Original Article

Synthesis And Characterization Of Hydrazide Based 1,3-Benzoxazole Derivatives As Antitubercular Agents

Rita D. Chakole[1], Manoj S. Charde[2*], Pralay A. Barhewar[3]

Department of Pharmaceutical Chemistry,
Government College of Pharmacy, Vidyanagar Karad, Dist: Satara,
Maharashtra, India, Pin: 415124
*For correspondence
Dr. Manoj S. Charde
Assistant Professor
Government college of Pharmacy, Vidyanagar, Karad,
Dist: Satara, Maharashtra, India, 415124
Email: manojudps@rediffmail.com
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Abstract

INTRODUCTION
Tuberculosis (TB) is one of the most common, communicable, and fatal diseases known to mankind. Tuberculosis is one of the world's great public health threats due to resistance to existing drugs. It is potentially serious infectious bacterial disease that mainly affects the lungs. The mycobacterium tuberculosis usually attack the lungs, but they can also damage other parts of the body. TB is spread through the air when a person with TB of lungs or throat coughs, sneezes, or talks.

MATERIALS AND METHODS
To Synthesize the hydrazide based Benzoxazole derivatives as Antitubercular agents and Study their biological activity along with structural assessment by using the various techniques of structural elucidation. All chemicals required for the synthesis were procured from Lobachem Laboratories, all chemical are synthetic grade.

REUSLT AND DISCUSSION
All the above compounds synthesized from 2-phenyl benzoxazole -5 carboxylic acid hydrazide with the reactive carboxylic group , acetophenone analogs to forming cyclization followed by peptide bond . In the synthetic pathway, during synthesis of intermediate 2 i.e, reduction of nitro group into amino group done by sodium dithionite this reaction was optimized by using various reducing agents like Zn/HCL, stannous chloride, copper sulphate and sodium borohydride but there is no desired product obtained. The purity of all synthesized compounds were confirmed by melting point and thin layer chromatography

CONCLUSION
The dissertation is concluded that the synthesized molecules are effective in inhibiting the mycolic acid cyclopropane synthase (cmaA1) which is play important role in mycolicbacterial cell wall growth. All compounds are showing the significant effect on target enzyme.

KEYWORDS: Anti tubuorculosis, Synthesis, Characterisation

INTRODUCTION

TB is spread through the air when a person with TB of lungs or throat coughs, sneezes, or talks. Millions of new cases occur every year throughout the world, and one-third of the world population is potentially infected with TB. Tuberculosis is caused by mycobacteria, predominantly Mycobacterium tuberculosis (MTB). MTB is slow-growing, intracellular organism known for its lipid rich cell wall. On top of this, various drug make resistant to TB and hence more critical forms of of tuberculosis has been reported and identified in patients. Therefore MDR_TB does not responds to the first-line of TB drugs. The first-line drugs Isoniazid (INH),

Rifampicin (RIF), Pyrazinamide, and Ethambutol are crucial therapeutics for the treatment of TB. Unfortunately, these drugs are becoming less effective due to the increasing prevalence of Multi-Drug-Resistant TB (MDR-TB) and extensively drug-resistant TB. Therefore, there is an urgent need to develop novel, effective, and fast acting Anti-tubercular drugs with low toxicity and activity against both actively growing and latent infections. Currently, there are three main approaches to developing novel Anti- tubercular agents:

Expanding the antibacterial spectrum by applying existing drugs to the treatment of TB. For example, Ciprofloxacin (CPFX) is currently recommended as second-line agent by the World Health Organization for the treatment of TB, primarily involving resistance or intolerance to first-line Anti-tubercular therapy.

Searching for novel structures and mechanisms that have never been used to act against the TB organism, such as TMC 207 for the treatment of MDR-TB.

Synthesizing new analogs or modifying existing drug compounds that can shorten and improve TB treatment. Another one requires extraordinary molecular diversity and chemical modification, this approach is easy and accessible through newly developed computational technique.[1]One modern concept in drug design is molecular hybridization, which is based on combining the pharmacophore moieties of different bioactive substances to produce a new hybrid compounds with improved affinity and efficacy compared to the parent drugs .

MATERIALS AND METHODS

Material Used

Materials used for work are listed below and their grades, also their procurements in the following table

Table no.1 Chemicals used

Sr. No.	Chemical names	Procured from	Grade
1	P-hydroxy methyl benzoate	Lobachem	Synthetic
2	Sodium dithionite	Lobachem	Synthetic
3	Con. Nitric acid	Lobachem	Synthetic
4	Con. Sulphuric acid	Lobachem	Synthetic
5	Benzoyl chloride	Lobachem	Synthetic
6	Benzoic acid	Lobachem	Synthetic
7	Hydrazine hydrate	Lobachem	Synthetic
8	Dimethyl formamide(DMF)	Lobachem	Synthetic
9	Dimethylacetamide (DMA)	Lobachem	Synthetic
10	Acetophenone	Lobachem	Synthetic
11	Acetic acid	Lobachem	Synthetic
12	Ethylacetoacetate	Lobachem	Synthetic
13	Dimethyl sulfoxide (DMSO)	Lobachem	Synthetic
14	Dichloromethane (DCM)	Lobachem	Synthetic
15	Diethyl ether	Lobachem	Synthetic

16	Ethanol	Lobachem	Synthetic
17	Methanol	Lobachem	Synthetic, analytical
18	Ethyl acetate	Lobachem	Synthetic
19	Hydrochloric acid	Lobachem	Synthetic
20	Benzene	Lobachem	Synthetic
21	Phosphorous oxychloride	Lobachem	Synthetic
22	1,4-dioxane	Lobachem	Synthetic
23	Potassium hydroxide	Lobachem	Synthetic
24	Carbon tetrachloride	Lobachem	Synthetic
25	3,5-dinitro benzoyl chloride	Lobachem	Synthetic
26	Potassium isocyanate	Lobachem	Synthetic

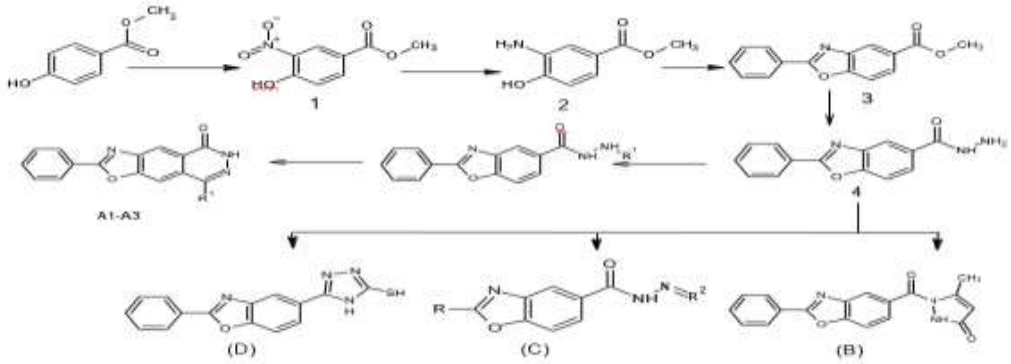
METHODS[1,2,3]

Methods used for the all research work framework and step by step explanation of work carried out.

- Synthetic scheme is highly susceptible for the reaction condition, all possible risk factors are taken into consideration and scheme is optimised time to time for good synthetic practices and safest way of result.
- Synthetic process is smart work and need to be cautions and time to time observing reaction so that avoid the errors and immediately came up with the alternative way for fluent reaction process. Chemical reaction depends upon many variables and monitored every time P^H, Temperature, etc.

