SYNTHESIS OF BIOACTIVE HETEROCYCLES WITH TWO HETEROATOMS

REPORT SUBMITTED TO
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BY

Puneet Chaucer
Registration no: 11500931

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M.Sc. (Honors) Chemistry

UNDER THE GUIDANCE OF

Dr. PRAVEEN KUMAR SHARMA

ASSOCIATE PROFESSOR

LOVELY PROFESSIONAL UNIVERSITY

PHAGWARA, PUNJAB (INDIA)

School of Physical Science, Department of chemistry
Lovely Professional University Punjab, India.
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CERTIFICATE BY GUIDE

This is to certify that this report for pre dissertation is a bonafide work done by **Puneet Chaucer** in partial fulfillment of the requirement for the Degree of Master of Science Hons. (Chemistry).

Date:
Place:

Supervisor
Dr. Praveen Kumar Sharma
Associate Professor,
Department of Chemistry,
Lovely Professional University

Acknowledgement

"It is not possible to prepare a project report without the assistance and encouragement of the other people. This one is certainly no exception".

On the very outset of this report, I would like to extend my sincere and heartfelt obligation toward the entire person's who helped me in the venture, without their active guidance, help, cooperation and encouragement, I would not have made progress in the project.

My profound gratitude and deep regards to my guide Dr. Praveen Kumar Sharma for her exemplary guidance, monitoring and constant encouragement throughout the course of this report. The blessing, help and guidance given by her time to time shall carry me a long way in the journey of life on which I am about to embark.

I extend my gratitude to Lovely Professional University for giving me this opportunity.

