Development of enzymatic aza-Michael addition reactions

A Dissertation

Submitted in the partial fulfilment of the Degree

Of

Masters of Science (Chemistry)

By

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July-2023

CERTIFICATE

This is to certify that the thesis entitled "**Development of enzymatic aza-Michael addition reactions**", submitted by **Ms. Isha Kansal (Reg. No. 302102024)** in the partial fulfilment of the degree of **Masters of Science in Chemistry** from Thapar Institute of Engineering & Technology, Patiala is an authentic piece of work carried out under the supervision of **Dr. Vikas Tyagi**, Assistant Professor, School of Chemistry & Biochemistry, Thapar Institute of Engineering & Technology, Patiala and no part of project has been submitted for award of any other degree in this or any other university or institute of learning.

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Place: TIET, Patiala

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DECLARATION

The work embodied in the project entitled "**Development of enzymatic aza-Michael addition reactions**" has been done by me in the partial fulfilment of the degree of **Masters of Science in Chemistry** and submitted at the **School of Chemistry & Biochemistry, Thapar Institute of Engineering & Technology, Patiala** is an authentic record of my own research work carried out under the supervision of **Dr. Vikas Tyagi**, Assistant Professor, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala. All the ideas and references has been acknowledged to best of my knowledge.

Date: 25-07-2023
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This is to certify that the above statement made by student concerned is correct and true to the best of my knowledge.

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ACKNOWLEDGEMENT

First of all, I would want to offer my sincere appreciation to the Supreme Almighty for protecting me and keeping me comfortable while I conducted my study. I am really grateful to **Dr. Vikas Tyagi,** Assistant Professor, School of Chemistry & Biochemistry, Thapar Institute of Engineering & Technology, Patiala for the worthy guidance, valuable suggestions, inspiration and leadership. He has always been very much concerned about my daily work and always sorted difficulties those came during my work.

I am thankful to **Dr. Satnam Singh**, Professor and Head, School of Chemistry and Biochemistry for giving me this golden opportunity and allowed me to avail various facilities in department. I would like to thank **Dr. Kamaldeep Paul** and **Dr. Davinder Kumar**, PG coordinators, all the faculty members and staff of School of Chemistry and Biochemistry who have helped me throughout my M.Sc. journey.

Next, I would like to thank **Ms. Parmjeet Kaur** for being there for me during my study. I appreciate that she corrected me every time I made a mistake while working on this assignment. Her friendliness made it easier for me to finish my job. Working with lab research scholars **Ms. Priya Kamboj, Ms. Pooja Soam, Ms. Kirti Singh and Mr. Abinash Mohapatra** has been a really positive experience for me, and I am grateful to them for their assistance.

I would like to acknowledge the financial support from Science and Engineering Research Board (SERB), India (CRG/2021/002077) and Sophisticated Analytical Instrument (SAI) laboratory, Thapar Institute of Engineering & Technology, Patiala for major findings of the work.

This work would not have been possible without my friends **Abhinav**, **Charu**, **Jaffica** and **Jahnvi** who made this trip enjoyable. Last but not the least, I would like to acknowledge my father **Mr. Raj Kumar**, my mother **Mrs. Sanju Rani** and my brother **Rohan Raj Kansal** for motivating me every single day.

Isha Kansal

CONTENTS

LIST OF ABBREVIATIONS

1	mg	Milligrams
2	mmol	Millimoles
3	ml	Millilitres
4	Eq.	Equivalent
5	MHz	Megahertz
5	ppm	Parts per million
6	v/v	Volume by volume
7	ºC	Degree-Celsius
8	δ	Chemical shift
9	$CDCl_3$	Deuterochloroform
10	DMSO	Dimethyl sulfoxide
11	EtOH	Ethanol
12	MeOH	Methanol
13	DCM	Dichloromethane
14	THF	Tetrahydrofuran
15	DCE	1,2-Dichloroethene
16	DMF	Dimethylformamide
17	TLC	Thin Layer Chromatography
18	^{1}HMR	Proton Nuclear Magnetic Resonance

19	^{13}CNMR	Carbon Nuclear Magnetic Resonance
20	HRMS	High Resolution Mass Spectroscopy
21	TMS	Tetramethylsilane

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ABSTRACT

Enzymes are the biocatalysts which are capable of accelerating biochemical reactions. The utilization of enzymes as catalysts for non-natural reactions has been recognized as a valuable and ecologically sustainable approach in the field of synthetic chemistry. In addition, these catalysts offer significant enantio-, chemo-, and regioselectivity, thereby decreasing the possibility of undesired side reactions. Enzymes have been classified into different categories, including isomerases, ligases, lyases, oxidoreductases, transferases, translocases and hydrolases. Among various types of hydrolases, α-amylase has been employed to facilitate a diverse range of non-natural organic conversions, in addition to its inherent ability to catalyse the hydrolysis of 1,4-glyosidic bonds found in starch. Furthermore, the Michael addition reaction is an important transformation in organic synthesis. Several reports have been published where a diverse range of catalysts have been employed for the aza-Michael addition of aromatic amines to α,β-unsaturated carbonyl compounds. Also, the obtained β-aminocarbonyls serve as active pharmaceutical precursors. Therefore, a green protocol for the aza-Michael addition of benzoyl hydrazine to chalcones using α-amylase from *Aspergillus oryzae* as biocatalyst in aqueous medium has been devised.

CHAPTER-1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Biocatalysis refers to the utilization of biologically derived molecules, primarily enzymes, for the efficient and selective transformation of organic compounds [1]. Enzymes, being nature's catalysts, exhibit active sites that are highly specialized and enhance specific chemical reactions by reducing the activation energy barrier, while also maintaining exceptional regio-, stereo- and chemo-selectivity. In contrast to conventional catalysts that can contain heavy metals or toxic compounds, enzyme-catalyzed processes offer enhanced environmental sustainability. Also, enzymes are derived from natural sources and typically function under gentle reaction conditions, that include ambient temperatures and neutral pH levels enabling reduced energy consumption and waste generation [2]. Enzymes facilitate a diverse array of reactions, including oxidation, hydrolysis, addition, halogenation, alkylation, and isomerization, spanning almost all categories of organic reactions. Hydrolase enzymes (lipase, amylase, amidase, etc.) were the first family of enzymes to be used as biocatalyst to explore novel abiotic organic transformations referring to their easy market availability, low cost, reusability, high temperature tolerance and stability in organic solvents. In recent decades, there have been several instances of promiscuous reactions facilitated by hydrolases [3]. Similarly, the hydrolase enzymes were reported by Lin's group to catalyse the Michael addition of imidazole with acrylates [4]. Recently, Chen et al. have reported the aza-Michael addition of amines to acrylates in the presence of lipase enzyme in supercritical carbon dioxide as a reaction medium [5]. The catalysis of the hydrolysis of 1,4-α-glycosidic bonds in polysaccharides such as starch or glycogen by α-amylase is a widely recognized phenomenon in the natural world. [6]. A variety of organic reactions have been catalysed by α-amylase over the years. Recently, Dutt *et al*. reported aza-Michael addition reaction of amines to enones using α-amylase from *Aspergillus oryzae* as catalyst [7]. Also, He *et al*. used α-amylase as catalyst for biocatalytic one-pot synthesis of highly substituted indoloquinolizines using tryptamines, β-ketoesters and α, β-unsaturated aldehydes [8].

Michael addition reaction also referred as 1,4 conjugate addition reaction, is among the most widely used functionalities in organic synthesis [9]. These reactions, named in honor of Arthur Michael, who first discovered this transformation during the early 1900s, involve the nucleophilic addition of carbanion or other nucleophile to electron-deficient Michael acceptors

usually α,β-unsaturated carbonyl compounds [10]. This results in formation of carbon-carbon or carbon-heteroatom bond, expanding the molecular complexity and introducing functional groups into organic molecules. The aza-Michael addition, which produces C-N bonds, is an important component of Michael addition [11]. In general, Michael additions are facilitated by strong acids or bases, resulting in the generation of environmentally harmful residues and undesirable by-products. To mitigate these issues, various types of organo- and organometallic catalysts have been devised for Michael addition reactions under milder conditions [12]. Furthermore, α-amylase has been proved to catalyse Michael addition with excellent results. Dutt *et al*. demonstrated formation of C-N bonds in quinoline derivatives via α-amylase catalysed one-pot sequential aza-Michael/aldol reaction [13]. The same authors demonstrated modification of β-aminocarbonyl using α-amylase enzyme and Pd-based catalyst in one-pot [14].

Further, chalcones, the α,β-unsaturated carbonyl compounds used as Michael acceptors in Michael addition reaction are open chain flavonoids that exhibit various biological capabilities, including antiulcer, anticancer, antidiabetic, anti-inflammatory etc. according to the previous scientific research **(Figure 1)** [15]. Based on the chalcone skeleton, some lead compounds with varied pharmacological characteristics have been created [16]. The aim of the present study is to develop acyl hydrazide derivatives using benzoyl hydrazine and chalcones in the presence of α- amylase *via* aza- Michael addition reaction.

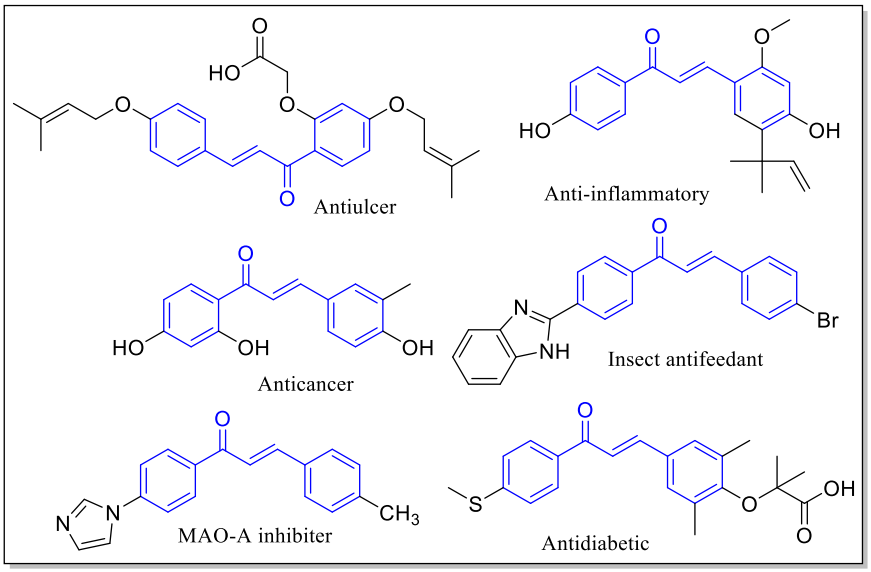
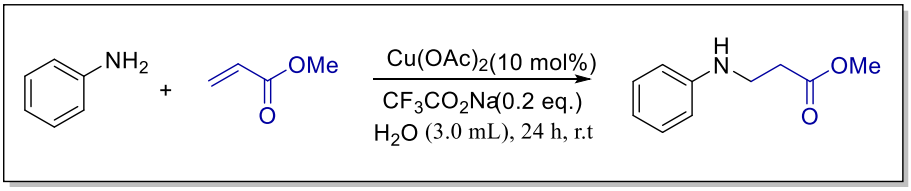


Figure 1: Chalcone containing pharmaceutically active drugs

1.2 Literature Review

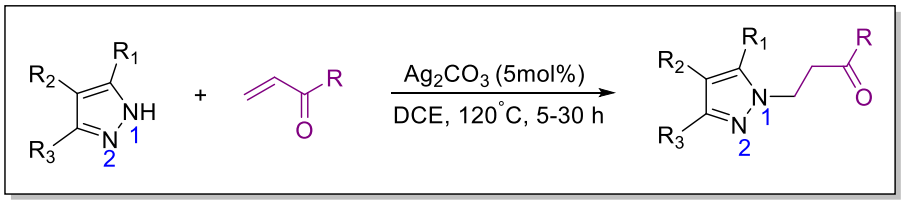
1.2.1 Metal catalysed aza-Michael addition

In a recent study, Masaeli *et al*. have successfully devised a modification of the copper-catalyzed aza-Michael reaction. This novel approach involves the utilisation of sodium trifluoroacetate in an aqueous medium, resulting in the efficient formation of new C-N bonds. The model substrates employed by the authors in this study were aniline and methyl acrylate **(Scheme 1)** [17]. The reaction was conducted in water at ambient temperature. The authors investigated that no reaction had been taken place both in the absence and presence of 10 mol % $Cu(OAc)_2$ as catalyst. A remarkable yield (95%) of the desired product was observed when employing the salt CF_3CO_2Na (2 eq.) as an additive complementary to catalyst. The present protocol offers a highly efficient approach for accessing hydroamination products, utilising optimised conditions that demonstrate compatibility with a diverse array of substrates.



Scheme 1: Copper acetate catalysed aza-Michael addition of aniline to methyl acrylate

During the same year, Che and coworkers introduced the concept of aza-Michael addition, focusing on the addition of pyrazoles to α,β-unsaturated carbonyl compounds. Under optimised conditions, a series of N-alkylated pyrazoles can be obtained in good to excellent yields by employing 5 mol% of Ag_2CO_3 in DCE at a temperature of 120°C **(Scheme 2)** [18]. Highly regioselective N^1-alkylated pyrazoles can be obtained from unsymmetrically substituted pyrazoles **(Figure 2)**.



Scheme 2: Ag_2CO_3 catalysed synthesis of N-alkylated pyrazoles

Pyrazoles bearing electron-donating groups, such as -Me, $-OCH_3$, and -Ph, exhibited a higher reaction rate compared to pyrazoles containing electron-withdrawing groups, namely CF_3, CO_2Et, $COCH_3$, and NO_2. In a similar manner, pyrazoles that are either 3, 5 or 3, 4, 5-

polysubstituted, exhibited a sluggish reactivity when subjected to unsaturated carbonyls, necessitating an extended reaction duration.

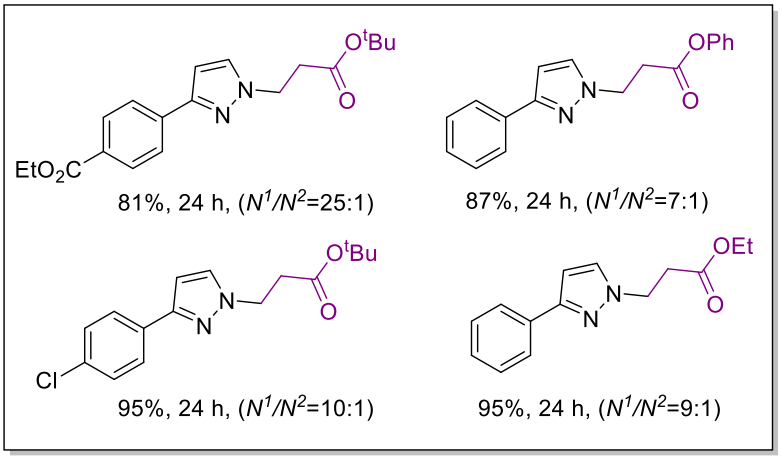
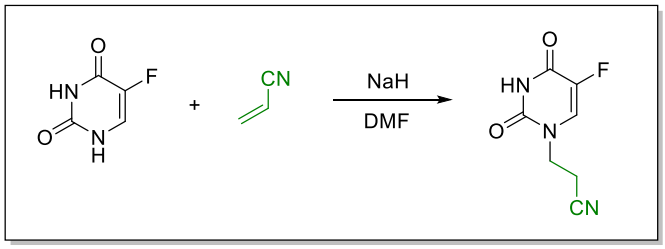


Figure 2: Substrate scope of the Ag_2CO_3 catalysed synthesis of N-alkylated pyrazoles

1.2.2 Base catalysed aza-Michael addition

Next, 5-Fluorouracil (5-FU) inhibits DNA synthesis to treat cancer. However, its short plasma half-life, limited affinity, myelosuppression, and intestinal toxicity necessitated the search for alternatives. Mushtaque *et al*. created a Carbonic Anhydrase IX inhibitor from 5-FU using acrylonitrile's cancer-fighting capabilities (Figure 3). The Aza-Michael addition reaction between 5-FU and acrylonitrile used NaH as a base and DMF as a solvent (Scheme 3) [19]. The reaction underwent reflux under mild heating conditions. The synthesised molecule targets the CAIX enzyme, exhibiting enhanced effectiveness against cancer cells.