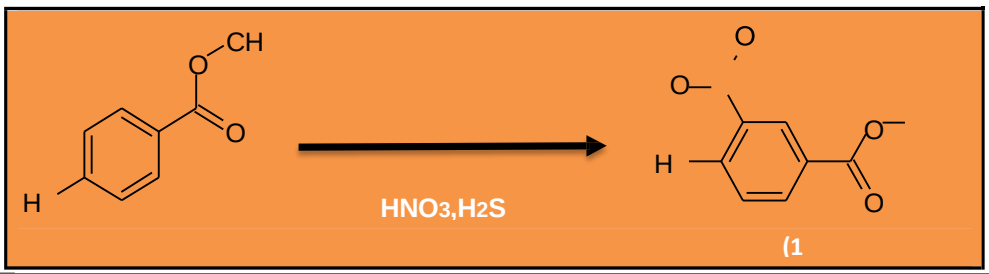
SCHEME[4,5]

Following scheme represent the general synthetic pathway and the possible end product, where R, R1, R2, represent the substituent to be added.



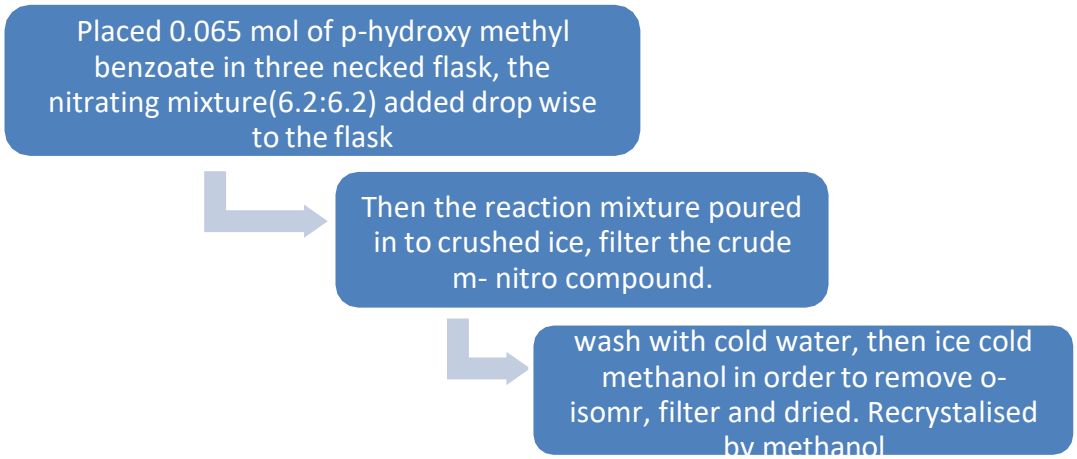
(**Figure No.1:**-Synthetic scheme of the Reaction (1,2,3,4 represents intermediates and A1-A3, B, C, D represents the products)

Step 1:-



(Fig. no. 2:- step 1 of synthesis**)**

In this step the p-hydroxy methyl benzoate react with the nitrating mixture with continuous stirring the reaction temperature does not exceed more than 10°C

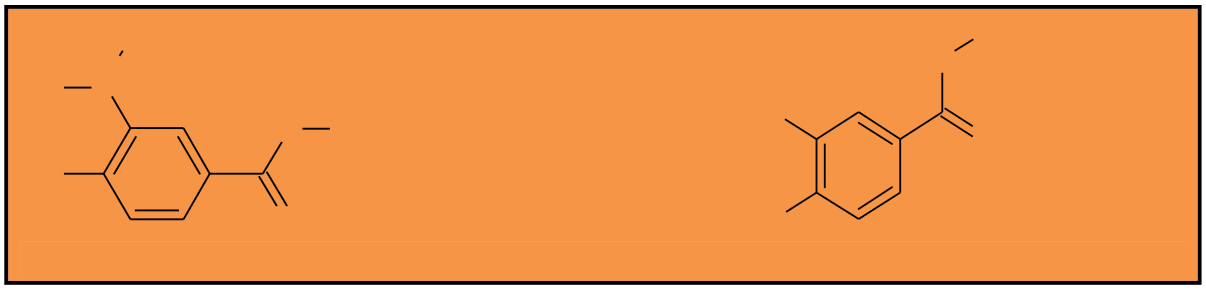


(Fig.no. 3:- flow chart of 1st step reaction procedure)

✓ **Characterization of compound**

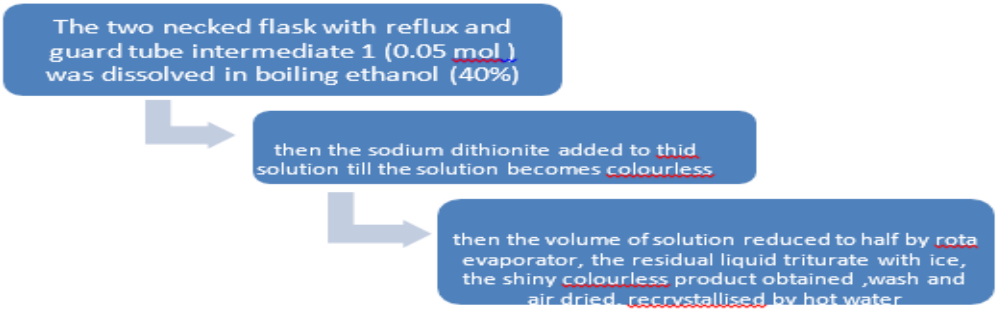
Name : methyl-4-hydroxy-3-nitro benzoate
Colour: Orange
Molecular formula: C8H7NO5 Melting point : 67-69°C
Solubility : ethyl acetate, dichloromethane, ether, etc.

Step 2:-



(Fig.no. 4:- Step 2 of synthesis)

The 3-amino-4-hydroxy methyl benzoate is formed by reduction process to convert nitro to amine group.



(**Fig.no.5**:- flow chard of 2nd step reaction procedure)

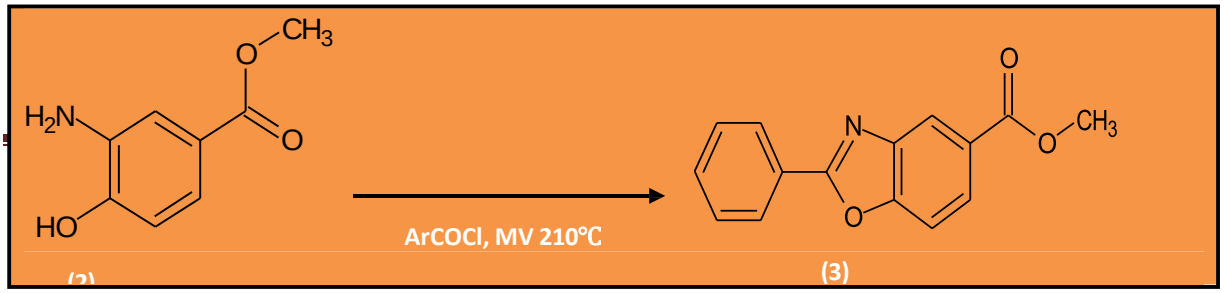
✓ **Characterization of compound Name : methyl-3-amino-4-hydroxy benzoate** Colour : white crystalline

