KUVEMPU UNIVERSITY

"SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL INVESTIGATION OF BENZOXAZOLE DERIVATIVES"

Thesis Submitted to the Faculty of Science,
Kuvempu University for the award of the Degree of

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

Submitted By

Mr. Ashok Kumar N. B.

Research Guide

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Feb-2019

DECLARATION

I hereby declare that the research work presented in this thesis entitled **"SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL INVESTIGATION OF BENZOXAZOLE DERIVATIVES"** is entirely original and was carried out by me in the Department of Chemistry under the supervision of **Dr. K. P. Latha,** Associate Professor, Department of Chemistry, Sahyadri Science College, Shivamogga, I further declare that the results presented in the thesis or any part thereof has not been submitted elsewhere for any other degree, diploma of similar title in any other Universities.

Date: 28-02-2019

Place: Shankaraghatta

Ashok Kumar N. B

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Certificate

This is to certify that the work reported in this thesis entitled **"SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL INVESTIGATION OF BENZOXAZOLE DERIVATIVES"** submitted by **Mr. Ashok Kumar N. B.** to the Faculty of Science, Kuvempu University, for the award of **Doctor of Philosophy in Chemistry** is a record of the bonafide and original research work carried out by him under my guidance and direct supervision. The work reported in this thesis has not formed the basis for the award of any degree or diploma or any other similar title.

Date: 28-02-2019

Place: Shivamogga

Dr. K.P. LATHA

(Guide)

DEDICATED TO MY BELOVED FAMILY MEMBERS, TEACHERS AND FRIENDS

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Finally, I am truly grateful for the many hands and hearts that helped me directly or indirectly during my research work.

Mr. Ashok Kumar N. B.

CONTENTS

Instrumentation details

Melting points

Melting points were recorded on electro-thermal melting point apparatus and are uncorrected.

IR spectroscopy

The FT-IR spectra of the compounds were taken in KBr pellet (100 mg) using Shimadzu Fourier Transformed Infrared (FT-IR) Spectrophotometer.

NMR spectroscopy

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer in IISc, Bangalore, Karnataka, IIIT Chennai and MIT Manipal, India. The chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal standard.

Mass spectroscopy

LC-MS were obtained using C 18 column on Shimadzu, LCMS 2010A, Japan. The column chromatography was performed using silica gel (230-400 mesh).

Thin Layer Chromatography (TLC)

Silica gel GF254 plates from Merck were used for TLC and spots located and identified by UV Chamber. The chemicals were purchased from Sigma-Aldrich Co and from SD Fine chemicals. The solvents for column chromatography were of reagent grade and were purchased from commercial source.

CHAPTER I

INTRODUCTION

1 General Introduction

The practice of medicinal chemistry is devoted to the discovery and development of new drugs for treating disease. An important role of medicinal chemistry has been to establish a relationship between chemical structure and pharmacological activity. The chemistry of heterocyclic compounds is the most important in the discovery of novel drugs. The study of these compounds is of immense interest both in theoretical as well as practical aspects.

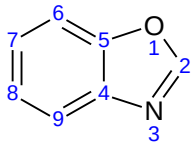
Various compounds like alkaloids, essential amino acids, vitamins, hemoglobin, hormones and a large number of synthetic drugs contain heterocyclic ring structure. There are large numbers of synthetic heterocyclic compounds like pyrrole, pyrrolidine, furan, benzoxazole, piperidine, pyridine and benzimidazole played an important role in medicinal filed and many are important intermediates in synthesis[1].

Oxazole derivatives are well-known five-membered nitrogen containing heterocyclic compounds. They are highly versatile intermediates used for the synthesis of numerous organic molecules, including amino acids, peptides, antimicrobial or antitumor compounds, immunomodulators, heterocyclic precursors for biosensors coupling and photosensitive composition devices for proteins[2-4]. The benzoxazole and its derivatives are known to exhibit high therapeutic efficiency as antibacterial, antifungal, antitumor, anti-tubercular, anti-inflammatory and HIV-1 reverse transcriptase inhibitory activities[5-9].

Introduction to benzoxazole

Benzoxazole is one of the most important heterocycle exhibiting remarkable pharmacological activities. Benzoxazole is an organic compound, which has benzene fused with an oxazole ring. Oxazole is having oxygen atom and

a pyridine type nitrogen atom at the 3-position in a five-member ring. A little change in the substitution pattern of benzoxazole nucleus causes a distinguishable difference in their pharmacological activities.



