

**Synthesis and Characterization of
bioactive triazine-chalcone hybrids**

Lovely Professional University

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PROJECT REPORT

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DECLARATION

I hereby affirm that the dissertation entitled "Synthesis and Characterization of bioactive triazine-chalcone hybrids" submitted for the award of Master of Science in Chemistry and submitted to the Lovely Professional University is the original and authentic study that I have carried out from August 2017 to November 2017 under the supervision of Dr. Harpreet Kaur. It does not contain any unauthorized works of other scholars, those referred are properly cited.

Name and Signature of the Student

Abhinav Sharma

Reg. No.: 11605060

CERTIFICATE

This is to certify that Abhinav Sharma has completed the dissertation report entitled, “Synthesis and Characterization of bioactive triazine-chalcone hybrids” under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study.

Date: 30 November, 2017

Dr. Harpreet Kaur

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Regards:

Abhinav Sharma

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M.Sc.Chemistry Hons.

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Introduction

Chalcones are the core of various biological compounds and is aromatic ketone and enone form collectively known as chalconoid or chalcones. Chalcones are the compounds where aromatic substituents are introduced in to the terminal position of the system $C = C-C=O$. So Chalcones are characterized by their position of $Ar(A) -CO-CH=CH-Ar(B)$ this type of structure in which two aromatic rings A and B are linked by an aliphatic three carbon chain. The main member of the chalcone family is Benzylideneacetophenone can be prepared by reacting various benzaldehydes and acetophenone in the basic medium under cold conditions. Chalcone and their derivative shows various important biological activities such as **antimicrobial, antibacterial, antiviral, anti-cancer** and etc. The name chalcone is given by Kostanecki and Tambor [1]. There are different methods available for the synthesis of chalcones such as Aldol condensation, Claisen-Schmidt Condensation and these methods are used to synthesis various derivatives of heterocyclic rings like pyrazolines, pyrimidines and isoxazoles. Chalcones can also use as artificial sweeteners as 3,2,4,6-tetrahydroxy-4-propoxy-dihydrochalcone-4- β' -neohesperdoside is 2200 times sweeter than the normal sugar [2]. In the chalcones presence of alpha and beta carbonyl system make the chalcones bioactive in nature [3]. s-Triazine based chalcones having three N-atom in the ring has been found in the many physiological agents [4] showing good therapeutic properties [5]. Chalcones are used to synthesize several derivatives like cyanopyridines, pyrazolines, isoxazoles and pyrimidines having different heterocyclic ring systems.

Chalcones has good resemblance with the flavonols, flavanones, and dihydroflavonols. They have good application as photosensitive material, stabilizer and fluorescent brightening agent as well as organic brightening agent. Keto ethylenic group present in the chalcones is has reactivity towards the reagent e.g. phenyl hydrazine hence are useful intermediate in the synthesis of the flavones, benzalcoumarones etc.

The triazine structure is a heterocyclic ring three carbon atom are replaced by the nitrogen atom in benzene ring. There are three isomers of the s-triazine on the basis of the position of the three nitrogen atom in the benzene ring and are referred to as 1,2,4-triazine, 1,2,3-triazine

and 1,3,5-triazine. These are endowed in the wide range of the **anti-inflammatory, anticancer** and in various other pharmacological activities. Also it has been reported that the derivative of the triazine i.e. 2,4,6-trisubstituted-1,3,5-triazine scaffolds has capability to inhibit the activity of the *Mycobacterium tuberculosis* (Mtb).

Molecular hybridization is a new concept in the drug design and the development based on the combination of the different pharmacophoric moieties of the bioactive substances to produce a new compound with improved efficacy and affinity. The molecular hybridization (MH) is a strategy of rational design of new ligands or prototypes based on the recognition of pharmacophoric sub-unities in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-unities, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates.

Molecular docking has become an important common component of the drug discovery toolbox, and its relative low-cost implications and perceived simplicity of use has stimulated an ever-increasing popularity within academic communities. Molecular Docking is defined as a study of how molecules or molecular structures like drug, enzyme, protein fit together. Or we can say that it is a molecular modelling technique that is used to detect how a protein or enzyme interacts with the small molecules.