Molecular formula : C8H9NO3

Melting point : 98-100°C

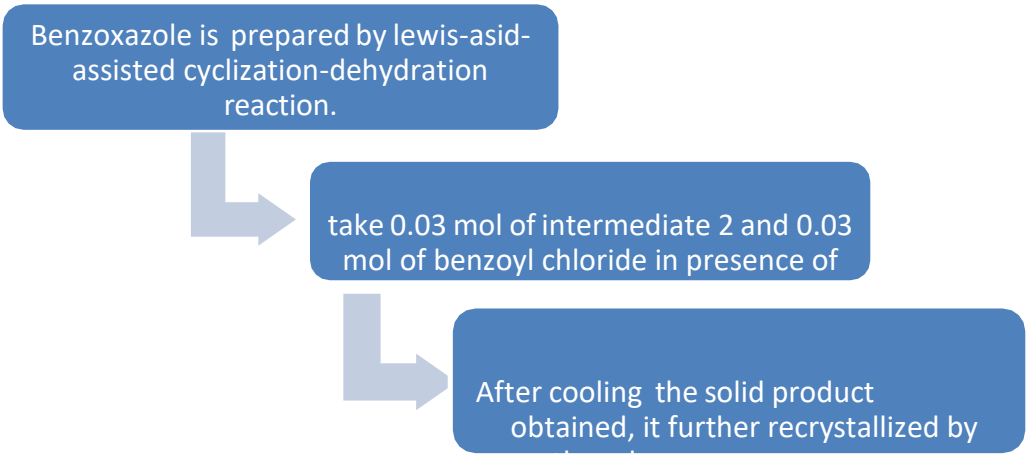
Solubility : soluble in ethanol, methanol, ethyl acetate Insoluble in water, dichloromethane, ether\

Step 3:-



(Fig.no. 6:- reaction procedure 3rd step)

In this reaction the 2nd intermediate is directly react with benzoyl chloride via microwave at 210°C under 1,4-dioxane to form 2-phenyl benzoxazole-5-carboxylic acid methyl ester.



(**Fig.no. 7:** Flow chart of 3rd step)

- Characterization of compound:

Name: 2-phenyl-benzoxazole-5-carboxylic acid methyl ester

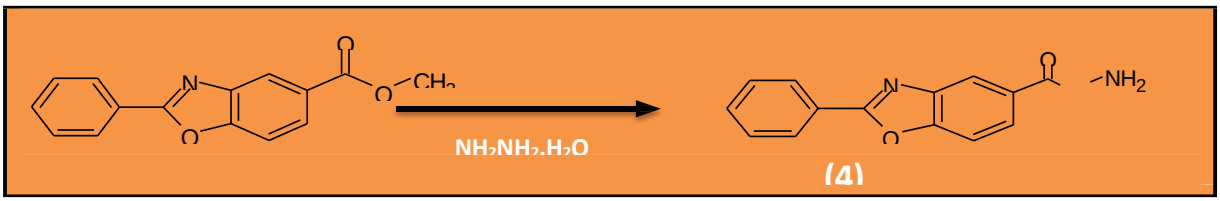
Colour : off white

Molecular formula : C15H11NO3

Melting point : 134-136°C

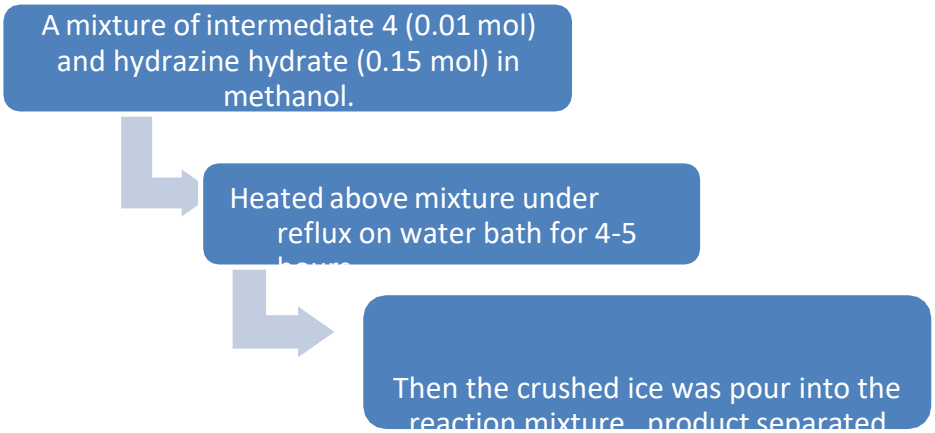
Solubility : Soluble in dichloromethane Insoluble in methanol, water, ethyl acetate, ether.

Step 4th :-



(Fig.no. 8:- reaction procedure 4th step)

In this process dissolve 0.01mol of intermediate 4 in 25 ml of ethanol and 0.15mol of hydrazine hydrate was heated under reflux on water bath for 8 hours.

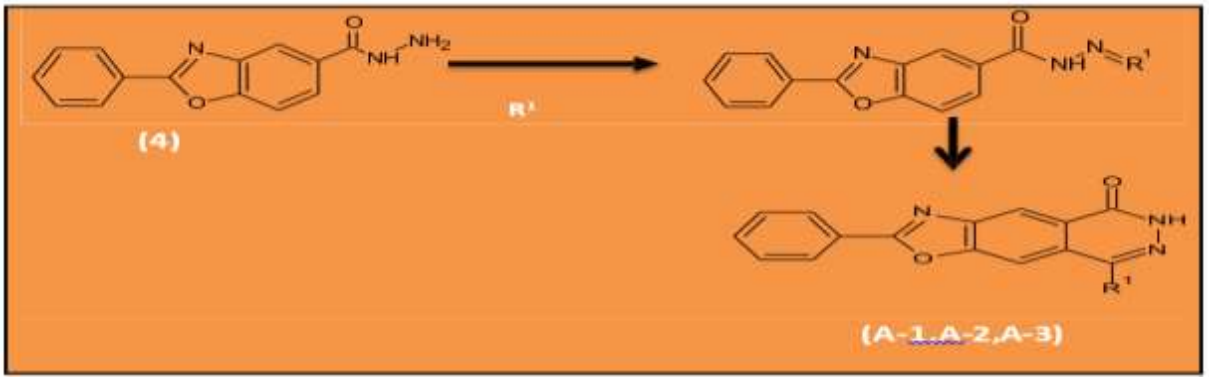


(**Fig.no. 9**:- flow chart of 4th step)