Benzoxazole is an aromatic organic compound having benzene fused oxazole ring structure with molecular formula of C_7H_5NO. It is white to light yellow in colour with an odour similar to pyridine. Benzoxazole is a planar molecule with conjugated π electrons sextets in the cyclic systems. In general the pharmaceutical field, new drugs are continuously discovered by molecular modification of lead compound of established activity. The molecular modification can possibly result in the activity which involves a combination of the separate group having similar activity in one compound by eliminating, substituting or adding new moiety to lead compound. In the survey of the literature, it was observed that drug design by molecular modification is a productive source of the new drug. Therefore the urgency to synthesize new molecules as potential medicinal agents is more relevant today[10-13].

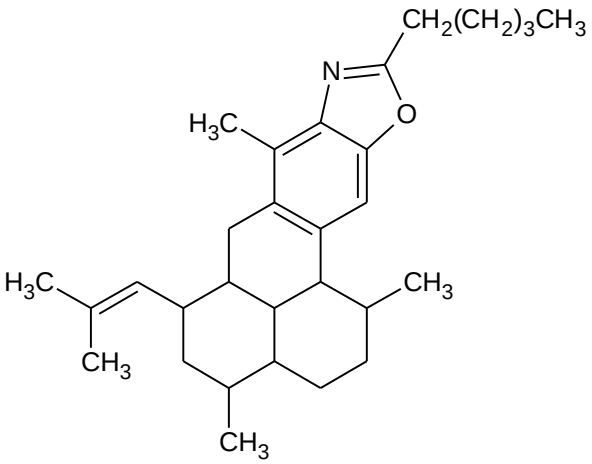
Among the verity of compounds studied, benzoxazole derivatives form an important class. The benzoxazole heterocycles were considered as ‘‘privileged scaffolds’’ in the design of pharmacological probes. Benzoxazoles are an important class of compounds and have exhibited a variety of biological activities such as antimicrobial[14], antiinflammatory[15], analgesic, antifungal[16], herbicidal[17], antiplatelets[18], anticonvulsants[29], antitumor[20], anticancer[21], CNS acitvity[22], antihyperglycemic potentiating activity[23], melatoninergic ligands[24], antitubercular[25], photophysical properties[26], anti HIV agents[27] and anthelmintic

agents[28]. There are several known strategies for the synthesis of 2-substituted benzoxazoles includes: coupling of carboxylic acids, acid chlorides, nitriles, aldehydes and orthoesters, 2-halophenols and aryl methanol with 2-aminophenol[29].

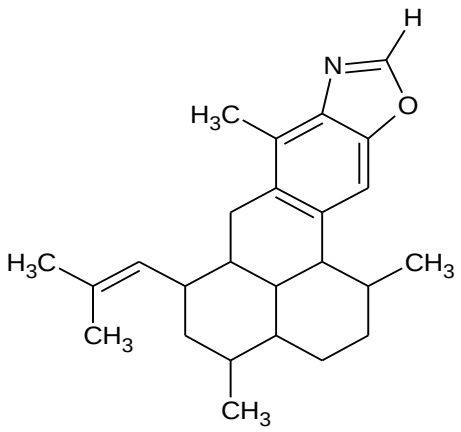
1.2 Naturally occurring benzoxazole derivatives

The benzoxazole structure is found in natural products and indicates a great number of pharmacological applications. The isolated benzoxazole exhibited many activities such as antibacterial antifungal and antitumor agent.

A new diterpene alkaloids, homopseudopteroxazole **1** pseudopteroxazole **2** were isolated by I. Ileana et al.,[30] from the sea plume *Pseudopterogorgia elisabethae*. The isolated compounds were evaluated for Biological screening. It indicated that homopseudopteroxazole **1** was a strong growth inhibitor of *Mycobacterium tuberculosis* H37Rv.

