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Synthesis and Characterization of Benzylidene Derivatives Using Various Solvents, Catalysts and Green Chemistry Methods

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Abstract: ***The work presented in thesis describes the synthesis of benzylidene as potential anti-bacterial agents. In the past few decades, intensive research experienced a significant development consequently leading to the availability of various anti-bacterial drugs in the market. However, these drugs suffer from some limitations and their use is limited due to their less bioavailability, immunogenicity and low stability. These shortcomings necessitated the development of new antibiotic drugs. The biological activity of these drugs is generally exerted by small regions in their zone of inhibition. In this context, we have designed and synthesized benzylidene by green chemistry techniques with good yields and created a data of advantages between the synthesis from regular techniques. The structures of all the compounds were unambiguously established on the basis of spectral data (IR spectra). In this context, we have screened the synthesized benzylidines for antibacterial activity and found that the molecules are comparatively active against several gram positive and gram negative bacteria which lead good source of developing benzylidenes as anti bacterial agents.***

Keywords: Benzylidenes, antibacterial, immunogenicity, IR spectra, antibiotics

1. Aim and Objectives of Study

1.1 Aim

Green chemistry, also called **sustainable chemistry**, is an area of chemistry and chemical engineering focused on the designing of products and processes that minimize the use and generation of hazardous substances. Whereas environmental chemistry focuses on the effects of polluting chemicals on nature, green chemistry focuses on technological approaches to preventing pollution and reducing consumption of nonrenewable resources.

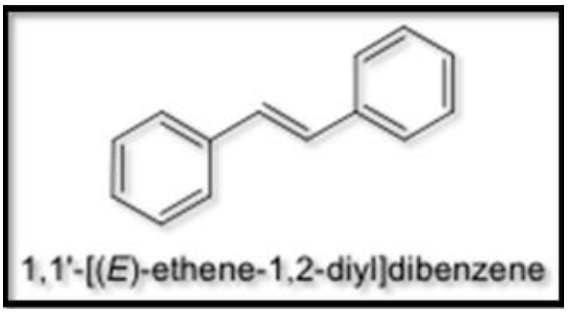
Green chemistry overlaps with all subdisciplines of chemistry but with a particular focus on chemical synthesis, process chemistry, and chemical engineering, in industrial applications. To a lesser extent, the principles of green chemistry also affect laboratory practices. The overarching goals of green chemistry—namely, more resource-efficient and inherently safer design of molecules, materials, products, and processes—can be pursued in a wide range of contexts.

Green chemistry addresses the environmental impact of both chemical products and the processes by which they are produced. In this project we shall be concerned only with the latter, i.e. the product is a given and the goal is to design a green process for its production. Green chemistry eliminates waste at source, i.e. it is primary pollution prevention rather than waste remediation (end-of-pipe solutions). Prevention is better than cure (the first principle of green chemistry, outlined above). An alternative term, that is currently favored by the chemical industry, is Sustainable Technologies.

The main objective of this work is to synthesis benzylidene derivatives using green solvents and green catalysts and characterize the physical parameters of the compounds obtained. Then to evaluate the antibacterial activity of the compound using in-vitro evaluation method i.e., by agar diffusion method.

1.2 Objectives of Study

1) To prepare Benzylidine from cyano esters.
2) To synthesize the title compounds, *1,1`-[(E)-ethene-1,2-diyl]dibenzene derivatives*.
3) To characterize all the synthesized compounds by physical (Molecular weight, Molecular formula, Melting point, Recrystallisation, Rf value) and IR spectral data.
4) To evaluate the title compounds for antibacterial activity.



Scheme

Scheme-I

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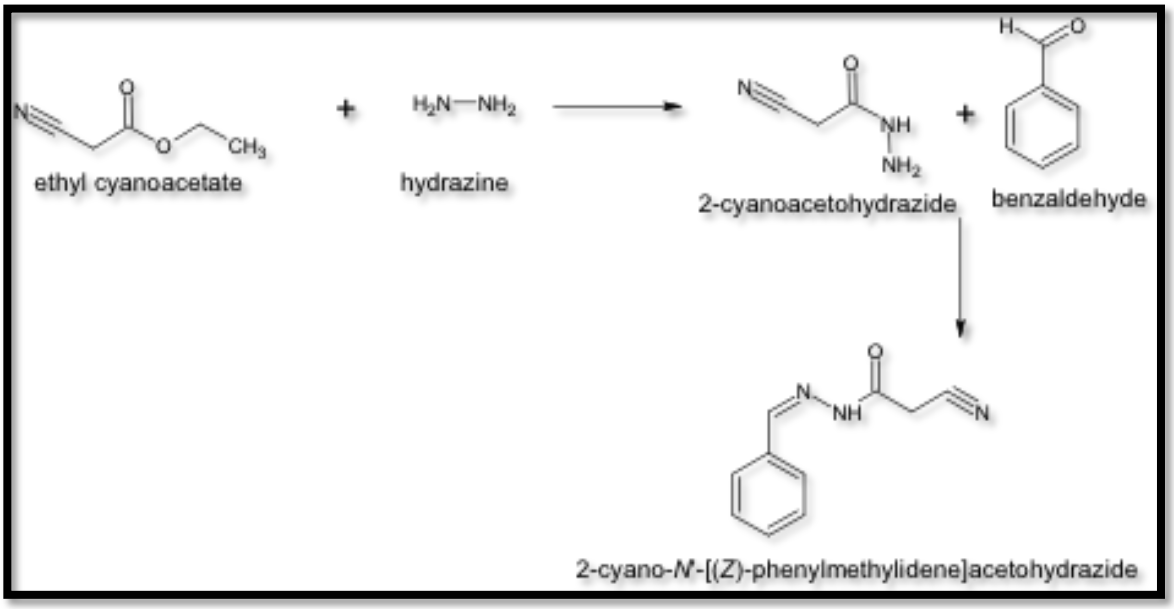
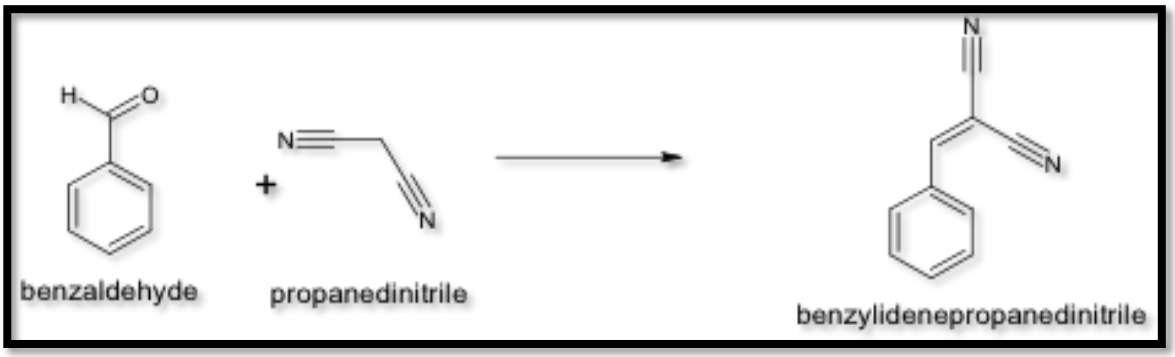
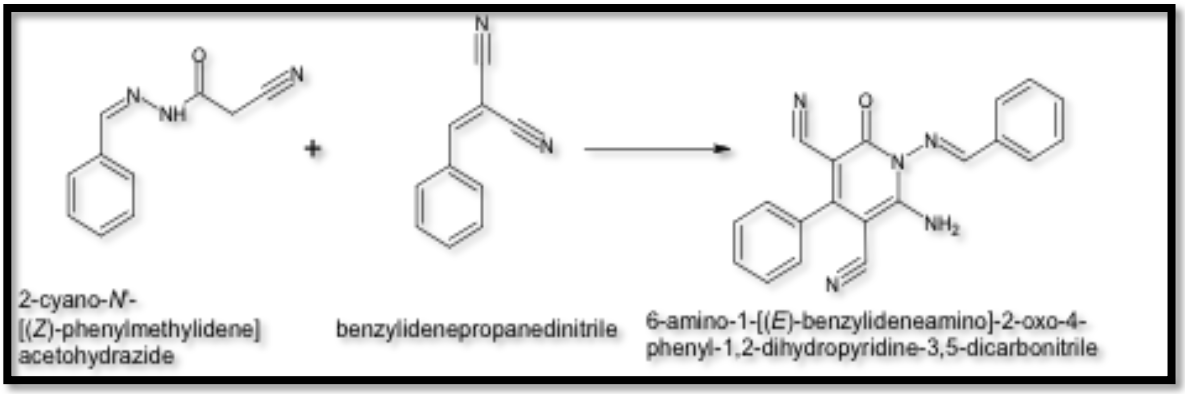


