SYNTHESIS OF SOME HECK COUPLED PYRIDINE DERIVATIVES OF ACRYLAMIDE AND ACRYLIC ACID AS POTENTIAL BACTERIAL FATTY ACID BIOSYNTHESIS INHIBITORS

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CERTIFICATE OF EXAMINATION

This is to certify that the dissertation titled "**Synthesis of some Heck coupled pyridine derivatives of acrylamide and acrylic acid as potential bacterial fatty acid biosynthesis inhibitors**" submitted by Ms Lilit Jacob (Reg No: MS10061) for the partial fulfilment of BS MS dual degree programme of Indian Institute of Science Education and Research Mohali, 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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Dated: 24th April 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 university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussion. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Lilit Jacob

(Candidate)

Dated:24th April 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)

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CONTENT

LIST OF ABBREVIATIONS

ACP:	Acyl Carrier Protein
FAB:	Fatty acid Biosynthesis
ToTP:	Tris(*o*-tolyl)phosphine
TEA:	Triethylamine
EDC:	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
CDI:	Carboxydiimidazole
HOBt:	Hydroxybenzotriazole
DMF:	Dimethyl Furan
DMSO:	Dimethyl Sulphoxide
$KMnO_4$:	Potassium permanganate
$CDCl_3$:	Deuterated chloroform
PPh_3/ TPP:	Triphenylphosphine
$CsCO_3$:	Cesium carbonate
K_2CO_3:	Potassium carbonate
HCl:	Hydrochloric acid
DIPEA:	Diisopropylethylamine
DMAP:	*N*,*N*'-Dimethyl-4-amminopyridine
NMR:	Nuclear magnetic resonance
TLC:	Thin layer chromatography
KBr:	Potassium bromide

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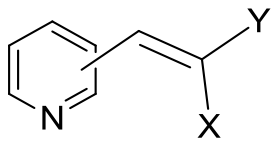
ABSTRACT

Antibiotics revolutionised medicine, by treating infections, which were considered to be fatal once. As a natural consequence of continuous use of antibiotics, bacteria started developing resistance. Indeed bacteria develop the resistance at a faster rate for almost all type of existing antibiotics. If this scenario continues, it is inevitable to come back to the same old situation, where bacteria will once again become life threatening pathogen to human population. This situation compels to develop novel methods for tackling those pathogens. In this regard, inhibition of bacterial fatty acid biosynthetic (FAB) pathway could be a viable strategy, which is relatively underexplored. Although there is cascade of processes and many enzymes are involved in FAB, our primary interest is to target Enoyl ACP Reductase enzyme, which reduces the olefinic group in the α,β-unsaturated ester. This enzyme is an attractive target as different drug molecules have been developed. To enhance and broadening of the activity spectrum, we considered a recent drug molecule (AFN-1252, currently in the clinical stage) as our lead, and designed few candidates based on pyridine derivative of acrylic acid/amide. The synthesis of new pyridine derivative of acrylic acid/amide will be presented in this work.



X= H, COOEt, COOMe, CN

Y= COOH, COOEt, $CONH_2$, SO_3Na

Chapter 1. INTRODUCTION

The idea of antibiotics and its synthesis has a long history in the field of medicinal chemistry and health care. However, the accidental discovery of penicillin and many related developments has revolutionized this field. Even in the current context, the discovery of new antibiotics is a crucial challenge[1], because of the development of antibiotic resistance in the bacteria.

An antibiotic is a chemical substance, which can either kill or inhibit the growth of pathogens like bacteria, fungus etc. Although antibiotics are a collective term for the treatment of many microbial infections, it is rather synonymously used for bacterial infections. Antibiotics are essential for human to treat the diseases caused by bacterial infection such as meningitis, cholera, influenza, etc. There are many different types of antibiotics, which can inhibit such infections. Examples of some antibiotics are given in Fig 1.[2] It is indeed fascinating to understand how those antibiotics work. Mostly they will work on inhibiting or preventing different cellular processes so that the bacteria cannot survive. Of all those cellular processes, the major targets are the following:

1) Bacterial cell wall biosynthesis (In bacteria, the cell wall is made up of cross linked peptide layer called peptidoglycan, which is being constructed with the use of an enzyme called transpeptidase. The antibiotic class of penicillin and cephalosporin act by inhibiting the functionality of transpeptidase enzyme.[3] There are other type of antibiotics, like vancomycin, which ties up the peptide substrate involved in the peptidoglycan and preventing its interaction with the transpeptidase enzyme.[4] Eventually these antibiotics disrupt the cell wall synthesis, causing bacterial death.)

2) Protein synthesis (When it comes to protein synthesis the RNA and protein machinery in prokaryote is distinct from eukaryote, which allows the targeting of different steps in protein synthesis of antibiotic action, erythromycin[5], tetracycline[6] and aminoglycosides[7] are example for this.)

3) Bacterial DNA replication and repair (In the case of DNA replication, fluroquinolones are a class of synthetic antibiotic that target the DNA Gyrase[8], an enzyme responsible for the uncoiling of intertwined double stranded DNA in bacteria.)

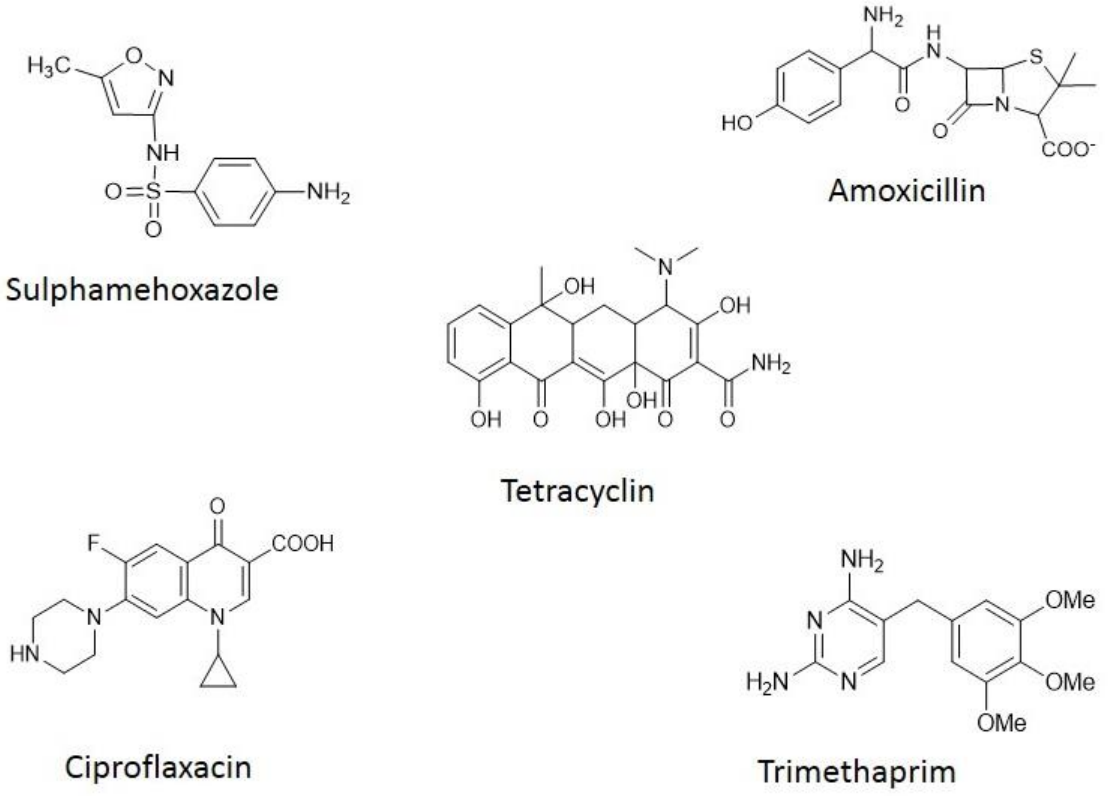


Fig 1: Examples of antibiotics

In spite of the tremendous growth in discovery and development of many different types of antibiotics, the field is not saturated yet. The primary reason for this situation is due to the bacteria started evolving resistance against these antibiotics.[9, 12] The resistance is a situation where the antibiotic become incapable of inhibiting bacterial growth and thus stands as a threat to human population. Enormous studies have been carried out to understand the mechanism of antibiotic resistance evolution that show there are mainly three ways through which the resistance is exhibited. The first one is through porin, a trans-membrane protein which allows the transport of small molecules in to the cell, whose size gets reduced. Generally large size antibiotics are incapable of passing through this size reduced porin, effectively fail to reach the target site of action. The second method of resistance is through efflux pump, situated in the membrane of the bacterial cell and helps in transporting the unwanted or toxic substance out of the cell. That means whenever the bacterial cell senses the antibiotic as a harmful substance it eliminates through efflux pump.[10] The third mode is chemical modification of either the target site or the antibiotic[11], which disrupts the functional integrity and the antibiotic remains ineffective.

Another important fact is that the rate of evolution of antibiotic resistance in bacteria was comparatively slow during pioneer days of the history, but now the resistance is increasing in an alarming rate.[1, 13] According to a recent study by World Health Organization (WHO) on antimicrobial resistance shows that antibiotic resistance is no longer a prediction for future, it is happening right now across the world, if we cannot find a proper solution to this we are going to a post antibiotic era in which small infectious diseases, which have been treatable for decades, can again kill.[14]

Eventhough the mechanism of antibiotic action is widely varying, the bacteria tend to evolve resistance through its own mechanism. As evolution of bacterial resistance is remaining as a curse to the human population, there is demand for a new methodology of tackling bacterial growth and survival[15] and in such scenario, the inhibition of fatty acid biosynthesis in bacteria is a validated target.[16] Fatty acid biosynthesis (**Fig 2**) is a biological process through which fatty acids are prepared from Acetyl CoA and malonyl ACP using an enzyme called FAB synthase. In mammals, the active sites of the FAB enzyme are present in a single protein in contrast to bacteria, where all the activity is found as discrete protein. This difference in active site organization makes this particular enzyme an interesting target for antibiotics.[17]

All the bacterial fatty acid synthesis genes were cloned from Escherichia coli. The enzymes are homologous and have similar biochemical activities, which make them good candidate for identifying broad spectrum antibiotic. Most of the FASII enzymes are important for bacterial survival and its growth, therefore they are interesting target for antibacterial drug development.[18]

The FAB synthase is composed of different enzymes like ketoacyl ACP synthase, Ketoacyl ACP reductase, Hydroxy acyl ACP Dehydrase and Enoyl ACP reductase. Among these Enoyl ACP reductase (FabI) is the most intense research area with the discovery of triclosan, widely used antibacterial drug and isoniazid, medicine for tuberculosis, both of them inhibit the FabI activity in fatty acid biosynthesis inhibition. The clear success in targeting the FabI enzyme and the determinant role of this enzyme in the elongation of each cycle make this the most attractive target for drug discovery and the future of FabI inhibitor as therapeutic agents seems very bright.[19]

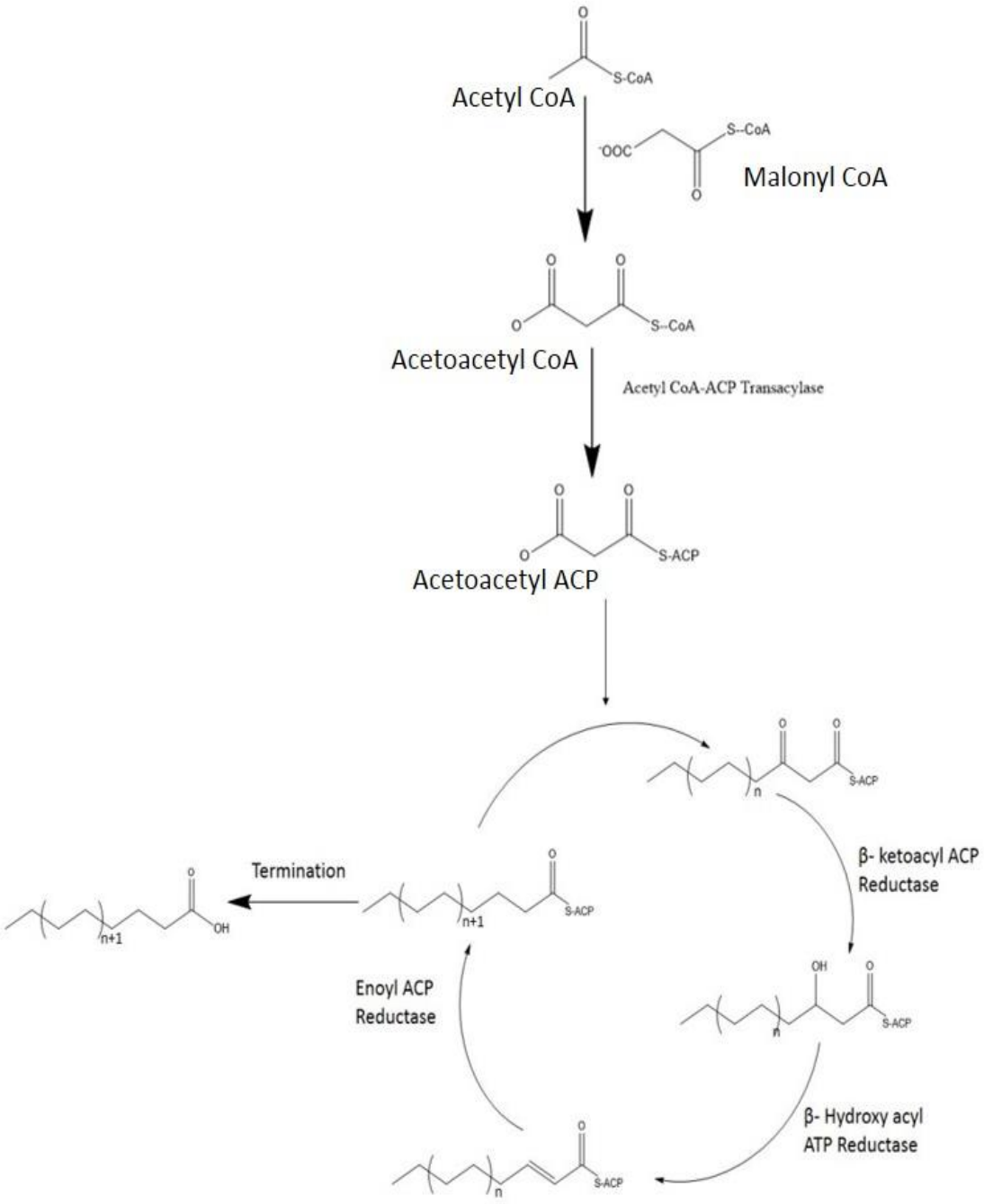


Fig 2: Fatty acid biosynthesis pathway in bacteria

The most important take in case of enoyl ACP reductase inhibition is a drug molecule (AFN-1252, **Fig 3)** synthesised by Affinium Pharmaceuticals, which is now in the phase III level of clinical trials.[20] As this drug has reached the phase III level, we can consider this as a promising lead compound for the development of further improved drug in this category. Based on the earlier developments on FAB inhibitors and detailed study of FAB pathway, we have designed our target molecules.

