl)acrylic acid (6b): off white solid, yield = 55 %; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.13 ppm (s, 1 H), δ 7.78 ppm (d, *J*=8.8 Hz, 1 H), δ 7.44 ppm (d, *J*= 16.1 Hz, 1 H), δ 6.58 ppm (s, 2 H), δ 6.47 ppm (d, *J*=8.8 Hz, 1 H), δ 6.23 ppm (d, *J*=16.1 Hz, 1 H); ^{13}C NMR(100 MHz, DMSO-d_6): δ 168 ppm, δ 161 ppm, δ 150 ppm, δ 142 ppm, δ 135 ppm, δ 114 ppm, δ 108 ppm ; MS (ESI) m/e 165 $(M+H)^+$.

5) Preparation of Ethyl (2-amino-1,3-thiazol-4-yl)acetate hydrochloride (4):

Thiourea (1 eq) was suspended in ethanol (5 mL) and ethyl-4-chloroacetoacetate (1 eq) was added to it dropwise. The reaction mixture was then refluxed overnight at 80 ^{o}C. Upon completion, the reaction mixture was concentrated in vacuo and ethyl acetate was added to it. The white solid was then filtered out and dried to get the product. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.48 ppm (br s, 2 H), δ 6.72 ppm (s, 1 H), δ 4.11 ppm (q, *J*=7.1 Hz, 2 H), δ 3.76 ppm (s, 2 H), δ 1.21 ppm (t, *J*=7.2 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 170 ppm, δ 169 ppm, δ 133 ppm, δ 106 ppm, δ 61 ppm, δ 33 ppm, δ14 ppm; MS (ESI) m/e 187 $(M+H)^{+}$.

6) General procedure for Knoevenagel condensation:

A mixture of 4-Pyridine carboxyldehyde (1 eq), active methylene compound (1.3 eq) and catalyst {PPh_3/ Piperidine} in necessary amounts were stirred at 75-80 ^{o}C for about 24h. The progress of reaction was monitored by TLC. After maximum conversion, as indicated by TLC, the reaction mixture was diluted with water and extracted with ethyl acetate (3x10 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude product was then purified by column chromatography (ethyl acetate/ Hexane) to afford pure product.

CHAPTER 5

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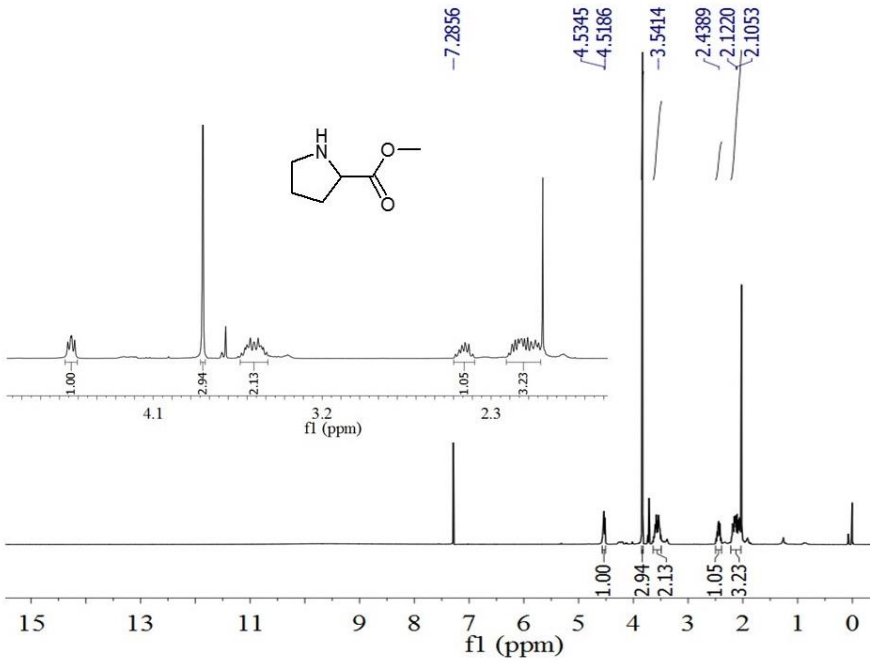
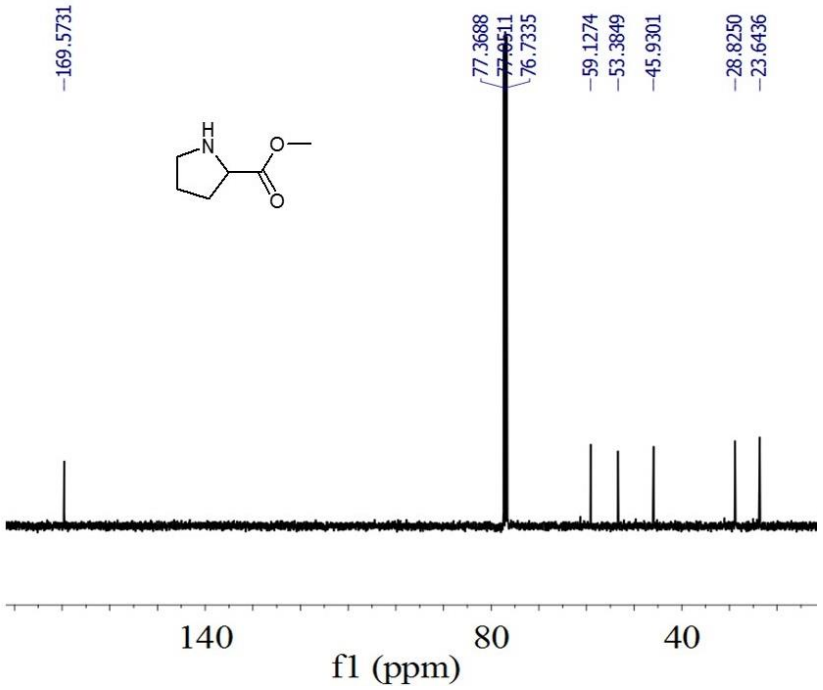
APPENDIX