Scheme II



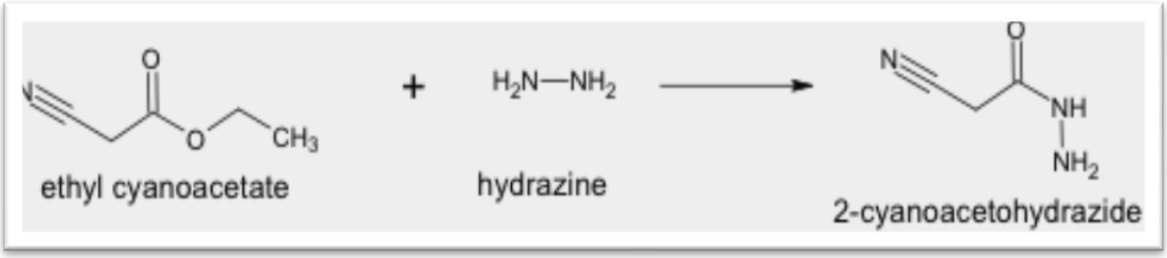
Scheme III



2. Experimental Methodology

Reaction

Synthesis of 2-cyanoacetohydrazide: C(1):



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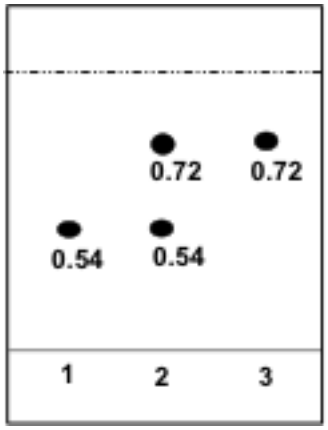
Procedure:
Ethyl cyanoacetate**(0.1 mol 11.1 ml),** hydrazine hydrate **(3.6 ml)** are taken separately and added to mortar drop by drop with continuous trituration until a clear precipitate is obtained then reaction quenched in to ice cold water. White precipitate was observed, which is filtered under suction, dried and recrystallised by hot ethanol.

TLC Studies of Compound (1):



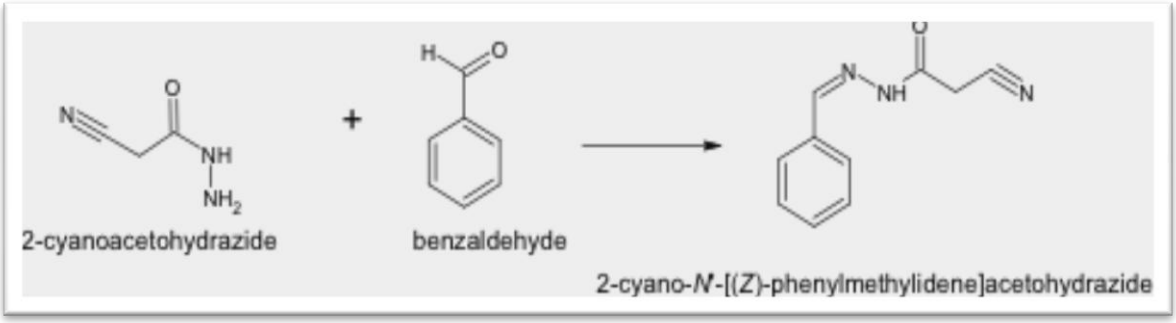
Solvent System: Chloroform: Methanol (9:1)
1. ethyl-cyanoacetate
2. Co spot of ethyl-cyanoacetate and Compound-1
3. Compound-1

Physical Properties:
Re-crystallization solvent : Ethanol
Yield : 64.75 %
M.P of pure product : $_{146}{}^{o}C$
Molecular Weight : 99.09
Molecular Formula: $C_3H_5N_3O$
Rf value of Compound (1) : 0.72

SYNTHESIS OF 2-cyano-N-[(Z)-phenylmethylidene]acetohydrazide:

Compound (2)

Reaction:



Procedure:
2-cyanoacetohydrazide(compound–1) **(0.1mol,1.0gm),** benzaldehyde **(4.2ml)** and ethanol 10 ml as a solvent are taken in a round bottomed flask, the mixture is refluxed for 4 hours. Reaction is monitored by TLC and after the completion of reaction solvent from the product is removed

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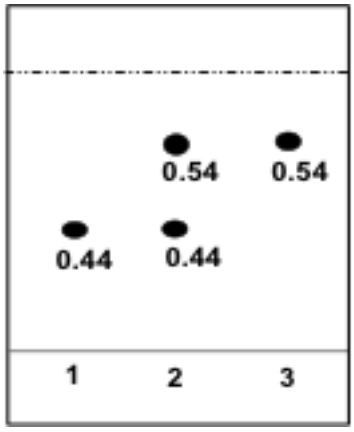
under pressure then product is quenched in to ice cold water. White precipitate was observed, which is filtered under suction, dried and recrystallised by hot ethanol.