Literature Review

From my literature review I have found that Chalcones and its heterocyclic analogues possess the number of biological properties like anticancer, antioxidant, antimicrobial and etc.[6].

Chalcones belongs to the flavonoid family. Biological Screening and synthesis of pyrrole and pyridine derivatives of Pyrozolo (3-4c) Pyrozoles is done by reaction of hydrazine hydrate with Chalcones of pyrazolone and phenyl hydrazine in the presence of NaOH/EtOH. With the cyclisation reaction of hydrate hydrazine and ethyl acetoacetate Compound 1 is prepared that is 5-methyl-2, 4-dihydro-3H-pyrazol-3-one and is used as starting material. The aim of the reaction is to prepare pyrozole that is 1,3a,4,5-tetrahydro-3-methyl-5-phenyl-4-(1H-pyrrol-2-yl)pyrazolo[3,4-c]pyrazole which show good IR value of intense band at 3480 and 3880 cm^{-1} corresponding to the NH_2 group, 2985 cm^{-1} , 1680 cm^{-1} indicate aromatic $=\text{C}-\text{H}$, $\text{C}=\text{O}$ groups and a weak absorption band at 1250 cm^{-1} , corresponding to $\text{C}=\text{S}$ group, confirming the occurrence of ring closure in the form of thioaminopyrazoline ring. These newly synthesised screened for their good antibacterial activity against certain Gram- as well Gram+ bacteria[7].

Synthesis of some new Chalcone is done by using s-Triazine derivatives by reaction of the 2-4-bis-tetrahydro-1, 4-oxazine-6-(4'-acetylphenylamino)-s-triazine with aromatic compounds which on cyclisation reaction with hydrazine hydrate gives pyrozolines in the presence of the acetic acid. Tetra hydro- 1,4-oxazine (0.01 mole, 0.87g in 10 ml acetone) and cynamaric acid is the starting which are reacted to take the product that is 2-4 bis (tetra hydro- 1,4-oxazine) 6- [4'- { 3''-(4'''-methoxyphenyl) -2''-propenon-1''-yl } phenyl amino]-s-triazine which shows IR(KBr in cm^{-1}) 1649 ($-\text{C}=\text{O}$), 1220 (C-O-C, ether), 806 (C-N, s-triazine), 786(=CH). ^1H NMR (CDCl_3) : 3.52 (t, 8H, oxzine ring), δ 3.92 (s, 3H,p-OCH3), δ 6.70 (s, 1H, -NH), δ 7.1 - 7.8 (m, 8H, Ar-H), δ 8.40 (d,1H,-CO-CH=), δ 8.7 (d, 1H,Ar-CH=).It has melting point 145 $^{\circ}\text{C}$ to 152 $^{\circ}\text{C}$ and yield is 75%[8].

Derivatives of triazine containing pharmacorophic group has contain various antiviral activity against Enecphalomyocarditics virus and etc. These derivatives can be prepare by reaction of 2-(4'-chlorophenylamino)-4-(4'-fluorophenylamino)-6-(4'-acetylphenylamino)-s-triazine(5) , different aromatic and heterocyclic aldehydes, which on cyclisation with phenyl hydrazine hydrochloride in the presence of alkali give phenyl pyrazolines like 2-(4'-chlorophenylamino)-4-(4'-fluorophenylamino)-6-[4'3 {3''(substitutedphenyl/2thienyl/2furanyl)-2''-propenon-1''-yl} phenyl amino]-s-triazine. Final product is having yield around 88% and

melting point is around 119⁰C. IR(KBr in cm⁻¹) 3468(-NH₂), 2207 (C=N), 1227 (C-O-C). 1013 (C-F), 803(C-N, s-triazine), 776 (C-Cl). The compound are above formed shows many antibacterial as well antiviral activity which is tested against *S.aureus* (MTCC 96) and *B.subtilis* (MTCC 441) Gram positive data and *E.Coli* (MTCC 443) and *S.paratyphi-B* (MTCC 773).Gram negative bacteria in nutrient agar medium[9].