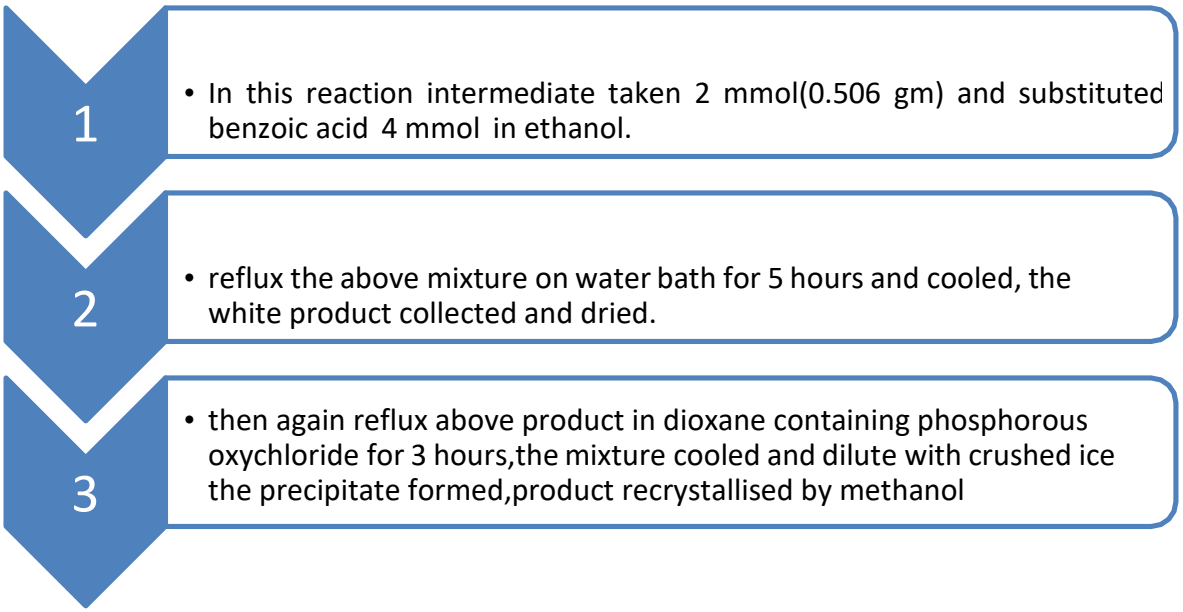
✓ **Characterization of compound:**

Name : 2-phenyl-benzoxazole-5-carboxylic acid hydrazide
Colour : white
Molecular formula : C14H11N3O2 Melting point :122-124^{0}C
Solubility : Soluble in acetic acid

Step 5[17]:-



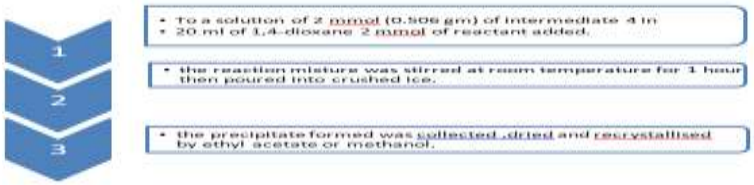
(**Fig.no. 10**: Procedure of 1st end product



(**Fig.no. 11** :- flow chart of 1st-3rd end products)

Step 6:-

(**Fig.no. 12**: procedure of 4nd end product)



(**Fig.no. 13** :- flow chart of 4thend products)

Step 7:-

(**Fig.no. 14**: procedure of 5th end product)

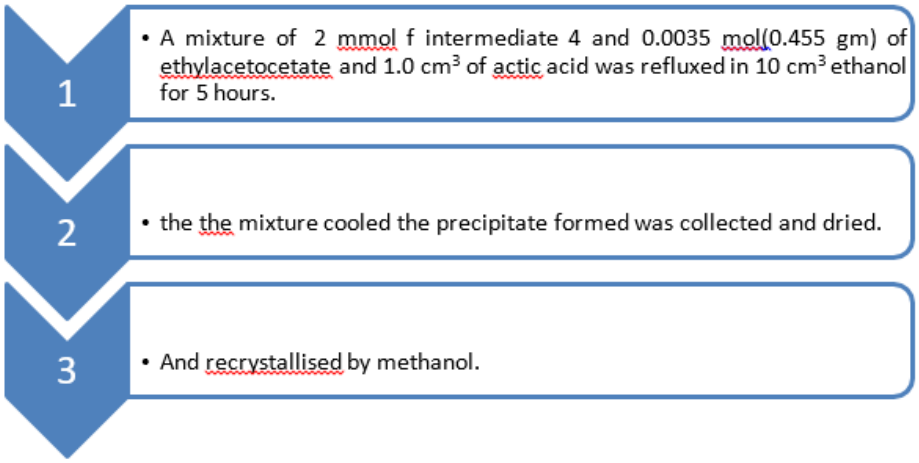
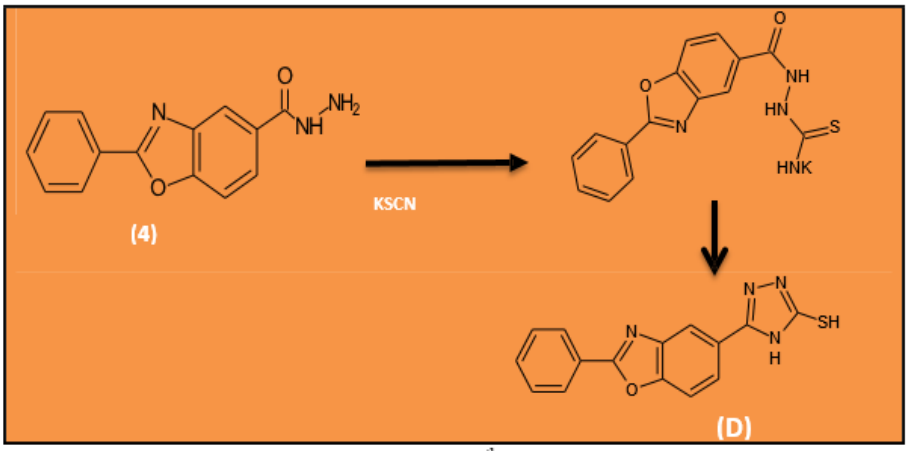
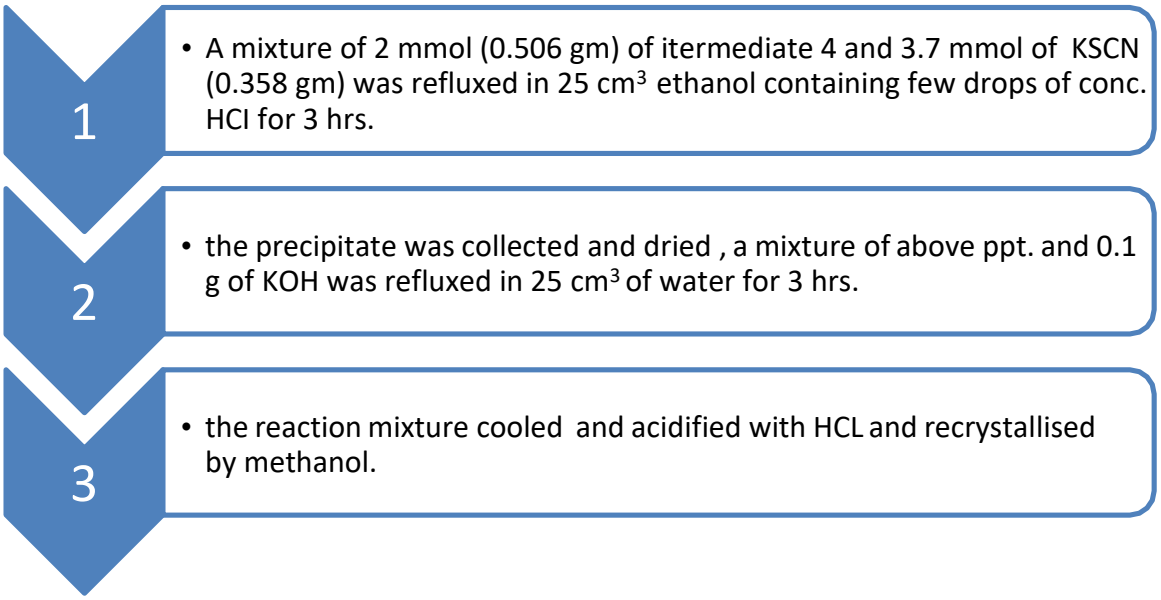