TLC Studies of Compound (2):



Solvent System: Chloroform: Methanol (9:1)
1) Compound-1.
2) Co spot of Compound-1 and Compound-2.
3) Compound-2.

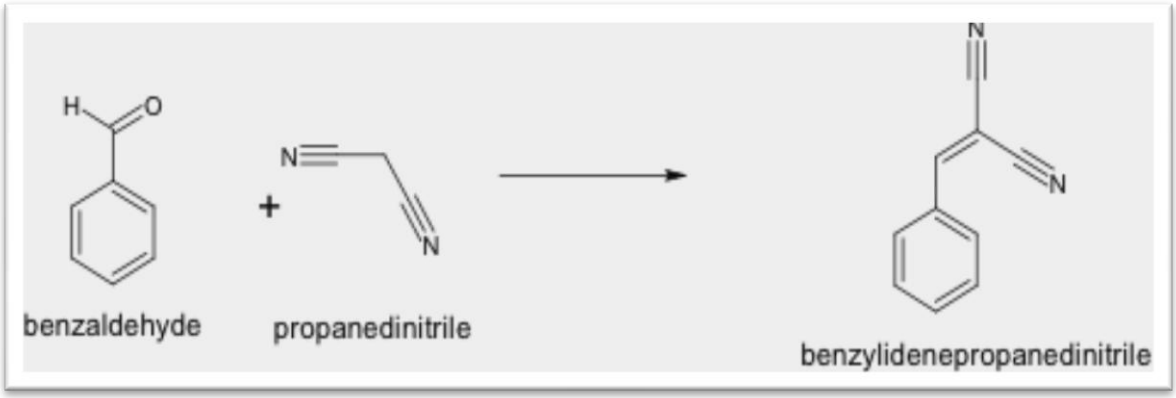
Physical Properties:

Recrystallization solvent	**:**	Ethanol
Yield	**:**	92.5%
M.P of pure product	**:**	$_{126}{}^{o}C$
Molecular Weight	**:**	187.19
Molecular Formula	**:**	$C_{10}H_9N_3O$
Rf value of Compound (1)	**:**	0.54

Synthesis of benzylidenepropanedinitrile:

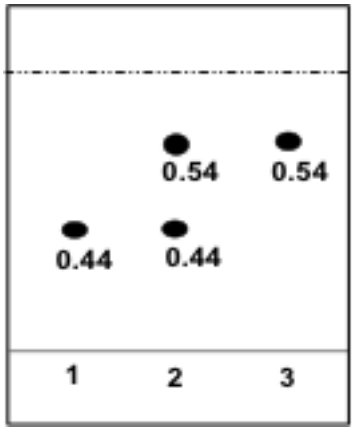
Compound (3)

Reaction:



Procedure
Benzaldehyde**(0.1mol,1.2 ml),** propanedinitrile**(1.5 ml)** are taken separately and added to mortar drop by drop with continuous triturating with an addition of base as catalyst Potassium Hydroxide **(0.5)gm** is added and the triturating is continued until a clear precipitate is obtained then reaction quenched in to ice cold water. White precipitate was observed, which is filtered under suction, dried and recrystallised by hot ethanol.

TLC Studies of Compound (3):



Solvent System: Chloroform: Methanol (9:1)
1Benzaldehyde –1.
2. Co spot of Benzaldehyde -1 and Product-3.
3. Product-3.
Physical Properties:

Recrystallization solvent :	Ethanol
Yield :	78.5%
M.P of pure product :	$_{84}{}^{o}C$
Molecular Weight :	154.16
Molecular Formula :	$C_{10}H_6N_2O$
Rf value of Compound (1):	0.54

SYNTHESIS OF 6-amino-1-[(E)-benzylidineamino]-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile: compound (4)

Reaction:

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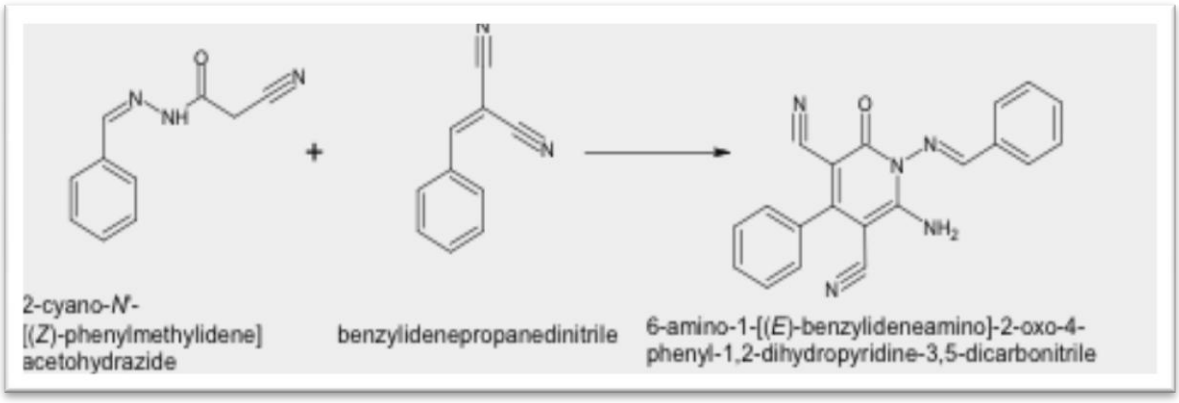
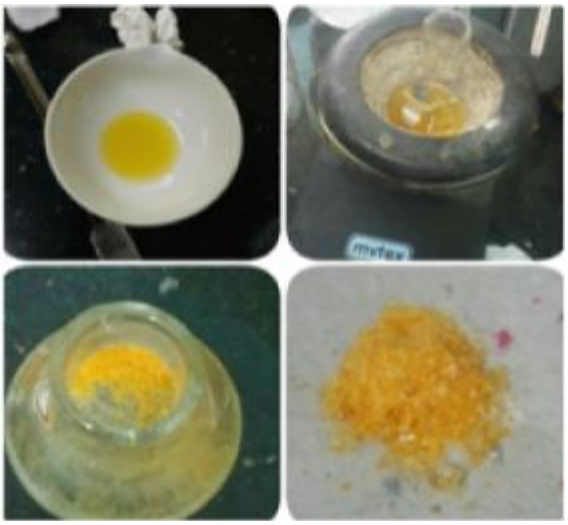
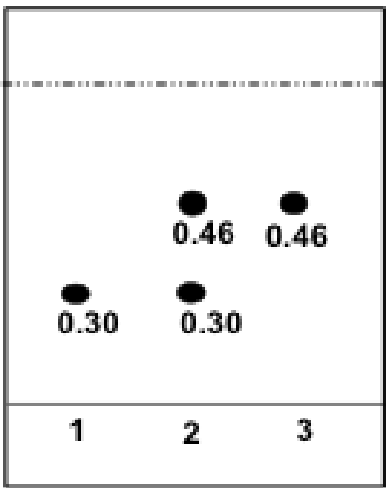


Procedure:
2-cyano-N-[(Z)-phenylmethylieine]acetohydrazide(compound–2)(**0.1 mol,1.04 gm),** benzylidenepropanedinitrile (**0.1 mol,0.9 gm),** and ethanol 10 ml as a solvent are taken in a round bottomed flask, the mixture is refluxed for 6 hours. Reaction is monitored by TLC and after the completion of reaction solvent from the product is removed under pressure then product is quenched in to ice cold water, a red precipitate was observed, which is filtered under suction, dried and recrystallised by hot ethanol.