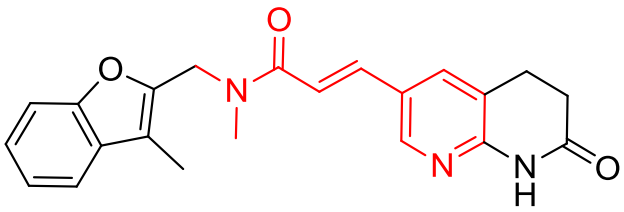
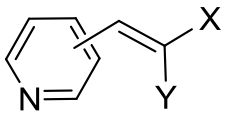


Fig 3: AFN-1252

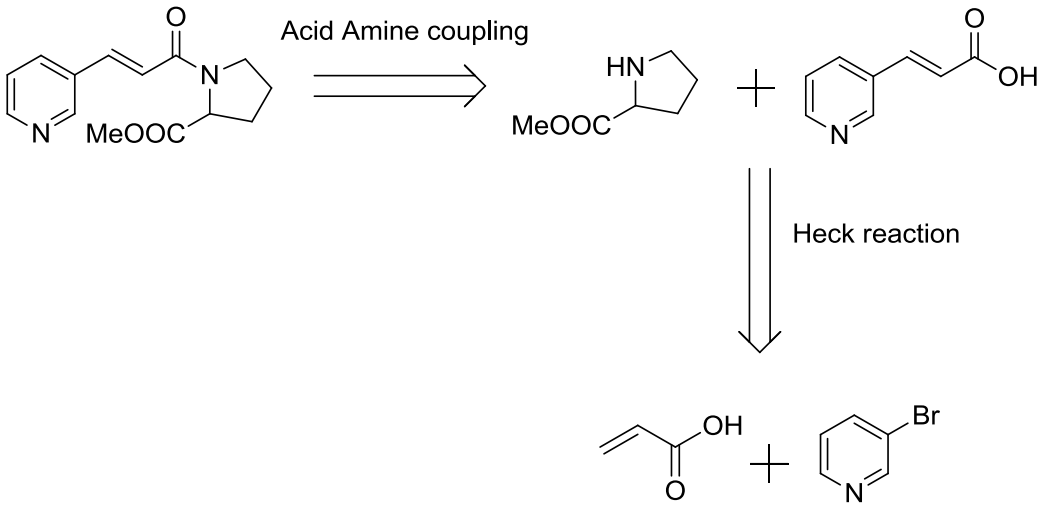
In the design part, we have adopted the analogous drug design approach and in this regard, we retained the pyridine moiety connected with acrylic acid and acrylamide. All the connections were done at 2, 3, 4-positions with respect to the pyridine nitrogen, in order to study the effect of substitution pattern. Apart from that we are also included cyclic amides to induce restricted conformational preferences, which we expect can be effective in binding as well as provide a non-planarity structure for the whole molecule (**Fig 4**). Besides this, the structural modification of AFN-1252 is necessary to convert the route of administration from topical (currently trials are performed at topical level) to oral.



X= COOH, COOR, $CONH_2$, SO_3Na, CONAr
Y= H, COOEt, COOMe, CN

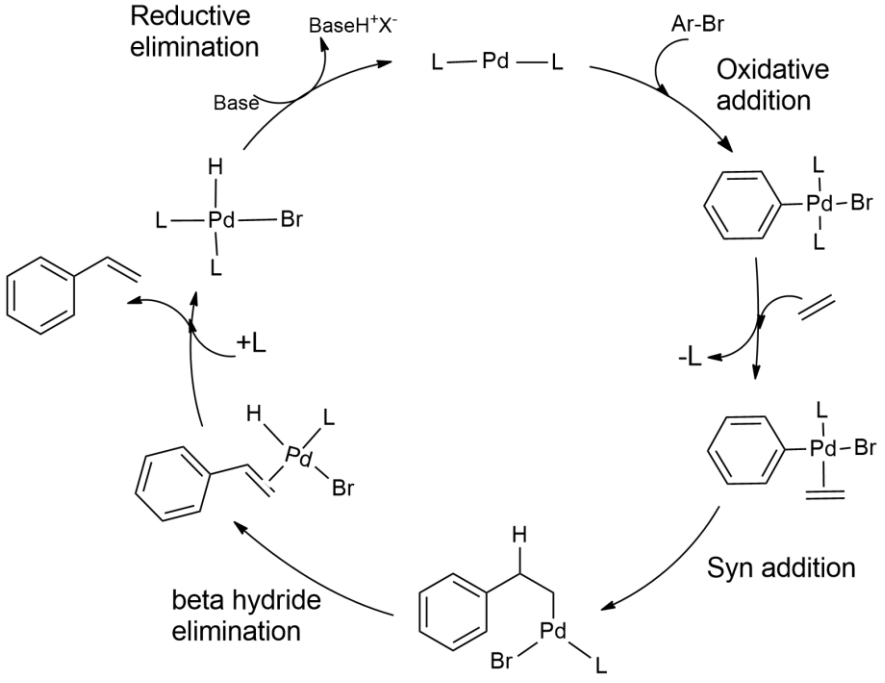
Fig 4: Theem of this work: Our target molecules

In order to synthesize our target molecules, we planned a synthetic pathway utilizing both the cross coupling reaction between haloarenes and vinyl substrates (Heck reaction), as well as acid-amine coupling reactions. The retrosynthetic pathway with respect to the synthesis of our target molecules are given in scheme 1.



Scheme 1: Retrosynthetic Pathway

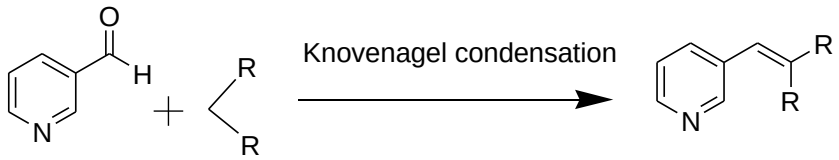
Heck reaction is the palladium catalysed C-C coupling between aryl or vinyl halides with activated alkenes in presence of a base.[21] The proposed mechanism of Heck reaction (**Scheme 5**) involves oxidative addition of an aryl halide into the Pd(0) complex followed by *syn* migratory insertion of alkene into Ar-Pd bond, *syn* β-hydride elimination of hydridopalladium, followed by the reductive elimination of hydropalladium halide to regenerate Pd (0) complex.[22]



Scheme 2: Mechanism of Heck coupling reaction

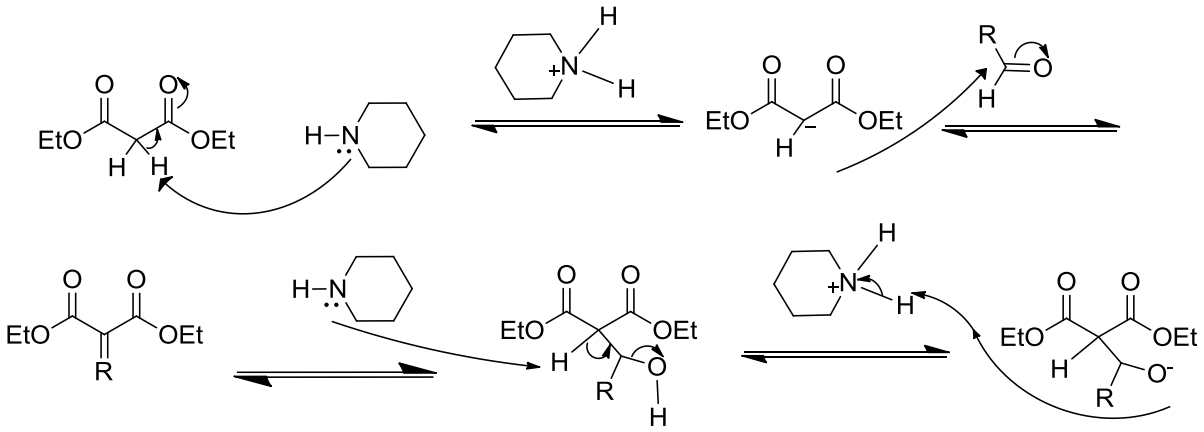
The next step is amide bond formation, which is thermodynamically unfavoured on mixing of acid and amine under normal conditions, and tend to form respective salts.[23] The condensation of the salt to achieve amide can be done using high temperarture[24], which is not compatible for other functionalities. Therefore activation of acid by the attachment of leaving group to the acyl carbon is necessary for the reaction to proceed to get amide. Usually thionyl chloride[25] or oxalyl chloride[26] are used to generate acyl chloride, which is then subjected to aminolysis. The reaction is promoted by addition of one drop of DMF to it.[27] We can also use coupling reagents like CDI, DCC, HOBt, EDC.HCl etc for the effective coupling of free acid and amine.[28] The general mechanism can be described as the reaction of coupling reagent with acid by the removal of the proton followed by the attack of the amine group at the carboxyl position to yield amide. Numerous coupling reagents were developed so far, but no comparative studies were reported on the efficiency in the coupling reaction till now.

We have also followed another approach to get our target molecule through Knoevenagel condensation. It is a kind of nucleophilic addition reaction between aldehyde or ketone with active methylene groups to yield α, β-unsaturated compounds in presence of an organic base. (**Scheme 3**) The reaction begins with deprotonation of the activated methylene group by the base to obtain resonance stabilized enolate. Enolate reacts with aldehyde forming aldol, and subsequently undergo base induced elimination to form the olefin product.[29] (**Scheme 4**) In accordance to our project, the Knoevenagel condensation of pyridine carboxaldehyde with different active methylene groups will give us unsaturated product, which on modification we can obtain our target molecule. There are also some reports, which depict the use of PPh_3 as a catalyst in Knoevenagel condensation.[30] The mechanism is depicted in scheme 5.

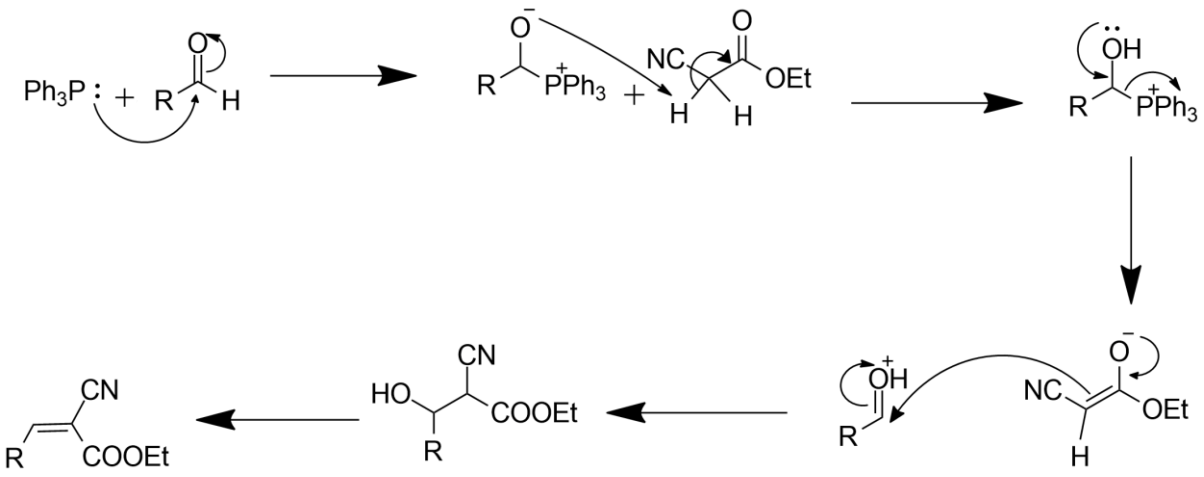


R= COOEt,COOMe,CN,COMe

Scheme 3: Knoevenagel condensation



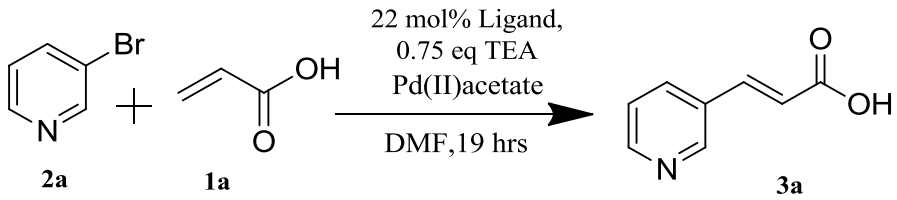
Scheme 4: Mechanism of Knoevenagel condensation with piperdidne



Scheme 5: Mechanism of Knovengel condensation with PPh_3

Chapter 2. RESULTS AND DISCUSSION:

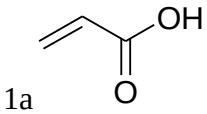
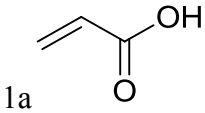
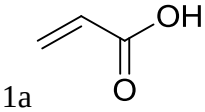
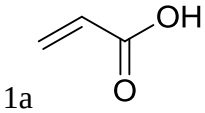
We commenced our project by optimizing the Heck reaction between acrylic acid (**1a**) and 3-bromopyridine (**2a**) to obtain the maximum yield of the product (**3a**). Table 1 comprises of different conditions that we have tried for the optimization of the product (**3a**). We started off with the conventional Heck reaction conditions[31] (**Table1**, Entry 1), but the yield of isolated product was not promising. Therefore we changed the conditions (**Table 1**, Entry 2) and reagents (**Table 1**, entry 3), still the yield did not improve. Surprisingly when we replaced the ToTp (tris-*o*-tolylphosphene) ligand with triphenylphosphene (PPh_3), there was a notable increase in the product yield (**Table 1**, entry 4).



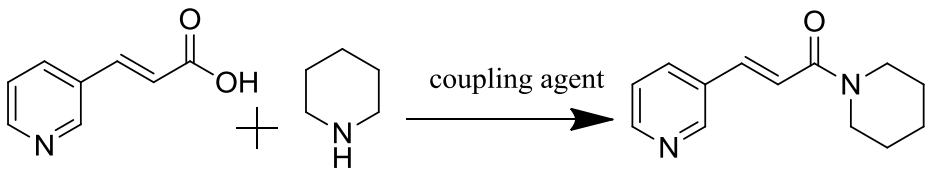
Scheme 6: General scheme for Heck coupling

After the characterisation and analysis of the isolated product (**3a**), we came to realize that the expected product was forming an adduct with triethylamine (TEA), the base in this reaction. According to the NMR spectra the free acid and TEA was present at 3:2 ratio respectively. And by analysing the two H peaks corresponding to the hydrogen at the carboxylic group, we came to conclude that the acid and TEA was forming a 1:1 adduct and rest of the compound remain as free acid. As this is the case, we tried to eliminate the triethylamine by adjusting the pH, checking the movement of the free acid by thin layer chromatography and extracting with an organic layer. But the complete removal of triethylamine remained as a challenge.