Fig.no. 15 :- flow chart of 5th end products)

Step 8:-



(**Fig.no. 16**: procedure of 6th end product)



(**Fig.no. 17** :- flow chart of 6th end products)

Synthesized compounds are characterized by following methods

Thin layer chromatography :

Thin layer chromatography of compounds carried out by using pre-coated TLC plate with silica gel GF 250[12]. Samples of reactant and products are prepared with its suitable solvents, various mobile phases used such as-

Stationary phase : Pre-coated silica gel GF 250

Mobile phases : methanol : dichloromethane (2:8), methanol: acetonitrile: ethyl acetate(4:4:4)

Detection : UV chamber, iodine chamber

Location of spots : spots were visualised by exposing the plates in UV camber and iodine chamber

Melting point :

The melting points of synthesized compounds were determined by open capillary tube method. The compounds loses its crystallinity at particular temperature were found.

Infrared spectroscopy

ATR-IR spectroscopy used for the IR spectra of synthesised compounds. These instrument does not need of any sample preparation as other instrument needs 'KBr pellets' in this solid sample directly placed on ATR crystal and the spectra is obtained.

R spectroscopy have finger print region (500 cm^{-1} to 1450 $cm^{-1)}$ and functional group region (1450 cm^{-1} to 4000 cm^{-1}) are powerful ranges for identifying the the pure organic and inorganic compounds functional groups, with exception of few diatomic molecules such as O_2, N_2, Cl_2 . with the exception of chiral molecules all the molecular species has a unique infrared spectrum.

NMR Spectroscopy-

To obtained "1H NMR" spectrum of any compounds the solvent used must be free from hydrogen because their the chances of solvent protons interfere with compounds proton.

NMR involves the interaction between electromagnetic radiation and the magnetic field of hydrogen nucleus. NMR enables us to number of equivalent protons and their different types of electronic environment in which proton is presents and help us to find structure of molecules. The number of signals denotes the number of equivalent proton in molecule. The position of signal help to know about nature of protons such as aromatic, aliphatic, vinyl etc. the signal splitting is due to different environment of protons not ith electrons.

MASS Spectroscopy:-

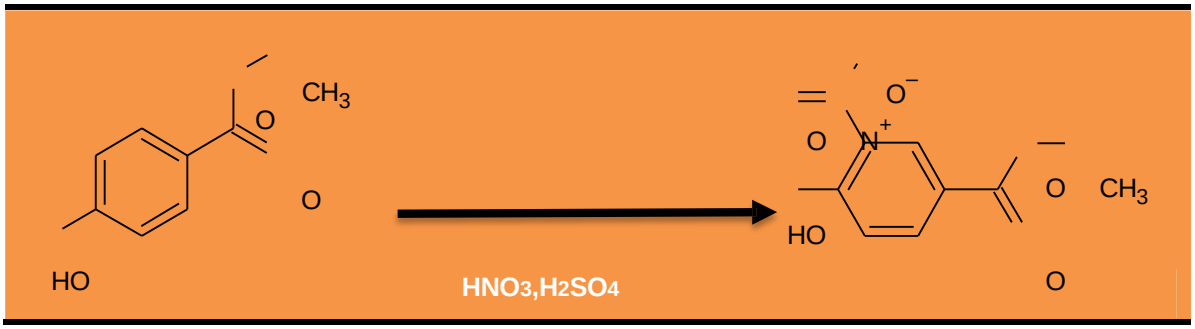
Mass spectroscopy is a technique to find exact molecular mass of compounds. Ionization source and magnetic detector. The commonly used methods as

- Soft ionization techniques
- Hard ionization techniques

In this technique, the compound is bombarded with a beam of electrons to produce ionic fragments of analyte. The relative abundance of fragmentation is depend on stability of ions. The charge particle then separated with their masses.

RESULTS AND DISCUSSION

Synthesis:- Step I:-



In this the p-hydroxy methyl benzoate taken 0.065 mol and treated with nitrating mixture at cold condition the percentage yield was found to be p-Hydroxy methyl benzoate = 4-Hydroxy-3-nitro methyl benzoate (1) 152 gm = 197 gm

10gm = ?

Step II :-

In this step 4-Hydroxy-3-nitro methyl benzoate (1) was taken 0.05 mol and its reduction done by sodium dithionate , the percentage yield was found to be 4-Hydroxy-3-nitro methyl benzoate = 3-Amino 4-hydroxy methyl benzoate

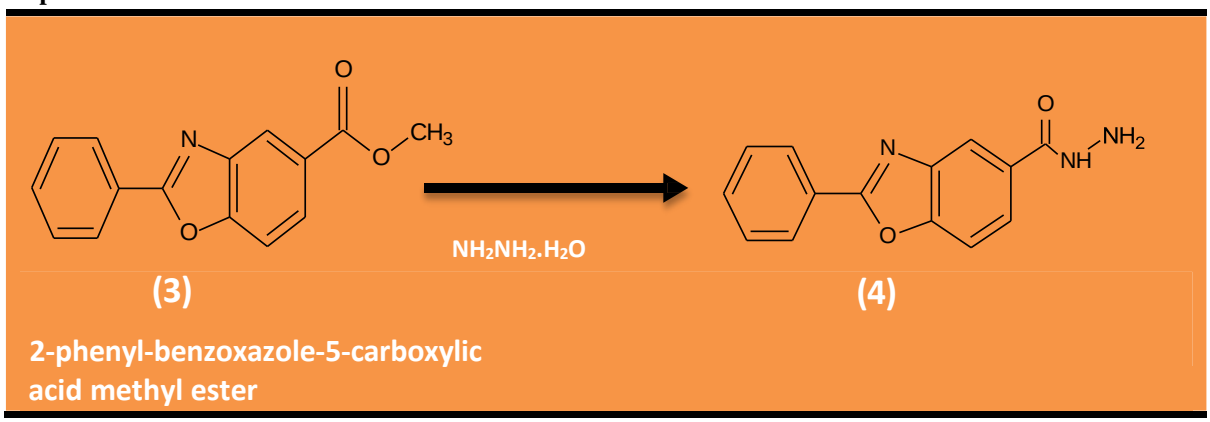
197 gm = 167 gm

9.85 gm = ?

Step III :-

In this step 3-Amino 4-hydroxy methyl benzoate(2) was taken 0.03 mol and benzoyl chloride 0.03 mol and reacted under microwave the resulting percentage yield was found to be 3-Amino 4-hydroxy methyl benzoate(2) =2-phenyl-benzoxazole-5-carboxylic acid methyl ester(3)

Step IV:-



In this step the 2-phenyl-benzoxazole-5-carboxylic acid methyl ester was taken 0.01 mol and 0.15 mol hydrazine hydrate reflux under methanol the percentage yield was found to be- 2-phenyl-benzoxazole-5-carboxylic acid methyl ester(3) = 2-phenyl-benzoxazole-5- carboxylic acid hydrazide

253 gm = 253 gm

2.53 gm = ?