TLC Studies of Compound (4):



Solvent System: Chloroform: Methanol (9:1)
1) Benzaldehyde -1.
2) Co spot of Benzaldehyde -1 and Product-3.
3) Product-3.

Physical Properties:
Recrystallization solvent : Ethanol
Yield : 78.5%
M.P of pure product : $_{84}{}^{o}C$
Molecular Weight : 339.3
Molecular Formula : $C_{20}H_{13}N_5O$
Rf value of Compound (1) : 0.46

Antibacterial Activity

3. Material and Methods

The synthesized compounds were screened for antibacterial activity. For determination of bacterial susceptibility test, both gram positive and gram negative organisms are used. All the bacterial strains were obtained from National Collection of Industrial Micro organisms A stock solution of amoxicillin was prepared and the dilutions are prepared. All the strains were maintained by weekly sub culturing on nutrient agar slant, stored at 4 0C after previous 24 h incubation at 37 0C. Before each experiment, the organism was activated by successive sub culturing and incubation. The activity is studied by using agar diffusion method.

Gram Positive Bacteria:
Bacillus subtilis : (NCIM-2545)
Staphylococcus epidermidis : (NCIM-2493)
Staphylococcus aureus : (NCIM-5021)

Gram Negative bacteria:
Escherichia coli : (NCIM-2803)

Procedure:
Composition of nutrient agar medium:
Peptone: 5.0g
Beef extract: 5.0g
NaCl: 5.0 mg
Agar: 2 %
Distilled water: up to 1000 mL

Standardization of test microorganisms:
A 10 mL volume of sterile water was added to the agar slant containing a 24 h old culture of purified test microorganism and shaken carefully to harvest the organism. Subsequently,

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dilutions were carried out to get microbial population of 105 cfu/mL by comparing with BaSO4, equivalent to McFarland 0.5 standard.

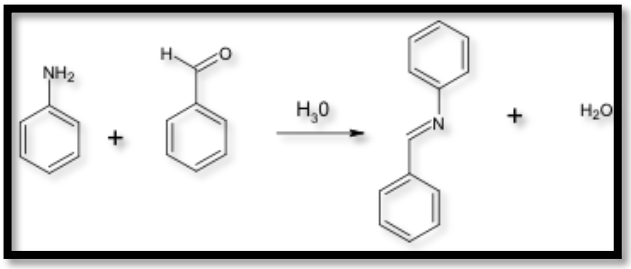
Preparation of BaSO4, suspension equivalent to McFarland 0.5 Standard:

To standardize the inoculums density for a susceptibility test, a BaSO4, turbidity standard, equivalent to a 0.5 McFarland standard is used. The BaSO4, McFarland 0.5 standard is prepared as follows. A 0.5 mL of 1.175 % w/v of BaCl2.2H2O is added to 99.5 mL of 1% w/v of H2SO4 with constant stirring to maintain suspension. The correct density of the turbidity standard is verified by using a UV-spectrophotometer by determining the absorbance. The absorbance at 625 nm is 0.08-0.10 for this standard. This suspension is used to standardize the inoculums density.

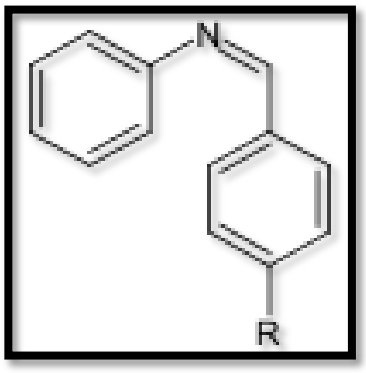
4. Review of Literature

1. M.O.BOUSSAID et.al, reported a solvent free procedure for preparation of benzylidene from amines and aldehyde by addition of simple catalyst in a green chemistry process given below.13

Reaction:



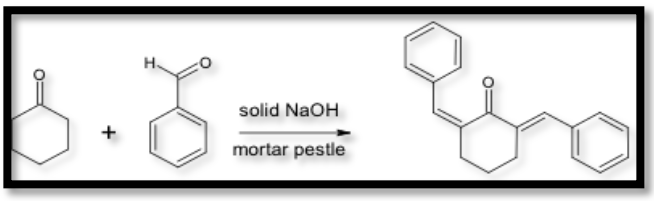
2. MAJED.M. HANIA et.al, reported benzylidine preparations from amines and different aldehyde with toluene as a solvent and prepared N-Substituted benzylidene.14



N-Substituted benzylidene

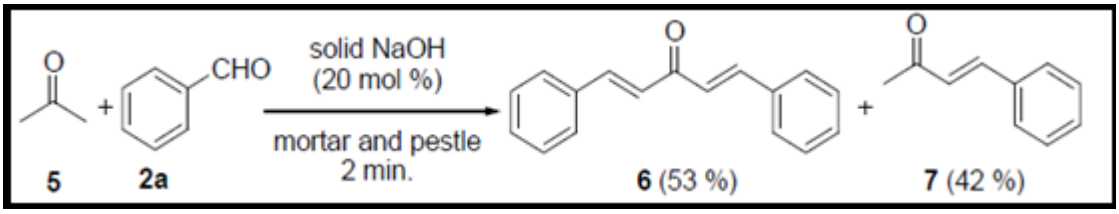
3. A.F.M. MOTIUR RAHAMAN et.al, Solvent free Claisen-Schmidt reactions of **1a**/**1b** with **2a** in presence of NaOH (100 mol%) by grinding with a mortar and pestle for 5 min.