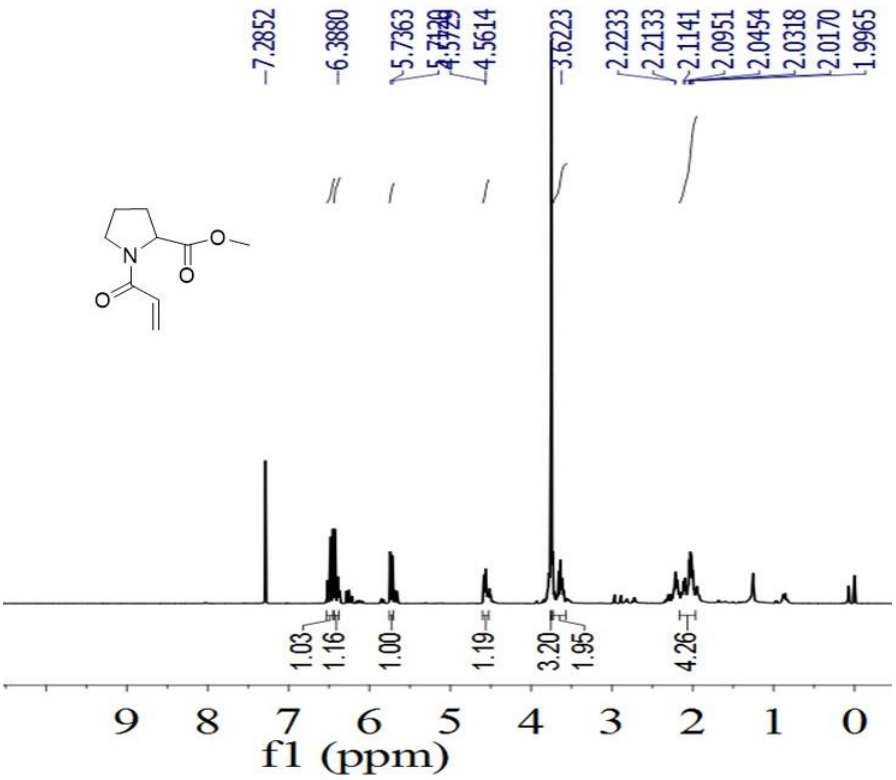
L-proline methyl ester (1): ^{1}H NMR



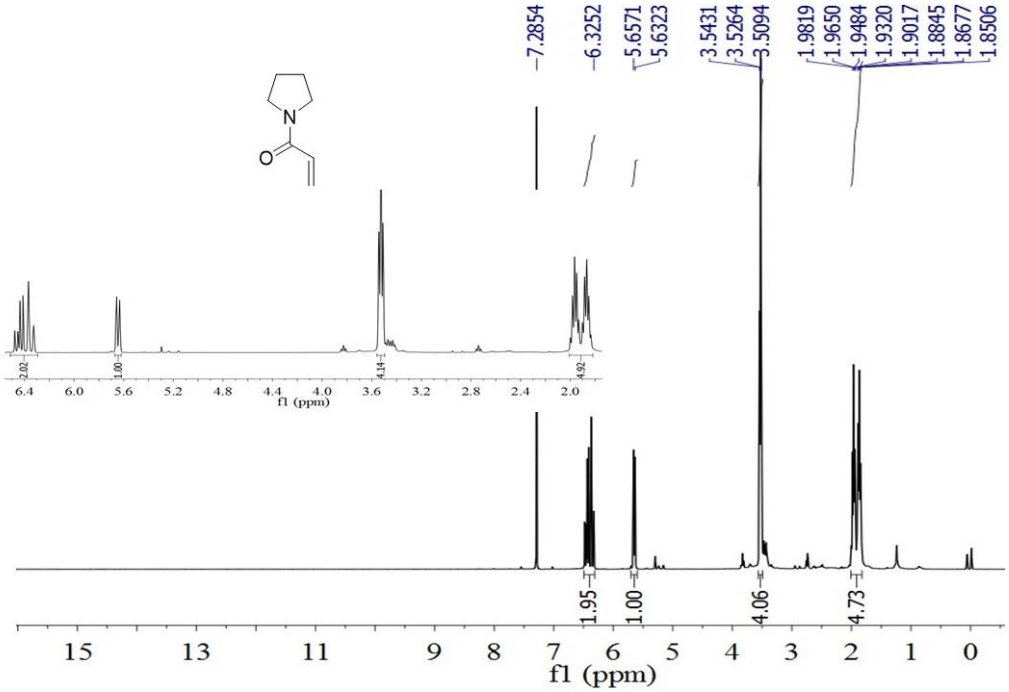
L-proline methyl ester (1): ^{13}C NMR