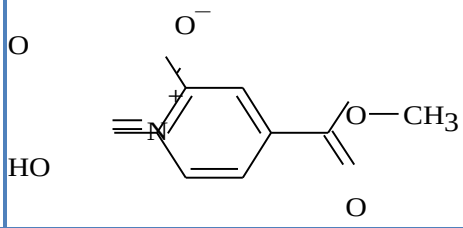
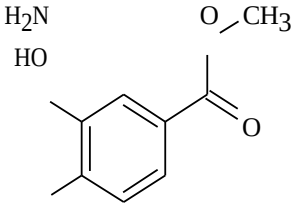
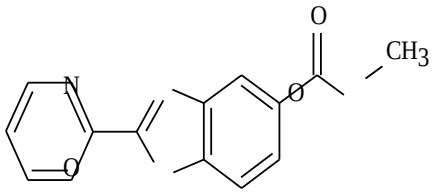
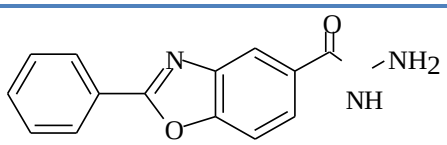
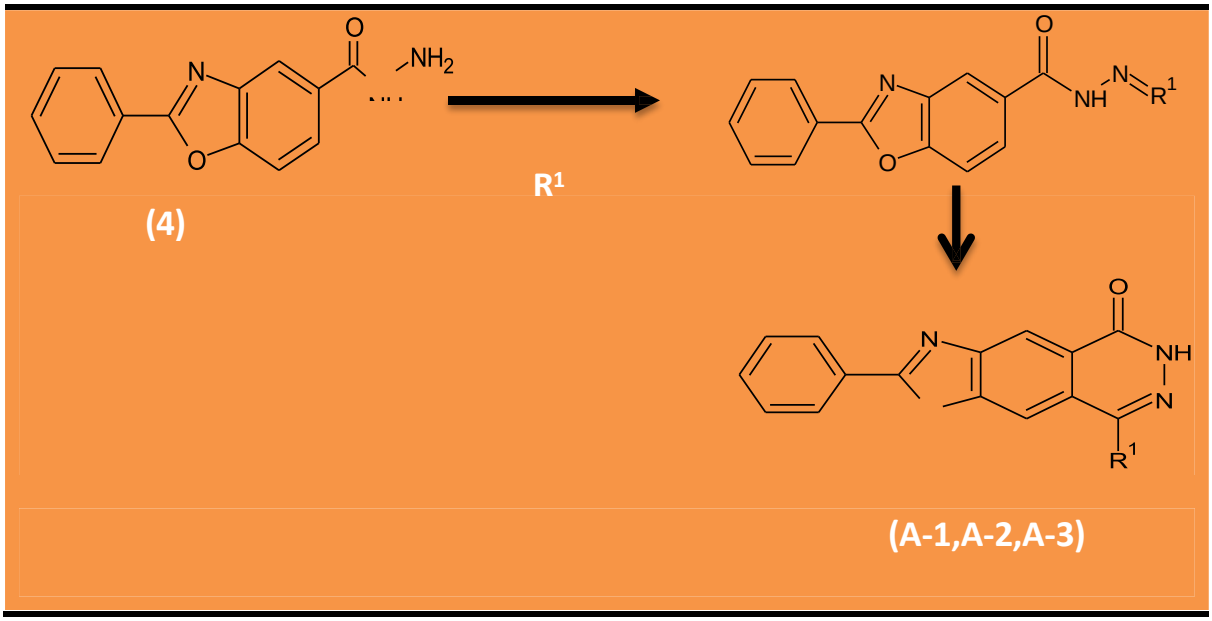
Intermediate	Structure	Yield and melting point
1(4-Hydroxy-3-nitro methyl benzoate)		Theoretical yield = 12.96 gm Practical yield = 10.1 gm Percentage yield = 77.93 % Melting point =67-69ºC
2 (3-Amino 4-hydroxy methyl benzoate)		Theoretical yield = 8.35gm Practical yield = 6.81gm Percentage yield = 81.55 % Melting point =98-100 ºC
3 (2-phenyl-benzoxazole-5-carboxylic acid methyl ester		Theoretical yield = 7.57gm Practical yield = 4.80 gm Percentage yield = 63.40 % Melting point = 134-136ºC
4 (2-phenyl-benzoxazole-5-carboxylic acid)		Theoretical yield = 2.53gm Practical yield = 2.17gm Percentage yield = 85.77 % Melting point = 122-126ºC

Table no.2:- Percentage yield data of intermediates

Step V:-



In ths step the 2-phenyl-benzoxazole-5-carboxylic acid hydrazide was taken 2 mmol and reacted with 4 mmol of substituted benzoyl acid the resulting meltig point was found to be 2-phenyl-benzoxazole-5-carboxylic acid hydrazide = product (A1,A2,A3)

253 gm = ? gm

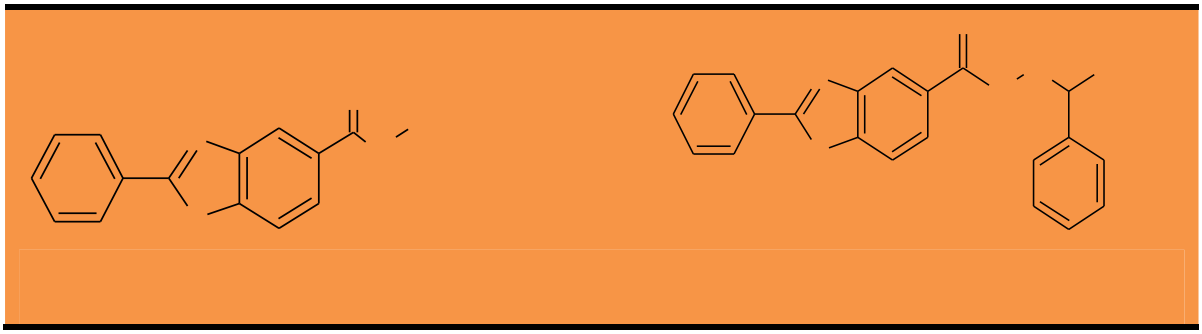
0.506 gm = 'B' gm

Theoretical yield was found to be = 'X'gm

Practical yield was found to be = 'Y'

gm Percentage yield was found to be = 'Z' %

For individual products calculation refer following table-

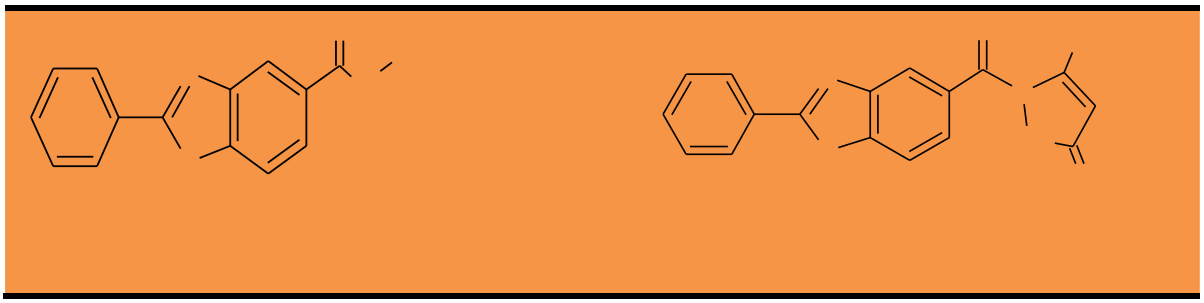


In this reaction 2-phenyl-benzoxazole-5-carboxylic acid hydrazide was taken 2 mmol and reacted with 2 mmol of acetophenne the resulting percentage yield was found to be