Reaction:



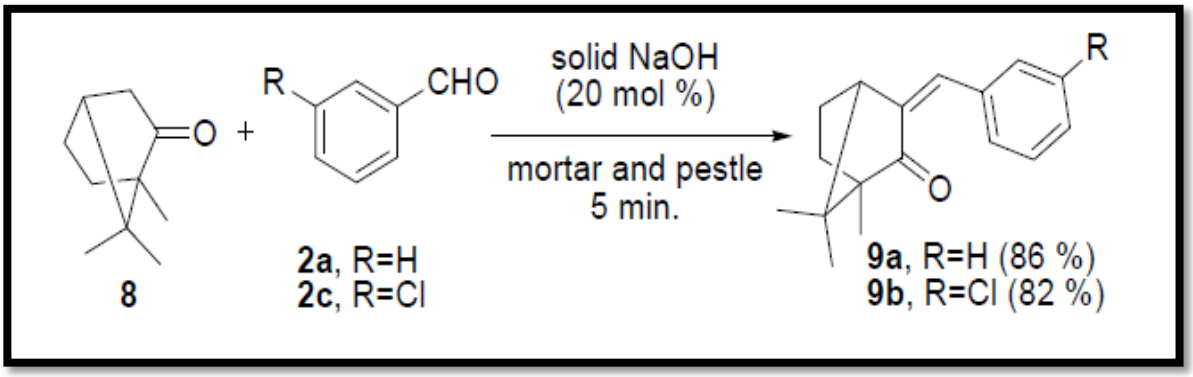
4. A.F.M. MOTIUR RAHAMAN et.al, The Claisen-Schmidt reaction of **1a–b** with **2a–h** in presence of solid NaOH (20 mol%) by grinding in a mortar and pestle for 5 min.

Reaction:



5. A.F.M. MOTIUR RAHAMAN et.al, Claisen-Schmidt reactions of 8 with **2a**/**2c** in the presence of solid NaOH(20 mol%) by grinding with a mortar and pestle for 5 min.

Reaction:



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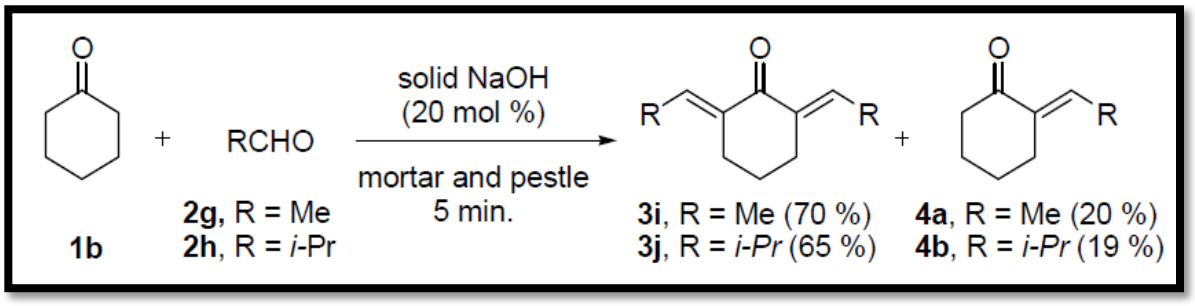
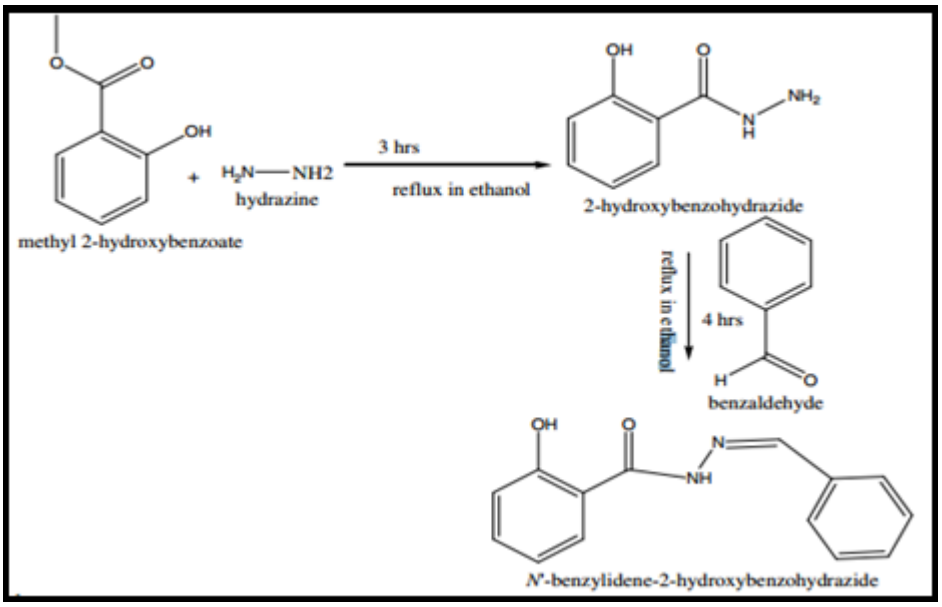
6. A.F.M. MOTIUR RAHAMAN et.al, Solvent free Claisen-Schmidt reactions of 1b with **2g**/**2h** in the presence of NaOH (20 mol%) by grinding with a mortar and pestle for 5 min.

Reaction:



7. TesfahunBufebo et.al, The Schiff base ligand was synthesized from 2- hyhdroxybenzohydrazide and benzaldehyde. The imines, very well known and popular as Schiff Bases. Recent developments on their "metallo-imines" variants have been described. The applications of Schiff bases in organic synthesis is recognizable Schiff bases are aldehyde or ketone like compounds in which the carbonyl group is replaced by an imine or azomethine group.

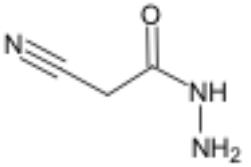
Reaction:



5. Results

5.1 Physical Properties

Table 1: Physical data of 2-cyanoacetohydrazide: (Compound 1)
* Solvent System:Chloroform: Methanol (9:1).

Compd. No	Compound structure	Mol. Formula	Mol. Wt	Recrys. Solvent	M.P (0C)	Yield (%)	Rf value*
1		C3H5N3O	99.09	Ethanol	146oC	64.75	0.72

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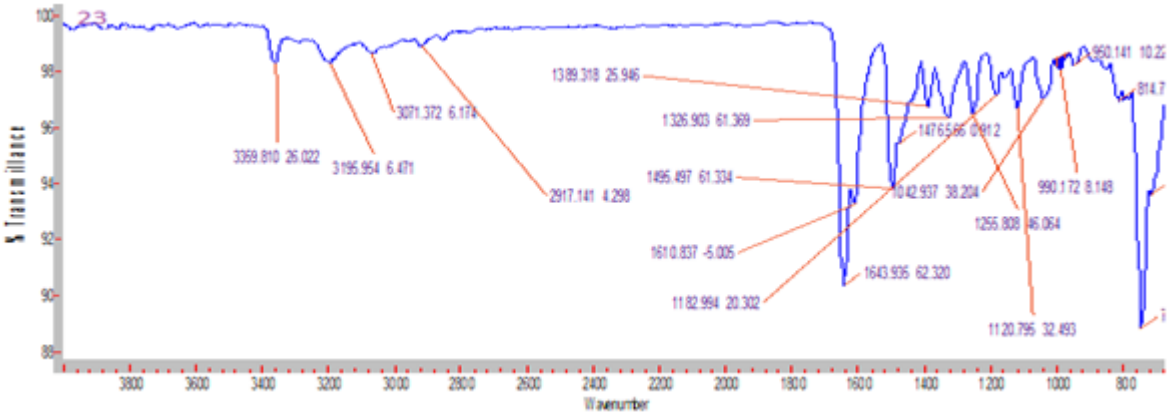
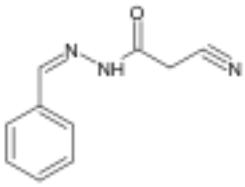


Figure 1: IR Spectra of Compound - 1

Table 2: Physical data of 2-cyano-N-[(Z)-phenylmethylidene]acetohydrazide: (Compound 2)
* Solvent System: Chloroform: Methanol (9:1).