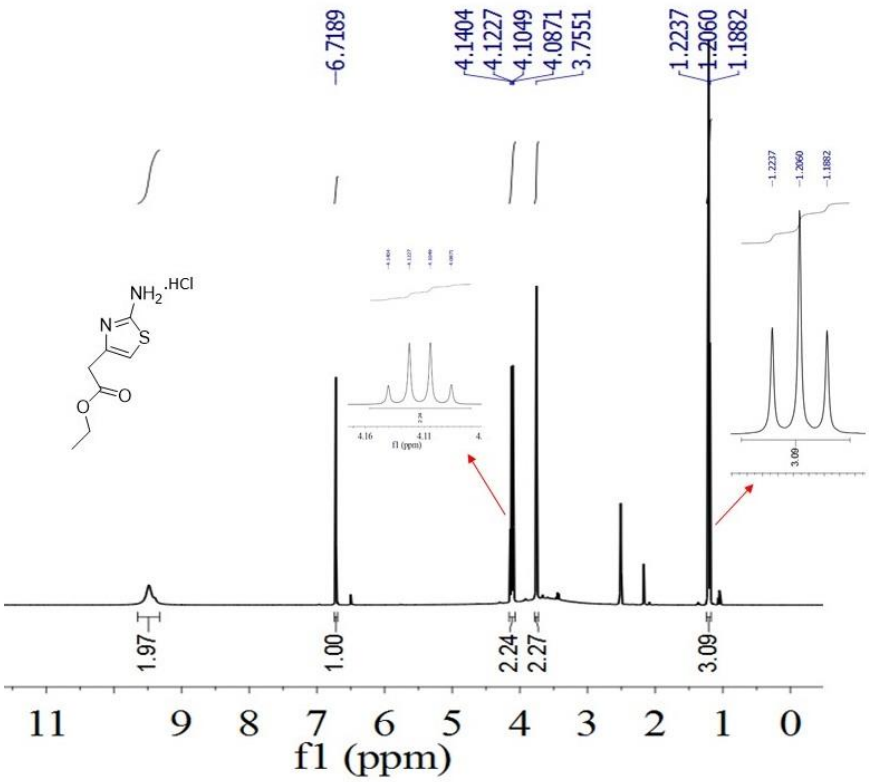
L-proline acryl amide (2): ^{1}H NMR



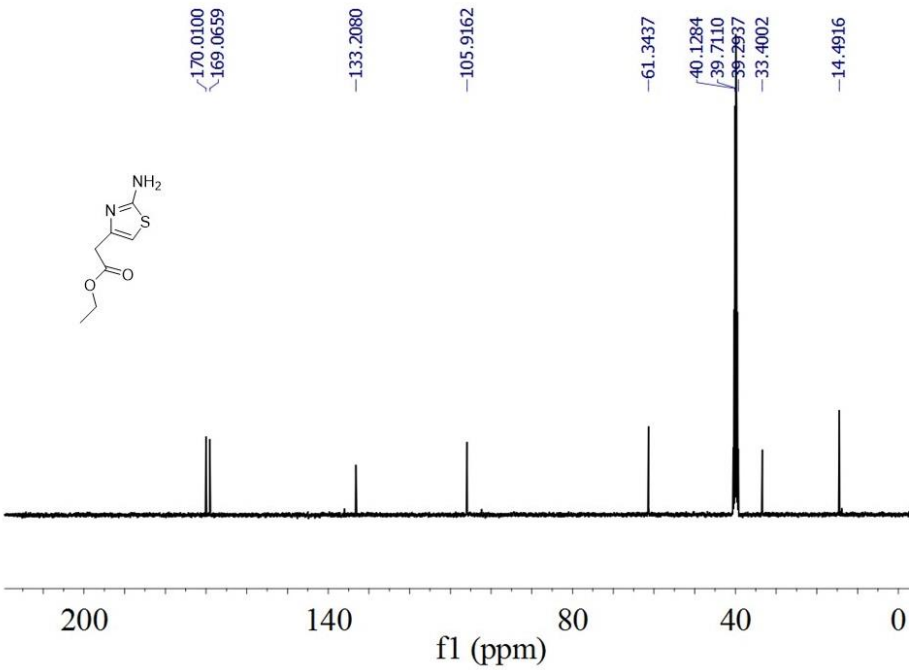