Table 1: Optimization of reaction conditions

No	Reagent	Ligand	3 Bromo-Pyridine	Temp	Pd(II) Acetate	Percentage Yield
1	 1a	TOTP	0.75eq	100	10 mol%	33
2	 1a	TOTP	1.5eq	100	10 mol%	35
3	 1a	PPh_3	1.5eq	130	10 mol%	96
4	 1a	PPh_3	1.5eq	130	1 mol%	63

Then we carried out the acid amine coupling reaction (**Table 2**) of the isolated product (**3a**) and piperidine (**4a**) with different coupling agents to form the respective amide (**5a**).[32]



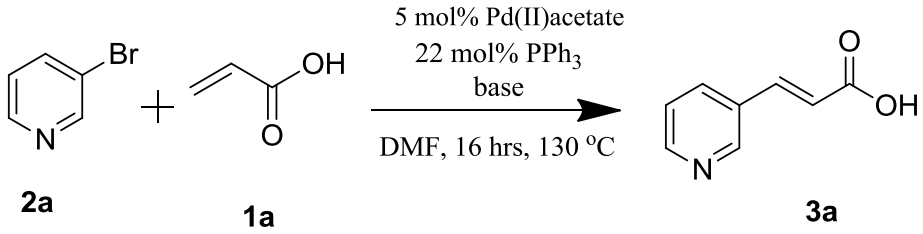
3a **4a** **5a**

Scheme 7: Acid amine coupling

Table 2: Acid amine coupling

No	Coupling reagent	Condition	Solvent	Base	Time (h)	Result
1	CDI (1.1 eq)	Reflux, 70 ^{o}C	THF	-	15	No reaction
2	CDI(1.1 eq)	Reflux, 70 ^{o}C	THF	-	24	No reaction
3	$SOCl_2$ (1.1 eq)	RT	DCM	TEA	5	No reaction
4	EDC.HCl/ HOBt (1.3eq)	RT	THF	DIPEA	18	No reaction

As the removal of triethylamine was not possible, we couldn't achieve the isolation of free acid, which might be necessary for the amide formation. Presumably this may be the reason for no formation of our expected amides. In order to obtain the free acid, we carried out the same Heck reaction between acrylic acid (**1a**) and 3-bromopyridine (**2a)** with different inorganic bases like $CsCO_3$, K_2CO_3 etc and hindered base like DIPEA (**Table 3**, entry 1-3).



Scheme 8: Screening of different base for Heck reaction.

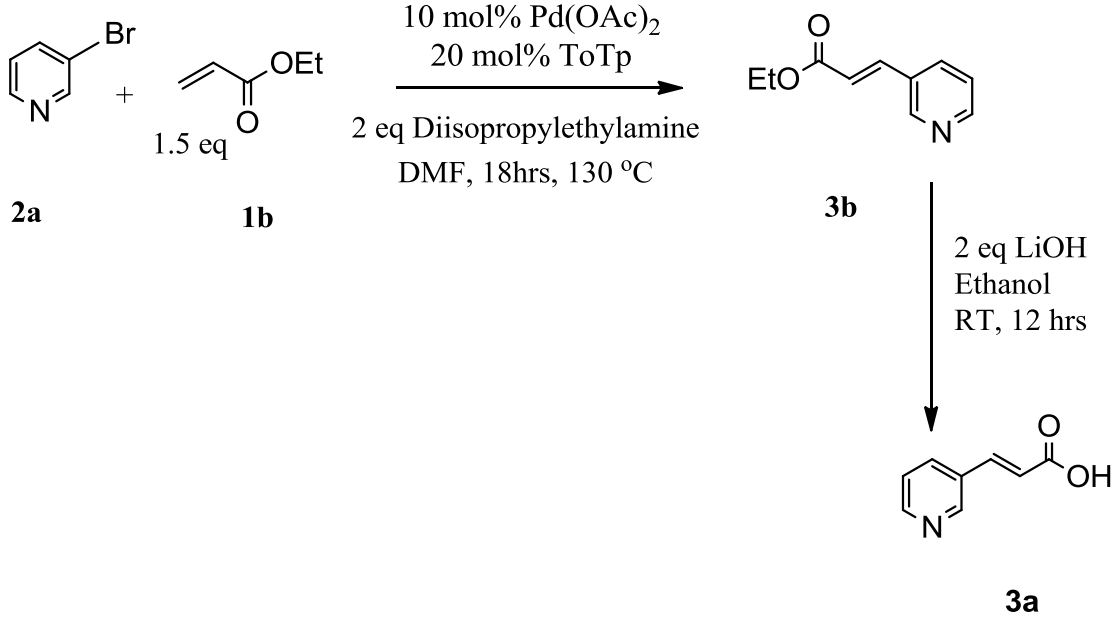
Table 3: Screening of different bases

Serial No	Base	Yield
1	K_2CO_3	21%

2	CS_2CO_3	24%
3	Diisopropyethylamine	17%

As expected, we have obtained a highly pure free acid through this reaction. However, the yield that we obtained for all the cases were poor.

After all these experiments, the synthesis of Heck product **3a** as a pure free acid with reasonable yield was not achieved. Therefore, we followed an entirely different strategy for the preparation of the same product **3a**, through the synthesis of its corresponding ester followed by hydrolysis[33] of it. Thus, we carried out the Heck reaction between ethyl acrylate **1b** and 3-bromopyridine **2a** using ToTp as a ligand and diisopropylethyamine as a base (**Scheme 5**). We were able to isolate the ester product **3b** in excellent yield. As expected, after the hydrolysis of ester product **3b**, we were able to isolate the free acid product **3a** with a reasonable yield.



Scheme 9: Heck reaction of ethyl acrylate followed by hydrolysis

After optimizing the synthesis of (2*E*)-3-(pyridin-2-yl) prop-2-enoic acid **3a**, we have tried to synthesize other α, β-unsaturated carbonyl derivatives as our targets. In this

regard, we have tried Heck reactions using different α, β-unsaturated carbonyl substrates, coupled with different aryl halides to obtain those targets.

In order to obtain the 4-pyridine derivatives, we performed the Heck reaction using 4-iodopyridine (**2b**) and acrylic acid (**1a**). The 4-iodopyridine was synthesized in our laboratory by the diazotization of 4-aminopyridne followed by nucleophilic substitution with iodide using potassium iodide.[34] In the cross coupling reaction, when we first used the triethylamine as base, the product was forming triethylammine adduct as in the case 3-pyridine derivative. Here again, we changed the base to caesium carbonate to obtain free acid product (**3c**), which we finally achieved (**Table 4**, entry 1).

Simillarly we performed the coupling of acrylamide (**1c**) with 4-iodopyridines (**2b**) using $Pd(OAc)_2$ catalysed Heck coupling. However, under these conditions, triethylamine was used as a base without any problem. After column purification we were able to obtain the pure product in good yield (**3d**) (**Table 4**, entry2).

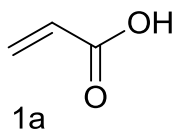
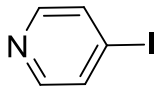
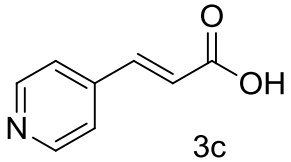
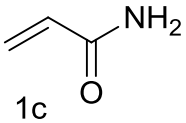
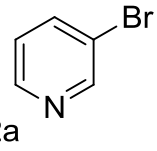
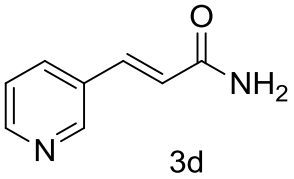
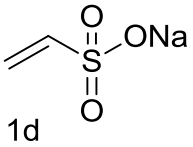
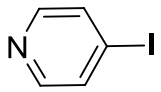
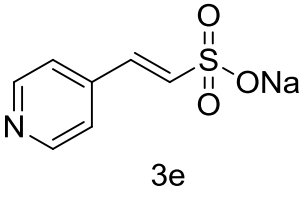
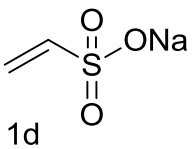
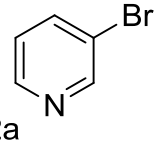
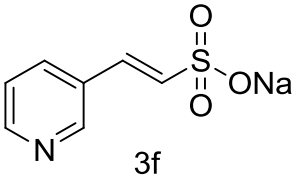
Since sulphonamides are one of the well-known bioisosteric group for the amides, we tried to replace α, β-unsaturated carbonyl group with the corresponding α, β-unsaturated sulphonyl group. In this regard, we performed the Heck reaction between commercially available sodium salt of vinyl sulphonic acid (**1d**) and 3-Bromopyridine (**2a**) with TEA as a base. Once again the isolated product that we obtain formed an adduct with TEA. Hence, we adopted the modified procedure that we adopted for getting pure free acid in the earlier cases by using caesium carbonate as a base. Here again, we were able to isolate the pure compound (**3f**) after workup (**Table 4**, entry 4). Furthermore, this method was extended to carry out the coupling between sodium salt of vinyl sulphonic acid (**1d**) and 4-iodopyrdine (**2b**), to get the analogous product (**3e**) (**Table 4**, entry 3).

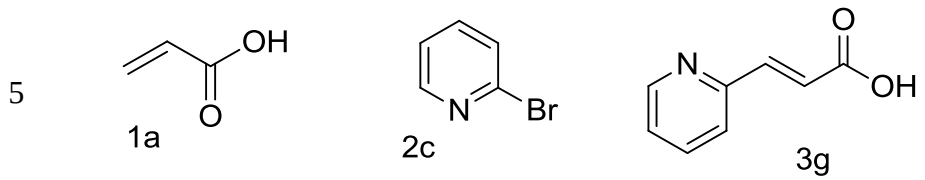
When we performed the same kind of Heck coupling with 2-bromopyridine (**2c**), using all the three vinyl substrates, we were not able to get the products. In each case, the reaction mixture was showing multiple spots (5-6) other than reactants, which were close lying to separate by column chromatography (**Table 4**, entry 5-6).

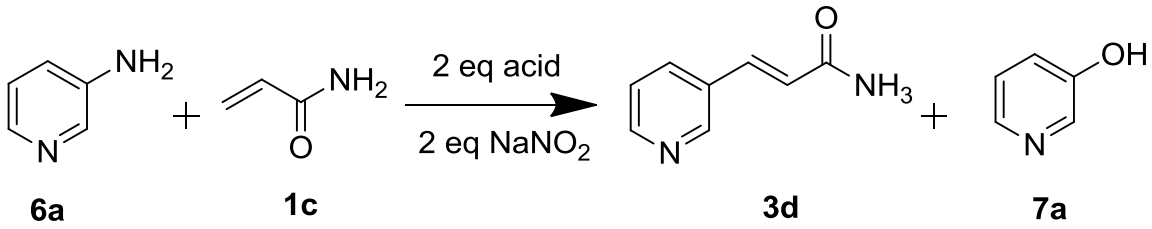
Meanwhile we have tried an entirely different strategy for the preparation of (2*E*)-3-(pyridin-2-yl) prop-2-enamide **3d**, through diazotisation. The diazonium salts have been reported as leaving groups and used for many cross-coupling reactions including Suzuki, Heck and Negishi reactions etc. [34] Also, under conditions favourable for radical generation, diazonium salts were able to form radical coupling with arenes.[35] In our

approach we coupled both these reports and tried to perform radical coupling of diazonium salts with acrylamide and acrylic acid. We have used ascorbic acid as a radical initiator. Not only the direct C-H arylation is of particular interest, but also it can avoid the use of metal catalyst. . Hence we followed the procedure and carried out reactions 4 aminopyridine (**6a**) with acrylamide (**1c**) in presence of ascorbic acid at different conditions (table 5, entry 1-3), but in each case the reaction was forming only 3- hydroxyl pyridine (**7a**) rather than the expected product **3d**. Probably the nucleophilic substation was the profound pathway under the experimental conditions instead of our expected C-C coupling. In a different report the metal free C-H arylation can also be achieved using PPh_3 as a reagent.[36] Again we tried the same reaction using PPh_3 as shown (**Table 5**,entry 4-6), but the results were same as in case above, i.e. it forms 3-hydroxy pyridine (**7a**).

Table 4: Substrate scope for Heck reaction of halopyridine and α,β-unsaturates amides and acids

S. No.	Vinyl substrate	Halopyridine	Product	Yield (%)
1	 1a	 2b	 3c	53
2	 1c	 2a	 3d	82
3	 1d	 2b	 3e	63
4	 1d	 2a	 3f	61



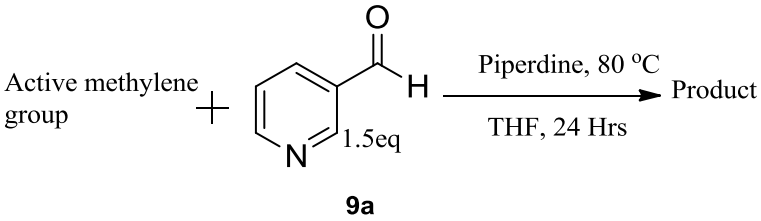


Scheme 10: C-C coupling through diazonium salt formation.

Table 5: *C*-arylation through diazotisation

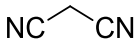
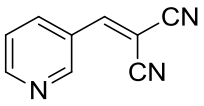
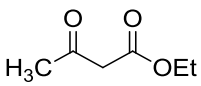
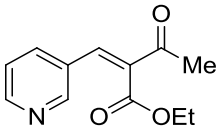
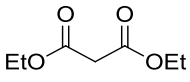
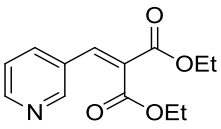
No	Acid	Water	Ascorbic acid	Acrylamide	PPh_3	Temp(^oC)	Result
1	HCl	1.2 mL	10 mol%	1 eq	--	-5	7a
2	HCl	1.2 mL	10 mol%	10 eq	--	-5	7a
3	HCl	0.2 mL	10 mol%	10 eq	--	-5	7a
4	HCl	0.2 mL	--	10 eq	1.1 eq	-7	7a
5	HBF_4	0.2 mL	--	10 eq	1.1 eq	-7	7a
6	HCl	0.2 mL	--	10 eq	1.1 eq	-10	7a

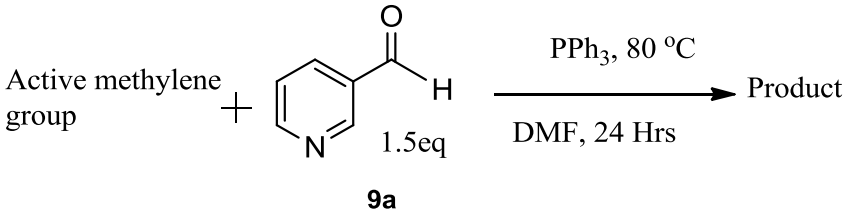
Apart from theses, we have also adopted another strategy to obtain our target, i.e. Knoevenagel condensation by reacting an aryl aldehyde with active methyene compounds. Herein, we utilized 3-pyridine carboxaldehyde (**9a**) as the aryl aldehyde and different active methylene groups. Knoevenagel condensation is the reaction of aldehyde or ketone with activated methylene groups to produce substituted olefins using an amine base. In our work the Knoevenagel condensation 3-pyridine carboxaldehyde with active methylene groups allows us to synthesise pyridine derivative of substituted olefins (**Scheme 11**), by which we can study the effect of different substituents. Additionally we can modify the substituent groups to obtain our targeted product. We followed the general procedure[38] for Knoevenagel condensation with piperidine as base and THF as solvent with heating (**Table 6,** entry 1-3), but the reaction didn't proceed as we expected. Even after two days most of the reactants were remaining in the reaction mixture.