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Thanking you

Puneet chaucer

Registration .No. 11500931

Table of Content

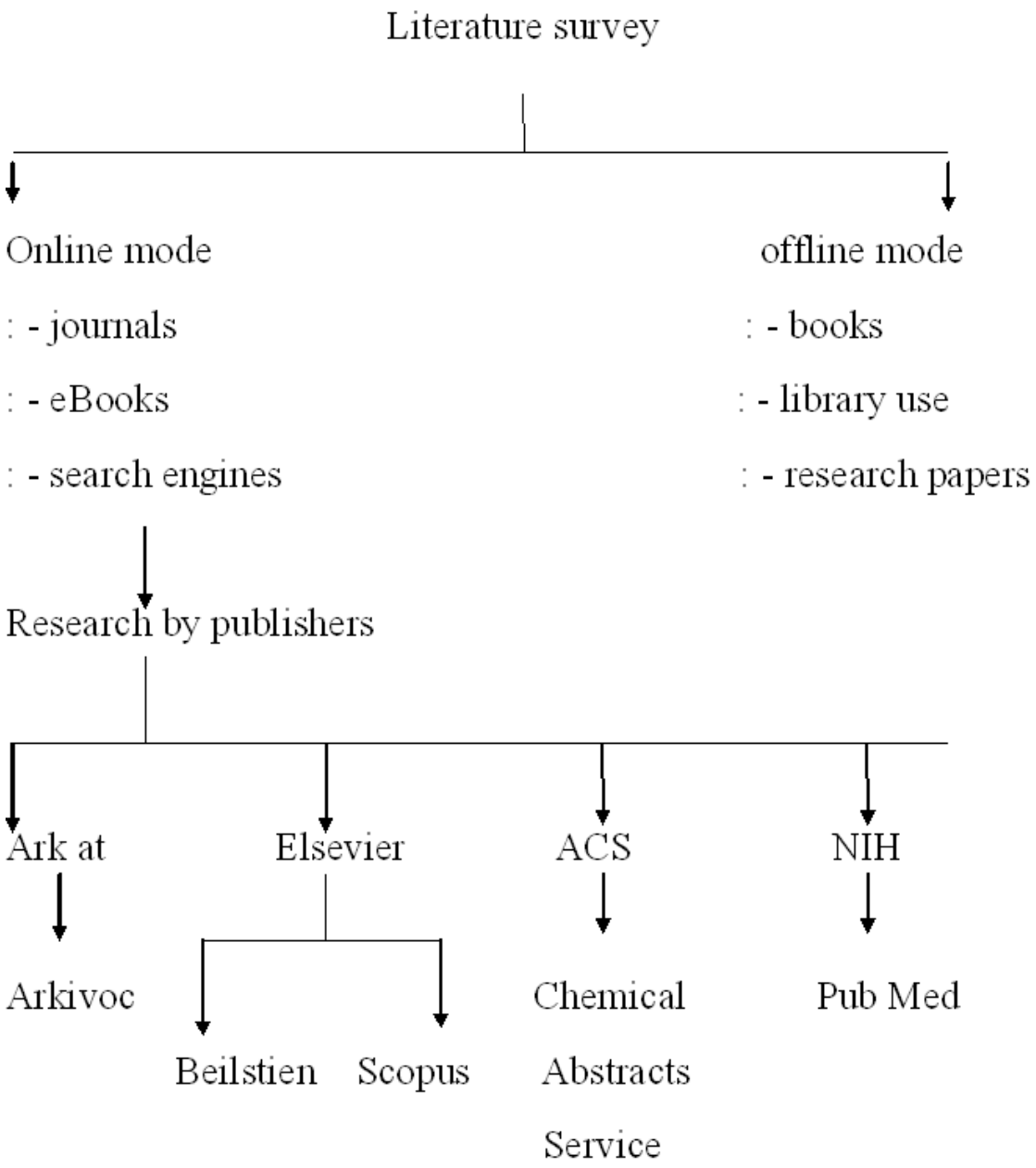
Introduction

Organic compounds plays very important role in living society[1] and have wide range of its application in different fields due to which research has been going on to synthesis new organic compounds with their derivatives. Heterocyclic compounds are the cyclic Compounds contain one or more heteroatom in a ring. These are aromatic and partially or fully saturated[2]. There are two types of heterocyclic compounds i.e. natural or synthetic both contains heteroatom such as N, S, O and many more but in this current review we are just talking about those elements that's mainly contains Nitrogen, Sulphur, and Oxygen atom[3]. These compounds have potential biological importance in our daily life products and show bioactivity[4] such as anti-tubercular, anti-bacterial, anti-microbial, anti-tumor, insecticidal, fungicidal, herbicidal agents, tranquilizer, anti-oxidant, anti-inflammatory and in various dyes etc. heterocyclic compounds also used to make some drugs. Thiazine- a heterocyclic compound containing four carbon atoms and one nitrogen and sulphur atom at various position in six member ring with different forms as 1,2; 1,3; 1,4;- thiazine and their derivatives examples are given in the table no.1. Morholine are also a type of heterocyclic compounds with four carbon atoms, one nitrogen and one oxygen atom in six member rings and found as major constituents of pharmaceutical drugs and heterocyclic systems. Thus for our ongoing interest of identifying biological important heterosystems we design a problem based on both heterosystems: Thiazine and Morholine incorporating antimicrobial and antioxidant activities.

Literature survey

Literature survey is the review of the papers, journals and, books which have the current knowledge as well as previous information about the term in theoretical and methodological contribution to a suitable topic. It also gives us a help to us for finding the best evidence for doing our work. A literature survey provides up-to-date information about the subject and its significance to practice. It also identifies the methods used in previous research on that particular topic and helps to work out on that then end with further work or conclusion that we will get from our survey. Literature survey includes the format of work done. Necessary things for work get by using online as well as offline mode. In offline literature search carried out by the use of books and review papers from library, as well as online mode carried out by the use of internet with the help of search engines and publisher websites for journals and eBooks.

The path used for literature survey is shown below



Search engines

Search engines is a system software that is use to search information on World Wide Web. Its generally contain pages, images, text and all other types of documents. Some of search engines contain database, and web directories which

are suitably maintained by human right. All search engines have their publisher as well owner. Types of search engines preferred by their publisher that we used for our plan.

Arkat

It was first established in year 2000. It is firstly get by personal donation from Alan R. Katritzky and Linde Katritzky. Arkivoc is being published by arkivoc. Its impact factor on 2014 is ranging of 1.165, and its rank is 37th out of 57 journals in the category of organic chemistry.