Pathogen have very good effect on the inhabitants of the tropical and subtropical area. Various semi synthetic and natural chalcone have good capability to inhibit the effects of the pathogen by showing antibacterial, antiviral activities. By Fredel Craft Acylation of the phenol chalcones showing antibacterial, antiviral activities can be directly prepared. By doing FCA 2,4-dimethyl-1,3,5-triolbenzene with cinnamoyl chloride the chalcone name 2,4,6-trihydroxy-3,5-dimethylchalcone can be easily fabricated. These synthetic chalcone have the capability to inhibit the molecular target of the virus as well as of parasites [10]. Derivatives of Phloretin is good antimicrobial agent. The growth of the microorganism is inhibited by the dihydrochalcones. *Staphylococcus aureus*, *Salmonella typhimurium*, *Listeria monocytogenes* and methicillin-resistant *S. aureus* are the various virus the growth of which is inhibited by these Phloretin. The chalcone inhibited energetic metabolism of *S. aureus* by decreasing the enzymatic activity of Lactate dehydrogenase (LDH) and Isocitrate dehydrogenase (IDH) [11].

Neutrophil has important effect in the inflammatory disorders. Different types of chalcones can be prepared by Claisen-Schmidt condensation of aldehydes and acetophenones to anti-inflammatory activity of the compound formed from these reactions can be easily observed. The chalcones obtained by this have good inhibitory effect on the activation of the neutrophils and Mast cells [12].

Consumption of the fruits, cereals, vegetables has been associated with the lower incidence of the chronic diseases and the cancer diseases. But how these dietary agent lowers the diseases is not still understood. Some of the most significant chalcones identified from these plants include flavokawin, butein, xanthoangelol, 4-hydroxyderricin, cardamonin, 2',4'-dihydroxychalcone, isoliquiritigenin, isosalipurposide, and naringenin[13].

Synthesis of various novel series of chalcones that is (2E)-1-[4-(2,4-dithio-3-phenyl-5-substituted-1,3,5-triazino-6-yl)aminophenyl]-3-(3,4-dimethoxyphenyl)prop-2-en-1-one by the isomerisation of series of (2E)-1-[4-(2-phenylimino-4-substitutedimino)amino-1,3,5-dithiazino-6-yl)aminophenyl]-3-(3,4-dimethoxyphenyl)prop-2-en-1-one is done in the presence of the sodium bicarbonate solution in the aqueous solution of ethanol. The method

used for this synthesis is cheaper and time consumptions is also low. The compound formed is cream yellow solid and yield is 72% and melting point calculated is 173^oC. FTIR in KBr (cm⁻¹) 3069.64 (ArC-H stretching), 3349.69 (N-Hstretching), 1687.26 (C=O stretching), 1139.82 (C=Sstretching), 1032.06 (C-O-C stretching) and 1212.66 (C-Nstretching) and this compounds shows good antimicrobial activities.[14]