Scheme 3: NaH catalysed synthesis of 5-FU derivatives

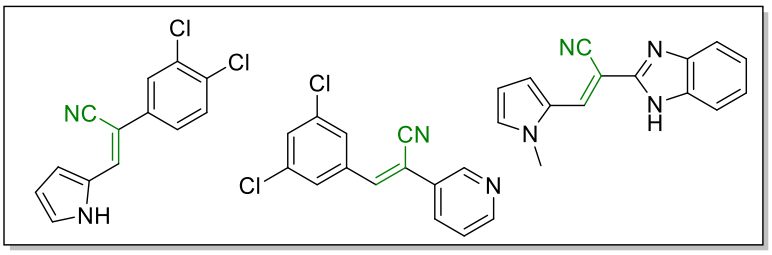
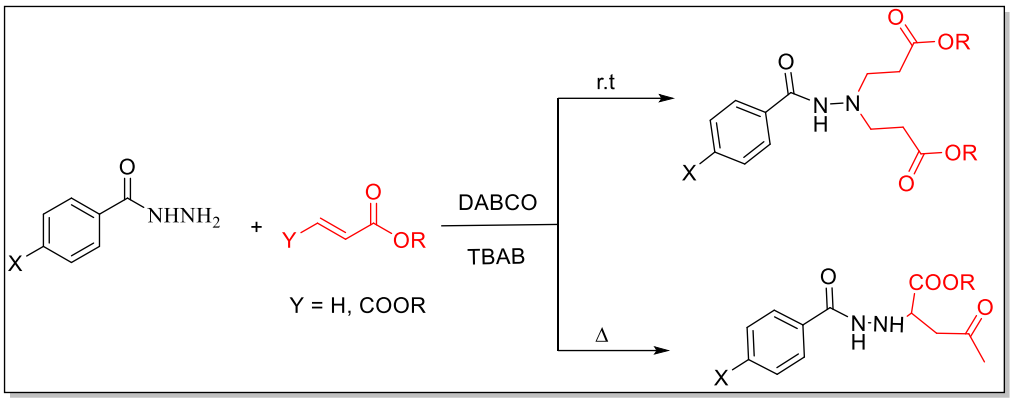


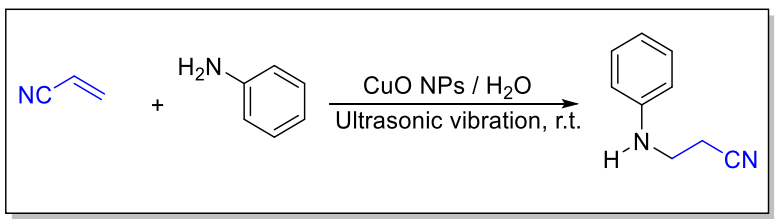
Figure 3: Acrylonitrile containing anti-cancer agents

Soltanzadeh and coworkers demonstrated the reaction of various acyl hydrazides and α,β-unsaturated esters in presence of ionic organic salt tetra *n*-butyl ammonium bromide (TBAB) and organic base catalyst of 1,4-diaza-bicyclo[2,2,2]octane (DABCO) for synthesis of new acyl hydrazide derivatives **(Scheme 4)** [20]. In presence of TBAB, the model reaction between n-butyl acrylate and acyl hydrazide was tested with various inorganic & organic bases and solvents like DMF, $CHCl_3$, DMSO, EtOAc giving best yield using DABCO as base under solvent free conditions. Surprisingly, where acrylic esters gave bis-Michael adduct at r.t, fumaric esters gave mono-adduct on increasing the reaction temperature to 70°C. The above protocol is green in nature due to recoverability of TBAB, inexpensive base DABCO and solvent free conditions.



Scheme 4: TBAB and organic base catalyst DABCO for synthesis of new acyl hydrazide derivatives

CuO nanoparticles (CuO NPs) were synthesised by Chowdhury *et al*. through the utilisation of copper (II) acetate and NaOH as the hydrolysing agent. This process was conducted in the presence of a flower extract derived from the Lantana camara plant, followed by calcination in an air environment at a temperature of 400°C. The catalytic activity of the synthesised CuO nanostructures (0.024 mmol) was demonstrated in the aza-Michael addition reaction between acrylonitrile (2 mmol) and aniline (2 mmol) **(Scheme 5)** [21].



Scheme 5: CuO nanocatalyzed aza-Michael addition reaction using acrylonitrile and aniline

The initial yield of the reaction conducted under solvent-free conditions at r.t was observed to be low as 30%. The utilisation of water and a water-ethanol mixture as solvents, along with an

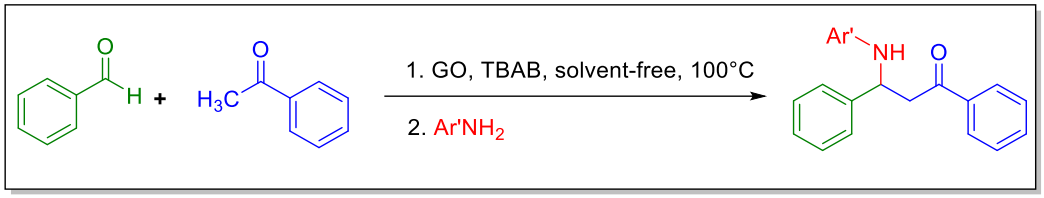
elevation in temperature to 60°C resulted in an enhancement in the yield of the reaction. In order to reduce energy consumption and promote environmentally sustainable reaction conditions, the experiment was repeated in water at r.t, using ultrasonic vibrations which gave maximum isolated yield of 80% **(Table 1)**. The optimised conditions resulted in the observation of good yields when electron withdrawing and donating groups were present at the *ortho*, *meta*, and *para* positions of aniline, as well as with aliphatic amines.

Table 1: Optimization of CuO catalyzed aza-Michael addition reaction

Entry	Solvent	Temperature	Time (h)	Yield (%)
1	Solvent-free	r.t	24	30
2	Solvent-free	60°C	9	50
3	Water	60°C	9	70
4	Water + Ethanol	60°C	9	70
5	Ethanol	60°C	9	65
6	Acetonitrile	60°C	9	30
7	**Water**	**r.t**	**4**	**80**

1.2.3 Acid catalysed aza-Michael addition

Khalili *et al*. demonstrated the use of graphene oxide (GO) in conjunction with tetra *n*-butyl ammonium bromide (TBAB) served as a highly efficient catalyst for the sequential aldol condensation and aza-Michael addition reactions, enabling the synthesis of amines from chalcone **(Scheme 6)** [22]. The initial yield of the reaction involving benzaldehyde and acetophenone as model substrates, with GO serving as the catalyst exhibited greater efficacy in solvents such as CH_2Cl_2, THF, EtOH, and CH_3CN when compared to water. The aza-Michael adduct was successfully synthesised with a high yield of 72% within a reaction time of 16 hours, using a solvent-free condition and a temperature of 100°C. The addition of 30 mol% of TBAB as an additive resulted in a further increase in the yield of the final product to 84%. Position of substituents on aniline played a major role. *Ortho* substituted anilines resulted in comparatively lower yield than *meta* or *para* substituted anilines due to steric hindrance **(Figure 4)**.



Scheme 6: GO catalysed sequential aldol condensation and aza-Michael addition reaction

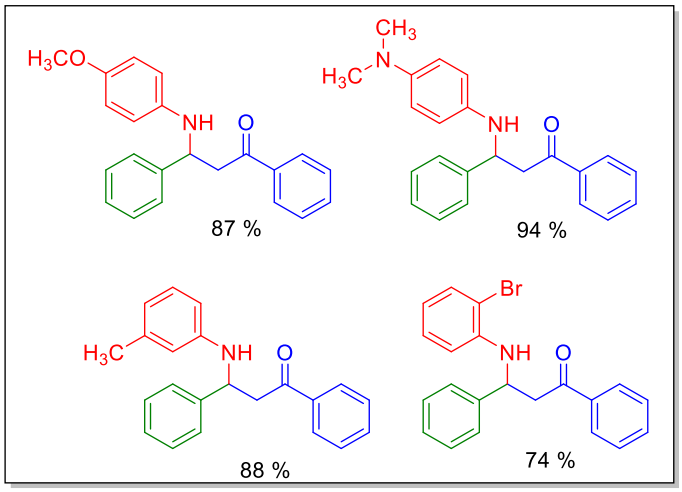
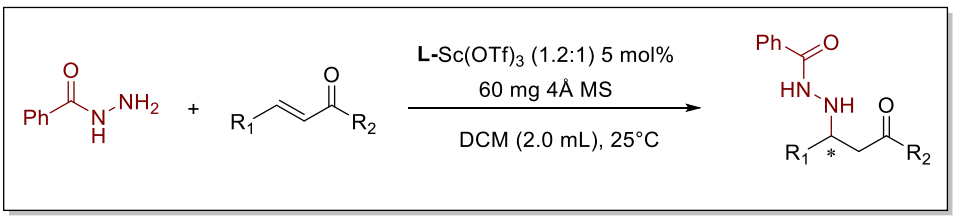


Figure 4: Substrate versatility of the GO catalysed reaction

The implementation of this protocol resulted in the exclusion of costly metals and facilitated the purification and isolation of the aza-adduct, primarily due to the heterogeneous nature of the GO catalyst.

Feng and coworkers demonstrated the catalytic asymmetric aza-Michael addition between model substrates benzoyl hydrazine and chalcone via the nonactivated amine moiety. The reaction was best catalysed by N, N'-dioxide ligand (**Figure 5**) complexed with $Sc(OTf)_3$ as Lewis acid. The reaction was further facilitated with combined use of DCM as solvent and 4Å molecular sieves (MS) **(Scheme 7)** [23]. Reducing the catalyst loading prolonged the reaction time. The products formed exhibit excellent enantioselectivity upto 97% and are pharmacologically useful.



Scheme 7: Scandium catalyzed Michael addition of benzoyl hydrazine and chalcone

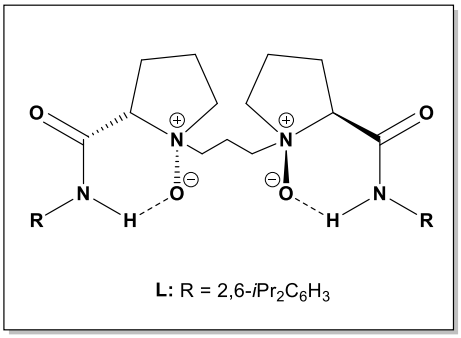
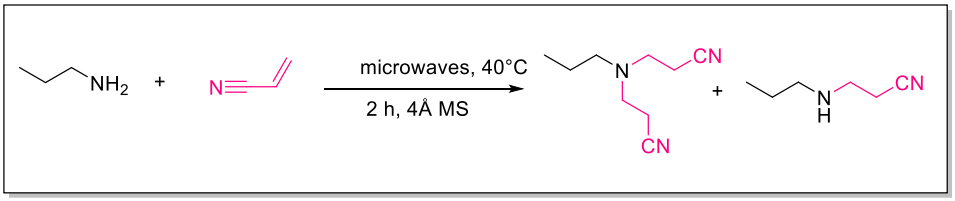


Figure 5: Optimised ligand for the synthesis

Bis-adducts of acrylonitrile and primary aliphatic amines which serve as useful building blocks for various biomedical compounds and as intermediates in the synthesis of surface-active materials. But formation of these adducts takes a lot of acrylonitrile, harsh acid catalyst, hazardous solvents and prolonged reaction duration. Das *et al*. described formation of mono- and bis-adducts of acrylonitrile with primary aliphatic and aromatic amines in one pot under microwave irradiation using ecofriendly molecular sieves (MS) (4Å) as catalyst **(Scheme 8)** [24]. The adducts have been synthesised by mixing the reactants containing MS with vortex mixture, heated to 40°C under microwave irradiation for 2 h, cooled and centrifuged to separate the catalyst. The catalyst was found to be reused upto 5 cycles without affecting the yield of reaction. Furthermore, the authors have proposed the plausible mechanism for the formation of the adducts (**Figure 6**).



Scheme 8: Formation of mono- and di-adducts of acrylonitrile using ecofriendly MS

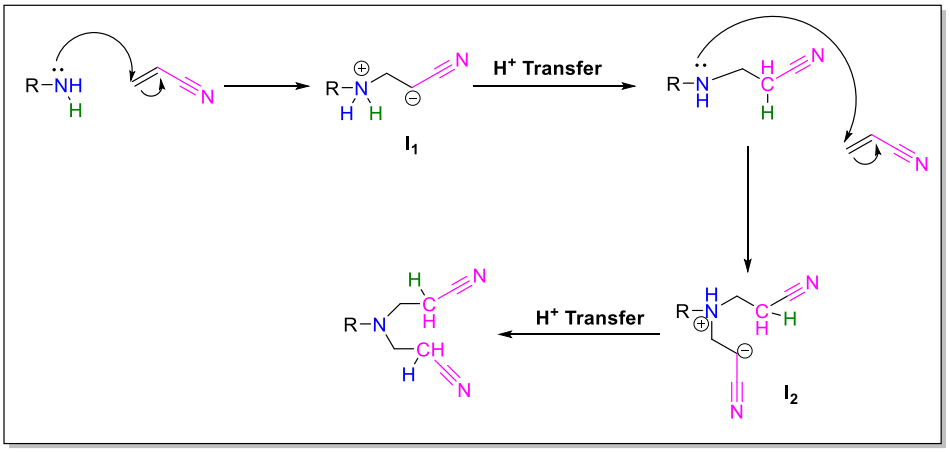
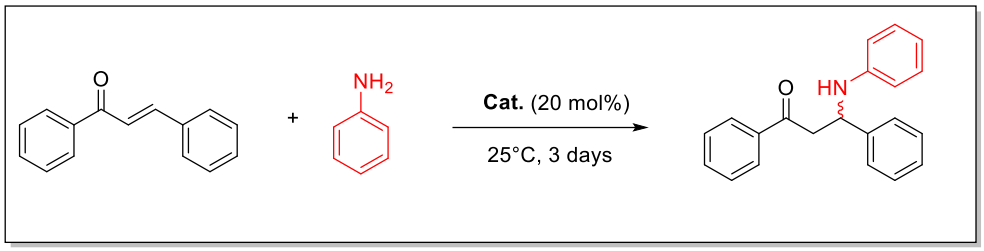


Figure 6: Plausible mechanism for synthesis of bis-adducts of acrylonitrile

1.2.4 Organocatalysed aza-Michael addition

In addition to Lewis acid catalysts like $Cu(OTf)_2$, $InCl_3$, $Yb(OTf)_3$, $LiClO_4$, $Bi(NO_3)$, $FeCl_3.6H_2O$ for aza-Michael addition, chiral organocatalysts derived from cinchona alkaloids have also been explored. Rahman *et al*. reported asymmetric aza-Michael of aniline to chalcone to form beta-amino ketones using squaramide linked cinchona alkaloid polymers **(PQ)** (**Figure 7**) **(Scheme 9)** [25].



Scheme 9: Cinchona alkaloid based organocatalyzed aza-Michael addition reaction

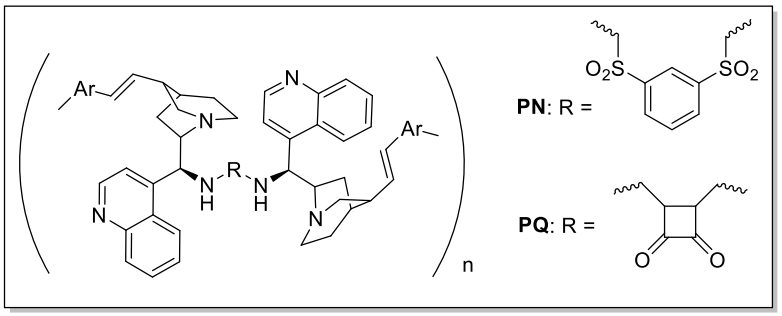
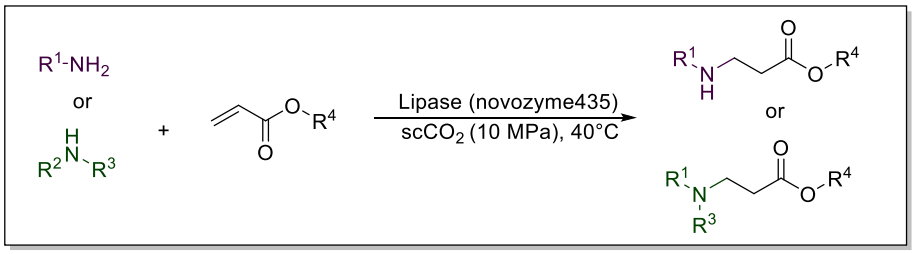


Figure 7: Catalysts used in the synthesis

The authors tested various sulfonamide **(PN)** and squaramide **(PQ)** catalysts. The catalyst with bulky anthracenyl added into its squaramide polymer structure had 95% enantioselectivity. Retro-aza-Michael reaction lowered enantioselectivity in organic solvents including MeOH, THF, and CH_2Cl_2, but solvent-free circumstances increased it. Enantioselectivity also reduced at 50°C. Low catalyst loading reduced reaction yield. Thus, no solvent, 25°C, and 20 mol% catalyst loading were optimal reaction conditions. Polymer catalyst's insolubility in common organic solvents helped its separation. Chalcones with electron donating groups (OH, OCH_3) had 94-99% enantioselectivity, while those with electron-withdrawing groups (NO_2, Cl, F) had 29-51%.