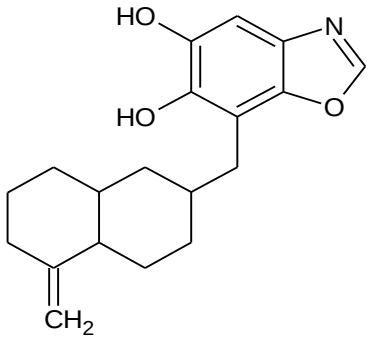


1



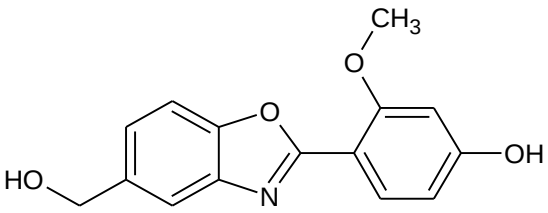
2

A new sesquiterpene benzoxazole, nakijinol B **3**, was isolated from the methanol extract of the marine sponge *Dactylospongia elegans.* The isolated compounds were subjected for their cytotoxicity against human tumor cell lines. The compound **3** displayed potent cytotoxic activity[31].



3

S. Mingwei et al.,[32] has been isolated nocarbenzoxazoles from the halophilic strain Nocardiopsis lucentensis DSM 44048. The isolated compounds were screened for their cytotoxic assay. Compound 4-[5-(hydroxymethyl)-1,3-benzoxazol-2-yl]-3-methoxyphenol **4** showed significant cytotoxic activity.

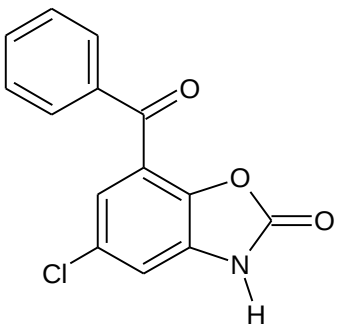


4

1.3 Synthetic benzoxazoles

Since biologically active benzoxazole derivatives have been known from long back and also it is considered as structural isosteres of the naturally occurring nucleic bases namely, adenine and guanine, which allows them to interact easily with polymers of living systems. Benzoxazoles occupies remarkable and a broad range of biological activities. Benzoxazole derivatives are potential synthons for building variety of chemical compounds known for their broader biological and pharmacological properties. The literature overview revealed that substituted benzoxazoles have been shown to exhibit activity against platelet aggregation, inflammation and cell proliferation[33], anti-inflammatory activity[34-41,42],COX-2 inhibitory[42], screened for their gastric ulceration on potential in tested animals[43], antimicrobial activity against some gram positive and gram negative bacteria and fungi[44-47], HIV-1 reverse transcriptase inhibitor activity[48], as DNA Topoisomerase I and II Inhibitors[49] anticancer activity[50,51] and anti diabetic[52] activities.

U. Serdar et al.,[53] synthesized a series of benzoxazole derivatives **5-6** and the compounds were evaluated for their anti-inflammatory activity. The test results indicated that [7-acyl-2-oxo-3H-benzoxazole-3-yl]alkanoic acids were showed more potent anti-inflammatory activity.



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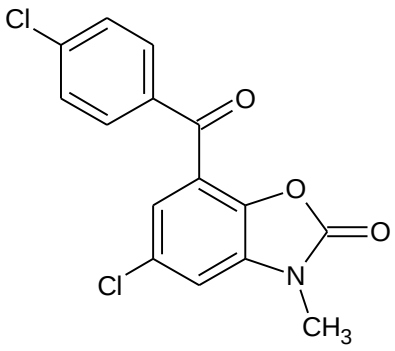
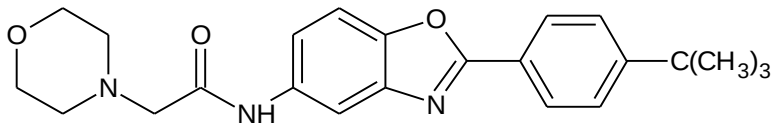


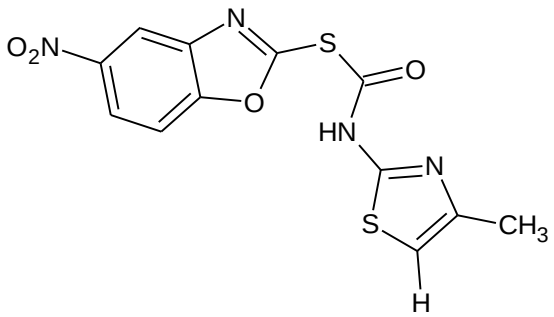
6

A.O. Temiz et al.,[54] synthesized a series novel benzoxazole derivative **7**, and evaluated for their antimicrobial activities. MIC values of synthesized compounds showing 3.12-50 μg/mL against the Candida species.