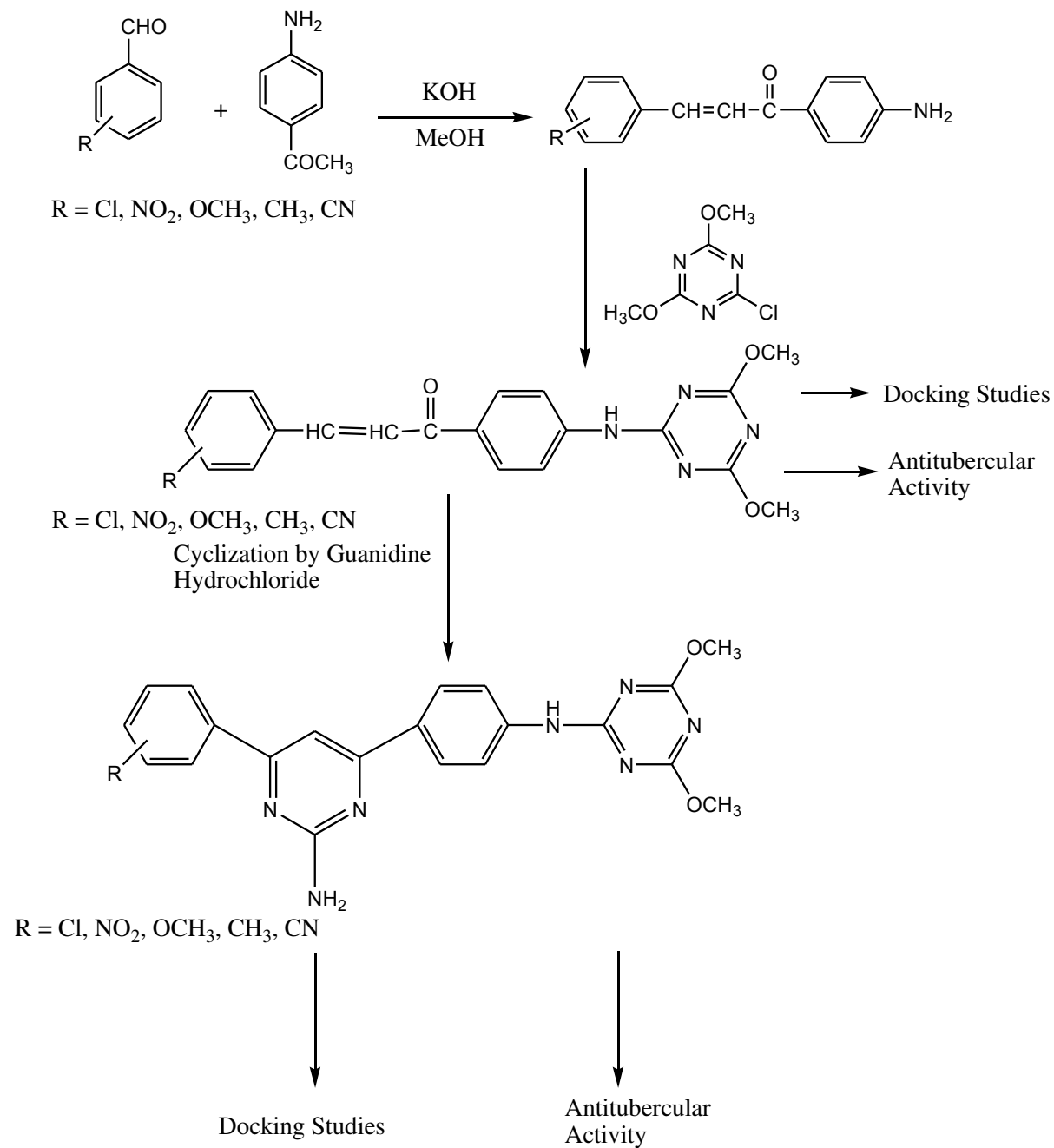
Isoxazoline possess the various application in the medicinal chemistry. Preparation of the 5-phenyl-3-[-2- Phynylvinyl]-4,5dihydro-1H-pyrazoline glycenate(II) is done by pyrazoline in ethanol having melting point -90^oC and characterization is done by IR (KBr cm⁻¹) i.e. The peak at 3059.10 cm⁻¹ indicates towards the presence of -NH₂group, 1647 cm⁻¹ (C=N stretching), 1492cm⁻¹ (C=C Stretching), 1712 cm⁻¹ (C=O stretching), 3026cm-1(Aromatic-CH-Stretching), 2891 cm⁻¹(aliphatic-CH Stretching), 758,802 and 869cm⁻¹(Aromatic -C-H Bending). The synthesised product shows the various spectral properties as well as biological properties[15].

(2E)-3-[5-(substituted phenyl)-furan-2-yl]-1-(aryl)prop-2-en-1-ones can be easily synthesised by condensation of the 5-Arylfuran-2-carboxaldehydes and 2-acetylpyrole or 2-acetylfuranx. The chalcone which is newly formed is characterized under FTIR and having yield of 78% and dark yellow in the colour and shown peaks at (KBr, v, cm-1): 3229 (pyrrole N-H), 3132 (Aromatic C-H), 2969 (aliphatic C-H), 1641 (C=O). The activity of newly formed chalcone is checked on *Escherichia coli*, *Klebsiella SPP*(Gram -) as well as *Staphylococcus aureus* and *Enterococcusfaecalis* (Gram +) using well diffusion method[16],[17].