1.2.5 Enzyme catalysed aza-Michael addition

Zhang *et al*. demonstrated an example of non-aqueous biocatalysis by using lipase as catalyst in the chemoselective aza-Michael addition of amines to acrylates in supercritical CO_2 ($scCO_2$) **(Scheme 10)** [5]. In this work, many organic solvents were tested like n-hexane, THF, toluene, ethanol, DCM resulting in formation of aminolysis product along with 1,4- addition product. Low yields were obtained using inactive enzyme, thermally denatured enzyme and in control reaction indicating the involvement of active site of enzyme in the reaction **(Table 2)**. Furthermore, 40°C temperature, 10MPa pressure, 20 mg catalyst loading, 1:1 molar ratio of substates and $scCO_2$ as reaction medium were ideal conditions to give 100% 1,4-addition products with high yield. Further it was observed that sterically hindered primary amines gave mono-adduct while linear primary amines gave a mixture of mono and bis-adduct.



Scheme 10: Chemoselective lipase catalyzed aza-Michael addition of amines to acrylates

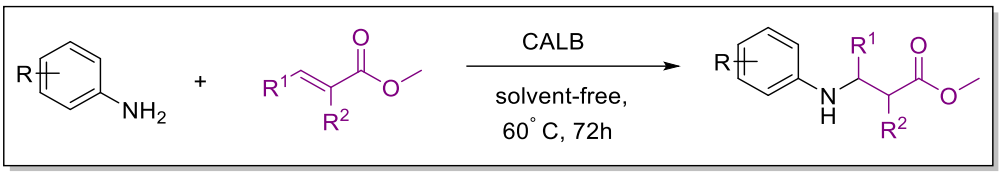
Table 2: Optimization of reaction conditions for aza-Michael addition of amines to acrylates

Entry	Solvent	Conversion (%)	1,4-Addition (%)	Aminolysis (%)
1	EtOH	46	28	72
2	DMF	35	30	70
3	THF	48	48	52
4	DCM	60	51	49
5	Toluene	65	65	35
6	n-Hexane	67	79	21
7	**$scCO_2$**	**93**	**100**	**0**
8	$scCO_2$[a]	17	100	0
9	$scCO_2$[b]	16	100	0
1.	$scCO_2$[c]	19	100	0

[a] Novozym 435 was inactivated by phenylmethanesulfonyl fluoride (PMSF)
[b] No enzyme
[c] Novozym was thermally deactivated by boiling in water for 12 h

Next, Yu and coworkers demonstrated CALB (Novozym435) as biocatalyst for aza-Michael addition of aniline and methyl acrylate as model compounds **(Scheme 11)** [26]. No aza-Michael adduct was observed by the authors in solvents like 2-propanol, 1-butanol, THF, DMF, DMSO etc. To their surprise, when methyl acrylate was used in excess as solvent, the yield of reaction

was 63% in 48 h probably due to increased binding of enzyme to substrates in absence of interference due to other organic solvents. Also, 20mg/ml was optimum catalytic loading with 60 °C as optimum reaction temperature. Formation of bis-Michael by product was observed if reaction time was increased to more than 3 days. After the reaction condition optimization authors went for substrate scope for the reaction.



Scheme 11: Solvent free CALB catalyzed aza-Michael addition reaction

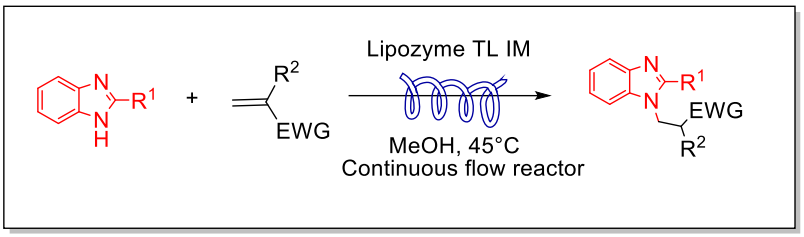
It was observed that anilines substituted with electron donating groups like hydroxyl at *meta* or *para* position facilitated the formation of aza-Michael adduct due to increase in nucleophilicity of aniline. In case of nitro, which is a strong electron withdrawing group, reaction hardly took place. Also, the yield was poor in case of methyl methacrylate and methylcrotonate **(Table 3)**.

Table 3: Substrate scope for the biocatalysed reaction

Entry	Donor	Acceptor		Yield (%)
	R	R^1	R^2	
1	3-OH	H	H	86
2	4-OH	H	H	78
3	2-NO_2	H	H	<1
4	3-NO_2	H	H	<1
5	4-Cl	H	H	27
6	2-Me	H	H	45
		H	Me	3
		Me	H	5
7	4-Me	H	H	70
		H	Me	4
		Me	H	9

Benzimidazole ring containing compounds are hot compounds in pharmaceutical industry due to their obvious anti-tumor, anti-HIV, anti-diabetic, anti-fungal and bacterial properties. Taking note of above properties of benzimidazole, Luo and coworkers proposed a microfluidic biocatalysis to construct N-substituted benzimidazole derivatives via aza-Michael addition reaction catalysed by lipase TLIM from *Thermomyces loauginosus* **(Scheme 12)** [27]. Among various investigated solvents such as CAN, DMF, DMSO, n-hexane, isopropanol and

methanol, methanol was optimum solvent for model reaction of benzimidazole with 2-chloroacrylonitrile with 1:6 as the optimised molar ratio. Reaction yield started to drop at temperature more than 45°C and residence time more than 35 mins. The use of continuous flow reactor and reusability of the immobilised lipozyme in this protocol provided good scalability. Substrate scope of the reaction is represented in **Table 4**.

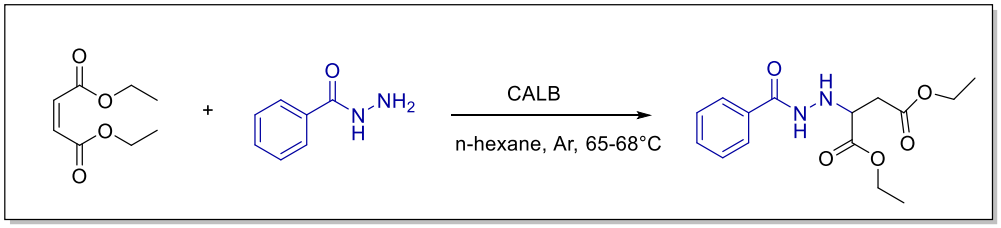


Scheme 12: Synthesis of N-substituted benzimidazole derivatives by TLIM lipase

Table 4: Substrate versatility of the lipase catalysed aza-Michael addition reaction

Entry	R^1	R^2	EWG	Yield (%)
1	H	Cl	CN	95.4 ± 1.6
2	H	H	CO_2Me	92.2 ± 1.5
3	H	H	SO_2Ph	97.1 ± 1.2
4	Cl	CH_3	CO_2Me	trace
5	CH_3	Cl	CN	95.6 ± 1.4
6	CH_3	H	CO_2Me	93.2 ± 0.6
7	CH_3	CH_3	CO_2Me	trace
8	CH_3	H	SO_2Ph	97.2 ± 0.8

Nazarian *et al*. investigated the activity of CALB immobilised on polyacrylin resin as a biocatalyst for mono aza-Michael addition of benzoyl hydrazine to diethyl maleate under argon **(Scheme 13)** [28].

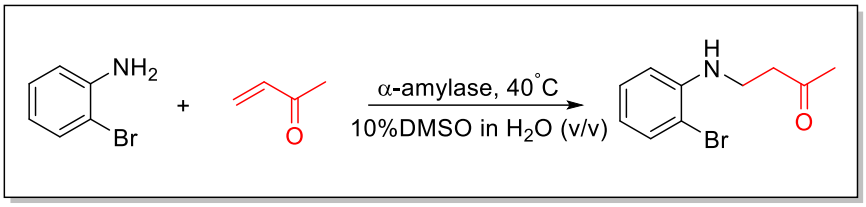


Scheme 13: Immobilised CALB used as a biocatalyst for mono aza-Michael addition of benzoyl hydrazine and diethyl maleate

This study investigates the influence of solvent on enzyme activity as solvent molecules binds to active site of enzyme and alter its performance. The studies demonstrated that CALB showed

its catalytic activity only in hexane, a non-polar solvent and failed in other solvents like DCM and THF. 65-68°C was the optimum temperature for reaction and reaction failed at lower temperatures. Also, the reaction did not occur with free enzyme, indicating lack of its long-term stability.

In the same year, Dutt *et al*. introduced a novel approach involving the utilisation of α-amylase as a catalyst for the biocatalytic aza Michael addition reaction. This reaction specifically focuses on the addition of less nucleophilic aromatic amines to enone. The optimised reaction conditions for achieving a high yield of the desired product involved 1:1.2 molar ratio of model substrates, 2-bromoaniline and methyl vinyl ketone with 10% DMSO in H_2O (v/v) as solvent at a temperature of 40°C. Additionally, a catalyst loading of 1.5 mg/ml was employed **(Scheme 14)** [7].



Scheme 14: α-amylase catalyzed aza-Michael addition of aniline to methyl vinyl ketone

The introduction of electron-donating groups, such as -Me and -OMe, onto the arene ring results in the formation of the desired products with high yields in contrast to electron-withdrawing groups, such as -NO_2 and -$COCH_3$ **(Figure 3)**. Furthermore, the enhanced efficiency of the catalyst was observed through the hybridization of amylase with Cu NPs. This hybrid catalyst exhibits the ability to be utilised multiple times while maintaining a high level of catalytic efficiency.

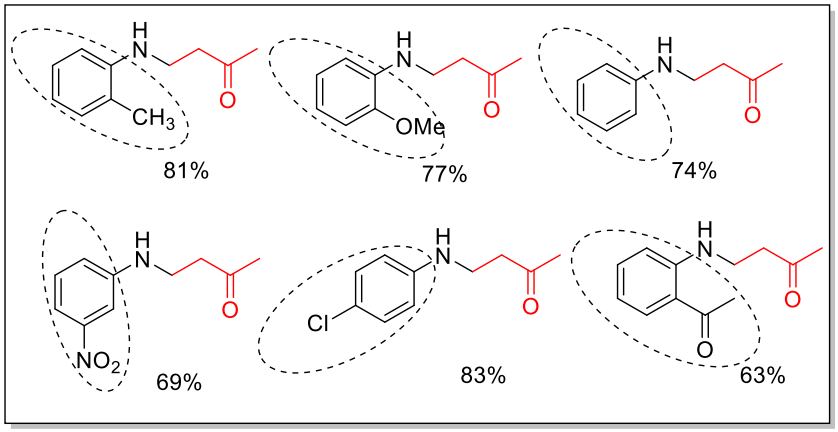
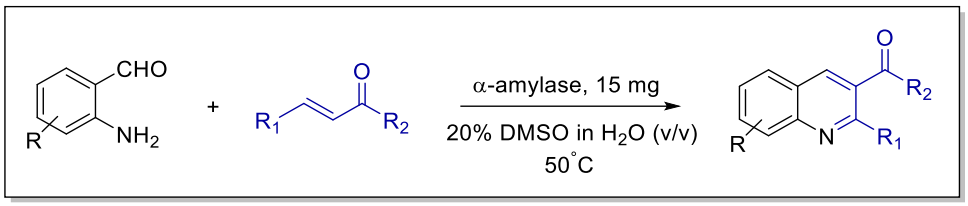


Figure 8: Substrate scope of the α-amylase catalysed aza-Michael addition reaction

The quinoline moiety is a biologically active component that is present in numerous pharmaceutical compounds as well as natural substances. The synthesis of substituted quinoline derivatives in satisfactory yield was reported by Dutt *et al*., utilising α-amylase as the catalyst **(Scheme 15)** [13]. The model substrates used in this study were 2-amino benzaldehyde and methyl vinyl ketone, with an optimised molar ratio of 1:1.2. The catalyst loading and solvent system were evaluated, and it was determined that a concentration of 7.5 mg/ml of α-amylase and a solvent mixture of 20% DMSO in water (v/v) yielded the most favourable results for the reaction **(Table 1)**. The reaction yield was relatively low while using pure DMSO or ethanol as solvents. Additionally, the optimised reaction temperature was determined to be 50°C.



Scheme 15: α-amylase used as a catalyst for synthesis of quinoline derivatives

Table 5: Optimization of reaction condition for synthesis of quinoline derivatives

Entry	Solvent	Enzyme loading	Substrate ratio	Temperature	Conversion
1	40% DMSO in H_2O (v/v)	5 mg	1:1	50˚C	59%
2	40% DMSO in H_2O (v/v)	10 mg	1:1	50˚C	81%
3	40% DMSO in H_2O (v/v)	15mg	1:1	50˚C	88%
4	40% DMSO in H_2O (v/v)	20 mg	1:1	50˚C	89%
5	40% DMSO in H_2O (v/v)	**15 mg**	**1:1.2**	**50˚C**	**91%**
6	40% DMSO in H_2O (v/v)	15 mg	1:1.5	50˚C	90%
7	40% DMSO in H_2O (v/v)	15 mg	1:1	40˚C	85%
8	40% DMSO in H_2O (v/v)	15 mg	1:1	60˚C	89%

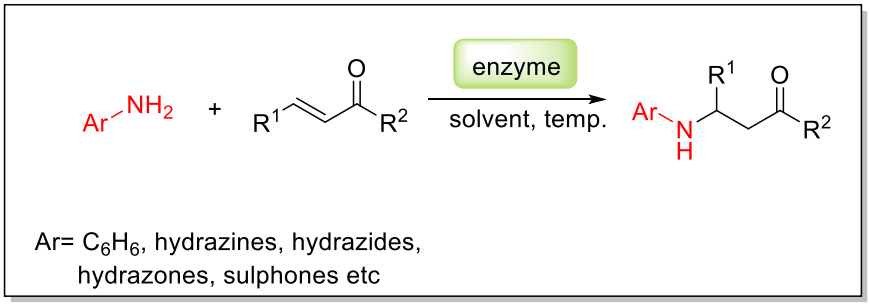
1.3 Research Gaps:

- Aza-Michael addition reaction is less explored using enzymes as catalysts.
- There is no previous report of biocatalytic aza-Michael addition of benzoyl hydrazine to chalcones.

1.4 Our Approach:

In light of the persistent interest and demand for pharmaceutically important β-amino carbonyl compounds, we have devised a methodology for their synthesis using enzyme as biocatalyst. Their synthesis involves the 1,4-aza-Michael addition reaction, wherein different amine containing moieties *i.e.* hydrazones, hydrazides and hydrazines work as Michael donors and α,β- unsaturated carbonyl compounds work as Michael acceptors. The catalytic activity of the enzyme is harnessed in a solvent system consisting of mixture of DMSO and water **(Scheme 16)**.

The utilisation of an environmentally friendly and non-toxic biocatalyst, along with water as the reaction medium, has resulted in the enhanced efficacy and sustainability of our experimental procedure.



Scheme 16: General representation of enzyme catalyzed aza-Michael addition reaction

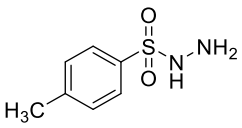
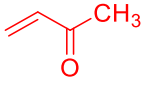
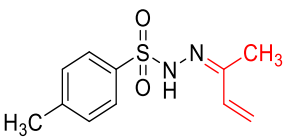
CHAPTER-2

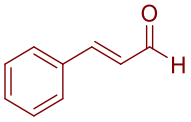
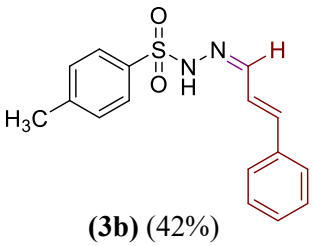
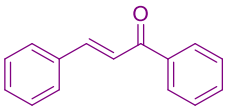
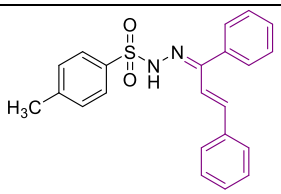
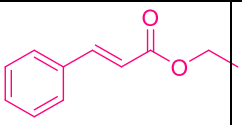
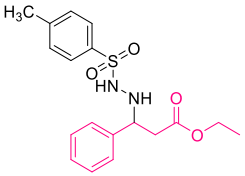
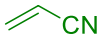
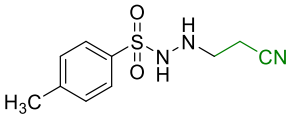
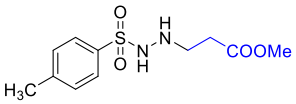
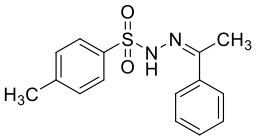
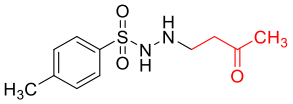
RESULTS AND DISCUSSION

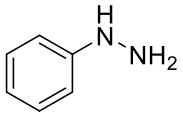
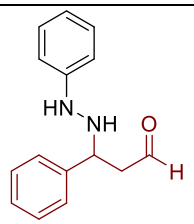
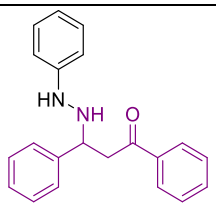
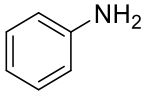
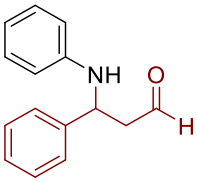
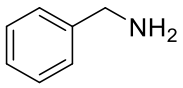
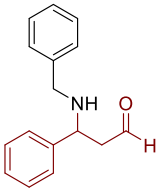
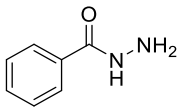
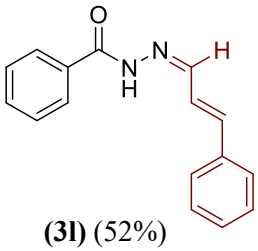
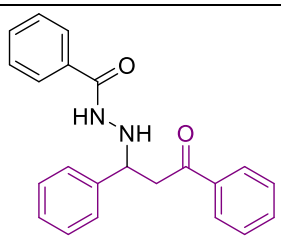
2.1 Investigation of various Michael donors and acceptors

Firstly, we have tried to explore the enzymatic aza-Michael addition with various Michael acceptors and donors using α-amylase as described in the following **Table 6**. Initially, we investigated the reaction of N-tosylhydrazine **(1a)** with methyl vinyl ketone **(2a).** Instead of aza-Michael adduct we encountered hydrazone formation **(3a)** due to condensation of carbonyl group of ketone with free amino group of hydrazine **(entry 1, Table 6)**. Similarly, the reaction of **(1a)** with cinnamic aldehyde **(2b)** and chalcone **(2c)** furnished the condensation product **(entries 2-3, Table 6)**. Furthermore, no product formation was observed with ethyl cinnamate **(2d)** and acrylonitrile **(2e)** as Michael acceptors **(entries 4-5, Table 6)**. To our surprise, when **(1a)** was made to react with methyl acrylate **(2f)**, 1,4-aza-Michael product was obtained **(entry 6, Table 6)**. This encouraged us to explore more N-tosylhydrazine like Michael donors. Then, we tested acetophenone N-tosylhydrazone **(1b)** as Michael donor with **(1a)** and got the aza-Michael adduct **(entry 7, Table 6)**. Sadly, both of the above mentioned aza-Michael addition reactions also worked in the absence of enzyme. Thus, we excluded the substrates **(1a)** and **(1b)** as Michael donors. We then moved to phenyl hydrazine **(1c)** which failed to react with **(2b)** and **(2c) (entries 8-9, Table 6)**. Also, aniline **(1d)** and benzylamine **(1e)** did not furnish any product with **(2b)** due to solubility issue of former in our solvent system **(entries 10-11, Table 6)**. Next, we have tried the reaction with benzoyl hydrazine **(1f)** which gave condensation product on reaction with **(2b) (entry 12, Table 6)**.