Scheme 11: General method for Knoevenagel condensation

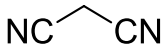
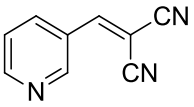
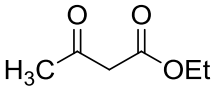
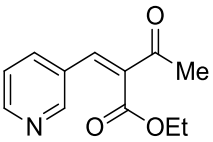
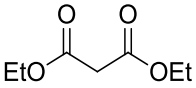
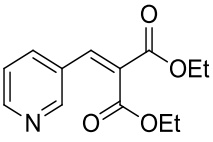
Table 6: Knoevenagel condensation of 3-Pyridine carboxaldehyde with different active methylene groups using piperidine

Sr. No.	Active methylene group	Base	Product	Time	Status
1.		Piperidine		32h	Slow reaction
2.		Piperidine		32h	Slow reaction
3.		Piperidine		32h	Slow reaction

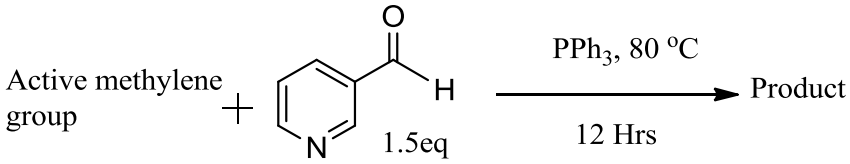


Scheme 12: Knoevenagel condensation using PPh_3

Table 7: Knoevenagel condensation of 3-Pyridine carboxaldehyde with different active methylene groups using PPh_3

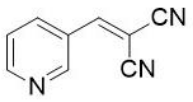
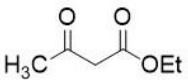
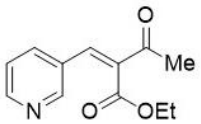
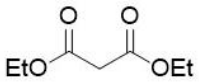
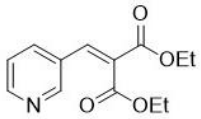
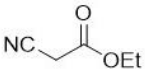
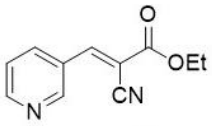
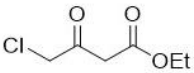
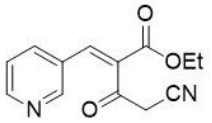
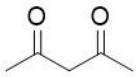
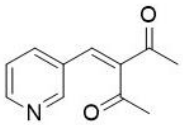
Serial No	Active methylene group	Product	status
1			New spot, not complete
2			New spot, not complete
3			New spot, not complete

Then we followed another paper, where they were also using PPh_3 as catalyst with heating, but in the absence of solvent.[30] So next we tried reaction under neat conditions (**Scheme 13**) and the product formed with the complete consumption of limiting reagent, active methylene group (**Table 8**). In the isolation part through column chromatography, the spot corresponding to the product and pyridine carboxaldehyde (**8a**) were close that the isolation of product became difficult. We were losing most of the yield as the product came as a mixture, even after changing the parameters of column chromatography to optimise the separation.

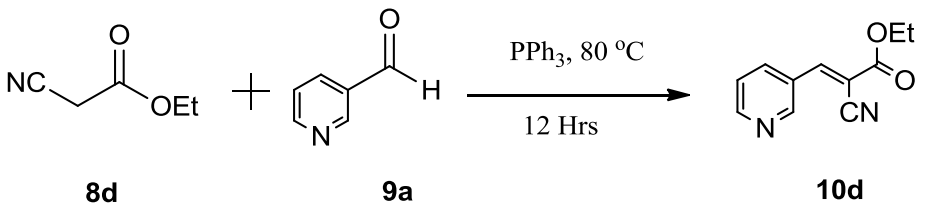


Scheme 13: Knoevenagel condensation under neat conditions

Table 8: Knoevenagel condensation of 3-Pyridine carboxaldehyde with different active methylene groups under neat conditions

Serial No	Active methylene group	product	STATUS
1			21% Yield
2			New Spot
3			New spot
4			New spot
5			New Spot
6			New spot

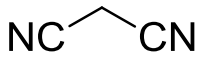
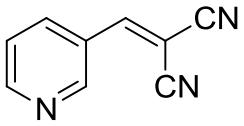
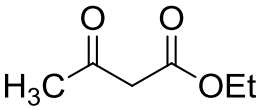
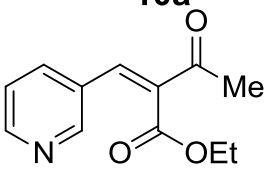
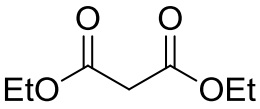
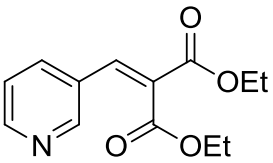
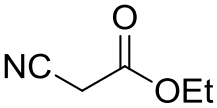
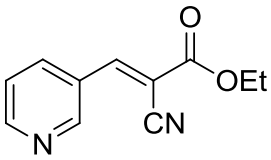
To check the reactivity of pyridine carboxaldehyde, we carried out two reactions of ethylcynoacetate with 3-pyridine carboxaldehyde, one with 3-pyridine carboxaldehyde as limiting reagent and in the other one ethylcynoacetate as limiting reagent, under the same conditions above; neat reaction with PPh_3 as catalyst (**Scheme 14**). In case of Reaction in which pyrine carboxaldehyde was the limiting reagent, after the completion of the reaction the carboxaldehyde was completely consumed and thus we were able to isolate the product with a reasonably good yield by column chromatography. We used this conditions for the other active methylene groups also (**Table 9)**.



8d **9a** **10d**

Scheme 14: Optimization condition for Knoevenagel condensation

Table 9: Substrate scope for Knoevenagel condensation

S. No.	Active methylene compound	Product	Crude Yield
1	 **8a**	 **10a**	81%
2	 **8b**	 **10b**	77%
3	 **8c**	 **10c**	72%
4	 **8d**	 **10d**	66%

Chapter 3 CONCLUSIONS AND OUTLOOK

The main objective of our work was to synthesise an active molecule that can perform inhibition of fatty acid biosynthesis in bacteria. Based on AFN-1252 as a lead compound, target molecules with pyridine connected α,β-unsaturated carbonyl compounds have been designed. Heck coupling and acid-amine couplings were planned in achieving the target molecules. Initially, we tried the Heck coupling using acrylic acid with 3-halopyridines. In all the cases the product formed an adduct with triethylamine, the base used in the reaction. Attempts were made to remove the triethylamine from the product by extraction at different pH, hydrolysis with strong acids and reaction with thionyl chloride etc. But none of the attempts resulted in the formation of the desired product. We have also tried the direct acid amine coupling (without removing TEA), once again, the desired amide product was not able to be prepared. The status was the same for Heck coupling reactions using isomeric halopyridine and acrylic acid or sodium vinyl sulfonate. In order to avoid the triethylamine adduct of the Heck coupled products, we have utilized the inorganic bases, however, we did not obtain good yields. In this regard, we modified the approach by performing the Heck coupling using the ethyl acrylate followed by the base hydrolysis. Using this method, we optimize the best conditions for the synthesis of pure form of (2*E*)-3-(pyridin-2-yl) prop-2-enoic. The optimized condition (mainly the change in base) was utilized for the Heck coupling using acrylic acid, acrylamide and sodium vinyl sulphonate with different halopyridines as well.

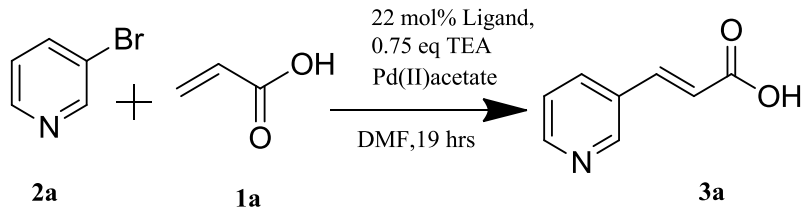
In order to introduce structural variation and study the effect of substituent in the medicinal effect of target molecules, we planned to prepare compounds having variation at different position. We tried to introduce variable substituent at terminal olefinic carbon and so we used Knoevenagel condensation reaction strategy. In this regard, we utilized pyridine-3-carboxaldehyde and various active methylene compounds. We obtained moderate to good yields of those desired compounds. The major limitation in our work is the unavailability of data from biological evolution of our target molecules through antibacterial activity studies, which will be done in near future.

Chapter 4. EXPERIMENTAL SECTION

General information:

All the reagents were commercial grade and used without purification. Reactions were monitored using TLC on silica gel and for detection UV (254 and 365 nm), $KMnO_4$ and iodine were used. Organic extracts were dried using anhydrous sodium sulphate and solvents were removed by rotary evaporation under reduced pressure. ^{1}H and ^{13}C NMR spectra were recorded in Avance-III, Bruker Biospin at 400MHz and 100MHz spectrometers, respectively with trimethylsilane as standard using $CDCl_3$/ D_2O/ DMSO-d_6 as a solvent. IR spectra (Perkin – Elmer FT IR spectrometer) were recorded on KBr plate or thin film. HRMS spectra were recorded in Bruker maxis spectrometer using ESI mode. Column chromatography was done using silica gel (60-120) mesh. All melting points are uncorrected.

General procedure followed for Heck reaction for the synthesis of (2*E*)-3-(pyridin-2-yl) prop-2-enoic acid (3a)



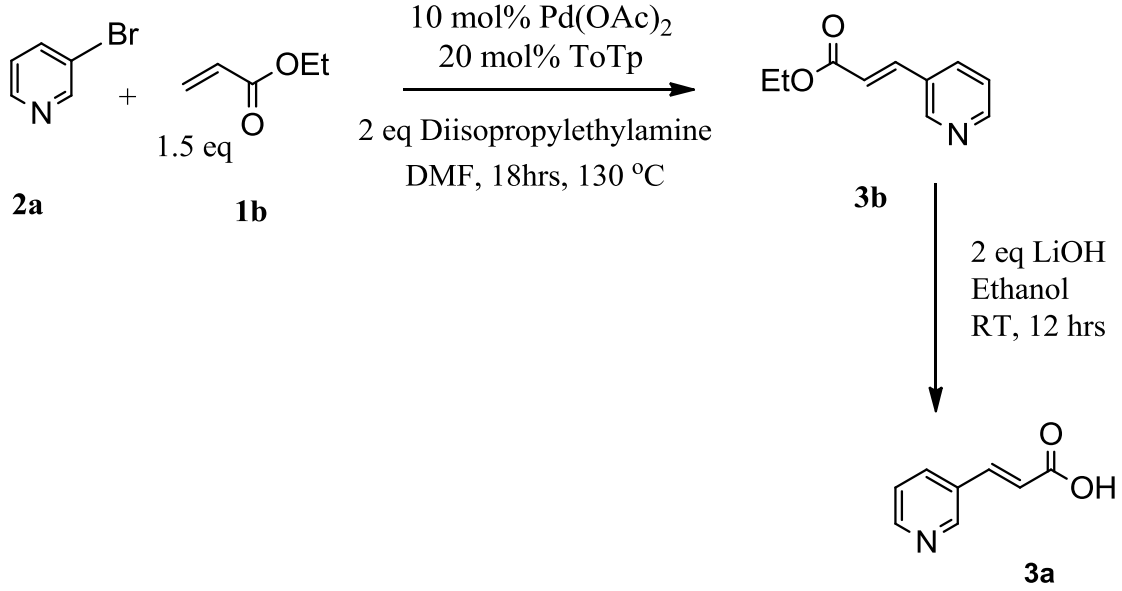
2a **1a** **3a**

Tripehenylphosphene (22 mol%) and Pd(II) acetate (5 mol%) were stirred in 1mL of dimethyl formamide under argon purging for 30 minutes. Simultaneously aryl halide (1.5eq) and base (2eq) were stirred at room temperature, and after 30minutes this mixture was transferred to the ligand-catalyst mixture and heated to 130^{o}C for about half an hour. Then the mixture was cooled to room temperature and the, β unsaturated acid/ amide (1eq) dissolved in 1 mL of dimethyl formamide was added to the mixture, followed by heating at 130 ^{o}C until the completion of the reaction. The reaction mixture was filtered through celite bed in Buchner funnel and the filtrate was concentrated by rotaryevaporation. The work up was carried out by first basification using sodium

bicarbonate and washing with ethyl acetate, adjusted the pH of aqueous layer to acidic (pH~1) followed by extraction using ethyl acetate, then again the pH was readjusted to ~5 and extracted using acetonitrile. The acetonitrile layer was dried over anhydrous sodium sulphate, and the solvent was evaporated under reduced pressure to afford respective products.

In case of reactions where acrylamide is used, the crude product after filtration and rotevaporation was purified over column chromatography using silica gel and eluted with ethyl acetate /hexane (50:50) to afford the product.

Synthesis by ester hydrolysis:



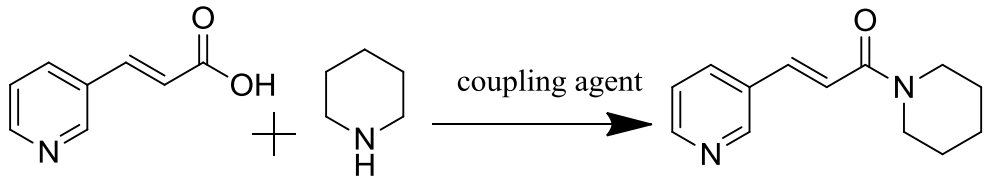
2a **1b** **3b** **3a**

Pd(II) acetate(142.6 mg, 0.63 mM, 5 mol%) and ToTP (1.2 g, 3.81 mM, 30 mol%) were stirred under argon atmosphere in DMF (5mL). 3-Bromopyridine (2 g, 12.7 mM, 1 eq) was added to the mixture followed by diisopropyethylamine (4.5 mL, 25.4 mM, 2 eq) and allowed to stir for 30 minutes at room temperature. Then ethyl acrylate (2.1 mL, 19 mM, 1.5 eq) was added to the above mixture and heated the reaction mixture at 130 ^{o}C for 16 hours.