Pyrrolidine acryl amide (3): ^{1}H NMR



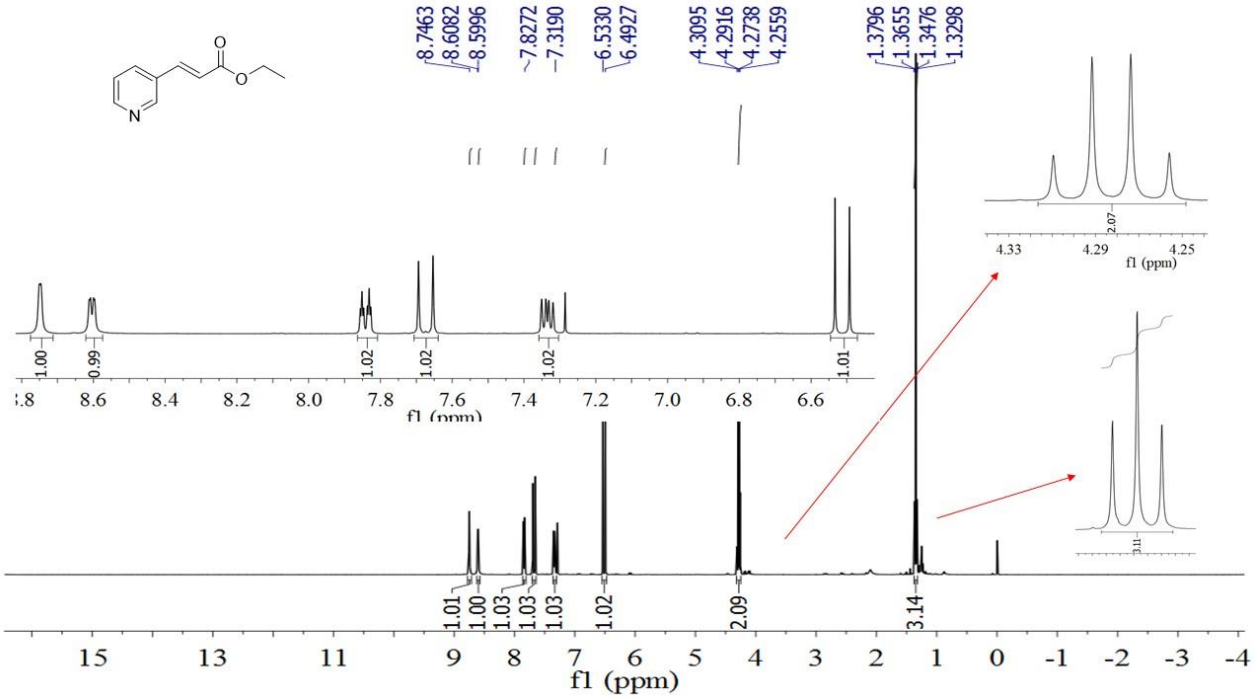
ethyl(2-amino-1,3-thiazol-4-yl)acetate hydrochloride (4): ^{1}H NMR