Table 6: Screening of various Michael donors and acceptors for aza-Michael addition reaction

Entry	Michael donor	Michael acceptor	Product (% isolated yield)	Reaction Conditions
1	 **(1a)**	 **(2a)**	 **(3a)** (35%)	α-amylase (15 mg), **1a** (1 eq., 0.536 mmol), **2a** (1.5 eq., 0.806 mmol), 40°C, 4 ml of 20% DMSO in H_2O (v/v), 24h

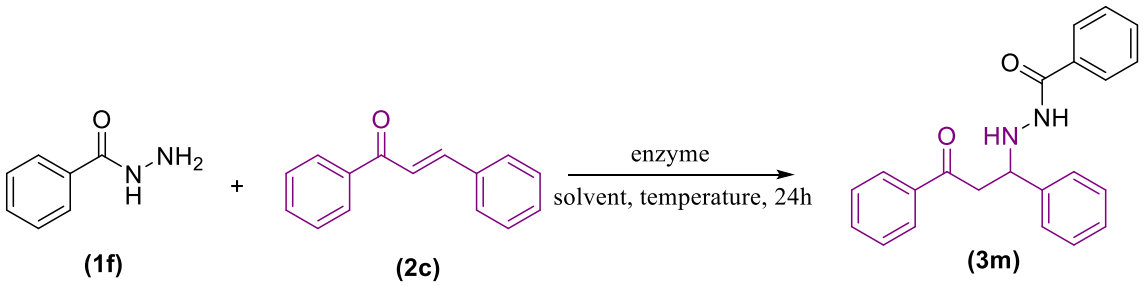
2	**(1a)**	 **(2b)**	 **(3b)** (42%)	α-amylase (15 mg), **1a** (1.0 eq., 0.536 mmol), **2b** (1.5 eq., 0.805 mmol), 40°C, 4 ml of 20% DMSO in H_2O (v/v), 2 h
3	**(1a)**	 **(2c)**	 **(3c)** (37%)	α-amylase (15 mg), **1a** (1.0 eq., 0.536 mmol), **2c** (1.0 eq., 0.538 mmol), 50°C, 4 ml of 10% DMSO in H_2O (v/v), 24 h
4	**(1a)**	 **(2d)**	 **(3d)** (0%)	α-amylase (3 mg), **1a** (1.0 eq., 0.053 mmol), **2d** (1.5 eq., 0.080 mmol), 40°C, 2 ml of 20% DMSO in H_2O (v/v), 24 h
5	**(1a)**	 **(2e)**	 **(3e)** (0%)	α-amylase (3 mg), **1a** (1.0 eq., 0.053 mmol), **2e** (1.5 eq., 0.080 mmol), 40°C, 2 ml of 20% DMSO in H_2O (v/v), 24 h
6	**(1a)**	 **(2f)**	 **(3f)** (46%)	α-amylase (15 mg), **1a** (1.0 eq., 0.536 mmol), **2f** (1.5 eq., 0.804 mmol), 40°C, 4 ml of 20% DMSO in H_2O (v/v), 24h
7	 **(1b)**	**(2a)**	 **(3g)** (30%)	α-amylase (15 mg), **1b** (1.0 eq., 0.346 mmol), **2a** (1.5 eq., 0.519 mmol), 40°C, 4 ml of 20% DMSO in H_2O (v/v), 24h

8	 **(1c)**	**(2b)**	 **(3h)** (0%)	α-amylase (3 mg), **1c** (1.0 eq., 0.092 mmol), **2b** (1.5 eq., 0.138 mmol), 40°C, 2 ml of 20% DMSO in H_2O (v/v), 24 h
9	**(1c)**	**(2c)**	 **(3i)** (0%)	α-amylase (3 mg), **1c** (1.0 eq., 0.092 mmol), **2c** (1.0 eq., 0.091 mmol), 40°C, 2 ml of 10% DMSO in H_2O (v/v), 24 h
10	 **(1d)**	**(2b)**	 **(3j)** (0%)	α-amylase (3 mg), **1d** (1.0 eq., 0.107 mmol), **2b** (1.0 eq., 0.107 mmol), 50°C, 2 ml of 10% DMSO in H_2O (v/v), 24 h
11	 **(1e)**	**(2b)**	 **(3k)** (0%)	α-amylase (3 mg), **1e** (1.0 eq., 0.093 mmol), **2b** (1.0 eq., 0.093 mmol), 40°C, 2 ml of 10% DMSO in H_2O (v/v), 24 h
12	 **(1f)**	**(2b)**	 **(3l)** (52%)	α-amylase (15 mg), **1f** (1.0 eq., 0.734 mmol), **2b** (1.0 eq., 0.712 mmol), 50°C, 4 ml of 10% DMSO in H_2O (v/v), 2h
13	**(1f)**	**(2c)**	 **(3m)** (30%)	α-amylase (15 mg), **1f** (1.0 eq., 0.734 mmol), **2c** (1.0 eq., 0.721 mmol), 50°C 4 ml of 10% DMSO in H_2O (v/v), 24 h

Delightfully, we have got 30% yield of product **(3m)** using benzoyl hydrazine **(1f)** and chalcone **(2c)** as Michael donor and acceptor respectively **(entry 13, Table 6)**. To get the higher product conversion we have optimized various parameters for the reaction.

2.2 Optimisation of reaction conditions:

Initially, we considered the reaction of benzoyl hydrazine and chalcone as template reaction to investigate different parameters of the reaction **(Scheme 17)**. First, we tested the reaction for different enzyme catalysts mainly amylases and lipases along with 4 ml of 10% DMSO in H_2O (v/v) as a green solvent system at 50°C for 24 h. After purification of product, we got maximum isolated yield of 42% using α -amylase from *A. oryzae.* The data obtained with different enzymes is represented in **Figure 9**.



Scheme 17: Optimisation of various reaction parameters

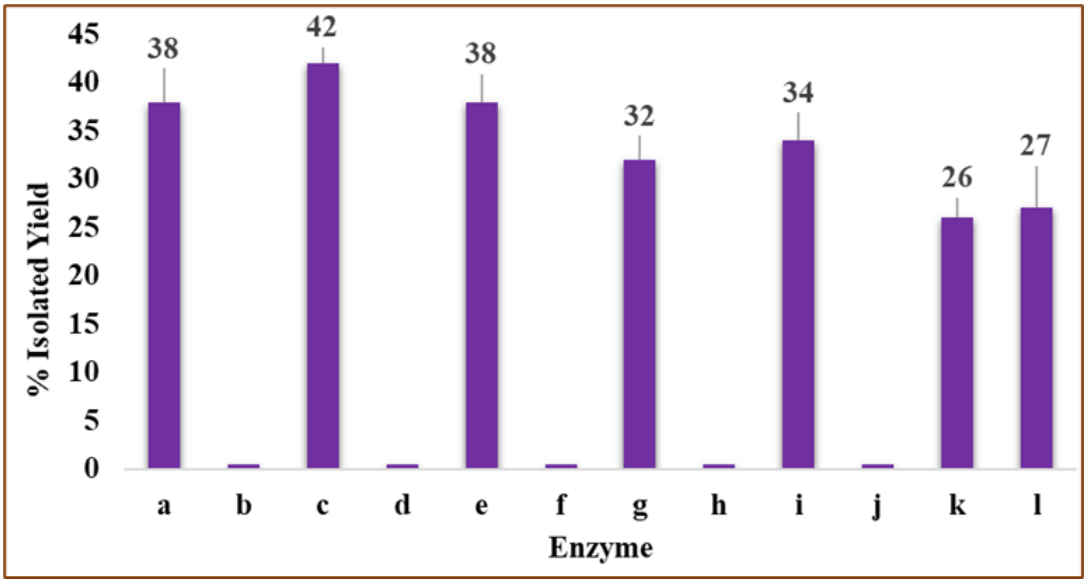


Figure 9: Optimisation of enzyme for the reaction[a]

(a) α-amylase from *Bascillus amyloliquefaciens* (b) α-amylase from *Bascillus sp.* **(c) α-amylase from *Aspergillus oryzae*** (d) α-amylase from *Hog pancreas* (e) β-amylase (f) Lipase from *Porcine pancreas* (g) *Candida antartica* lipase B (h) Lipase from *Aspergillus niger* (i) Lipase from *Rhizomucour miehei* (j) Lipase from *Candida sp.* (k) Lipase from *Candida rugosa* (l) Lipase from *Pseudomonas flourescens.*

[a]**Reaction Conditions**: Benzoyl hydrazine (**1f**) (1 eq., 0.734 mmol), Chalcone (**2c**) (1.0 eq., 7.211 mmol) 50°C, 4 ml of 10% DMSO in H_2O (v/v), 24 h

Results obtained from catalyst optimisation gave pathway for further optimisation of different parameters. The solvent is regarded as a crucial factor in enhancing the reaction yield. We fixed the enzyme α- amylase from *Aspergillus oryzae* and repeated the reaction with different solvents like MeOH, EtOH, THF, Dioxane etc and obtained low product yield. Then we used mixture of solvent *i.e.* DMSO and H_2O which give considerable amount of yield of desired product. We found that the mixture of 10% DMSO in H_2O (v/v) served as the perfect solvent system for the reaction with 42 % yield. Higher ratio of DMSO in water as solvent resulted in no product formation might be due to denaturation of enzyme. Also, the partial miscibility of substrates in some solvents resulted in poor yields (**Figure 10**).

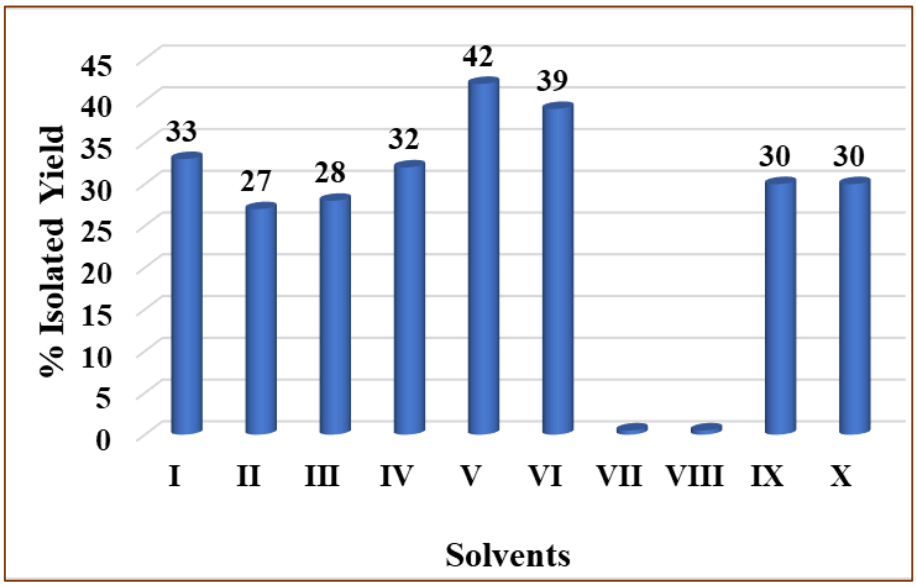


Figure 10: Optimisation of solvent for the reaction[a]

I- DMF, II-Toluene, III-THF, IV-1,4- Dioxane, **V-10% DMSO in H2O (v/v)**, VI-20% DMSO in H2O (v/v), VII-30% DMSO in H2O (v/v), VIII-40% DMSO in H2O (v/v), IX-EtOH, X-MeOH

[a]**Reaction Conditions**: Benzoyl hydrazine (**1f**) (1 eq., 0.734 mmol), Chalcone (**2c**) (1.0 eq., 7.211 mmol), α-amylase from *Aspergillus oryzae* (15 mg), 50°C, solvent (4 ml), 24 h.

After having the optimised biocatalyst and solvent in hand, we further stepped towards temperature optimisation. The reaction yield was examined at different temperatures as depicted in **(Figure 11)**. At r.t there was no product formation. On increasing the temperature upto 50°C, there was increment in the reaction yield. We noticed that at 60°C, yield slightly decreased. Further increase in temperature significantly decreased the product yield.

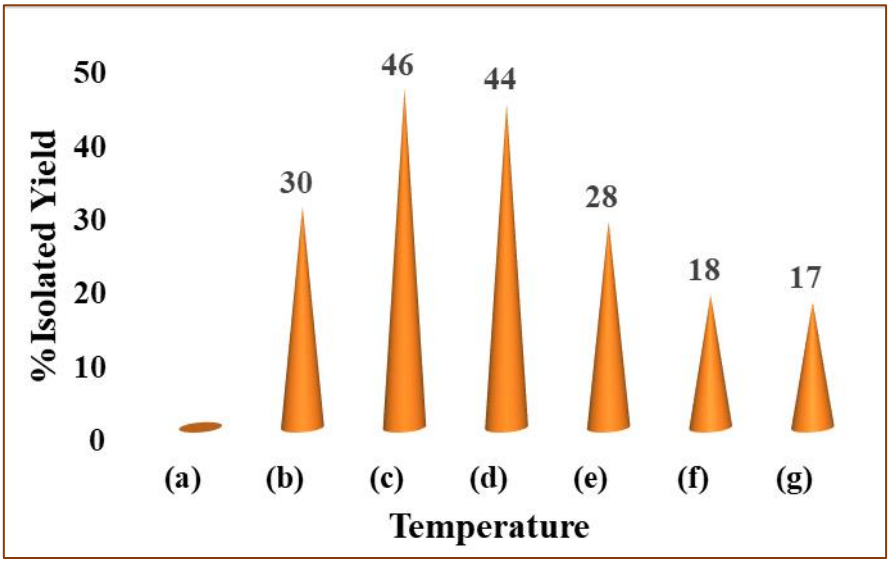


Figure 11: Optimisation of temperature for the reaction[a]

(a)30ºC, (b) 40ºC, **(c) 50ºC**, (d) 60ºC, (e) 70ºC, (f) 80ºC, (g) 90ºC

[a]**Reaction Conditions**: Benzoyl hydrazine (**1f**) (1 eq., 0.734 mmol), Chalcone (**2c**) (1.0 eq., 7.211 mmol), α-amylase from *Aspergillus oryzae* (15mg), 4 ml of 10% DMSO in H_2O (v/v), 24 h

All the previous optimisations encouraged us to further optimise the molar ratio of the reactants. The reaction was examined using different molar ratios of reactants benzoyl hydrazine (**1f**) and chalcone (**2c**) and to our delight 1:1 molar ratio of **1f**:**2c** gave maximum yield. Increasing the moles of **2c** decreased the yield of product due to solubility issue in the solvent **(Figure 12)**.