Synthesis, antitubercular and antibacterial evaluation of the pyrrolyl derivatives having phenyl thiourea, isoxazole and pyrazoline moieties. Surfex-Dock[18, 19] is used to carry out molecular docking of the enoyl ACP reductase from *Mycobacterium Tuberculosis* and is useful to design antitubercular agents. Docking analysis is done using surflex dock and compounds 1-(2-(2,5-dimethyl-1Hpyrrol-1-yl)phenyl)-3-(substituted phenyl)thioureas shows the maximum antitubercular activity and is synthesis by using mixture of the 2-(2,5-dimethyl-1H-pyrrol-1-yl)aniline and phenyl isothiocyanates in chloroform and reflux for the 20-22 hrs and yield calculated is 78% and characterization is done using FTIR(KBr in cm⁻¹) 3308.39 &3165.87 cm⁻¹(N-H), 2978.35 (Ar-H) cm⁻¹[20].

Experimental Work

Methodology



Equipment to be used

Condenser, Round bottom flask(250 ml), Magnetic stirrer, Beakers(500 ml), Tripod stand, Thermometer, Guard tube, Separating funnel, Magnetic bead, Measuring cylinder, test tubes, TLC plate, U.V chamber, Column.

Chemical and Solvents used

p-Nitroacetophenone, Ethanol, Methanol, Ethylacetate, 2-chloro-4,6-dimethoxy-1,3,5-triazine, Potassium hydroxide, Conc.HCl., Dry acetone, Guanidine Hydrochloride, chloroform, hexane, distilled water, *anisaldehyde*, *benzaldehyde*, *p-chloro benzaldehyde*, *p-cyano benzaldehyde*.

Methods:

1. Synthesis of 1, 3 -diphenylprop-2-en-1-one. 1(a)

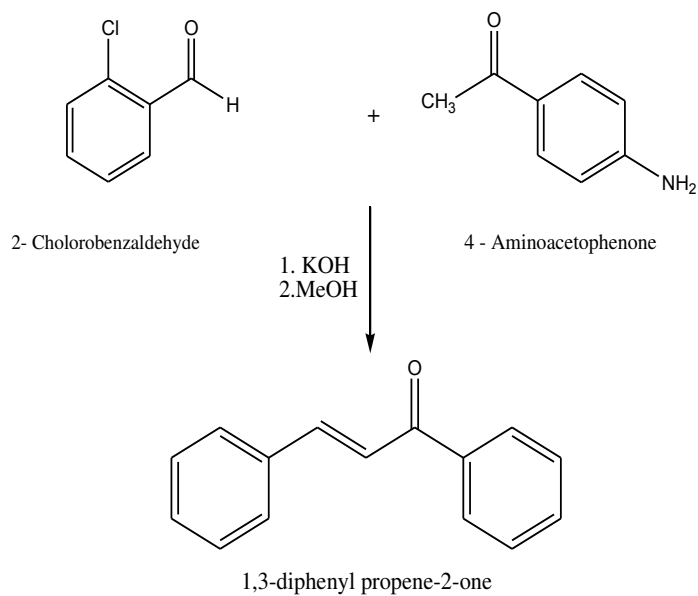
3gm of potassium hydroxide pellets (KOH) was dissolve in small amount of distilled water in a beaker. In round bottom flask (RBF) and add 10ml of methanol at room temperature. Stir the solution for around 5 minutes. 1.35gm of amino acetophenone(0.01 mol) is added to the 250 ml RBF and continued the stirring. Slowly start adding 1.6ml of the 2 chlorobenzaldehyde(0.01 mol) drop wise in the RBF. Continue the stirring for 48 hours in cold conditions and reactions done in the basic medium. Monitor the reaction using Thin Layer Chromatography (TLC). Precipitation takes place with the stirring. As reaction occurs in the basic medium, add dil. HCl in the beaker drop wise. Filter out the precipitates using Watt man filter paper and Buckner funnel. Wash the product using distilled water and ethanol. Recrystallise the product.