NIH

IT is publisher of PubMed. It is free search engine accessing the MEDLINE database. It contains the reference and information on life sciences and biomedical topics. The national institutes of health maintain the database of information retrieval. MEDLINE online accessed to MEDLARS online by 1971 to 1997. It is first released in January 1996 and then its freely used by public from June 1997.

Elsevier B. V.

It is an academic publisher company. Which is mainly published the medical and scientific literature. It has an operation in UK, US, Spain, and elsewhere. It is yearly publishes approximately 350,000 articles in 2000 journals. It is found in 1880 and also it is oldest and largest company contains this much large database.

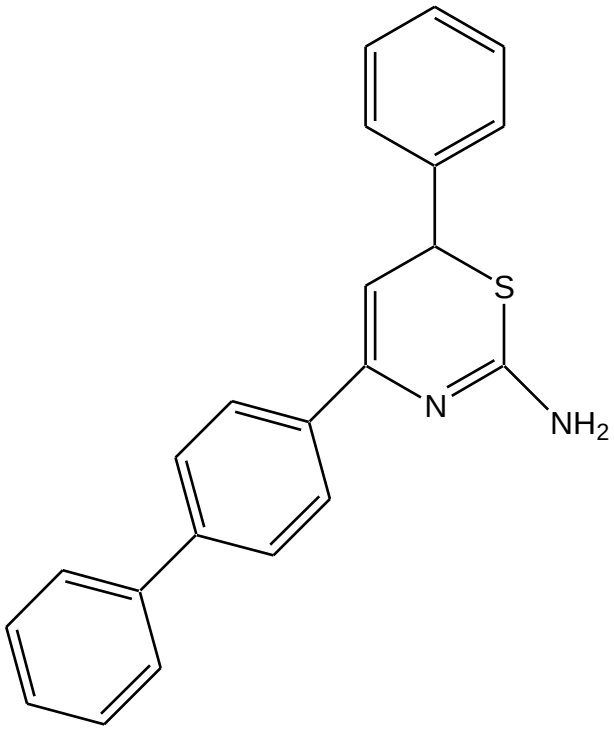
Elsevier has two main operating divisions i.e. science & technology and health sciences. Science direct is Elsevier's path for research. It has numbers of journals and 6000 eBooks.

By using above search engines we review our biological activity of heterocyclic compounds.

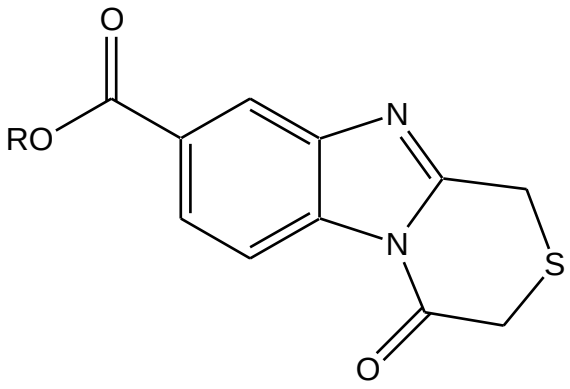
Biological activity of thiazine based on literature review.

Thiazines have numbers of biological activities some important are give as:-

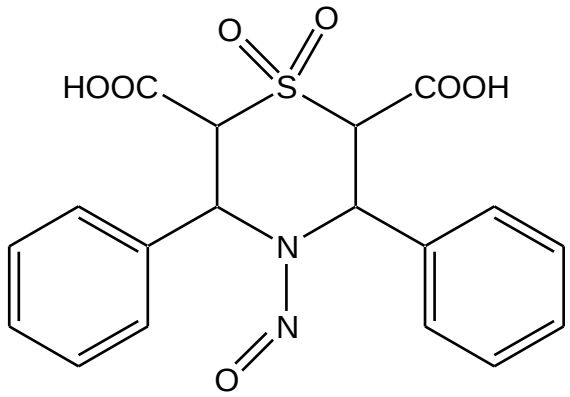
Ingarsal, N et.al;(2006) A series of new 2-amino-4-(1,1_-biphenyl-4-yl)-6-aryl-6H-1,3-thiazines has been synthesized, characterized by IR, 1HNMR, 13C NMR, mass and elemental analyses and evaluated for *in vitro* antibacterial activity against some Gram-positive and Gram-negative bacteria. The antibacterial data revealed that the compounds had better activity against tested organisms than the reference norfloxacin [5]



Shadia A G et.al; (2011) reported about 1,3-dihydro-4H-benzo[4',5']imidazole [2,1 c][1,4]thiazine -4-one-8-carboxylic acid derivatives. These compounds were tested against antiviral activity they were also tested against their herpes simplex virus[6].