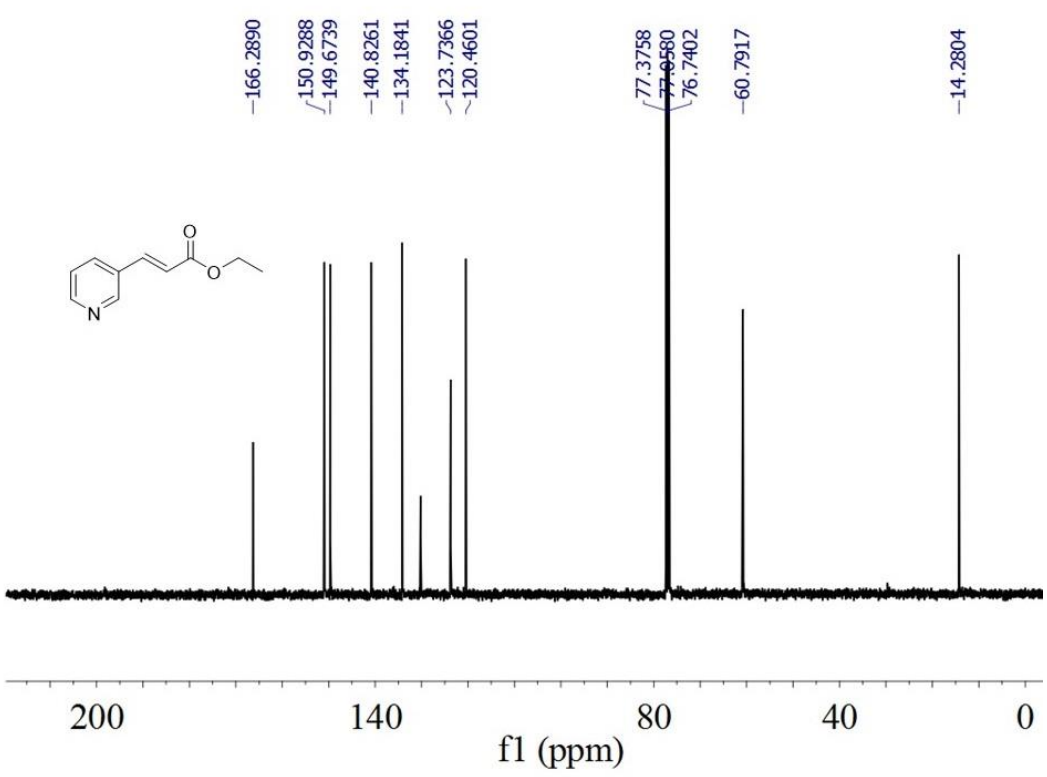
ethyl(2-amino-1,3-thiazol-4-yl)acetate hydrochloride (4): ^{13}C NMR