2-phenyl-benzoxazole-5-carboxylic acid hydrazide = product (B)

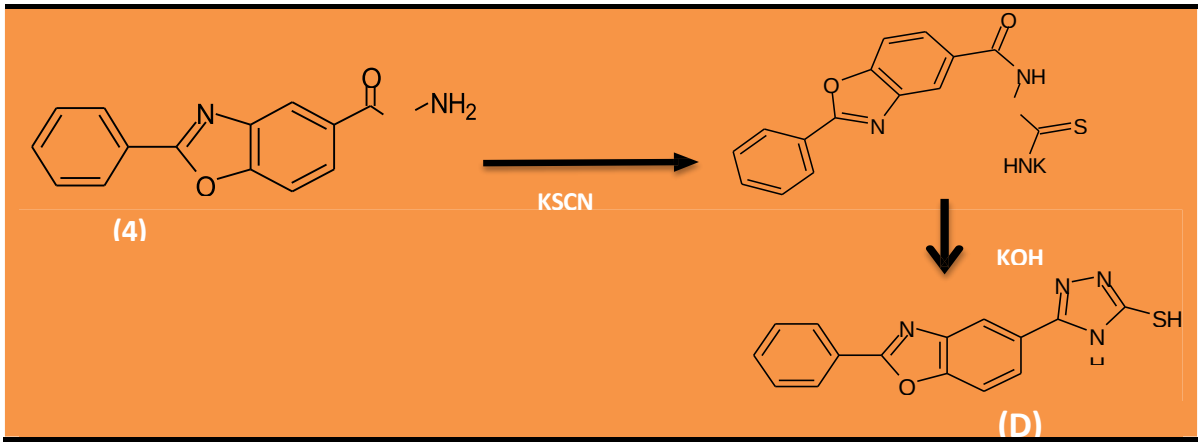
253 gm = 355 gm

0.506 gm = ?



In this reaction intermediate 4 react with ethylacetoacetate and refluxed the resulting percentage yield was found to be - 2-phenyl-benzoxazole-5-carboxylic acid hydrazide = product (C)

253 gm = 319 gm



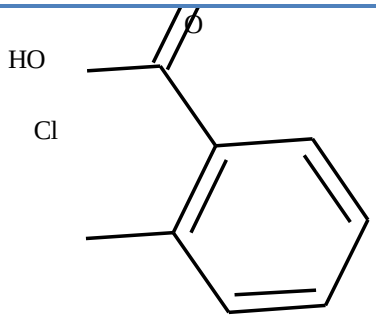
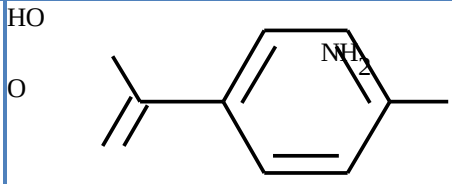
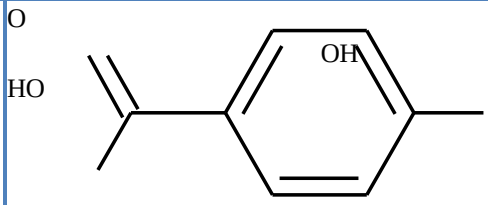
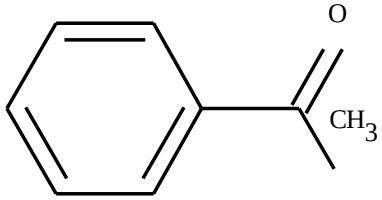
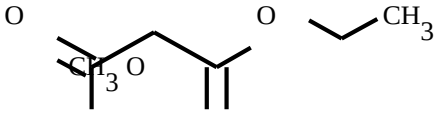
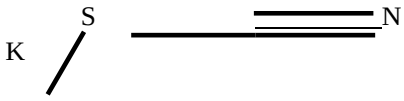
0.506 gm = ?

In this step the intermediate 4 react with potassium thiocyanate and treated with potassium hydroxide the percentage yield was found to be 2-phenyl-benzoxazole-5-carboxylic acid hydrazide = product (D)

253 gm = 294 gm

0.506 gm = ?

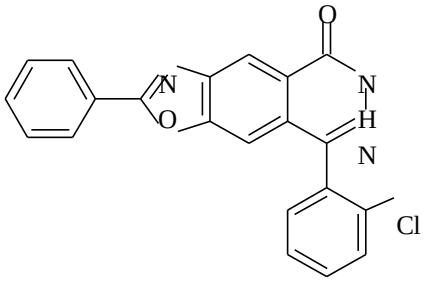
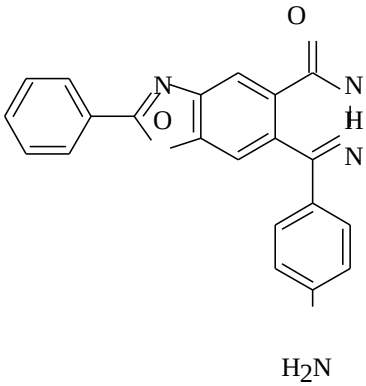
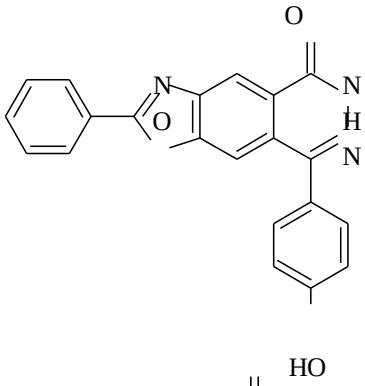
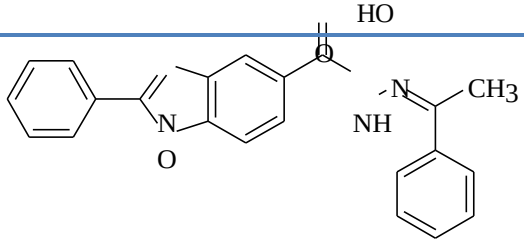
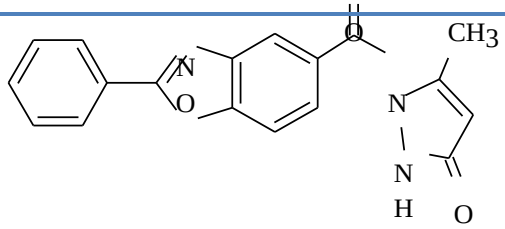
Table No. 3:- End products percentage yields

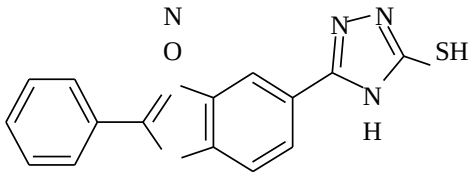
Compound No.	Reactants	Yield and melting point
A1		Theoretical yield = 0.746 gm Practical yield = 0.361 gm Percentage yield = 48.39 % Melting point = 166-168 ºC
A2		Theoretical yield = 0.708 gm Practical yield = 0.479 gm Percentage yield = 67.65 % Melting point =132-134 ºC
A3		Theoretical yield = 0.710gm Practical yield = 0.438 gm Percentage yield = 61.69 % Melting point = 224-226 ºC
B		Theoretical yield = 0.71gm Practical yield = 0.481 gm Percentage yield = 67.74 % Melting point =198-202 ^{O}C
C		Theoretical yield = 0.638 gm Practical yield = 0.421 gm Percentage yield = 66.98 % Melting point = 312-314 ^{O}C
D		Theoretical yield = 0.588 gm Practical yield = 0.543gm Percentage yield = 92.34 % Melting point = 326-328 ^{O}C