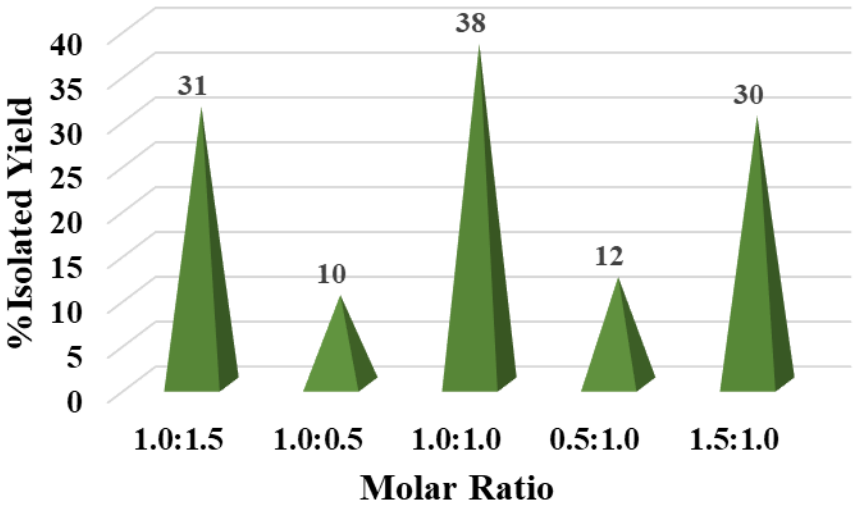
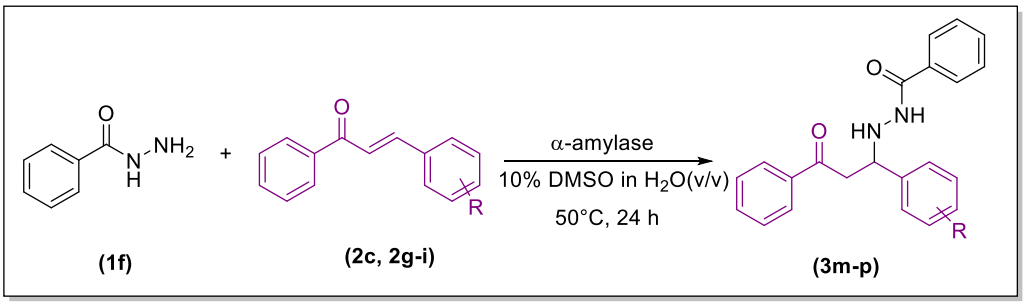


Figure 12: Optimisation of molar ratio of substates for the reaction[a]

[a]**Reaction Conditions**: Benzoyl hydrazine (**1f**):Chalcone (**2c**) , α-amylase from *Aspergillus oryzae* (15 mg), 4 ml of 10% DMSO in H_2O (v/v), 50°C, 24 h.

2.3 Substrate scope of reaction:

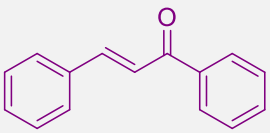
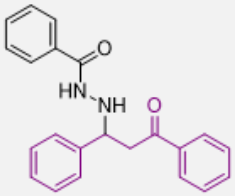
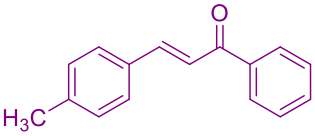
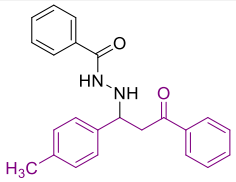
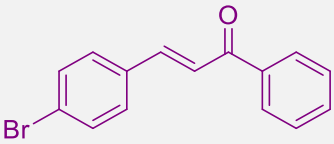
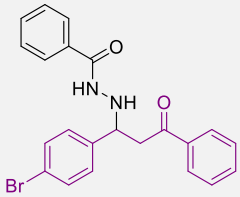
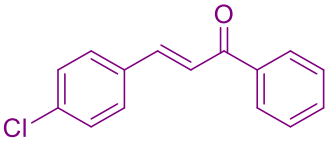
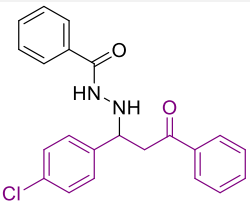
After having the optimum reaction parameters, we assessed the formation of Michael adducts with different chalcone derivatives and results were listed in **Table 7**. The reaction proceeded well with different substituted chalcones to convey the product in decent yields. The reaction of unsubstituted chalcone gave product in remarkable yield. We observed no significant effect of electron donating (-CH_3) and electron withdrawing (-Br, Cl) substituents on the reaction yield.



Scheme 18: α- amylase catalyzed reaction of benzoyl hydrazine and chalcones[a]

[a]**Reaction conditions**: α-amylase (15 mg), Benzoyl hydrazine (0.734 m moles, 1.0 eq.), Chalcone (7.211 m moles, 1.0 eq.), 50°C, 4 ml of 10% DMSO in H_2O (v/v), 24 h

Table 7: Substrate scope of the α- amylase catalyzed aza-Michael addition reaction of benzoyl hydrazine and chalcones

Entry	Michael Donor	Michael Acceptor	Product (% isolated yield)
1	**(1f)**	 **(2c)**	 **(3m)** (46)
2	**(1f)**	 **(2g)**	 **(3n)** (42)
3	**(1f)**	 **(2h)**	 **(3o)** (41)
4	**(1f)**	 **(2i)**	 **(3p)** (44)

CHAPTER-3

MATERIALS AND METHODS

3.1. General material

The chemicals and solvents were procured from readily accessible sources and utilised without undergoing any purification process. NMR spectra were acquired by employing TMS as an internal reference in deuterated solvents. The measurements were conducted on Jeol spectrometers operating at frequencies of 400 MHz for proton ^{1}H NMR and 100 MHz for ^{13}C NMR, respectively. The chemical shifts, denoted as δ, are typically expressed in parts per million (ppm), while the coupling constant, represented as *J*, is commonly reported in hertz (Hz). The peaks are variably denoted as singlet (s), doublet (d), triplet (t), doublet of doublet (dd) multiplet (m), and broad singlet (brs). Silica gel plates were employed for the purpose of conducting TLC in order to assess the degree of reaction completion. The process of column chromatography was employed to purify the product, utilising silica gel particles with a size range of 60-120 microns. The synthesised compounds were characterised utilising ^{1}H NMR, ^{13}C NMR, and HRMS techniques.

3.2. General methods

3.2.1 Procedure for preparation of Chalcones:

The chalcone was synthesized using aldol condensation reaction using aldehyde and ketone [29]. In this synthesis, acetophenone (1 eq., 8.32 mmol) was added in a round bottom flask containing 10 ml ethanol and made to react with ethanolic solution of benzaldehyde (1.0 eq., 8.32 mmol) with aq. KOH (3.0 eq., 24.9 mmol) as catalyst. The reaction mixture was stirred initially at 0°C and then at r.t. The solution started precipitating indicating the formation of chalcone. The degree of completion of reaction was checked by TLC. After the complete consumption of the starting materials, the reaction mixture was filtered and washed with cold water three times. The precipitates were dried and recrystallised in ethanol to furnish the chalcone product.

3.2.2 Procedure for aza-Michael addition reaction:

The synthesised chalcone (1 eq.,0.721 mmol) was reacted with benzoyl hydrazine (1.0 eq., 0.734 mmol) in a sealed reaction tube in the presence of α-amylase (15 mg) as catalyst and 4 ml of 10% DMSO in H_2O (v/v) as reaction medium at 50°C for 24 h. The course of reaction

was monitored by TLC. The crude mixture was subjected to separatory funnel for work up using ethyl acetate and water. Afterwards, the desired compound was isolated and purified using column chromatography and formation of 1,4-aza-Michael adduct was confirmed by NMR spectroscopy.

3.3 NMR Data of compounds

(*Z*)-*N*'-(but-3-en-2-ylidene)-4-methylbenzenesulfonohydrazide (3a):

^{1}H NMR (400 MHz, $CdCl_3$) δ 7.85 (d, *J* = 8 Hz, 2H), 7.62 (brs, 1H), 7.31 (d, *J* = 12 Hz, 2H), 6.44(q, *J* = 12 Hz, 1H), 5.53(d, *J* = 16 Hz, 1H), 5.42 (d, *J* = 12 Hz, 1H), 2.41 (s, 3H), 1.84 (s, 3H) ppm.

4-methyl-*N*'-((1*Z*,2*E*)-3-phenylallylidene)benzenesulfonohydrazide (3b):

^{1}H NMR (400 MHz, $CdCl_3$) δ 8.26 (s, 1H), 7.85 (d, *J* = 8.2 Hz, 2H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.38 (dd, *J* = 8.2, 1.8 Hz, 2H), 7.34 – 7.27 (m, 6H), 6.79 (d, *J* = 5.8 Hz, 1H), 2.40 (s, 3H) ppm.

***N*'-((1*E*,2*E*)-1,3-diphenylallylidene)-4-methylbenzenesulfonohydrazide (3c):**

^{1}H NMR (400 MHz, $CdCl_3$): δ 7.81(d, *J* = 8 Hz, 2H),7.53-7.51(m, 2H), 7.48(s, 1H), 7.41-7.35 (m, 2H), 7.30 (t, *J* = 8 Hz, 3H), 7.28 - 7.24 (m, 2H), 7.10 - 7.03 (m, 3H), 6.27 (d, *J* = 16 Hz, 1H), 2.43 (s, 3H) ppm.

methyl 3-(2-benzoylhydrazinyl)propanoate (3f):

^{1}H NMR (400 MHz, $CdCl_3$): δ 7.78 (d, *J* = 8 Hz, 2H), 7.36 (d, *J* = 8 Hz, 2H), 3.62 (s, 3H), 3.39 (t, *J* = 8 Hz, 2H), 2.72 (t, *J* = 8 Hz, 2H), 2.44 (s, 3H) ppm.

(*Z*)-*N*'-(but-3-en-2-ylidene)-4-methylbenzenesulfonohydrazide (3g):

^{1}H NMR (400 MHz, $CdCl_3$): δ7.77(d, *J* = 8 Hz, 2H),7.36(d, *J* = 8 Hz, 2H), 3.33(t, *J* = 8 Hz, 2H), 2.90 (t, *J* = 8 Hz, 2H), 2.41 (s, 3H), 2.13 (s, 3H) ppm

***N*'-((1*Z*,2*E*)-3-phenylallylidene)benzohydrazide (3l):**

^{1}H NMR (400 MHz, $CdCl_3$): δ 11.73 (s, 1H), 8.22 (d, *J* = 8 Hz, 2H),7.88 (d, *J* = 8 Hz, 2H), 7.60 (d, *J* = 8 Hz, 2H), 7.55 (d, *J* = 8 Hz, 1H), 7.48 (t, *J* = 8 Hz, 2H), 7.35 (t, *J* = 8 Hz, 2H), 7.29 (t, *J* = 8 Hz, 1H), 7.04(d, *J* = 4 Hz, 2H) ppm. HRMS $(ESI)^+$ calculated for $C_{16}H_{15}N_2O$ $[M+H]^+$: 251.1184; found: 251.202.

(*E*)-chalcone (2c):

^{1}H NMR (400 MHz, $CdCl_3$) δ 8.03 (d, *J* = 8 Hz, 2H), 7.83 (d, *J* = 16 Hz, 1H), 7.64 (d, *J* = 8 Hz, 2H), 7.60 - 7.57(m, 1H), 7.55 (s, 1H), 7.52 (d, *J* = 4 Hz, 1H), 7.50 (d, *J* = 8 Hz, 1H), 7.41 (t, *J* = 4 Hz, 3H) ppm.

(*E*)-1-phenyl-3-(p-tolyl)prop-2-en-1-one (2g):

^{1}H NMR (400 MHz, DMSO) δ 8.12 (d, *J* = 8 Hz, 2H), 7.87 (d, *J* = 16 Hz, 1H), 7.75 (d, *J* = 8 Hz, 2H), 7.71 (d, *J* = 16 Hz, 1H), 7.62 (t, *J* = 8 Hz, 1H), 7.53 (t, *J* = 8 Hz, 2H), 7.24 (d, *J* = 8 Hz, 2H), 2.31 (s, 3H) ppm.

(*E*)-3-(4-bromophenyl)-1-phenylprop-2-en-1-one (2h):

^{1}H NMR (400 MHz, DMSO) δ 8.13 (d, *J* = 8 Hz, 2H), 7.97 (d, *J* = 8 Hz, 1H), 7.83 (d, *J* = 8 Hz, 2H), 7.70 (d, *J* = 20 Hz, 1H), 7.64 - 7.60 (m, 3H), 7.55 (t, *J* = 8 Hz, 2H) ppm.

(*E*)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one (2i):

^{1}H NMR (400 MHz, DMSO) δ 8.13 (d, *J* = 4 Hz, 2H), 7.91 (t, *J* = 8 Hz, 3H), 7.72 (d, *J* = 16 Hz, 1H), 7.64 (t, *J* = 8 Hz, 1H), 7.54 (t, *J* = 8 Hz, 2H), 7.50 (d, *J* = 8 Hz, 2H) ppm.

***N*'-(3-oxo-1,3-diphenylpropyl)benzohydrazide (3m):**

^{1}H NMR (400 MHz, DMSO) δ 9.93 (d, *J* = 8 Hz, 1H), 7.90 (d, *J* = 8 Hz, 2H), 7.65 (d, *J* = 8Hz, 2H), 7.57 (t, *J* = 8 Hz, 1H), 7.47 (d, *J* = 8 Hz, 2H), 7.44 – 7.41 (m, 2H), 7.39 – 7.34 (m, 3H), 7.26 (t, *J* = 8 Hz, 2H), 7.20 (d, *J* = 8 Hz, 1H), 5.53 (t, *J* = 4 Hz, 1H), 4.65 (q, *J* = 8 Hz, 8 Hz, 1H), 3.59 (dd, *J* = 8 Hz, 1H), 3.43 (dd, *J* = 8 Hz, 1H) ppm. ^{13}C NMR δ 198.64, 166.08, 141.99, 137.24, 133.75, 133.61, 131.76, 129.23, 128.76, 128.63, 128.55, 128.40, 127.63, 60.70, 44.07 ppm.

***N*'-(3-oxo-3-phenyl-1-(p-tolyl)propyl)benzohydrazide (3n):**

^{1}H NMR (400 MHz, DMSO) δ 9.92 (d, *J* = 8 Hz, 1H), 7.88 (d, *J* = 8 Hz, 2H), 7.65 (d, *J* = 4 Hz, 2H), 7.56 (t, *J* = 8Hz, 1H), 7.46-7.42 (m, 3H), 7.35 (t, *J* = 8 Hz, 2H), 7.27 (d, *J* = 8 Hz, 2H), 7.06 (d, *J* = 8 Hz, 2H), 5.45 (t, *J* = 8 Hz, 1H), 4.59 (q, *J* = 8 Hz, 8 Hz, 1H), 3.55 (dd, *J* = 4 Hz, 1H), 3.40 (dd, *J* = 8 Hz, 1H), 2.14 (s, 3H) ppm. ^{13}C NMR δ 198.40 166.16 141.50 137.11 133.83 133.53 131.80 131.43 130.75 129.23 128.78 128.54 127.61 120.88 60.00 43.78 ppm.

***N*'-(1-(4-bromophenyl)-3-oxo-3-phenylpropyl)benzohydrazide (3o):**

^{1}H NMR (400 MHz, DMSO) δ 9.90 (d, *J* = 8 Hz, 1H), 7.90 (d, *J* = 8 Hz, 2H), 7.64 (d, *J* = 8 Hz, 2H), 7.58 (t, *J* = 8 Hz, 1H), 7.47 – 7.42 (m, 5H), 7.37 – 7.35 (m, 4H), 5.60 (t, *J* = 8 Hz, 1H), 4.62 (q, *J* = 8 Hz, *J* = 4Hz, 1H), 3.59 (dd, *J* = 4Hz, 1H), 3.43 (dd, *J* = 8 Hz, 1H) ppm.