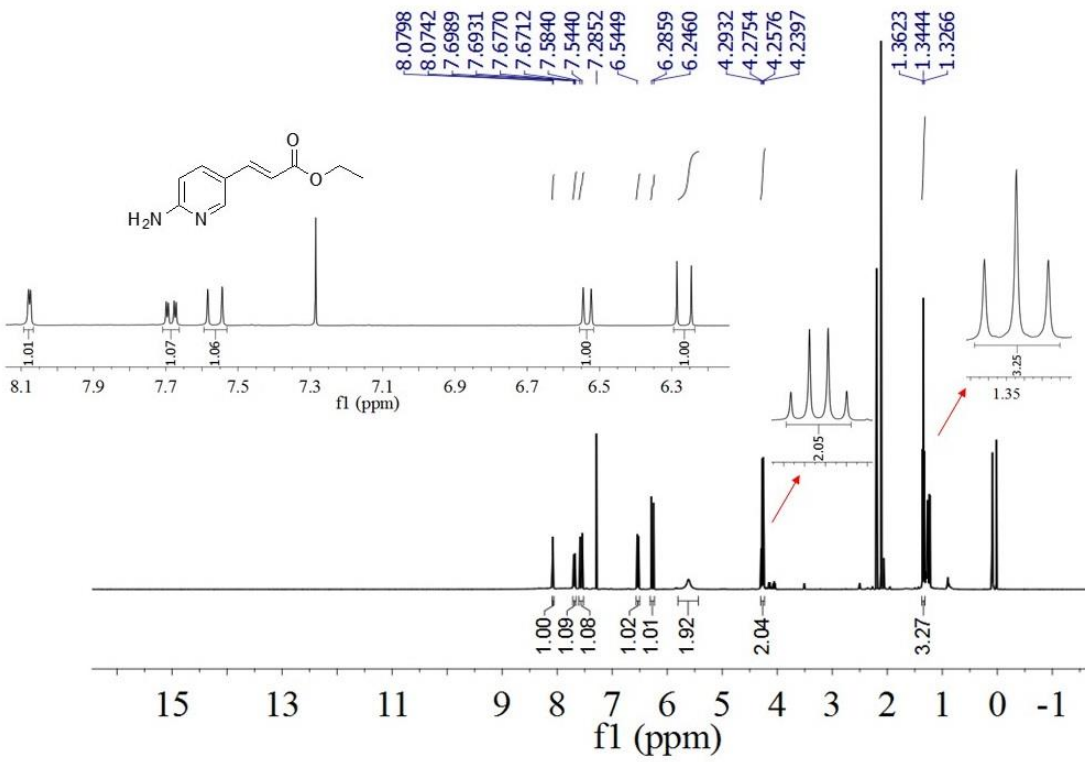
(E)-ethyl 3-(pyridin-3-yl)acrylate (5(b)): ^{1}H NMR



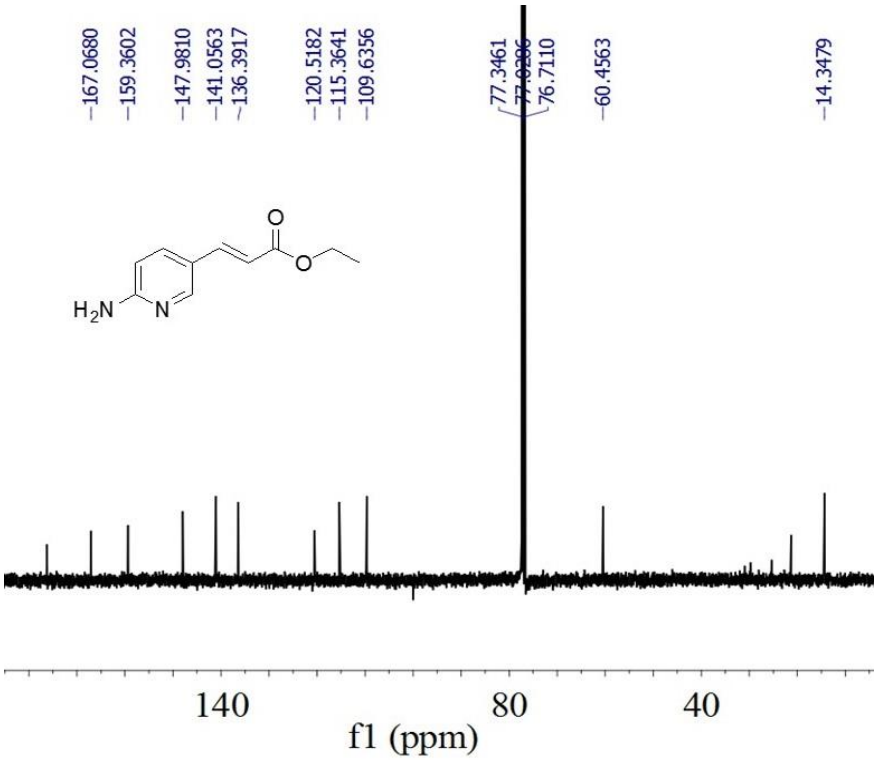
(E)-ethyl 3-(pyridin-3-yl)acrylate (5(b)): ^{13}C NMR