Characterization:-

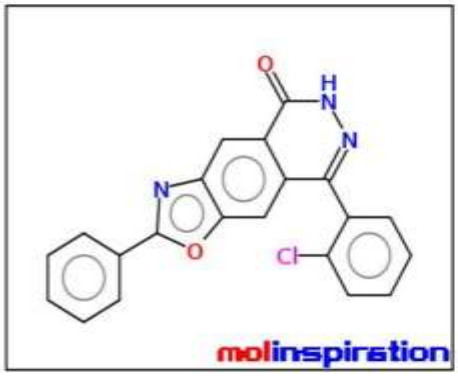
Results include the characterization of all synthesized compounds as depicted below. All the synthesized compounds are analyzed by TLC, IR, MASS spectroscopy.

Table no. 4:- synthesized compounds

Compounds	Structures	IUPAC name
A1		8-(2-chlorophenyl)-2-phenyl[1,3]oxazolo[4,5-*g*]phthalazin-5(6*H*)-one
A2		8-(4-aminophenyl)-2-phenyl[1,3]oxazolo[4,5-*g*]phthalazin-5(6*H*)-one
A3		8-(4-hydroxyphenyl)-2-phenyl[1,3]oxazolo[4,5-*g*]phthalazin-5(6*H*)-one
B		2-phenyl-*N*'-[(1*Z*)-1-phenylethylidene]-1,3-benzoxazole-5- carbohydrazide
C		5-methyl-1-(2-phenyl-1,3-benzoxazole-5-carbonyl)- 1,2-dihydro-3*H*-pyrazol-3- one

D		5-(2-phenyl-1,3-benzoxazol- 5-yl)-4*H*-1,2,4-triazole-3- thiol

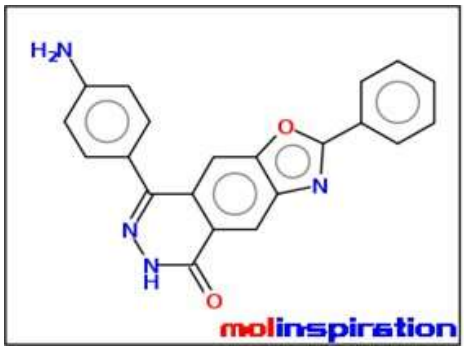
1. **For molecule -A1**



Molinspiration property engine v2018.10

miLogP	5.15
TPSA	71.79
natoms	27
MW	373.80
nON	5
nOHNH	1
nviolations	1
nrotb	2
volume	305.60

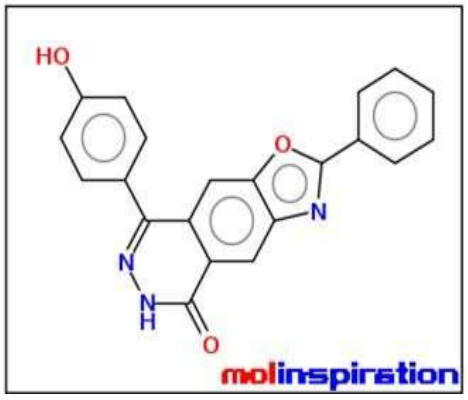
2. **For molecule- A2**



Molinspiration property engine v2018.10

miLogP	3.59
TPSA	97.81
natoms	27
MW	354.37
nON	6
nOHNH	3
nviolations	0
nrotb	2
volume	303.36

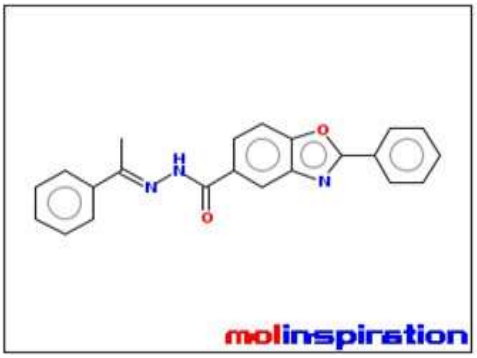
3. **For molecule- A3**



Molinspiration property engine v2018.10

miLogP	4.04
TPSA	92.01
natoms	27
MW	355.35
nON	6
nOHNH	2
nviolations	0
nrotb	2
volume	300.09

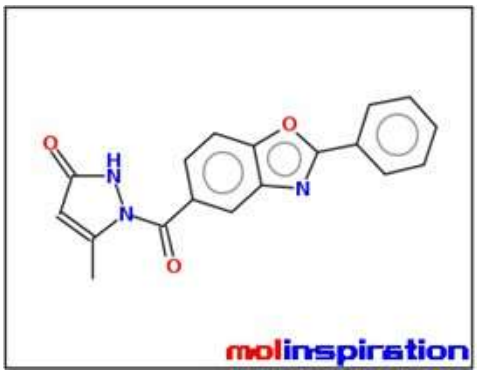
4. **For molecule- B**



Molinspiration property engine v2018.10

miLogP	4.70
TPSA	67.49
natoms	27
MW	355.40
nON	5
nOHNH	1
nviolations	0
nrotb	4
volume	319.47

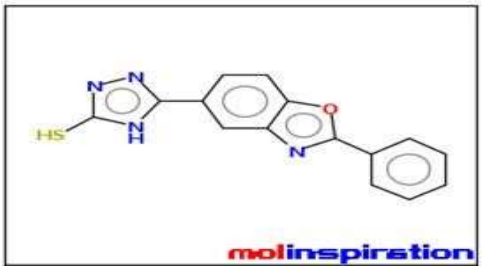
5. For molecule -C



Molinspiration property engine v2018.10

miLogP	3.21
TPSA	80.90
natoms	24
MW	319.32
nON	6
nOHNH	1
nviolations	0
nrotb	2
volume	273.14

6. For molecule D



Molinspiration property engine v2018.10

miLogP	3.91
TPSA	67.61
natoms	21
MW	294.34
nON	5
nOHNH	1
nviolations	0
nrotb	2
volume	242.60

DISCUSSION:

All the above compounds synthesized from 2-phenyl benzoxazole -5 carboxylic acid hydrazide with the reactive carboxylic group , acetophenone analogs to forming cyclization followed by peptide bond .

In the synthetic pathway, during synthesis of intermediate 2 i.e, reduction of nitro group into amino group done by sodium dithionite this reaction was optimized by using various reducing agents like Zn/HCL, stannous chloride, copper sulphate and sodium borohydride but there is no desired product obtained.

The purity of all synthesized compounds were confirmed by melting point and thin layer chromatography

Synthesized compounds chemical structure were confirmed by various spectroscopical techniques such as IR, NMR, MASS. And before the synthesis of this molecule first confirmed their conformational structure , chirality and then check their toxicity using tool OSIRIS toxicity predictor.

CONCLUSION

The selecting molecule were synthesized by microwave and conventional method and its purity were determined by the TLC and their physical constant, the structure of molecules were assessed by the various spectroscopy like IR and MASS and confirmed.

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