After the completion of the reaction, the reaction mixture was filtered through celite bed in a Buchner funnel, and the filtrate was dried under vacuum. The crude product was purified over silica gel column using ethyl acetate/hexane (30:70) mixture. The product was concentrated by rotaevaporation under reduced pressure to afford ethyl (2*E*)-3-(pyridin-2-yl) prop-2-enoate (**3b**) (2.19g, 97% yield).

The compound ethyl (2*E*)-3-(pyridin-2-yl)prop-2-enoate (**3b**) (96 mg, 0.54 mM, 1 eq) was dissolved in ethanol (0.5 mL) and Lithium hydroxide (56.9 mg, 1.4 mM, 2.5 eq) was added to it and allowed to stir at room temperature overnight. After the reaction got completed, the pH was adjusted ~6, so that the product was precipitated. The product was filtered out using a cintered funnel and washed with hexane. Some of the product got dissolved in the mother liquid, which was then recovered by evaporation under reduced pressure. The collected solid was dried under high vacuum to afford (2*E*)-3-(pyridin-2-yl) prop-2-enoic acid (**3a)** (46.7 mg, 61% yield).

General procedure for acid amine coupling for synthesis of (2*E*)-1-(piperidin-1-yl)-3-(pyridin-3-yl) prop-2-en-1-one (5a)



3a **4a** **5a**

Using acid chloride

Thionyl chloride (27 µl, 0.37 mM, 1.1 eq) was added slowly to (2*E*)-3-(pyridin-2-yl) prop-2-enoic acid (**3a)** (50 mg, 0.33mM) in dichloromethane (1mL) and allowed the reaction to stir at room temperature for half an hour. In another round bottom flask, pyrrolidne (29 µL, 0.35 mM, 1.05 eq), triethylammine (98 µL, 0.07 mM, 2.1 eq), catalytic amount of dimethylamminopyridine (DMAP) and one drop of dimethyl formamide were allowed to stir in dichloromethane (1mL) at room temperature. After half an hour the amine mixture was transferred to acid chloride solution, drop wise slowly and allowed the reaction to stir at room temperature for 3 hours.

The reaction mixture was quenched with dil.HCl and extracted with ethyl acetate. The aqueous layer was the basified (pH~8) using sodium bicarbonate and extracted using acetonitrile. The acetonitrile layer was dried using anhydrous sodium sulphate, and the solvent was evaporated under reduced pressure.

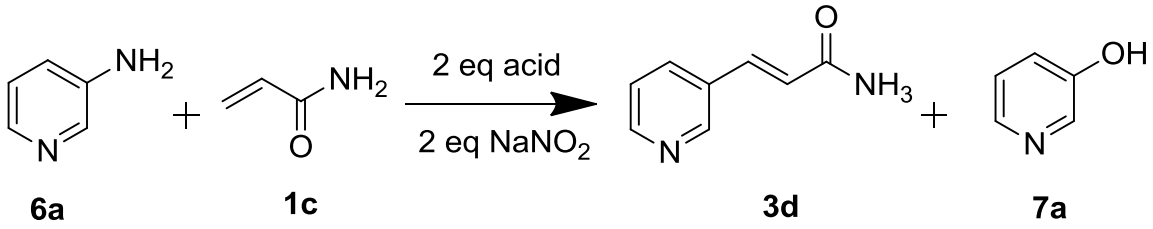
CDI coupling:

(2*E*)-3-(pyridin-2-yl) prop-2-enoic acid (**3a)** (25 mg, 0.168 mM, 1 eq) and CDI (30 mg, 0.185 mM, 1.1 eq) were stirred in a two necked round bottom flask at room temperature in trihydrofuran (2mL). After half an hour piperidine was added drop wise to the above mixture and refluxed at 80 ^{o}C for 15 hours.

EDC. HCl/ HoBt:

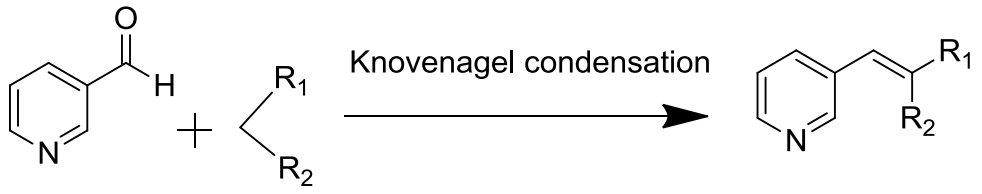
HoBt (29.5 mg, 0.218 mM, 1.3 eq) followed by EDC.HCl (42 mg, 0.218 mM, 1.3 eq) was added to a solution of (2*E*)-3-(pyridin-2-yl) prop-2-enoic acid (**3a**) (25 mg, 0.168 mM, 1 eq) in Dimethyl formamide (1mL). After 15 minutes piperidine (17µL, 0.168mM, 1eq) was added to the above mixture followed by DIPEA (73 µL, 0.42 mM, 2.5 eq) drop wise and allowed the reaction to continue at room temperature for 48 hours.

General procedure for synthesizing (2*E*)-3-(pyridin-2-yl) prop-2-enamide (3d) through diazotisation.



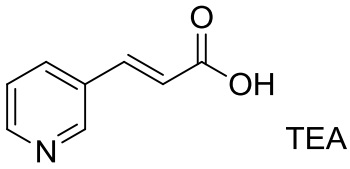
3-Aminopyridine (100 mg, 1.062 mM, 1 eq) was taken in two necked RB with ice bath and dissolved in minimum amount of water (~0.2 mL) followed by addition of conc.HCL (140 µL, 2.124 mM, 2 eq) and ascorbic acid (18.7 mg, 0.11 mM, 10 mol%). After stirring for 10 minutes, sodium nitrate (147 mg, 2.124 mM, 2 eq) was added to the mixture slowly in about half an hour while maintaining the temperature to be -5 to -7 ^{o}C. When the diazonium salt formation was completed (~30 minuts), the acrylamide was added (754.5 mg, 10.62 mM, 10 eq) to the mixture. The reaction got completed in about 2 hours.

General procedure for Knoevenagel condensation:

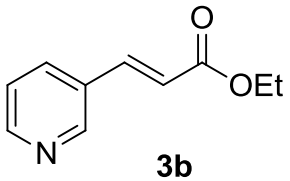


R1= CN, COOEt.
R2= CN, COOEt, COOMe

3-pyridine carboxaldehyde (1eq), active methylene group (1.5 eq) and TPP (20 mol %) were taken in 5 mL vial and heated at 80^{o}C for 12 hours. The reaction mixture was purified through column chromatography using silica gel with ethyl acetate: hexane (30:70) mixture. The fraction was concentrated using rotary evaporator under reduced pressure to afford the respective product.

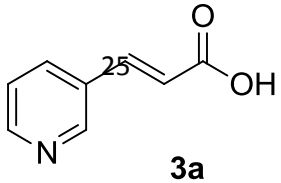


(2*E*)-3-(pyridin-2-yl) prop-2-enoic acid TEA adduct: Following the general procedure through ester hydrolysis, the product 8a was obtained after filtration as light brown colour solid; yield: 97%; ^{1}H NMR (400MHz, DMSO): δ 12.6 (s, 0.9H), 10.7 (s, 0.6 H), 8.9 (s, 1H), 8.5 (d, 1H, J= 3.4 Hz), 8.1 (d, 1H, J= 8 Hz), 7.6 (d, 1H, J= 16.1 Hz), 7.4 (m, 1H), 6.7 (d, 1H, J= 16.1 Hz), 3.0 (q, 4H, J_1=7.3 Hz, J_2=14.6 Hz, J_3= 21.8 Hz), 1.2 (t, 6H, J_1=7.3 Hz, J_2=14.6 Hz); ^{13}C NMR (100 MHz, DMSO): δ 167.7, 151.2, 150.2, 141.0, 130.5, 124.4, 121.8, 110.4, 45.6, 8.8; IR (KBr): 3421, 2939, 2675, 2493, 1656, 1637, 1579, 1418, 1286, 976, 806, 686 cm^{-1} ; ESI-TOF: $(M+H)^{+}$ of molecular formula $C_8H_7NO_2$: calculated 150.0557; found 150.0550; melting point: 159 ^{o}C.



3b

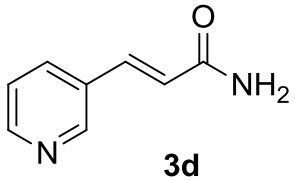
Ethyl (2*E*)-3-(pyridin-3-yl)prop-2-enoate (3b): Following the general procedure for Heck reaction the product was obtained after column chromatography using Ethyl acetate: Hexane (50:50) as brown sticky liquid; Yield: 97%; ^{1}H NMR (400MHz, DMSO): δ 8.6(s, 1H), 8.5 (d, 1H, J=4.8 Hz), 7.7 (d, 1H, J=7.79 Hz), 7.6 (d, 1H, J=16.12



3a

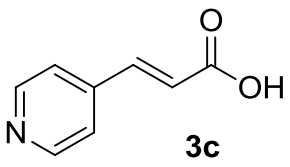
Hz), 7.3 (m, 1H), 6.4 (d, 1H, J=16.12 Hz), 4.2 (q, 2H, J_1=5.6 Hz, J_2=7.12 Hz, J_3= 8.64 Hz), 1.2 (t, 3H, J_1=8.68 Hz, J_2=14.12 Hz) ; ^{13}C NMR (100 MHz, DMSO): δ 166.2, 150.8, 149.6, 140.7, 134.1, 130.1, 123.6, 120.4, 60.7, 14.2; IR (KBr): 3039, 2985, 2877, 1728, 1638, 1564, 1407, 1291, 920, 824, 702 640 cm^{-1}; ESI-TOF: $(M+H)^+$ of molecular formula $C_{10}H_{11}NO_2$: calculated 178.0871; found 178.0873.

(2*E*)-3-(pyridin-2-yl) prop-2-enoic acid (3a): Following the general procedure through ester hydrolysis, the product 8a was obtained after filtration as white colour solid; yield: 61%; ^{1}H NMR (400 MHz, DMSO): δ 12.5(s, 1H), 8.8 (s, 1H), 8.5 (d, 1H, J=3.4 Hz), 8.1(d, 1H, J=7.9 Hz), 7.6(d, 1H, J=16.12 Hz), 7.4 (dd, 1H, J_1=2.9Hz, J_2=7.9Hz, J_3=1.97Hz), 6.7 (d, 1H, J = 16.12 Hz); ^{13}C NMR (100 MHz, DMSO): δ 167.7, 151.2, 150.2, 141.1, 135.0, 130.3, 124.3, 121.7; IR (KBr); 3446, 3054, 2675, 2493, 1709, 1637,1579, 1419, 1311, 1285, 974, 810, 640 cm^{-1}: ESI-TOF: $(M+H)^+$ of molecular formula $C_8H_7NO_2$: calculated 150.0557; found 150.0560; melting point : 236 ^{o}C



3d

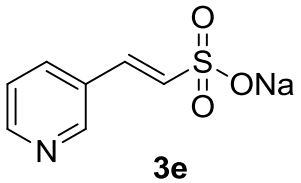
(2*E*)-3-(pyridin-3-yl) prop-2-enamide (3d): Following general procedure for Heck coupling, the product was obtained after column chromatography as a white solid; Yield 82%; ^{1}H NMR (400 MHz, DMSO): δ 8.7(s, 1H), 8.5 (d, 1H, J=3.52 Hz), 8.0 (d, 1H, J=7.96 Hz), 7.61 (s, 1H), 7.47-7.43 (m, 2H), 7.21 (s, 1H), 6.7 (d, 1H, J=16 Hz); ^{13}C NMR (100 MHz, DMSO): δ 166.8, 150.6, 149.6, 136.3, 134.4, 130.3, 124.8, 124.4.; IR (KBr): 3336, 3088, 2966, 1650, 1555, 1462, 1336, 1245, 1237, 1115, 544 cm^{-1} ; ESI-TOF: $(M+H)^+$ of molecular formula $C_8H_8N_2O$: calculated 149.0717; found 149.0710; Melting point: 209 ^{o}C.



3c

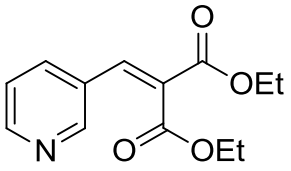
(2*E*)-3-(pyridin-4-yl) prop-2-enoic acid (3c): Following general procedure for Heck reaction using $CsCO_3$ as base, the product was obtained after acid-base work up as brown

colour solid; yield: 53%; ^{1}H NMR (400 MHz, DMSO): δ 8.69 (d, 2H, J= 6.04 Hz), 7.6 (d, 2H, J= 6.16 Hz), 7.5 (d, 1H, J=16.08 Hz), 6.8 (d, 1H, J=16.08 Hz); ^{13}C NMR (100 MHz, DMSO): δ 167.2,162.7, 150.8, 142.2, 140.6, 125.8, 122.5; IR (KBr); 3432, 3024, 2664, 2472, 1705, 1638,1579, 1422, 1308, 1284, 954, 820 cm^{-1} ; ESI-TOF: $(M+H)^{+}$ of molecular formula $C_8H_7NO_2$: calculated 150.0557; found 150.0559; Melting point : 244 ^{o}C.



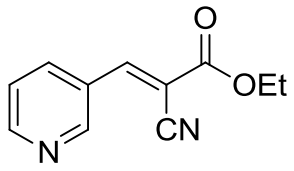
3e

Sodium (*E*)-2-(pyridin-3-yl) ethane sulfonate (3e): Following the general procedure of Heck coupling using CsCO3 as a base, the product was obtained after acid base workup as white solid; Yield: 61%; ^{1}H NMR (400 MHz, D_2O): δ 8.6 (s, 1H), 8.4 (s, 1H), 8.0 (d, 1H, J=9.68 Hz), 7.4 (m, 1H), 7.2 (d, 1H, J= 15.8 Hz), 7.0 (d, 1H, J= 15.8 Hz); ^{13}C NMR (100 MHz, D_2O): δ 167.9, 162.8, 150.7, 142.2, 140.6, 125.8, 122.5; IR: 3451, 3055, 2594, 1311, 1270, 1086, 1003, 707, 554 cm^{-1}; ESI-TOF: $(M+H)^{+}$ of molecular formula $C_7H_6NNaO_3S$: calculated 208.0046; found 208.0047.