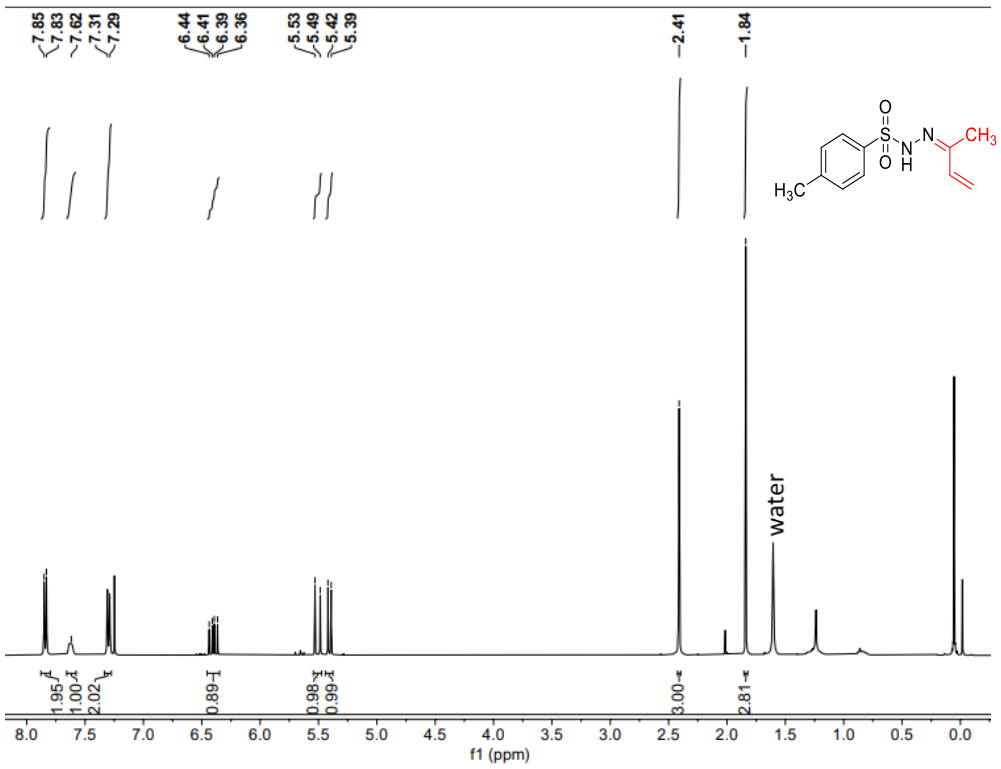
^{13}C NMR δ 198.40 166.16 141.50 137.11 133.83 133.53 131.80 131.43 130.75 129.23 128.78 128.54 127.61 120.08 60.00 43.78 ppm.

***N*'-(1-(4-chlorophenyl)-3-oxo-3-phenylpropyl)benzohydrazide (3p):**

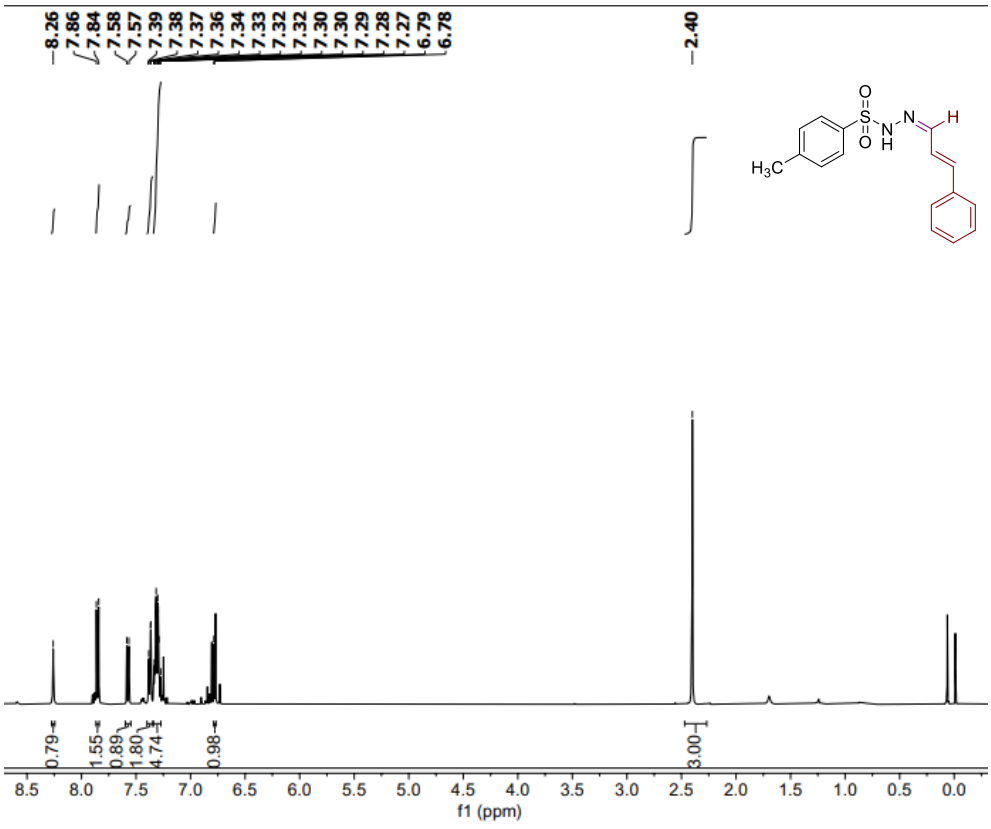
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 8 Hz, 2H), 7.58 (d, *J* = 8 Hz, 2H), 7.53 (d, *J* = 8 Hz, 1H), 7.45 (d, *J* = 8 Hz, 2H), 7.41-7.36 (m, 4H), 7.35 (d, *J* = 8 Hz, 1H), 7.31 (d, *J* = 8 Hz, 2H), 5.81 (brs, 1H), 4.79 (t, *J* = 8 Hz, 1H), 3.49 (dd, *J* = 8 Hz, 1H), 3.38 (dd, *J* = 4 Hz, 1H) ppm.

3.4 NMR spectra of compounds

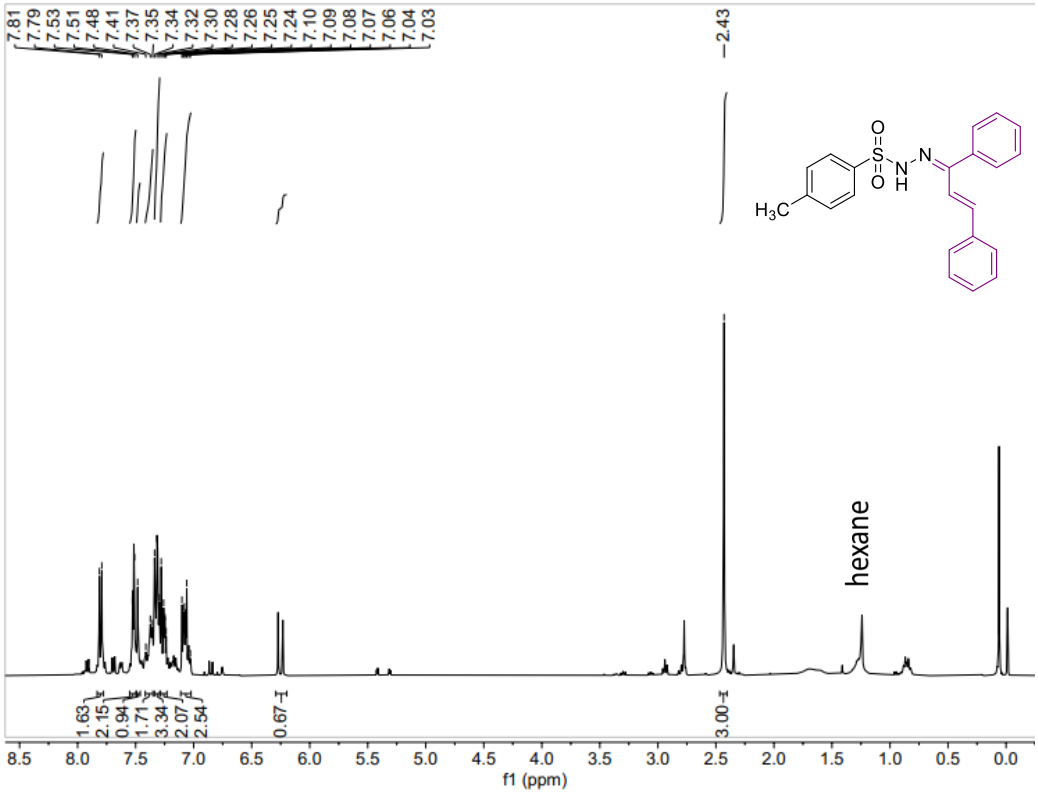
^{1}H NMR of **3a**



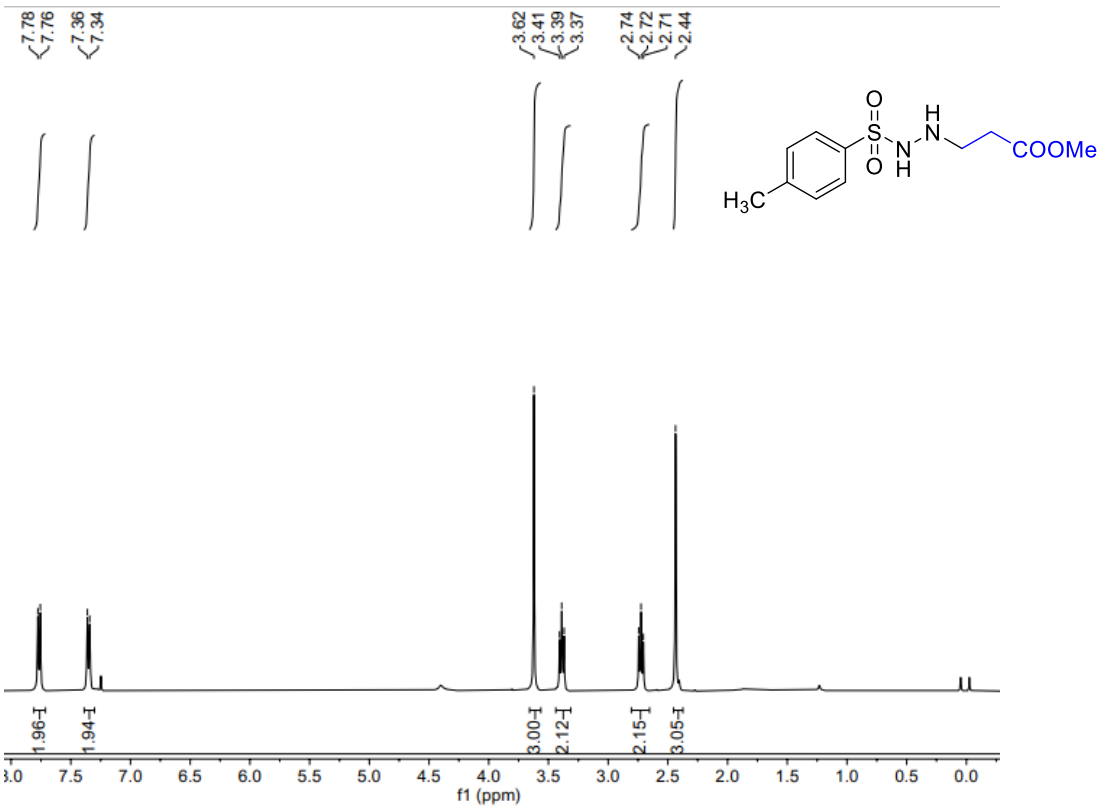
^{1}H NMR of **3b**



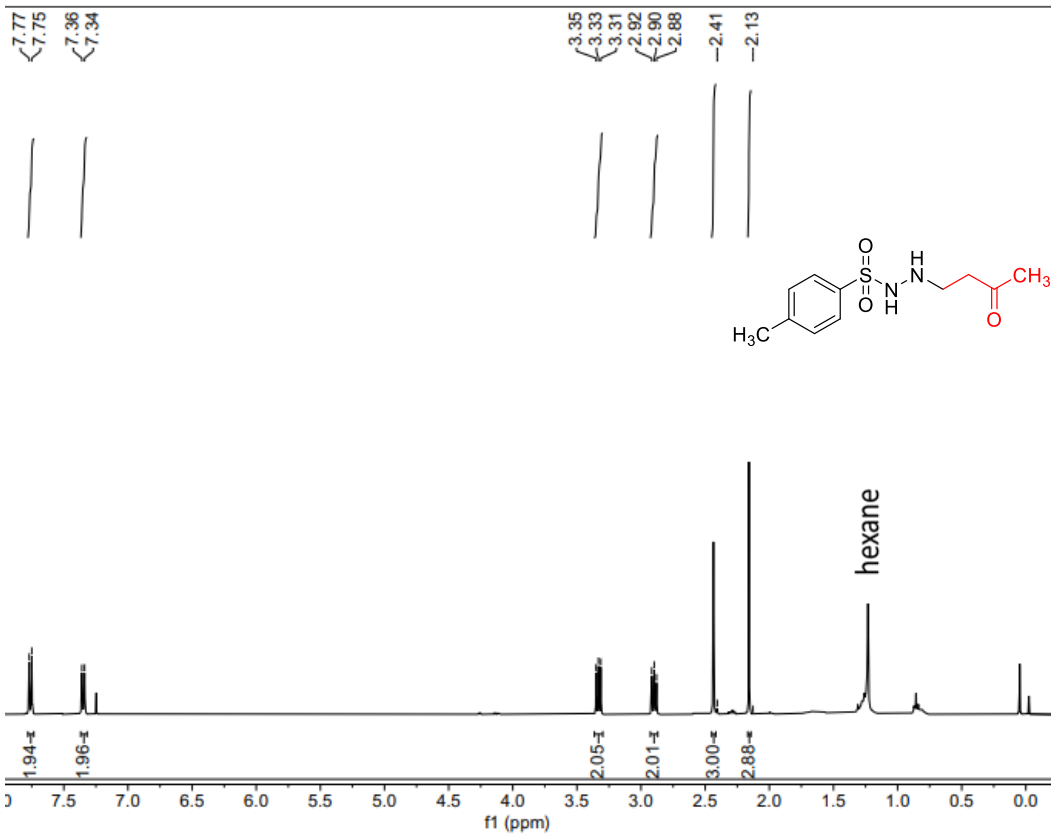
^{1}H NMR of **3c**



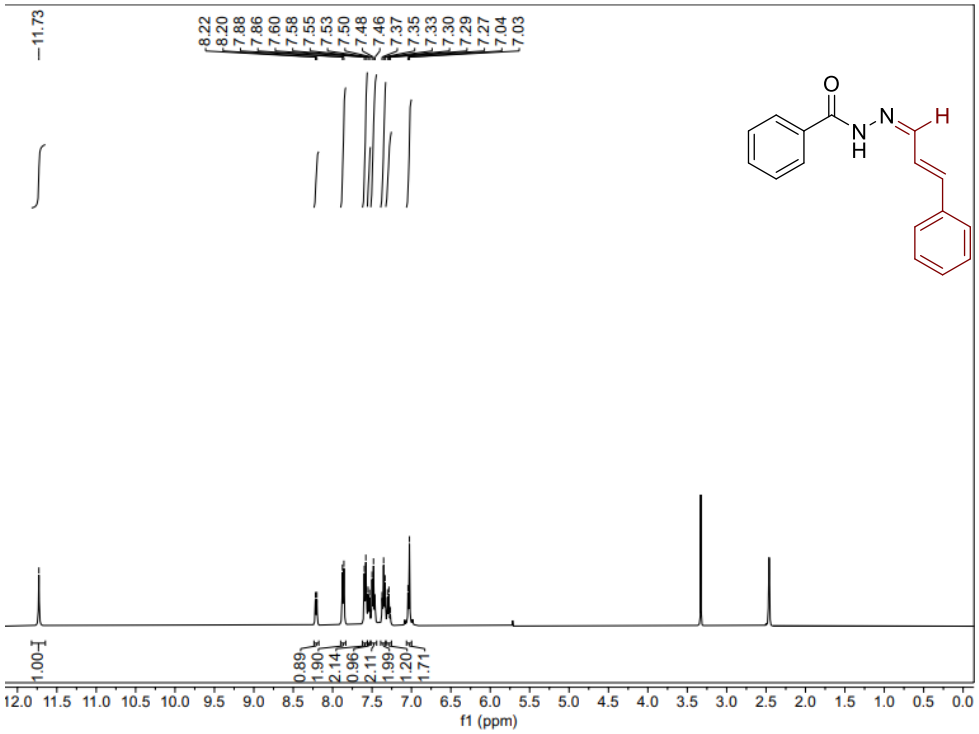
^{1}H NMR of **3f**



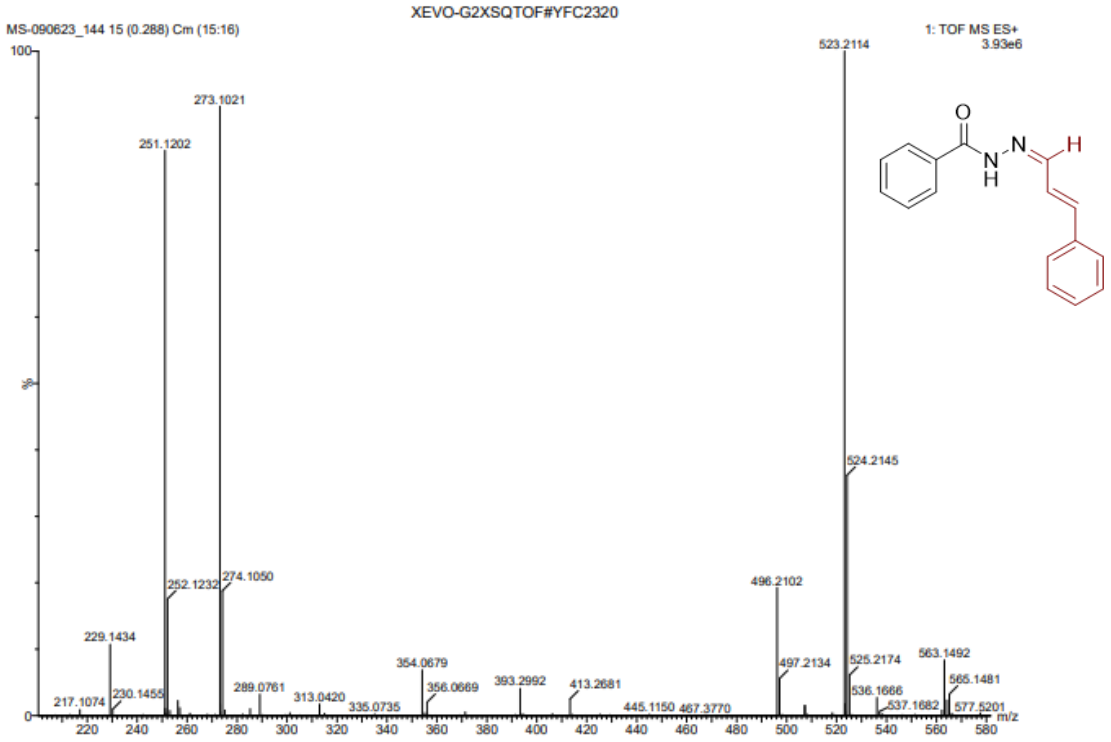
^{1}H NMR of **3g**


7.77
7.75
7.36
7.34
3.35
3.33
3.31
2.92
2.90
2.88
2.41
2.13
O
S
O
N
H
N
H
CH3
O
H3C
hexane
1.94
1.96
2.05
2.01
3.00
2.88
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