Compd. No	Compound structure	Mol. Formula	Mol. Wt	Recrys. Solvent	M.P (0C)	Yield (%)	Rf value*
2		C10H9N3O	187.19	Ethanol	126	92.5%	0.54

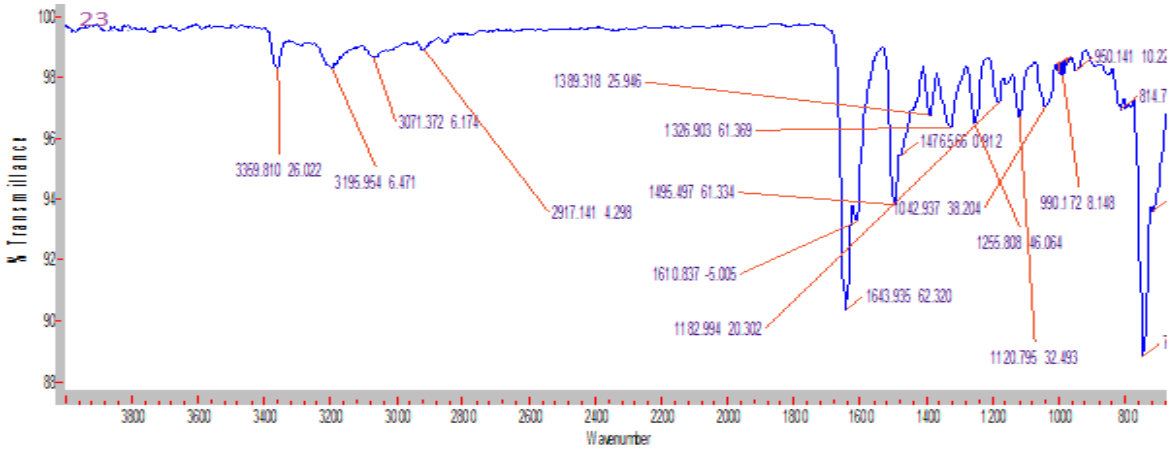
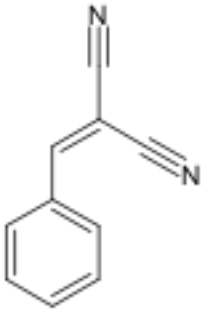


Figure 2: IR Spectra of Compound – 2

Table 3: Physical data of benzylidenepropanedinitrile: (Compound 3)
* Solvent System:Chloroform: Methanol (9:1).

Compd. No	Compound structure	Mol. Formula	Mol. Wt	Recrys. Solvent	M.P (0C)	Yield (%)	Rf value*
3		C10H6N2O	154.16	Ethanol	128	84	0.54

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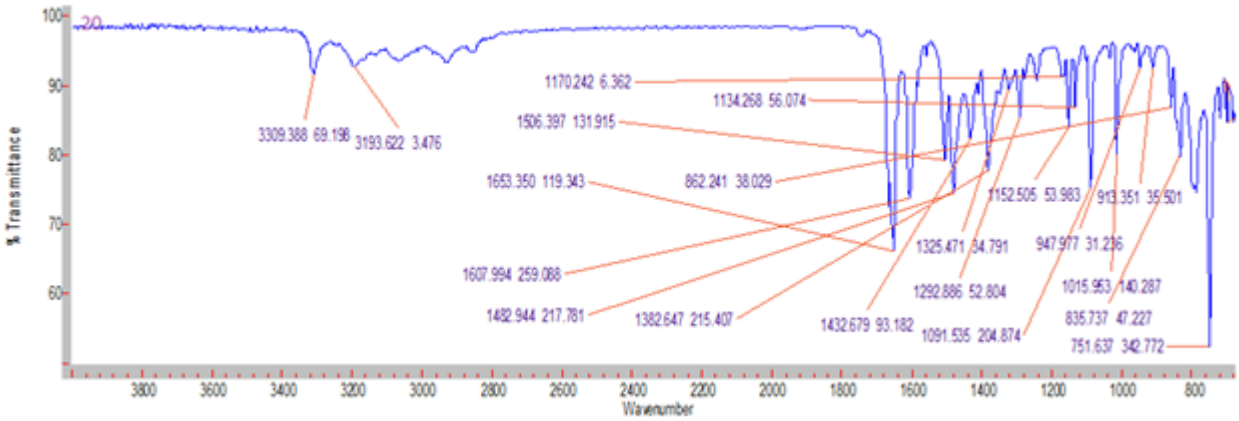
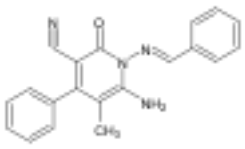


Figure 3: IR Spectra of Compound - 3

Table 4: Physical data of 6-amino-1-[(E)-benzylidineamino]-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile: (Compound 4)

* Solvent System:Chloroform: Methanol (9:1).

Compd. No	Compound structure	Mol. Formula	Mol. Wt	Recrys. Solvent	M.P (0C)	Yield (%)	Rf value*
4		C20H13N5O	339.3	Ethanol	84	78.75	0.46

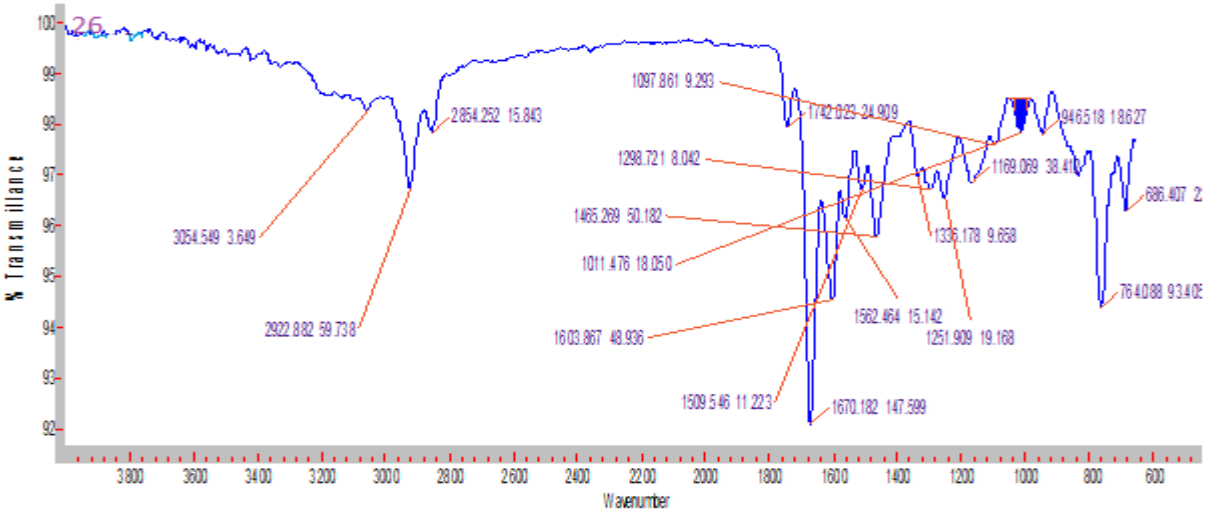
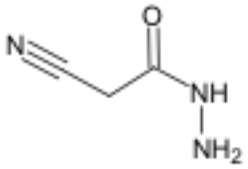
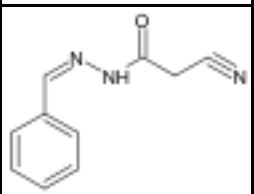