10c

4-(pyridin-3-ylmethylidene) heptane-3,5-dione (10c): Following the general procedure for Knoevenagel condensation, the product was obtained after the column chromatography using ethyl acetate: hexane (30:70) as brown liquid; Yield: 72% ;^{1}H NMR (400 MHz, $CDCl_3$): δ 8.7 (s, 1H), 8.6 (d, 1H, J=4.8 Hz), 7.8 (d, 1H, J=12.4 Hz), 7.3 (s, 1H), 7.5 (m, 1H), 4.3 (q, 4H, J_1= 1.92 Hz, J_2=9.04 Hz, J_3=16.16 Hz), 1.3 (t, 6H, J_1= 7.12 Hz, J_2=14.12 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166, 163.6, 151, 150.6, 138.4, 135.8, 128.9, 128.5, 123.5, 62, 14.1, 13.9; IR: 2986, 1753, 1742, 1596, 1467, 1042, 1037, 993, 929, 684, 601 cm^{-1}; ESI-TOF: $(M+H)^{+}$ of molecular formula $C_{13}H_{15}NO_4$: calculated 250.2705; found 250.2707.



10d

Ethyl (2*E*)-2-cyano-3-(pyridin-3-yl) prop-2-enoate (10d): Following the general procedure for Knoevenagel condensation, the product was obtained after the column chromatography using ethyl acetate: hexane (30:70) as yellow colour solid; Yield: 66% ; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.9 (s, 1H), 8.7 (d, 1H, J=3.28 Hz), 8.6 (d, 1H, J=8.16 Hz), 8.3 (s, 1H), 7.5 (m, 1H), 4.4 (q, 2H, J_1= 7.12 Hz, J_2=14.28 Hz, J_3=21.44 Hz), 1.4 (t, 3H, J_1= 7.16 Hz, J_2=14.28 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.7, 153.5, 151.3, 135.5, 124.0, 114.2, 104.9, 63.2, 14.0; IR (KBr): 3012, 2955, 2235,1739, 1494, 1478, 1394, 1027,975, 879, 720, 586 cm^{-1}; ESI-TOF: $(M+H)^+$ of molecular formula $C_{11}H_{10}N_2O_2$: calculated 203.0822; found 203.0825; Melting point: 203 ^{o}C.

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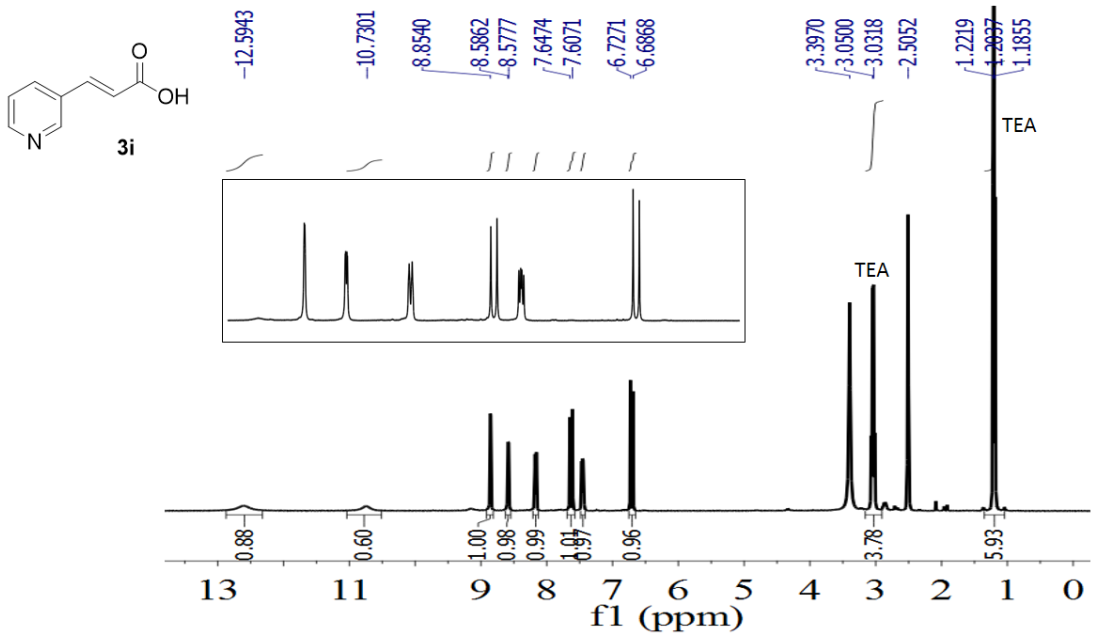
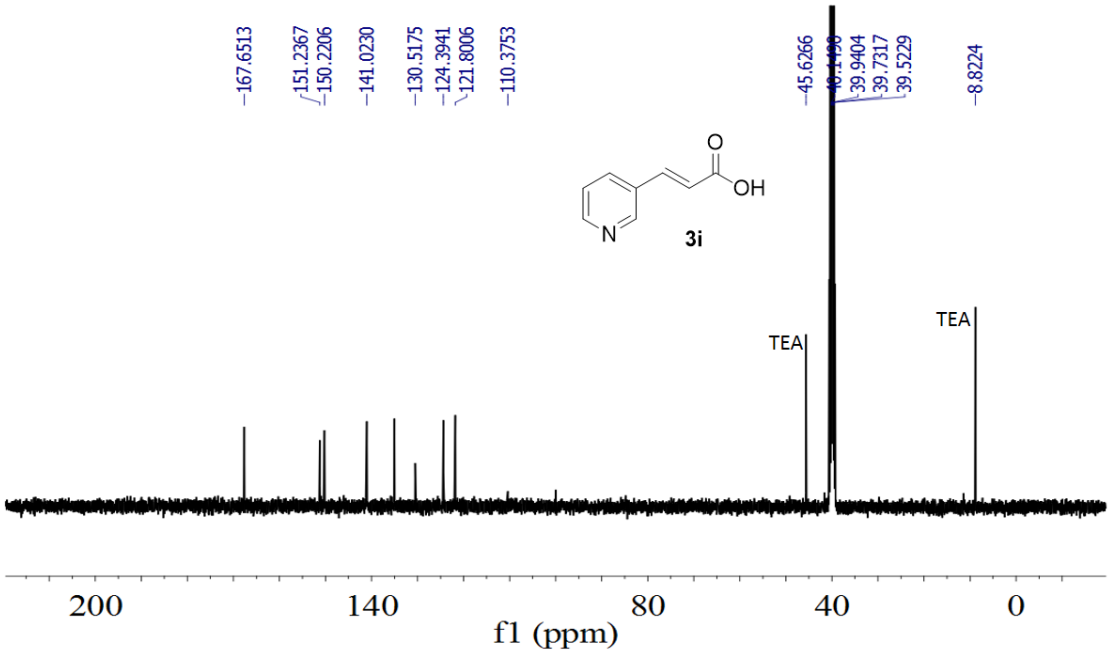
APPENDIX

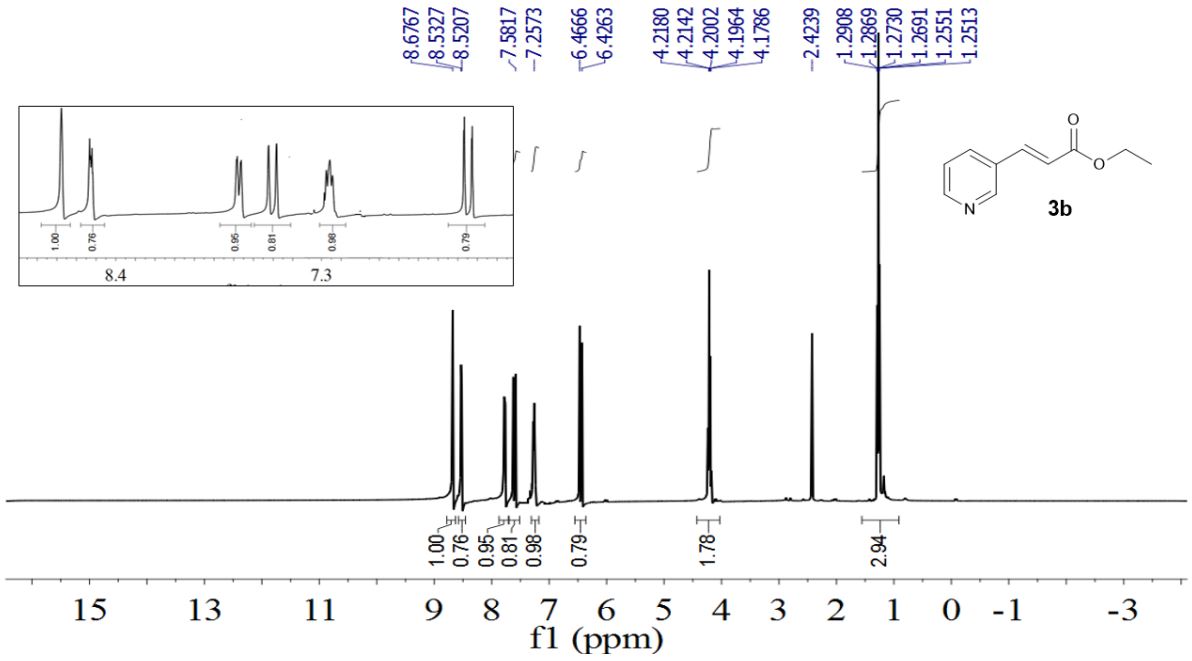
(2*E*)-3-(pyridin-2-yl) prop-2-enoic acid TEA adduct: ^{1}H NMR, DMSO-d$_6$



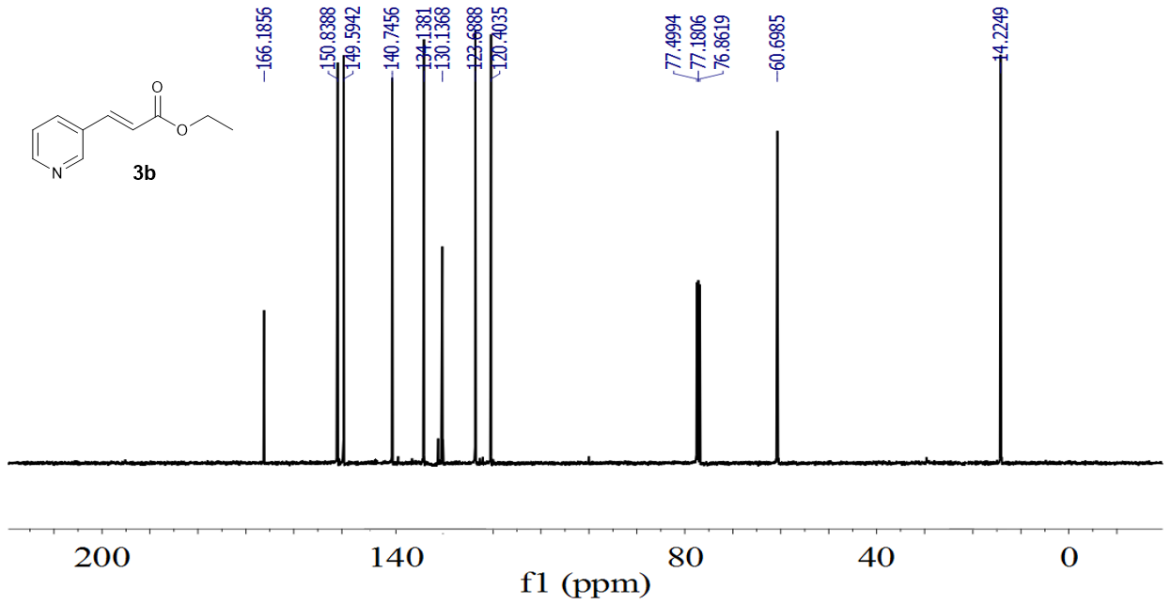
(2*E*)-3-(pyridin-2-yl) prop-2-enoic acid TEA adduct: ^{13}C NMR, DMSO-d$_6$



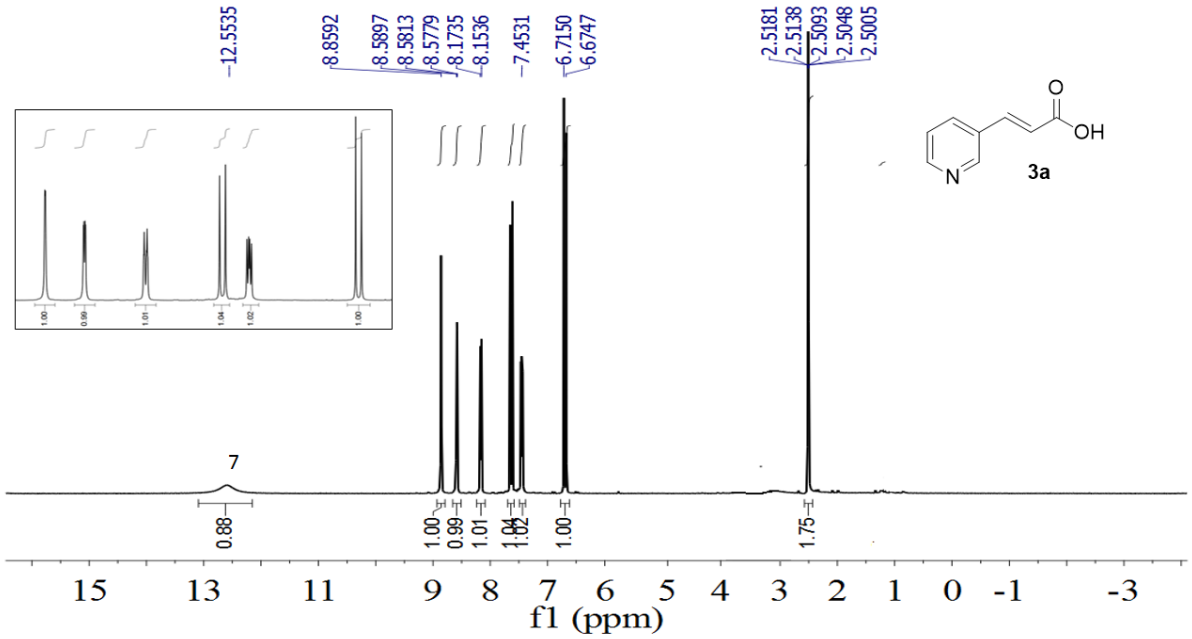
Ethyl (2*E*)-3-(pyridin-3-yl) prop-2-enoate (3b): ^{1}H NMR, DMSO-d$_6$