2. Synthesis of 1, 3-diphenylprop-2-en-1-one. 2(a)

3gm of KOH was dissolve in small amount of distilled water in beaker and dissolve it in 10ml of methanol in RBF. Stirred for 5 minutes on the magnetic stirrer and add 1.35gm of amino acetophenone(0.01 mol) to it. Slowly 1.50gm of 2-nirobenzaldehyde (0.01 mol) is added and string is continued for 48 hours around 15⁰C. Dil. HCl is added to the solution to make the medium acidic. Precipitation takes place with the stirring while adding HCL.

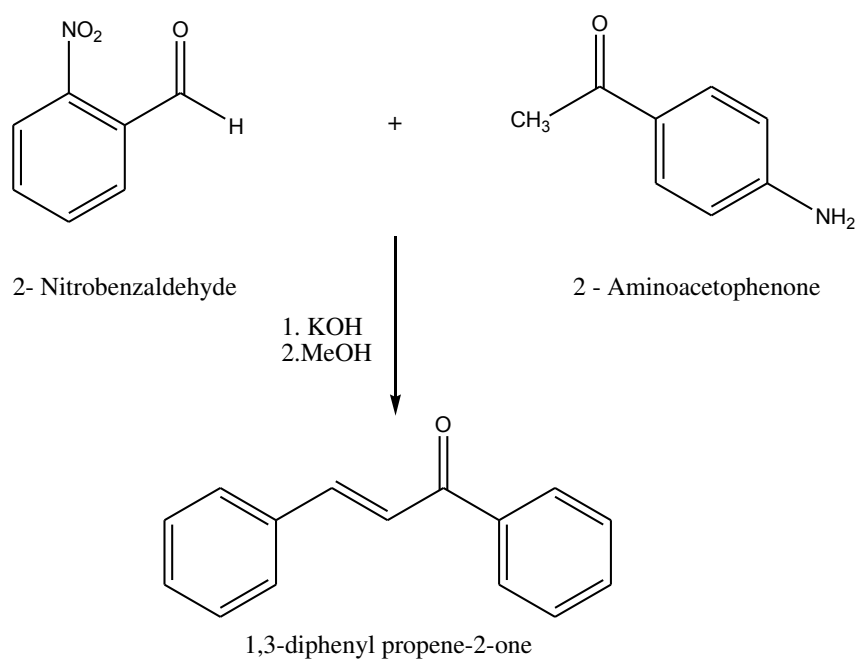
Filtered the content in Buckner's funnel using walt man filter paper and washed with distilled water and ethanol. Dry the product.

Reaction-1



1(a)

Reaction-2



2(a)

Result and Discussion

The colour of the compound (**1a**) obtained from first reaction is yellow having yield of 68%. TLC is used to check the purity of the sample giving two spots and R_f calculated is 0.475. TLC as in **Figure 1**. This showed that the compound was not pure. Column Chromatography is done order to purify the compound.



Figure 1

For the separation column chromatography is used and pure compound is obtained that is checked by TLC as shown in **Figure 2**

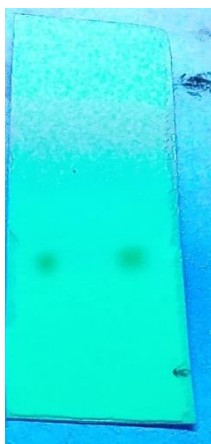


Figure 2

The colour of the compound **2(a)** is light brown and yield is 78% and purity of the compound was checked by putting TLC and is impure that is shown in the **Figure 3**.

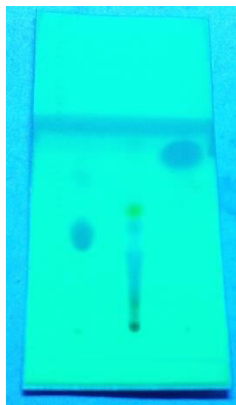


Figure 3

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