Naushad E et al; (2012) synthesized N-nitroso-2, 6-dicarboxy-3, 5 diaryltetrahydro-1,4 thiazine-1,1dioxides . They are tested against anti-bacterial and antifungal activity. This compound also exhibit antifungal activity[7].



S. P. Rathod et al (2010; The synthesis, spectral analysis and biological activities of some 4-phenyl-2-hydroxy-chlorosubstituted-2-imino-1, 3-thiazene with phenyl thiourea and diphenyl thiourea have been carried out in two series.. The Antibacterial activities of this compound were studied[8].

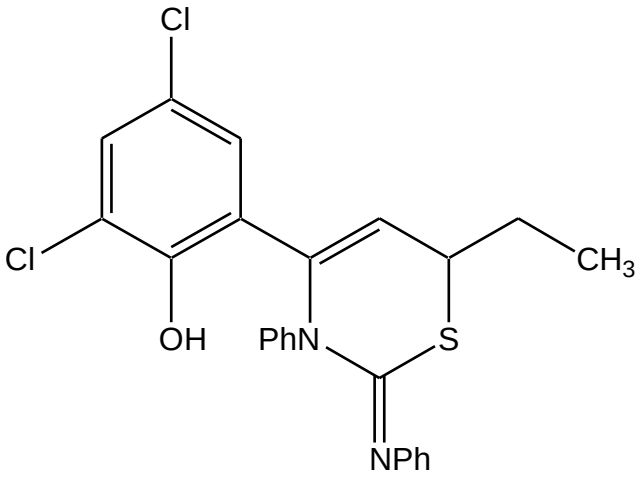


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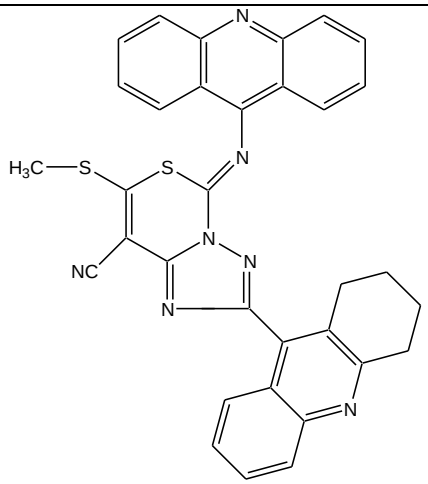
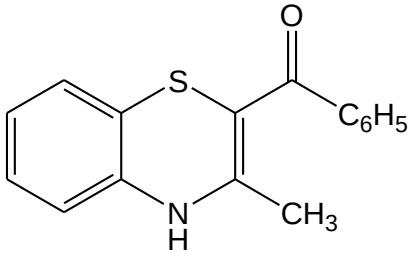
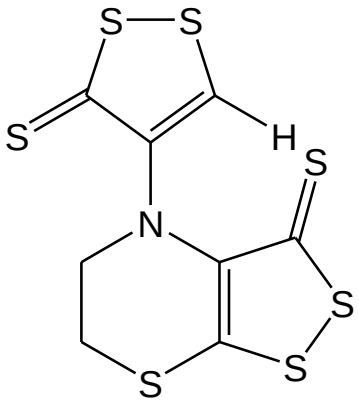
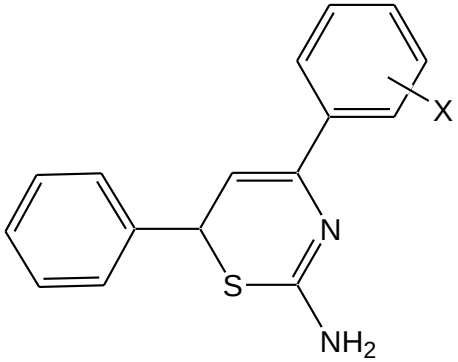
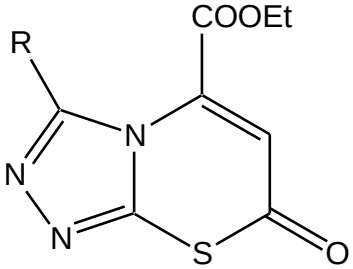
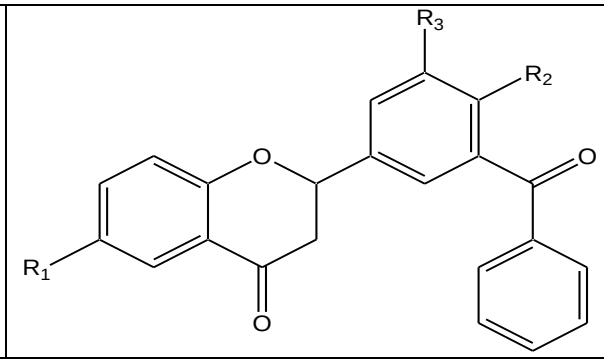
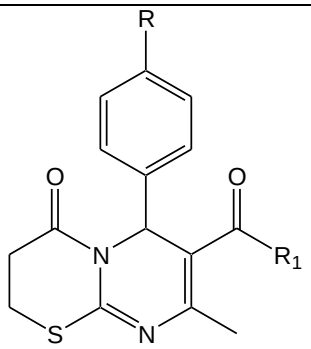
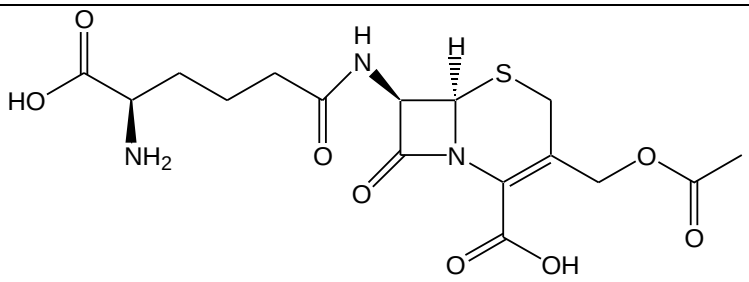
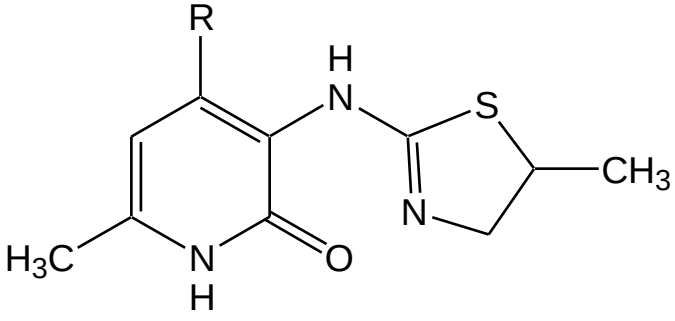
Table no.1 Important examples of activity of thiazine compounds

Sr. no,	Structure	Activity	Reference
1.		Anti fungal	9
2.		Anti fungal	10

3		Antimicrobial	11

4.		antibacterial	12
5.		antibacterial	13

6.		antimicrobial	14

7.		antibacterial	15
8.		antimicrobial	16
9.		antioxidant	17

Scope of Study

On the basis of literature survey, we identify some heterocyclic compounds containing nitrogen and sulphur atoms exhibit potential biological and pharmacological activities. The heterocycles showing biological/Pharmacological activities are used in various drugs that are useful for mankind. Their derivatives possess various types of biological activities such as antibacterial, anticancer, anti tubercular, antifungal, analgesic, and anti-HIV activities anti-oxidants etc.

Work Plan/Methodology

Work plan further classified in two major classes.

a) Synthesis of heterocycles containing two or more heteroatom.

b) Biological evaluation of synthesized heterocycles.