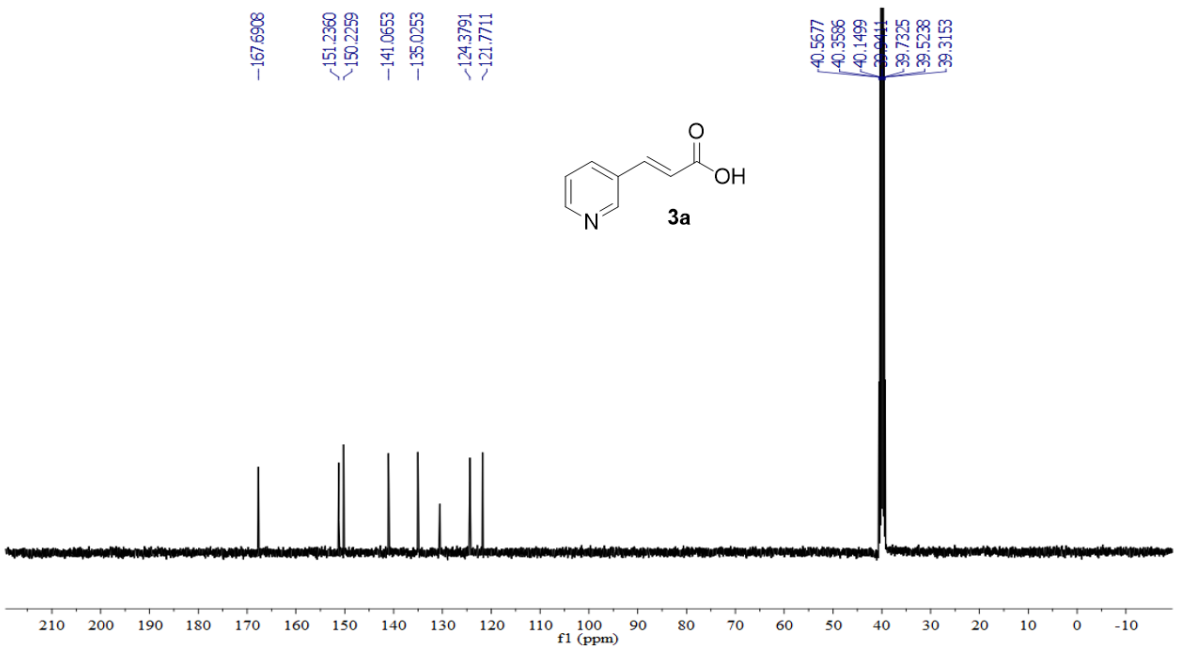
Ethyl (2*E*)-3-(pyridin-3-yl) prop-2-enoate (3b): ^{13}C NMR, DMSO-d$_6$



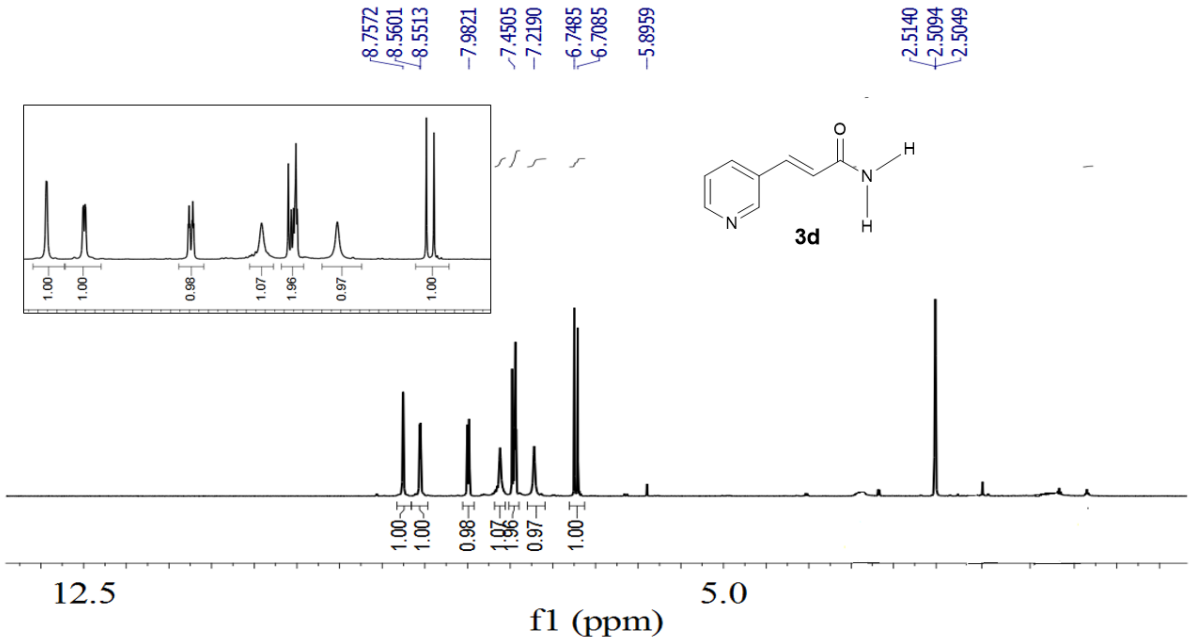
(2*E*)-3-(pyridin-2-yl) prop-2-enoic acid (3a): ^{1}H NMR, DMSO-d$_6$



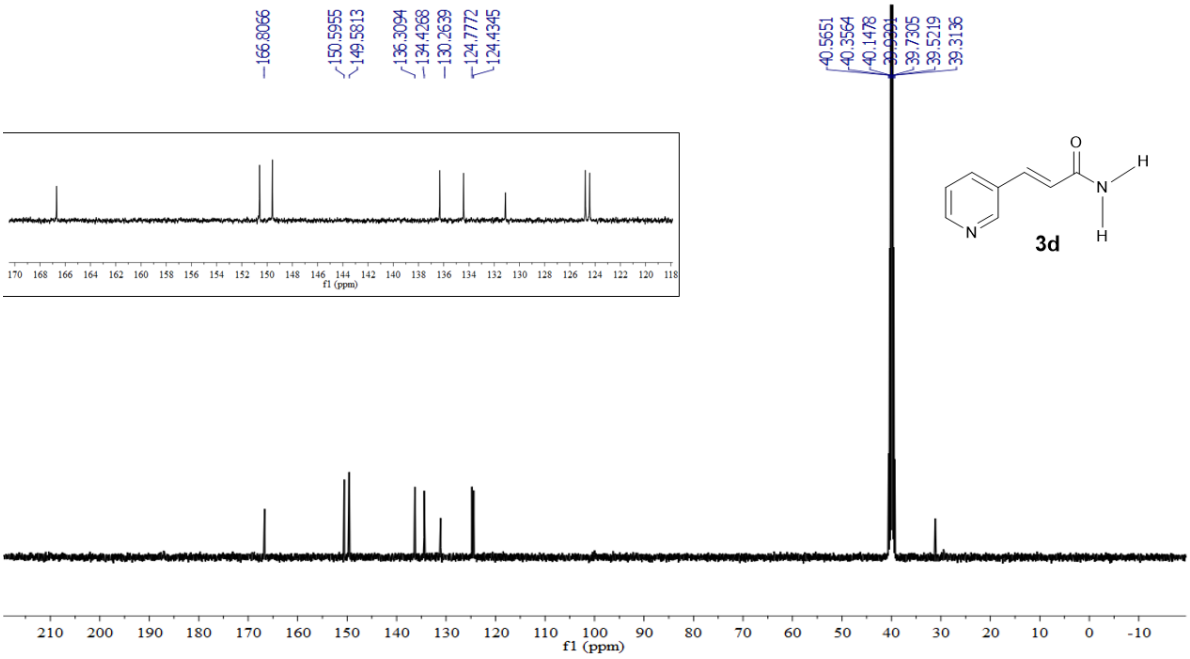
(2*E*)-3-(pyridin-2-yl) prop-2-enoic acid (3a): ^{13}C NMR, DMSO-d$_6$



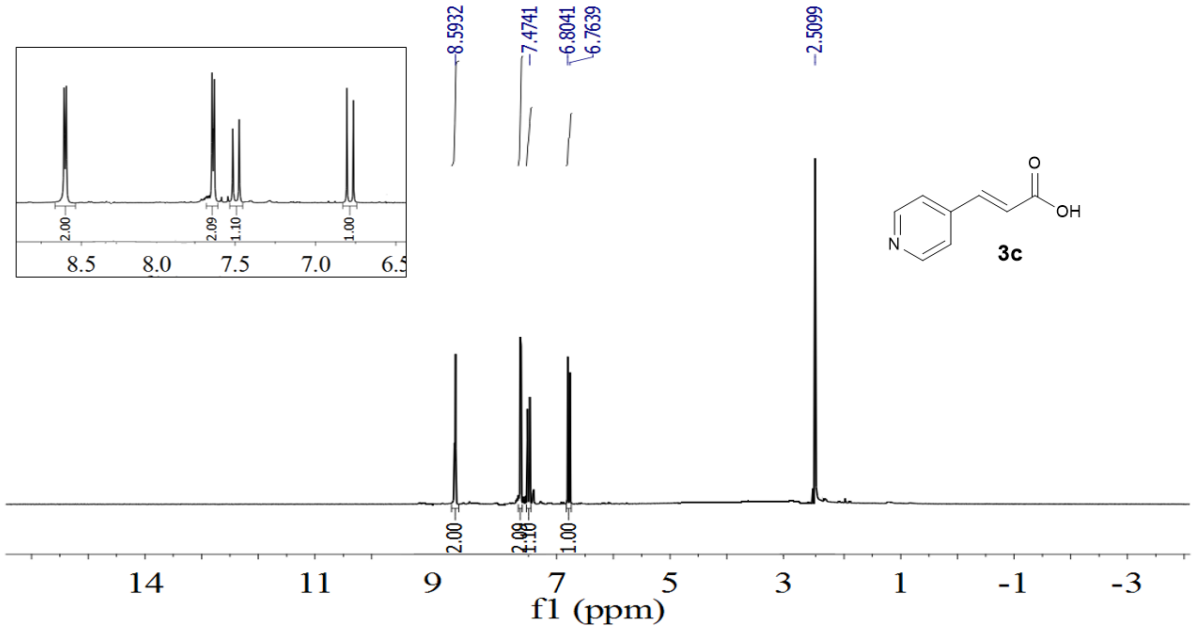
(2*E*)-3-(pyridin-3-yl) prop-2-enamide (3d): ^{1}H NMR, DMSO-d$_6$



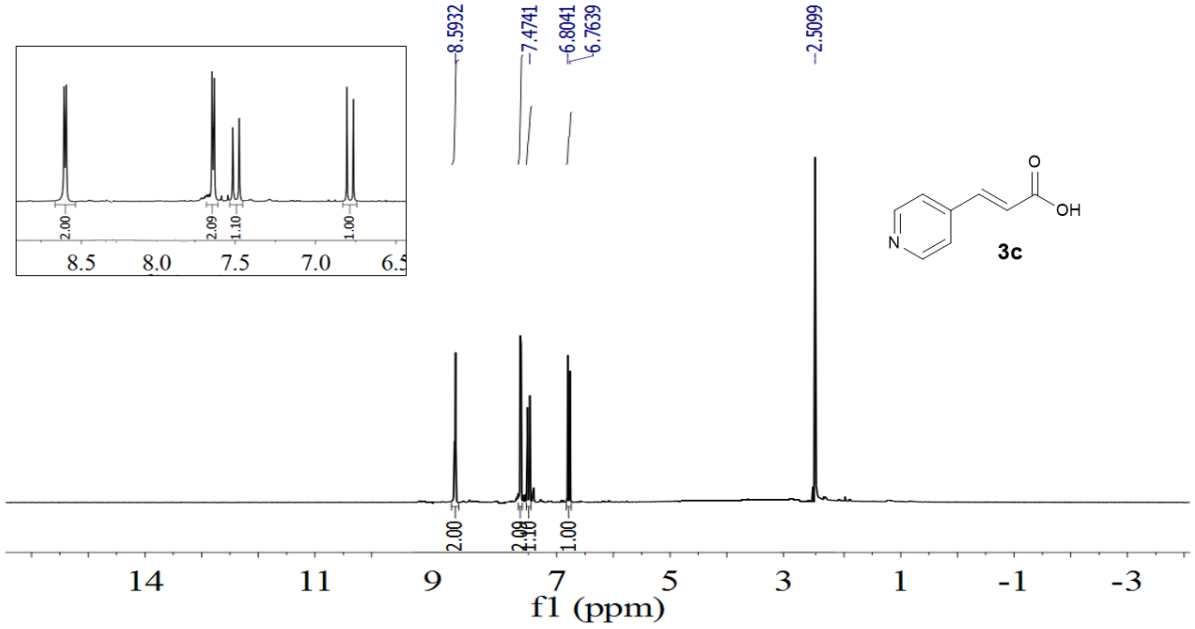
(2*E*)-3-(pyridin-3-yl) prop-2-enamide (3d): ^{13}C NMR, DMSO-d$_6$



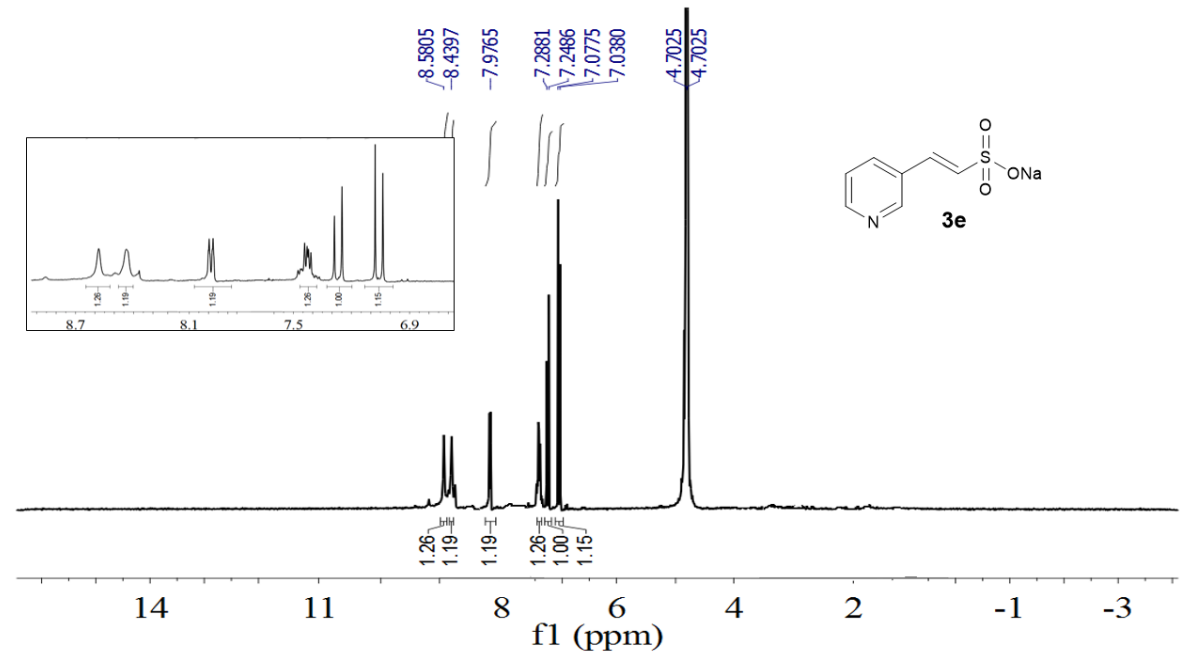
(2*E*)-3-(pyridin-4-yl) prop-2-enoic acid (3c): ^{1}H NMR, DMSO-d$_6$