Figure 4: IR Spectra of Compound - 4

Table 5: Spectral Data

Compound code	Compound structure	IR (Cm-1)
1		IR (Cm-1) (KBr): 3359 (NH2),3195 (C-NH)1643 (C=O), 1610(CN).
2		IR (Cm-1) (KBr): 3309 (C-NH),3193 (C-NH)1653 (C=O), 1607(CN).

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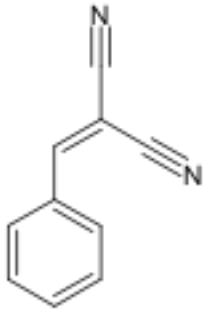
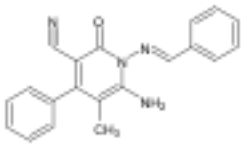
3		IR (Cm-1) (KBr): 1643 (C=C), 3195 (CN), 3369 (CN)
4		IR (Cm-1) (KBr): 3054 (CN),2854 (C=N)1562 (C-N) 1670 (C=O)2922 (C-NH2)

Antibacterial Activity Results

Antibacterial activity profile of 1,1'-[(E)-ethene-1,2-diyl]dibenzene for Escherichia *coli*
Category: Gram Negative.
Control : Amoxicillin.

Table 6

Compound no.	R	Escherichia *coli (µg/mL)*			
		10	20	40	50
6a	Compound-1	--	13.3 ± 0.05	16.6 ± 0.05	17.3 ± 0.05
6b	Compound-2	--	13.3 ± 0.05	16.3 ± 0.05	17.3 ± 0.05
6c	Compound-3	--	13.3 ± 0.05	16.0 ± 0.05	18.0 ± 0.08
6d	Compound-4	--	13.3 ± 0	16.0 ± 0	18.0 ± 0.08
Amox	--	15.5 ± 0.07	18.7 ± 0.06	21.2 ± 0.09	24.8 ± 0.10

Zone of inhibition in millimeters. (Average ± SEM) (n=9)

Antibacterial activity profile of 1,1'-[(E)-ethene-1,2-diyl]dibenzene for *Bacillus subtilis:*
Category : Gram Positive
Control : Amoxicillin

Table 7

Compound no.	R	*Bacillus subtilis(µg/mL)*			
		10	20	40	50
6a	Compound-1	--	12.7 ± 0.08	16.3 ± 0.05	18.3 ± 0.05
6b	Compound-2	--	13.6 ± 0.05	15.3 ± 0.05	18.0 ± 0.08
6c	Compound-3	--	13.6 ± 0.05	15.3 ± 0.05	16.6 ± 0.13
6d	Compound-4	--	13.6 ± 0.05	15.3 ± 0.05	17.0 ± 0.05
Amox	--	16.3 ± 0.05	19.0 ± 0.08	20.8 ± 0.09	25.2 ± 0.09

Zone of inhibition in millimeters. (Average ± SEM) (n=9)

Antibacterial activity profile of 1,1'-[(E)-ethene-1,2-diyl]dibenzene for *Staphylococcus aureus:*
Category : Gram Positive.
Control : Amoxicillin.

Table 8

Compound no.	R	*Staphylococcus aureus (µg/mL)*			
		10	20	40	50
6a	Compound-1	--	12.7 ± 0.08	16.3 ± 0.05	18.3 ± 0.05
6b	Compound-2	--	13.6 ± 0.05	15.3 ± 0.05	18.0 ± 0.08
6c	Compound-3	--	13.6 ± 0.05	15.3 ± 0.05	16.6 ± 0.13
6d	Compound-4	--	13.6 ± 0.05	15.3 ± 0.05	17.0 ± 0.05
Amox	--	16.3 ± 0.05	19.0 ± 0.08	20.8 ± 0.09	25.2 ± 0.09

Zone of inhibition in millimeters. (Average ± SEM) (n=9)

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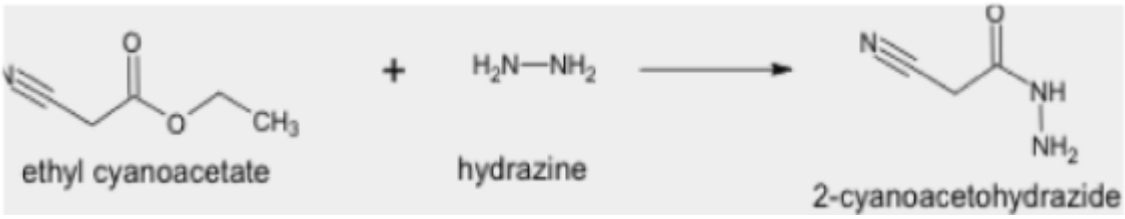
6. Discussion

Synthesis of 2-Cyanoacetohydrazide:

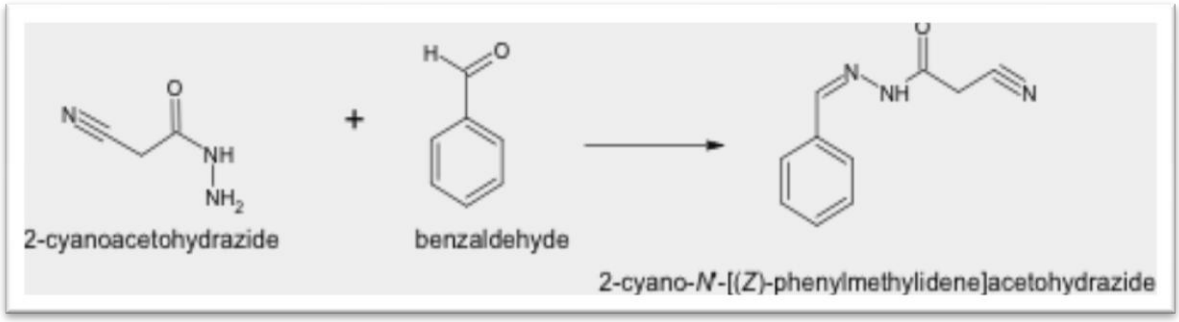
Reaction:



In the above reaction ethyl cyanoacetate was taken in mortar in equimolar concentration with hydrazine hydrate and then triturated continuously until a white crystalline precipitate was obtained then the mixture is quenched in to ice cold water and then filtered and washed with water to remove the non reacted reagents, then the product is recrystallized by hot ethanol to remove the impurities.