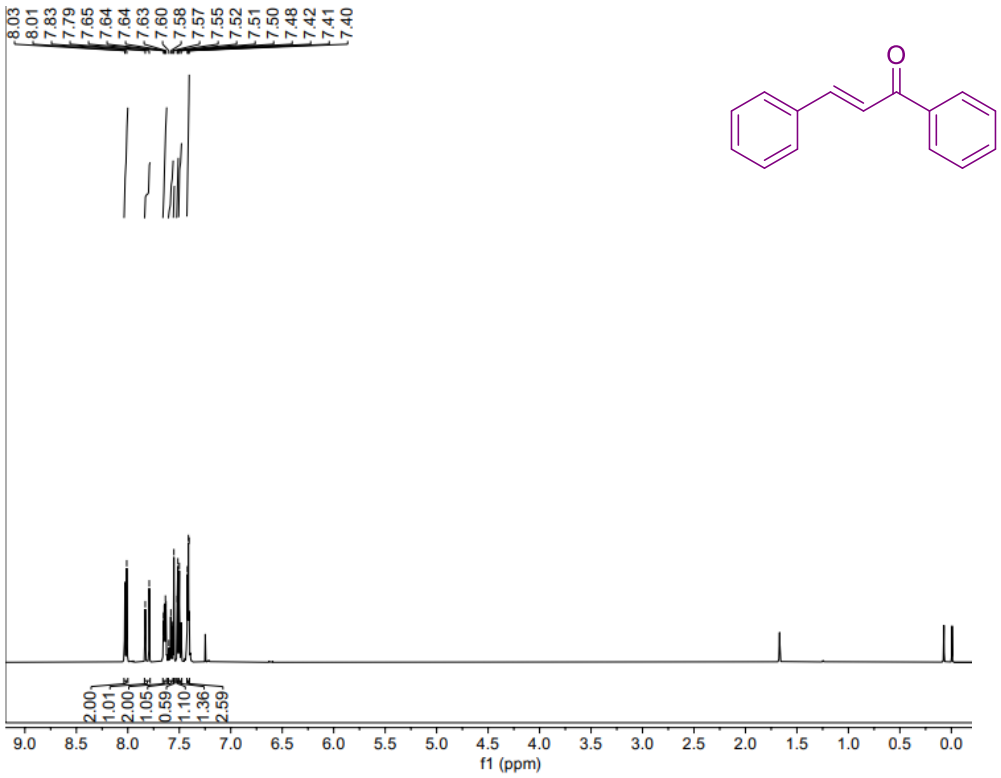
^{1}H NMR of **3l**



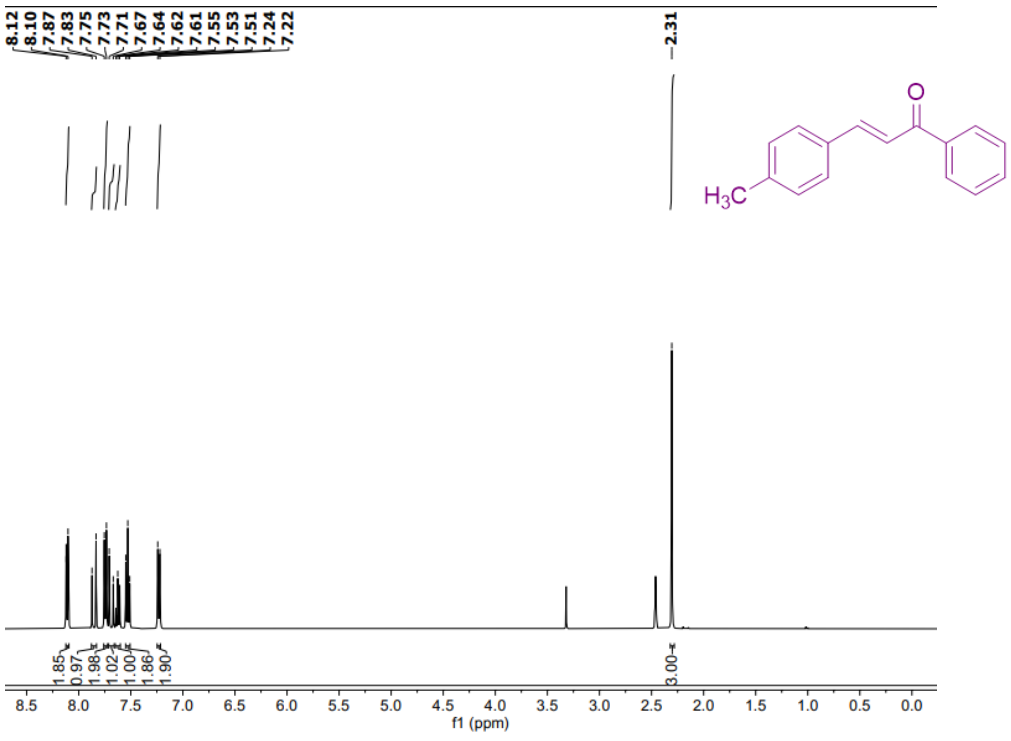
HRMS of **3l**



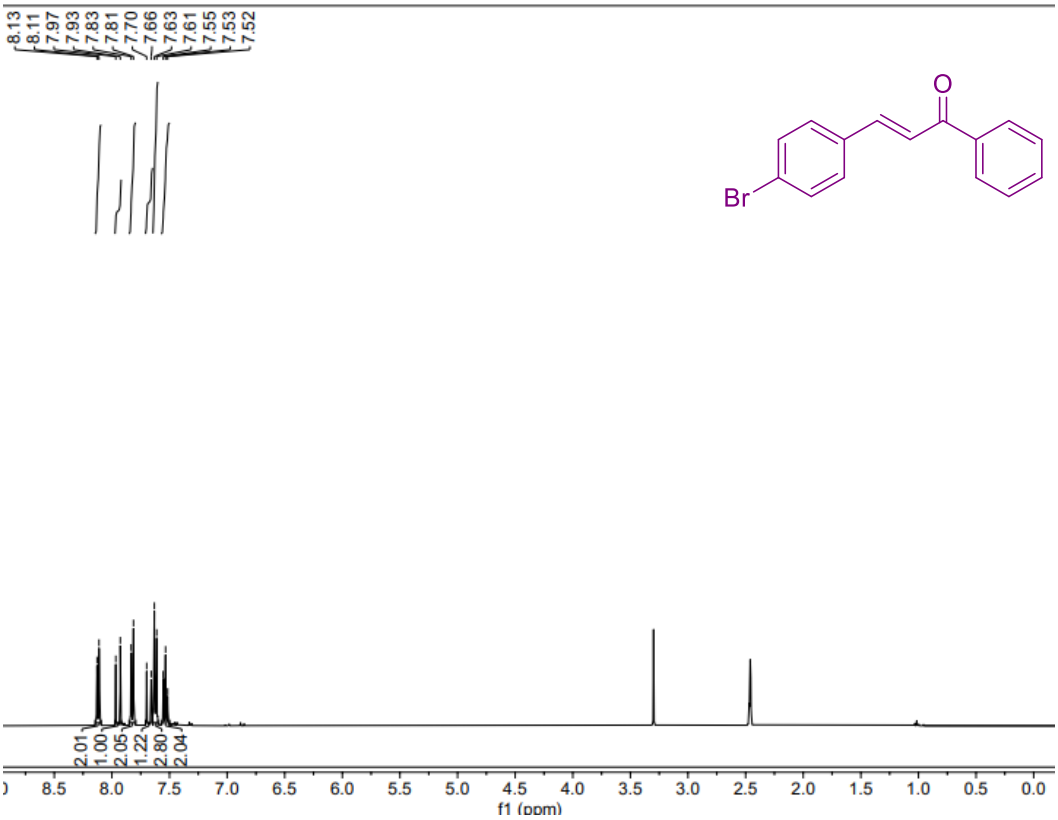
^{1}H NMR of **2c**



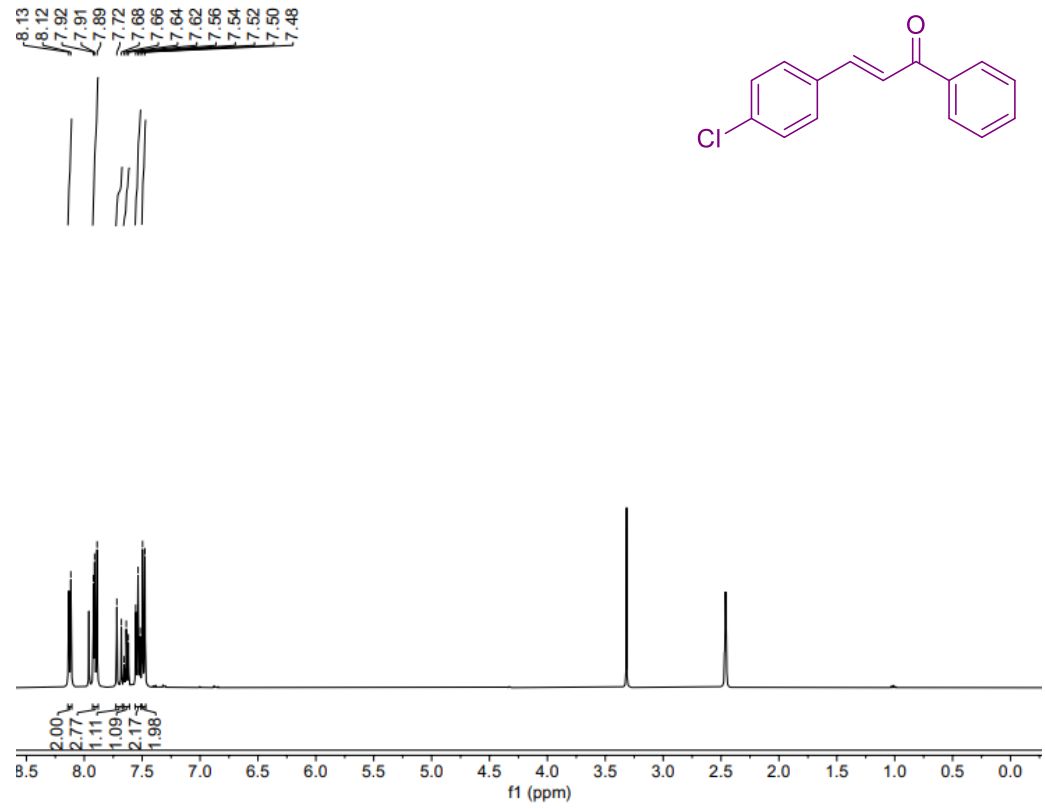
^{1}H NMR of **2g**



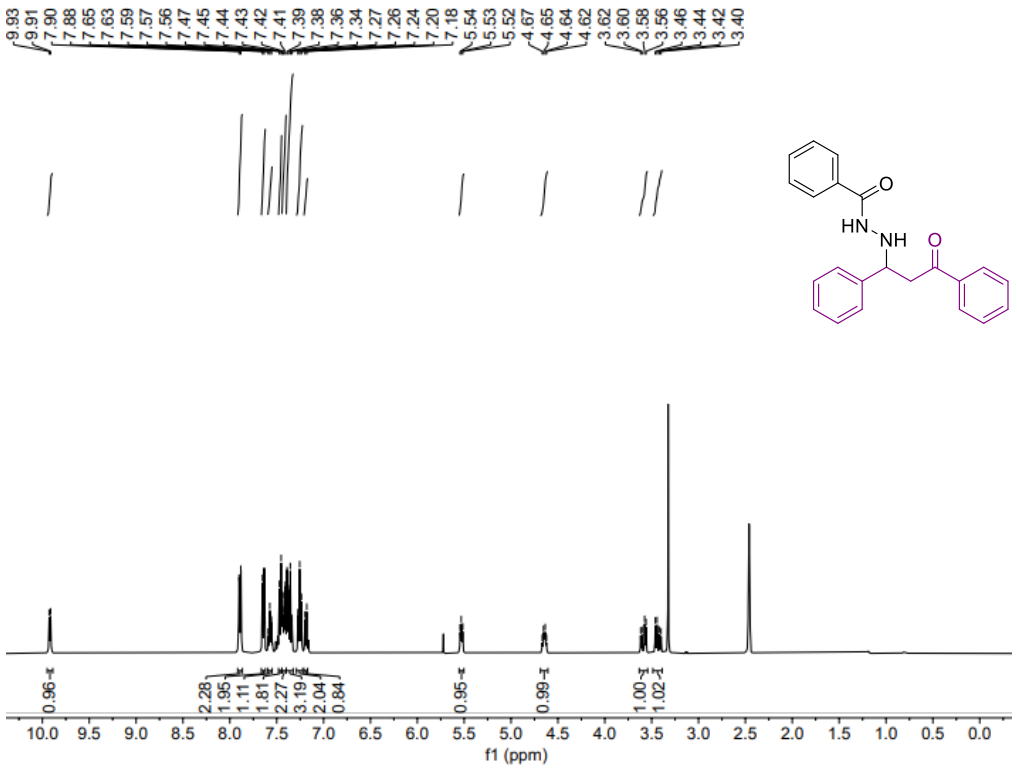
^{1}H NMR of **2h**



^{s1}H NMR of **2i**



^{1}H NMR of **3m**



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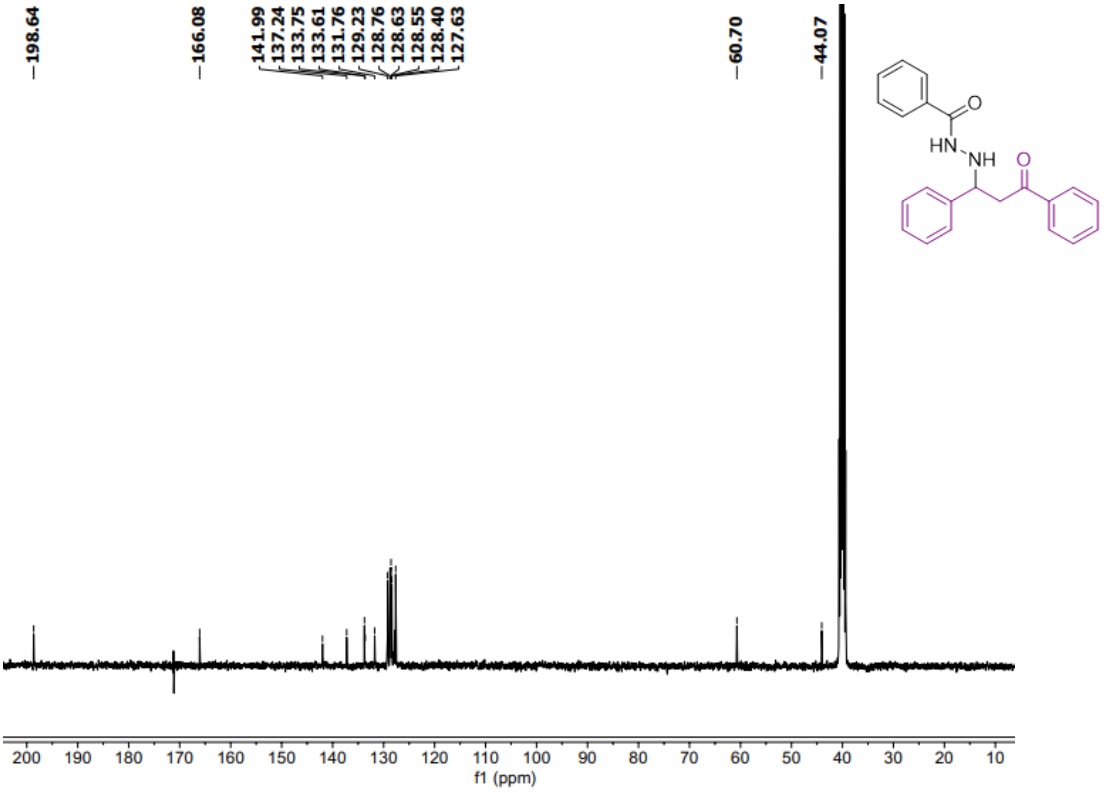