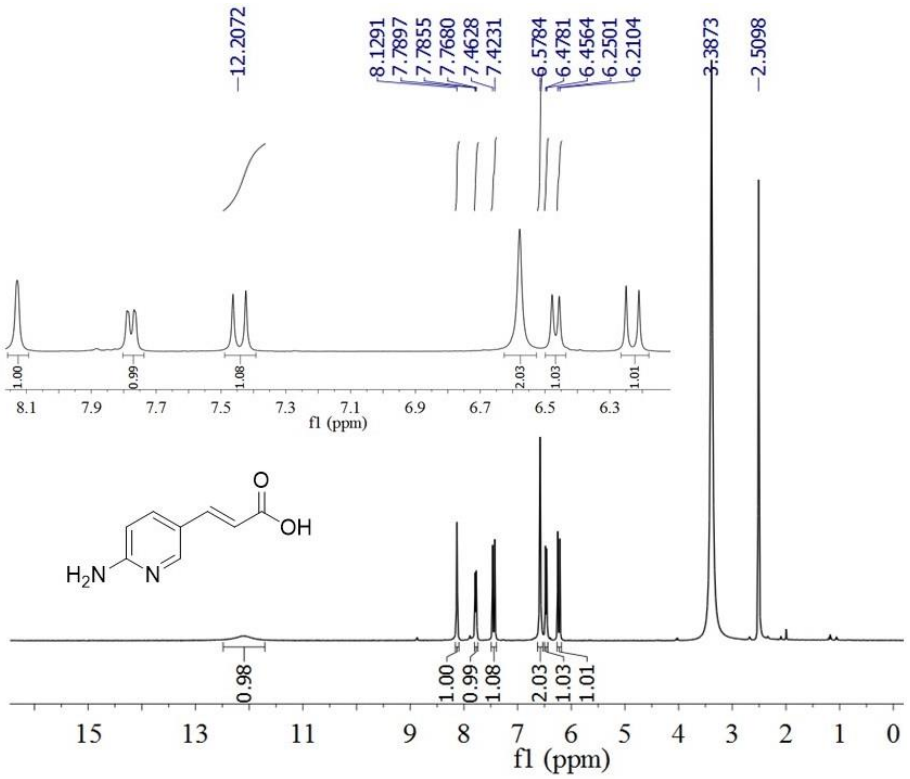
(E)-ethyl 3-(6-aminopyridin-3-yl)acrylate (5(c)): ^{1}H NMR