Research Methodology of biological activity of heterocyclic compounds

Heterocycles generally contains two or three heteroatoms. By their presence they show enormous behavior so due to this they have numbers of biological activity in their real use such as anti-tubercular, anti-bacterial, anti-microbial, anti-tumor, insecticidal, fungicidal, herbicidal agents, tranquilizer, anti-oxidant, anti-inflammatory

Preparation of morpholine substituted 4H-1,4-benzothiazines II

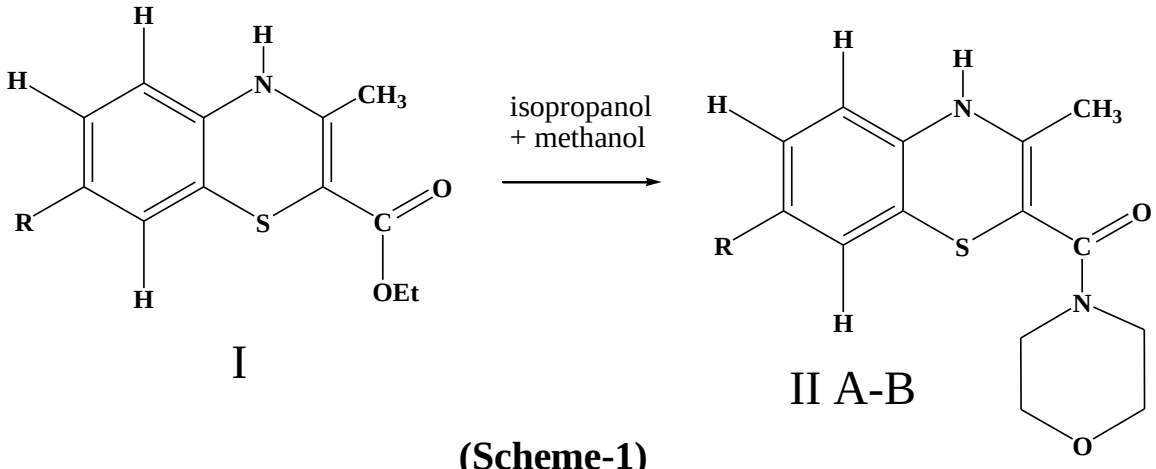
Experimental Methods

1. Preparation of morpholine substituted 4H-1,4-benzothiazines II

Substituted 4H-1,4-benzothiazine IIA-B were synthesized by the use of method present in literature[18-25].

IIA 3, 7-Dimethyl-2-(4'-morpholinylcarbonyl)-4H-1,4-benzothiazine

IIB 3-Methyl-2-(4'-morpholinylcarbonyl)-4H-1,4-benzothiazine



(Scheme-1)

IIA, R =CH_3

IIB, R = H

Scheme-1:- Schematic presentation for the synthesis of substituted 2-(4'-morpholinylcarbonyl)-4H-1,4-benzothiazine

2. Antimicrobial Activities

Antibacterial is a type of drug that use to destroys bacteria or control their growth. Also antibacterial drug used to treat some skin problems. Antibacterial agents are classified into two terms bactericidal agents, who generally kill bacteria, and bacteriostatic agents, which slow down the bacterial growth[26, 27].

Compound (IIA) were screened for their antimicrobial activity against bacteria *Bacillus alkalophilus* (MTCC No. 7913), *Bacillus subtilis* (MTCC NO. 411), *Bacillus flexus* (MTCC NO. 7024), as well as their antifungal activity against *A. Flexus, A. Nigrum.* Except this compound (IIB) were also be screened for their antimicrobial activity against bacteria *Halobacteria salinarium* (NCIM NO. 2854) by disc diffusion method.

Material, Chemicals and Instrumentation Required

Potato-dextrose agar + Broth
Nutrient Agar + Broth
Agar-Agar
Ethanol
Bacteria Used: *Halobacteria,Bacillius mageterium*
Fungi Used: *Trichoderma,Aspergillius*
Petri plates