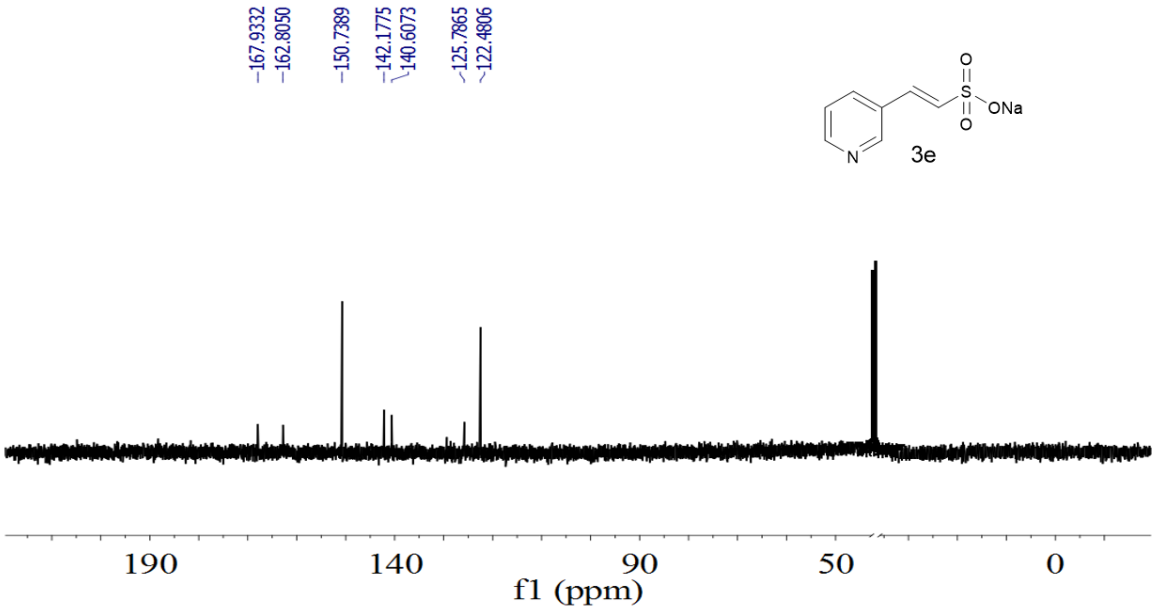
(2*E*)-3-(pyridin-4-yl) prop-2-enoic acid (3c): ^{13}C NMR, DMSO-d$_6$



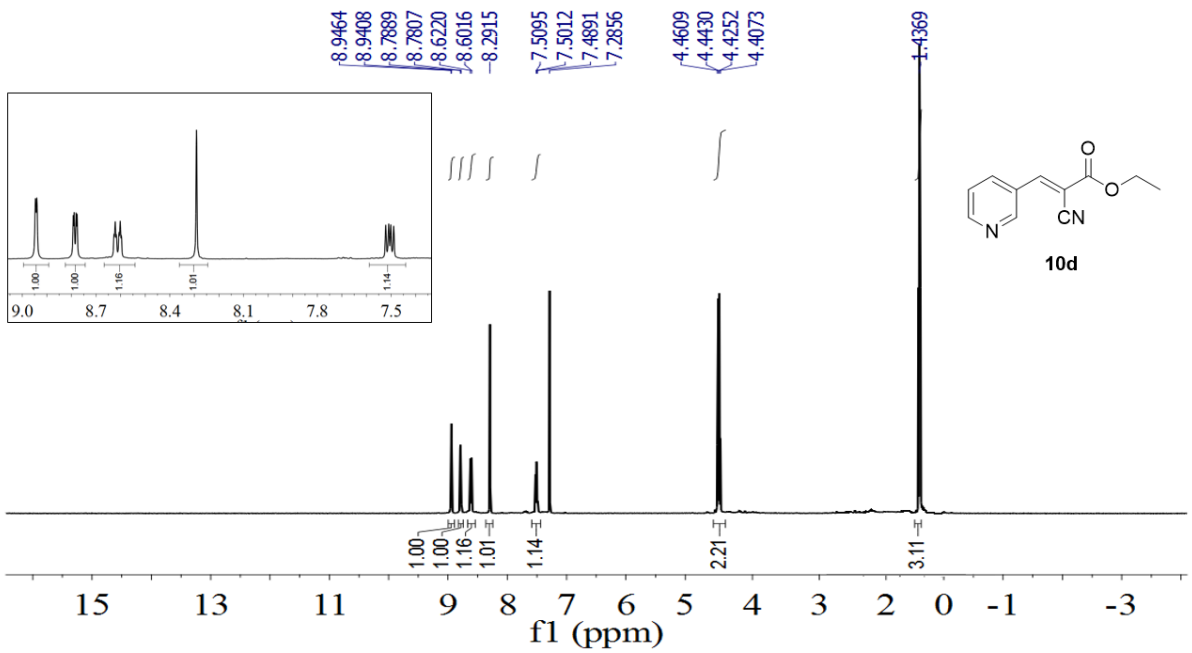
Sodium (*E*)-2-(pyridin-3-yl) ethane sulfonate (3e): ^{1}H NMR, D_2O



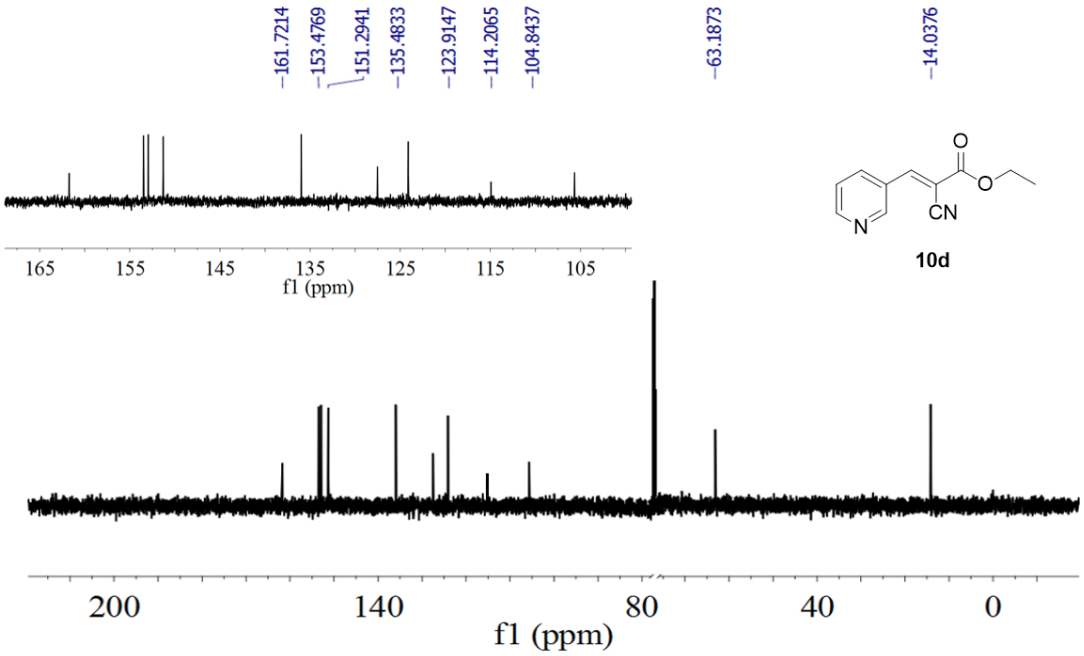
Sodium (*E*)-2-(pyridin-3-yl) ethane sulfonate (3e): ^{13}C NMR, D_2O



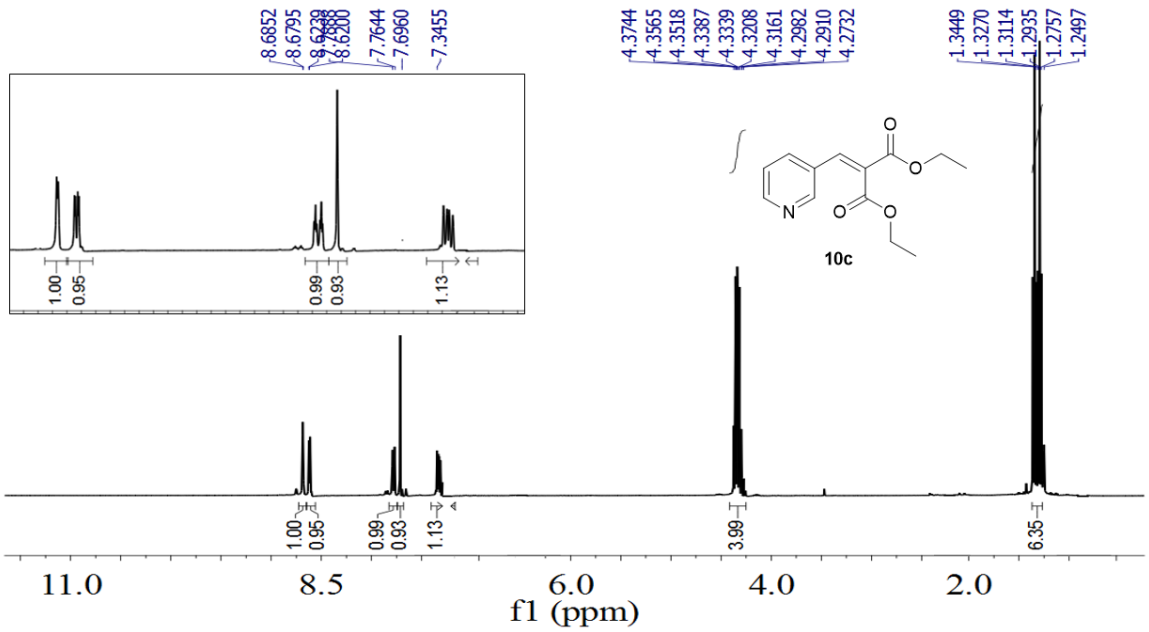
Ethyl (2*E*)-2-cyano-3-(pyridin-3-yl) prop-2-enoate (10d): ^{1}H NMR, $CDCl_3$



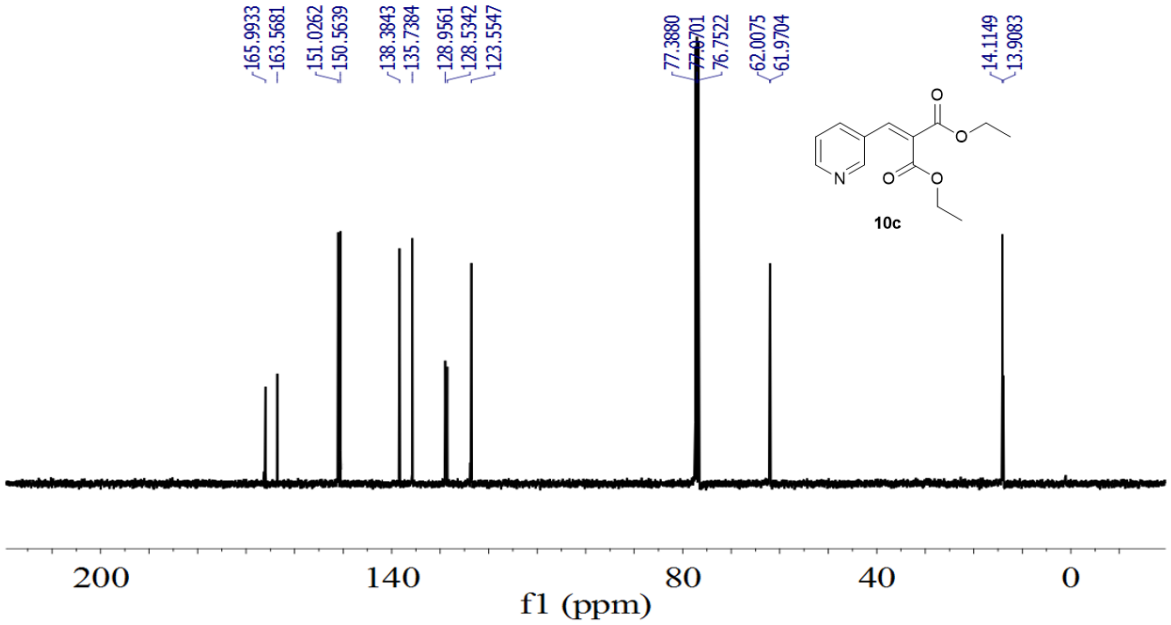
Ethyl (2*E*)-2-cyano-3-(pyridin-3-yl) prop-2-enoate (10d): ^{13}C NMR, $CDCl_3$



4-(pyridin-3-ylmethylidene) heptane-3, 5-dione (10c): ^{1}H NMR, $CDCl_3$



4-(pyridin-3-ylmethylidene) heptane-3, 5-dione (10c): ^{13}C NMR, $CDCl_3$



CURRICULUM VITAE

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OBJECTIVE

To outshine in research and development in the field of medicinal chemistry by acquiring knowledge from the practical experiments and projects.

EDUCATION

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2010-2015 Integrated M S (5th year chemistry major)
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Punjab, Indi
Current CPI: 8.0
HSC
2008-2010 SEETHI SAHIB HR.SEC.SCHOOL, Kannur, Kerala, India
Marks obtained – **93%**
SSLC
2007-2008 SACRED HEART HIGH SCHOOL, Kannur, Kerala, India
Marks obtained – **94%**

AREAS OF INTEREST

- ✓ Medicinal Chemistry
- ✓ Organic chemistry
- ✓ Medicinal Biology
- ✓ Biochemistry
- ✓ Human Physiology

EXPERIENCE WITH TECHNIQUES, SCIENTIFIC EQUIPMENT AND EXPERIMENTS:

CHEMISTRY

Organic synthesis methodologies, characterization techniques like IR, NMR spectroscopy, Mass spectrometry and UV-VIS spectroscopy , Separation techniques like column chromatography, acid base extraction and thin layer chromatography, synthesis of metal ligand complexes.

BIOLOGY:

Protein crystallization, Gel electrophoresis, PCR, SDS page, Restriction Digestion and cloning, RNA isolation, Plasmid isolation, cDNA synthesis, protein expression, primer designing, competent cell preparation, protein purification Ni-NTA column .