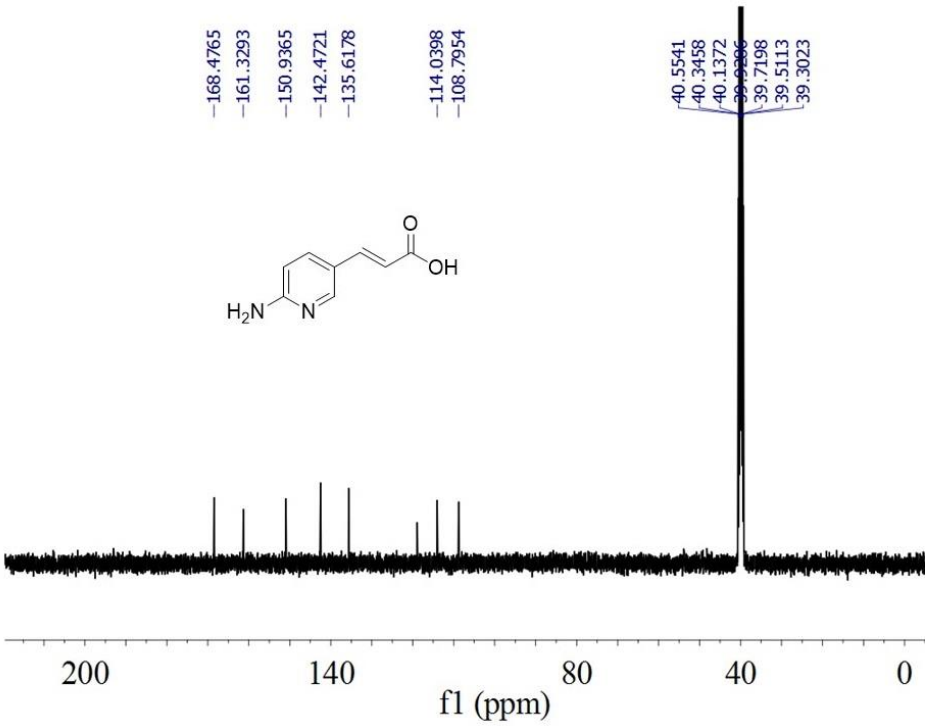
(E)-ethyl 3-(6-aminopyridin-3-yl)acrylate (5(c)): ^{13}C NMR



(E)-3-(6-aminopyridin-3-yl)acrylic acid (6(b)): ^{1}H NMR



(E)-3-(6-aminopyridin-3-yl)acrylic acid (6(b)): ^{13}C NMR



Curriculum Vitae

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  Overall: 92.8%

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RESEARCH EXPERIENCE:

- Project on *"Diels Alder Reactions and their Synthetic and Commercial Applications"*, in IISER Mohali under the guidance of Dr. R. Vijaya Anand (Asst. Professor IISER Mohali) during May-July, 2011.

- Project on *"Applications of Heterogeneous Palladium Catalyst in Suzuki-Miyaura Cross-Coupling Reaction using different techniques",* in Institute of Himalayan Bioresource Technology (IHBT-CSIR), Palampur (Himachal Pradesh) under the guidance of Dr. Pralay Das (Scientist-C, Natural Plants Products Division, IHBT-CSIR Palampur) during May-July, 2012.

- Project on "*Synthesis and characterization of acyclic Phosphazene type complexes",* in IISER Mohali under the guidance of Dr. Sanjay Singh (Asst. Professor IISER Mohali) during May-July, 2013.

- Project on *"Synthesis of pyridine based Bacterial fatty acid synthesis inhibitors"*, in IISER Mohali under the guidance of Dr. Sugumar Venkataramani (Asst. Professor IISER Mohali) during May-July, 2014.

- MS Thesis Project: *"Synthesis of some pyridine substituted α,β unsaturated amides as potential Bacterial fatty acid biosynthesis enoyl ACP reductase inhibitors."* Under Dr. Sugumar Venkataramani (Asst. Professor, Department of Chemistry, IISER Mohali) during August, 2014- April, 2015.

FELLOWSHIPS AND DISTINCTIONS:

- Inspire Fellowship for a Period of Five Years. (August 2010 – May 2015) under Department of Science & Technology (DST), India.
- Summer Research Fellow (2012) - Institute of Himalayan Bioresource Technology (IHBT-CSIR), Palampur (Himachal Pradesh).
- Summer Research Fellowship- IISER Mohali (2011, 2013, 2014).
- Scholarships for being Top 0.1% in Central Board of Secondary Education (CBSE) in Class 10^{th} and 12^{th}.

LANGUAGES

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- Hindi- speak fluently and read/write with high proficiency.

COMPUTER & INSTRUMENTAL SKILLS:

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- Basic knowledge of C and C++ Programming languages.
- Operating systems: Linux & Windows.