Para film foil
Graduated pipettes
Hot Air oven
Autoclave
Laminar
Incubation

Procedure

The bacterial inoculums repair to definite concentration was inoculated on to the total face of a plate through a sterile cotton-tipped pad to form an even lawn. The above components were dissolved and sterilized in an autoclave at 121 ^{o}C for fifteen minutes at *15lbs pressure*. The sterilized medium was release into different sterilized Petra-plates in laminar, and was endorsed to solidify. The paper disks saturate with diluted sample compound solution was placed on the surface of each nutrient agar plate using a sterile pair of tongs. Plates were aerobically incubated and the diameter of zone inhibition was observed by a calliper. Based on inhibition zone and the results were assigned to three categories, resistant susceptible or intermediate. Bigger the inhibition zone, more susceptible is the microorganism to the antimicrobial compound**.** Cultures having 10^{5}CFU/mL were used against each concentration levels. The impregnated disks were placed on the medium suitably spaced apart, and the plates were incubated at 37 ^{o}C for 24 h and 28^{o}C for fungal species[28].

Table-2 Antibacterial activities of IIA at different concentration in ethanol as control.

Name of Bacteria	Zone of inhibition in different concentration in (mm)			
	250ppm	200ppm	150ppm	100ppm
Bacillus alkalophillus (MTCC NO. 7913)	12mm	13.5mm	8mm	7mm
Bacillus subtilus (MTCC NO. 411)	9mm	7mm	7mm	7mm
Bacillus flexus (MTCC NO. 7024)	7mm	8mm	8mm	6mm

Table-3 Antifungal activity of IIA at different concentrations in ethanol as control

Name of Fungal	Zone of inhibition in different concentration (in mm)		
	200ppm	150ppm	100ppm
Aspergillus nigrum	17mm	12mm	15mm
Aspergillus flexus	19mm	17mm	21mm

Table-4 Antibacterial activities of IIB at different concentration in ethanol as control.

Name of bacteria	Zone of inhibition in different concentration in (mm)			
	1000ppm	500ppm	250ppm	125ppm
Halobacteria salinarium (NCIM NO. 2854)	15mm	7mm	9mm	14mm
Bacillus megaterium (MTCC NO. 8510)	0mm	0mm	0mm	0mm

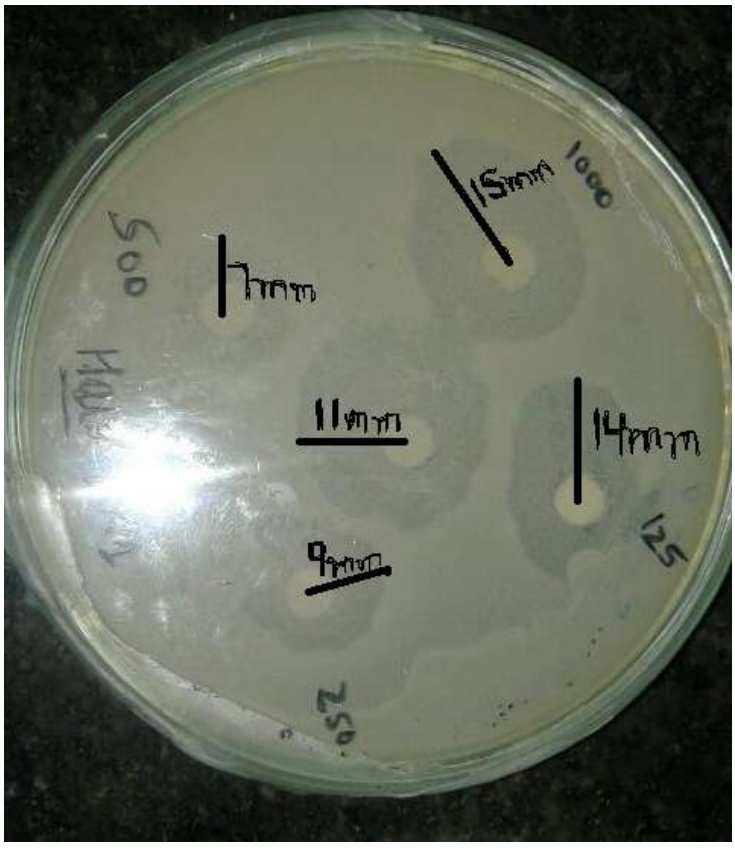


Fig 1. Antibacterial activity of compound IIA on *Halobacteria salinarium* (NCIM NO. 2854)

Table- 5 Antibacterial activities of FCN (Flucanzole) standard at different concentration in ethanol as Control.