SYNTHESIS OF 2-CYANO-N-[(Z)-PHENYLMETHYLIDENE] ACETO HYDRAZIDE:

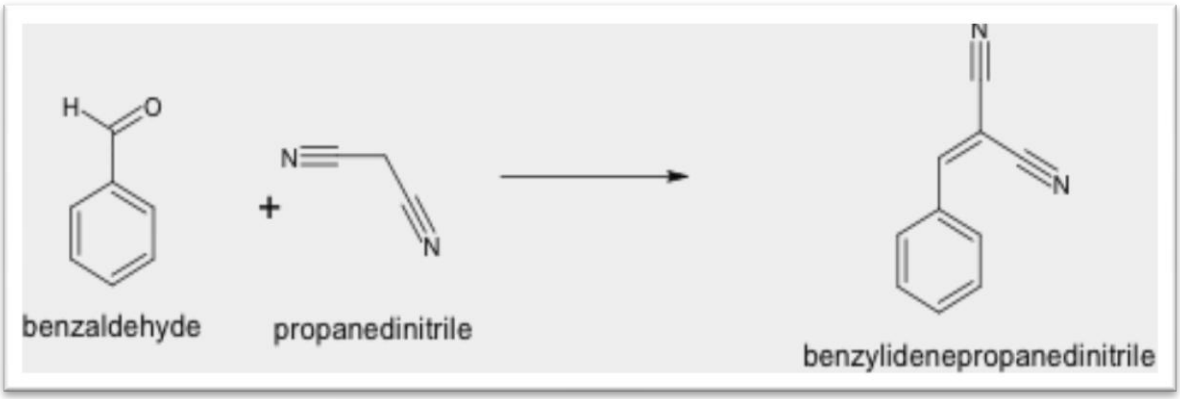
Reaction:



In the above reaction 2-cyanoacetohydrazide product of reaction 1 and benzaldehyde are taken in a round bottomed flask with ethanol as solvent and refluxed for 4 hours and then reaction process is monitored by TLC studies and then after completion of reaction the solvent ethanol was removed under reduced pressure and the mixture is quenched in to ice cold water and then filtered and washed with water to remove the non reacted reagents, then the product is recrystallized by hot ethanol to remove the impurities.

SYNTHESIS OF BENZYLIDENEPROPANEDINITRILE:

Reaction:



In the above reaction benzaldehyde and propanedinitrile (malanonitrile) are taken in a mortar and the triturated with base as catalyst potassium hydroxide has been used as base catalyst and then trituration is continued until a thick paste is prepared and then paste is quenched into ice cold water and then obtained precipitate was filtered and washed with water and dried and the product is recrystallized with hot ethanol.

Synthesis of 6-AMINO-1-[(E)-BENZYLIDENE AMINO]-2-OXO-4-PHENYL -1, 2-DIHYDROPYRIDINE-3,5-Dicarbonitrile:

Reaction:

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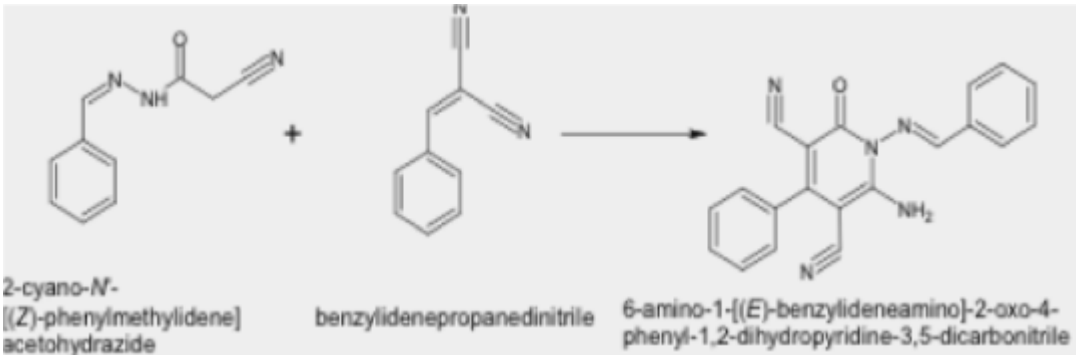

In the above reaction 2-cyano-phenylmethylidineacetohydrazide and benzylidenepropanedinitrile are taken in a round bottomed flask with ethanol as solvent and refluxed for 4 hours and then reaction process is monitored by TLC studies and then after completion of reaction the solvent ethanol was removed under reduced pressure and the mixture is quenched in to ice cold water and then filtered and washed with water to remove the non reacted reagents, then the product is recrystallized by hot ethanol to remove the impurities.

Antimicrobial Activity

The newly synthesized compounds were screened for antibacterial activity against Gram positive [Bacillus subtilis (NCIM-2545), Staphylococcus epidermidis (NCIM-2493), Staphylococcus aureus (NCIM-5021)] and Gram negative [(Escherichia coli (NCIM-2803)] bacteria by agar diffusion method. All the compounds were found to be active against all the four bacterial strains at 20 µg/mL concentration. And the activity of these compounds varied with the kind of organism. All compounds (**compound 1-4)** showed similar zone of inhibitions. The compound 1,3-benzothiazole-2-amine exhibited activity against *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*at a concentration of 20 µg/mL. All the activity is done with Amoxicillin as reference.

7. Summary and Conclusion

- 2-cyanoacetohydrazide was prepared from ethyl cyano acetate
- **2**-cyano-N-[(Z)-phenylmethylidine]acetohydrazide was prepared from 2-cyanoacetohydrazide.
- benzylidenepropanedinitrile: was prepared from benzaldehyde
- 6-amino-1-[(E)-benzylidene amino]-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile was prepared from benzylidenepropanedinitrile and 2-cyano-N-[(Z)-phenylmethylidene]acetohydrazide
- A facile method under mild conditions has been developed for the synthesis of the title compounds.
- All the compounds synthesized were characterized by physical (Rf values, Melting point, Molecular weight, Molecular formula) and spectral data (IR spectra).
- The title compounds were screened for antibacterial activity by agar diffusion method.
- The Minimum Inhibitory Concentration (MIC) of the synthesized compounds was also determined. The obtained antibacterial results were analyzed statistically.
- This acts as a lead for further optimization.

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