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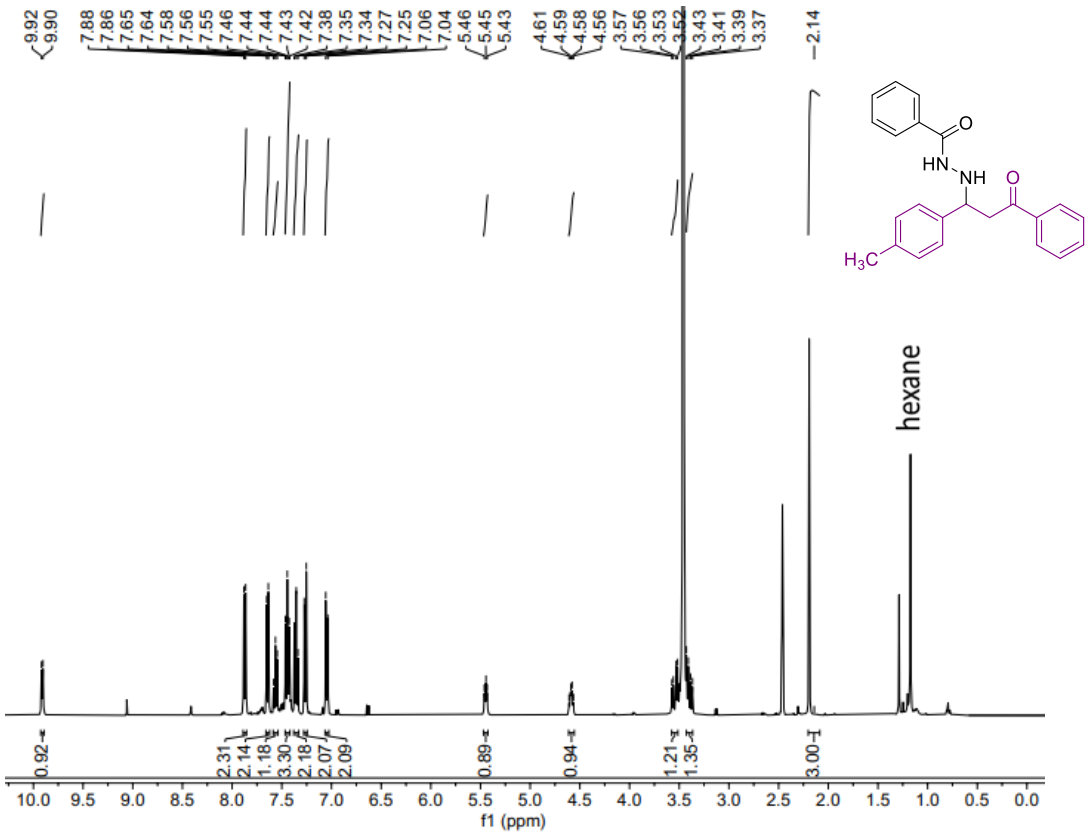

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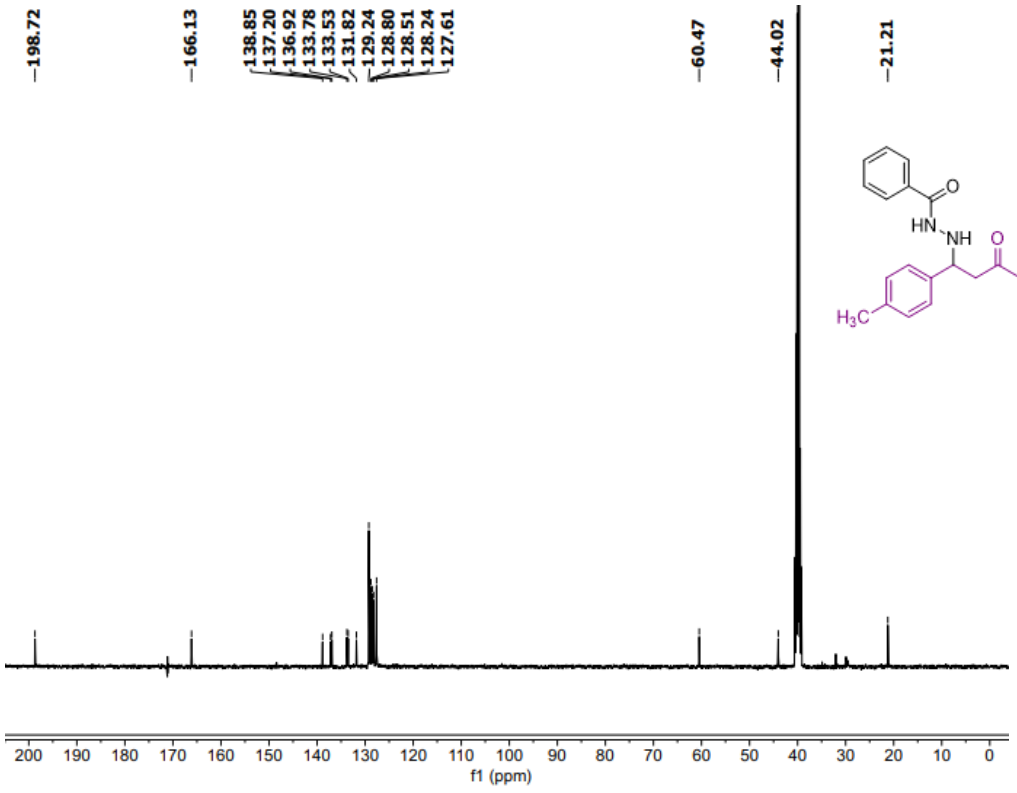

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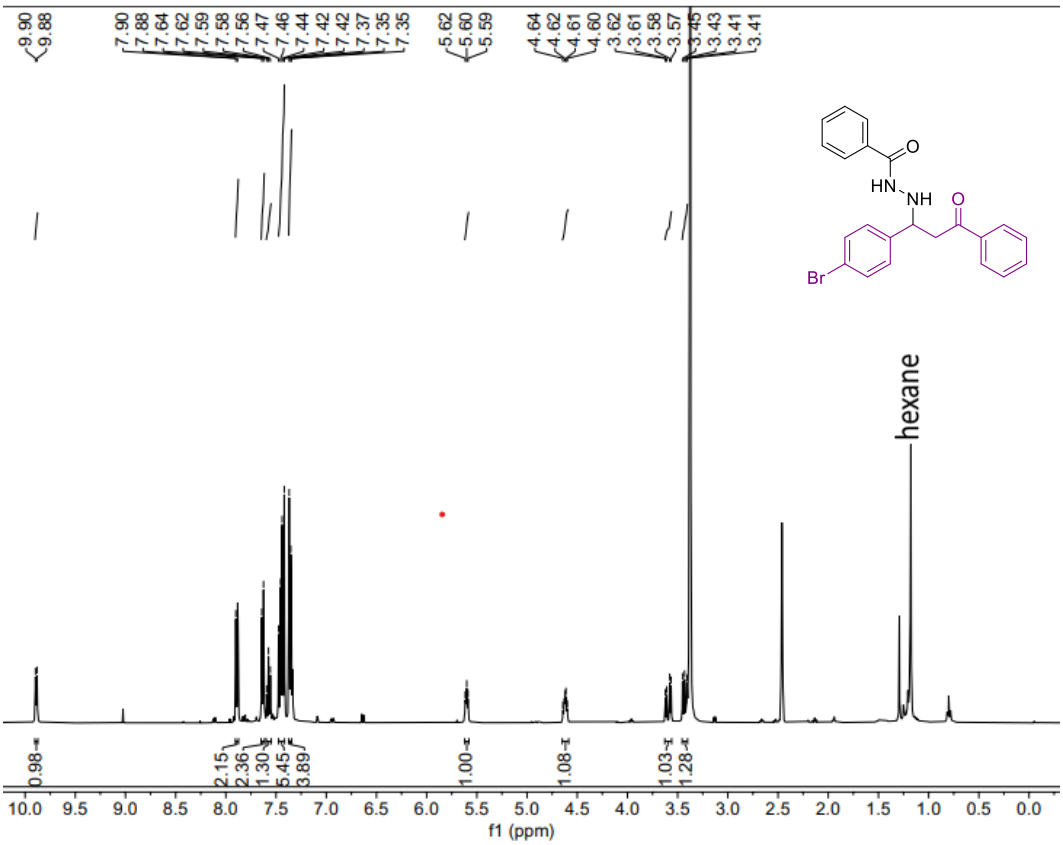

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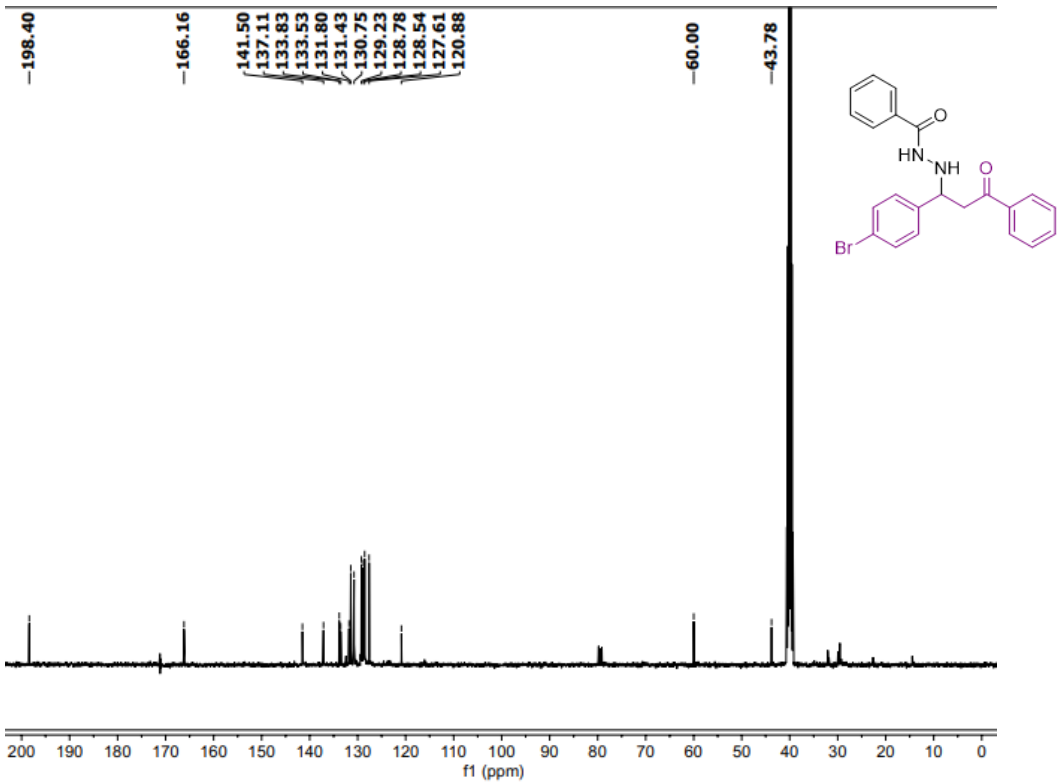

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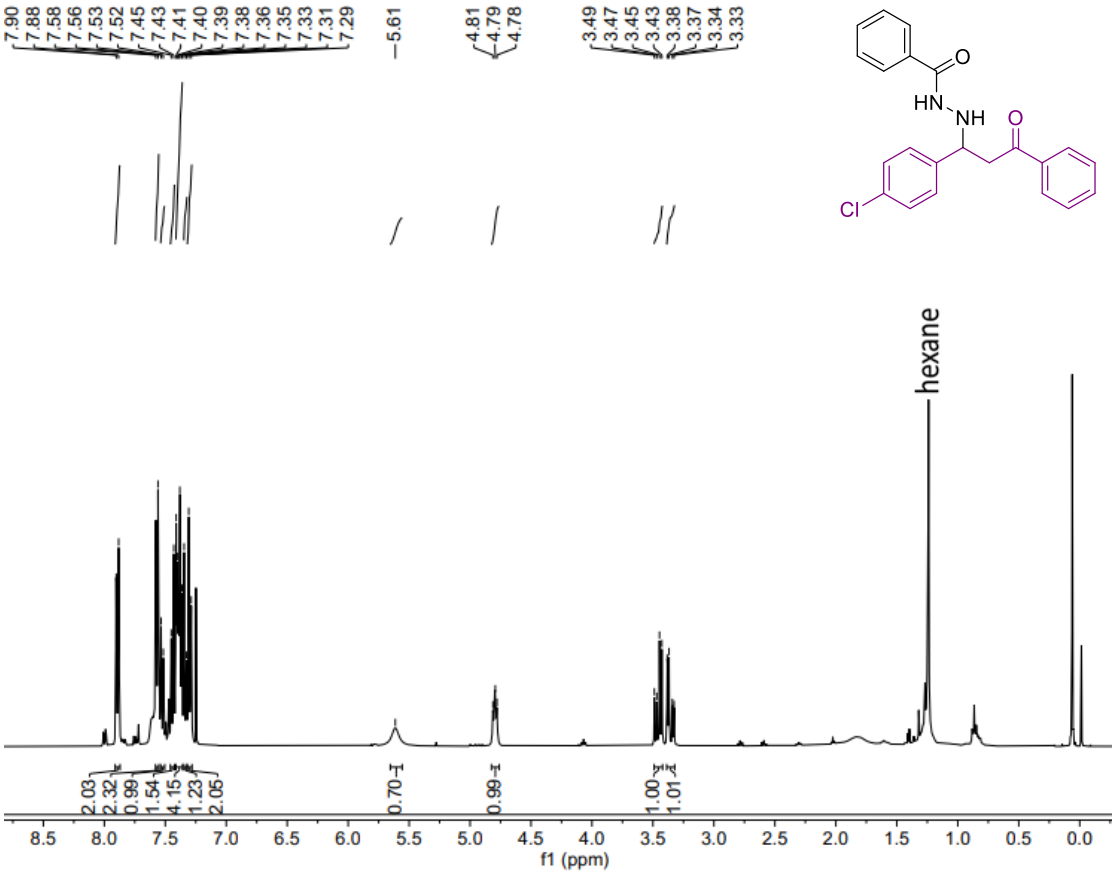
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7.88
7.58
7.56
7.53
7.52
7.45
7.43
7.41
7.40
7.39
7.38
7.36
7.35
7.33
7.31
7.29
5.61
4.81
4.79
4.78
3.49
3.47
3.45
3.43
3.38
3.37
3.34
3.33
O
HN
NH
O
Cl
hexane
2.03
2.32
0.99
1.54
4.15
1.23
2.05
0.70
0.99
1.00
1.01
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

CONCLUSION

In this study, we have demonstrated the first approach of employing α-amylase as a catalyst for the biocatalytic aza-Michael addition reaction of benzoyl hydrazine to chalcones. The reaction was conducted in an aqueous medium, resulting in the synthesis of acyl hydrazine derivatives based on the chalcone skeleton. The synthesized products are biologically valuable. This study broadens the scope of α-amylase utilization for catalysing significant transformations in the field of organic chemistry.

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Development of enzymatic aza-Michael addition reaction A Dissertation Submitted in the partial fulfilment of the Degree Of Master of Science (Chemistry) By Isha Kansal (302102024) Under the supervision of Dr. Vikas Tyagi (Assistant Professor)

School of Chemistry & Biochemistry Thapar Institute of Engineering & Technology Patiala-147004, Punjab, India July-2023

CERTIFICATE This is to certify that the thesis entitled "Development of enzymatic aza-Michael addition reaction", submitted by Ms. Isha Kansal (Roll no. 302102024) in the partial fulfilment of the requirement for the degree of Masters of Science in Chemistry from Thapar Institute of Engineering and Technology, Patiala is an authentic piece of work carried out under the supervision of Dr. Vikas Tyagi, Assistant Professor, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala and no part of project has been submitted for award of any other degree in this or any other university or institute of learning.

Supervisor Dr. Vikas Tyagi Assistant Professor School of Chemistry & Biochemistry Thapar Institute of Engineering & Technology, Patiala

DECLARATION The work embodied in the project entitled "Development of enzymatic aza-Michael addition reaction" has been done by me in the partial fulfilment of the requirement for the award of degree of Masters of Science in Chemistry and submitted at the School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala is an authentic record of my own research work carried out under the supervision of Dr. Vikas Tyagi, Assistant Professor, School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala. All the ideas and references has been acknowledged to best of my knowledge.

Date: Isha Kansal Place:

This is to certify that the above statement made by student concerned is correct and true to the best of my knowledge.

Supervisor Dr. Vikas Tyagi Assistant Professor School of Chemistry & Biochemistry Thapar Institute of Engineering & Technology, Patiala