Name of bacteria	**Zone of inhibition in different concentration in (mm)**			
	1000ppm	**500ppm**	**250ppm**	**125ppm**
Bacillus megaterium (MTCC NO. 8510)	10mm	8mm	9mm	0mm
Halobacteria salinarium (NCIM NO. 2854)	0mm	0mm	0mm	0mm

Antioxidant activities

Antioxidant activities of the heterocyclic compounds are very beyond. Antioxidant is a molecule that carries the oxidation of other molecules. Oxidation is a chemical reaction that involves the loss of electrons which can produce free radicals. Antioxidant compounds in our diet play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk form those diseases which cause cancer and heart diseases. To measure antioxidant activity is well documented for our need.[29]

Antioxidant activity of **IIA** were tested by DPPH method

Sample preparation for the antioxidant activity of compound (IIA)

Add 0.0197 milligram of DPPH to the 100 mL of methyl alcohol and centrifuged to prepare 0.5 milimolar of DPPH solution[30].

Principal solution prepared by adding up of 2.5 milligram of compound IIA to 100 mL of methyl alcohol. Dilution was prepared by addition of 2, 4, 6, 8, 10 microgram/ml solution from principal solution.

A spectrum of DPPH solution and sample solution was taken separately. Then from each of the sample solution 4.5 ml of solution is to be taken and to that of the above diluted sample solutions, 0.5 ml of DPPH solution is to be added. Measure the wave length of each of the sample solution separately by UV.

Analysis of Antioxidant activities:-

Percentage inhibition is calculated by given principle.

% of inhibition = $\{(control - sample) \div control.\}$* 100

Control = absorbance of DPPH solution

Sample=DPPH +sample

Calculation for compound (II)

Absorption of control at 517nm= 0.1123

Absorption of sample at same wavelength= 0.004

% of inhibition= 96.43%

Result and Discussion

Substituted-(4'-morpholinylcarbonyl)-4H-1,4-benzothiazine II(A-B) shows antimicrobial as well as antioxidant activity against different strains of microbes. Compound 3,7-dimethyl-2-(4'-morpholinylcarbonyl)-4H-1,4-benzothiazine (IIA) shows superior activity against *Bacillus alkalophillus* in comparison with *B. subtilus and B. flexus*. Except this 3,7-dimethyl-2-(4'-morpholinylcarbonyl)-4H-1,4-benzothiazine (IIA) exhibit better result against *Aspergillus flexus* in comparison to *A. nigrum* It has been found that the combination of two biodynamic heterosystems: morpholine and thiazine, results in the formation of a new heterocyclic scaffold through significant bioactivity. 3,7-dimethyl-2-(4'-morpholinylcarbonyl)-4H-1,4-benzothiazine (IIA) exhibit antioxidant activity by DPPH process. Thus from the results, 3,7-dimethyl-2-(4'-morpholinylcarbonyl)-

4H-1,4-benzothiazine(IIA) shows sufficient variety of antimicrobial and antioxidant activities

Compound 3-methyl-2-(4'-morpholinylcarbonyl)-4H-1, 4-benzothiazine (IIB) screened for their antimicrobial activity against bacteria *Halobacteria salinarium* significantly shows better results than standard thereby standard shows better results rather than compound IIB, for *Bacillus megaterium*

Conclusion

On the basis of literature and above results. It has been confirmed that morpholine containing benzothiazine heterocycles has an enormous conformity of attention to the chemist and medicinal chemist as potential antimicrobial agents. Besides having antimicrobial activities, morpholinyl-benzothiazines have also been recognized as antioxidant agents

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List of Publication

1. Puneet Chaucer, Praveen Kumar Sharma, Gulshan Kumar, and Leena Parihar; "*Antimicrobial and Antioxidant activities of substituted 4H-1, 4-benzothiazines*" **AIP Conference Proceedings (RAFAS)** communicated.

2. Puneet Chaucer, Praveen Kumar Sharma; *A review: "Thiazine consider as potential anticancer agents"*; **International journal of pharmaceutical quality assurance (IJPQA)** Communicated.