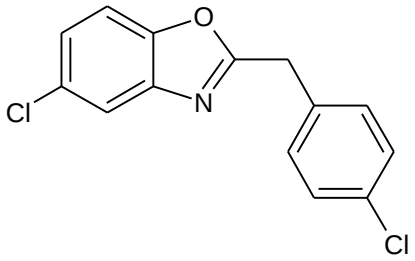
7

G.T. Zitouni et al., have been synthesized 2-[(benzoxazole-2-yl) thioacetylamino]thiazole derivative **8**. The prepared compound exhibited significant antimicrobial activity and toxicity[55].



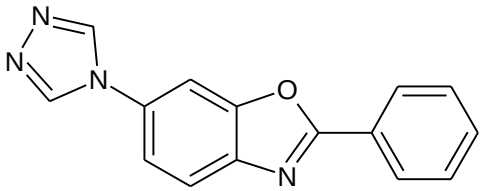
8

D. Ozden et al.,[56] reported and synthesized a series of substituted 2-anilinobenzimidazoles benzoxazoles derivatives. The molecule was evaluated for their anti-staphylococcal activity, result was found that the compound **9** displayed considerable anti-staphylococcal activity.



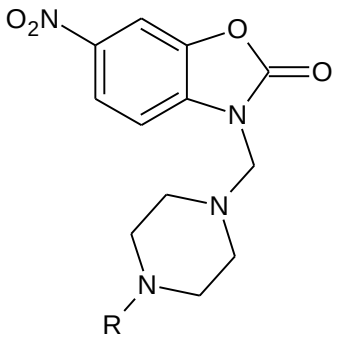
9

C.X. Wei et al., reported a series of 2-substituted-6-(4*H*-1, 2, 4-triazol-4-yl) benzo[*d*]oxazoles. The anticonvulsant effect and neurotoxicity of the compound was evaluated with the maximal electroshock (MES) test, it was indicated that 2-phenyl-6-(4*H*-1,2,4-triazol-4-yl)benzo[*d*]oxazole **10** was the most active and also had the lowest toxicity[57].



10

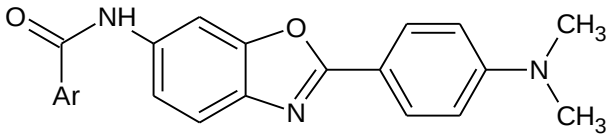
M. Koksal et al., synthesized a novel series of 5-nitro-3-substitutedpiperazinomethyl-2-benzoxazolinones **11**. The compounds were evaluated for their anti-inflammatory activity. Among the tested compounds most promising results were obtained for the compounds having electron withdrawing substituent (F, Cl, $COCH_3$), exhibited higher anti-inflammatory activity[58].



R= 4-F C_6H_5, 4-Cl C_6H_5

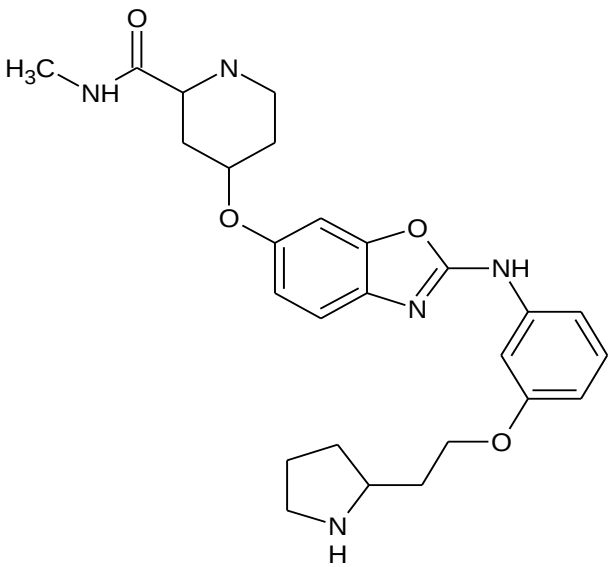
11

H.S. Hausner et al., have been synthesized 5- and 6-substituted 2-(4-dimethylaminophenyl)-1,3-benzoxazoles **12** and tested for *in vitro* and *in vivo* imaging agents for Alzheimer's disease (AD)-related amyloid plaque[59].



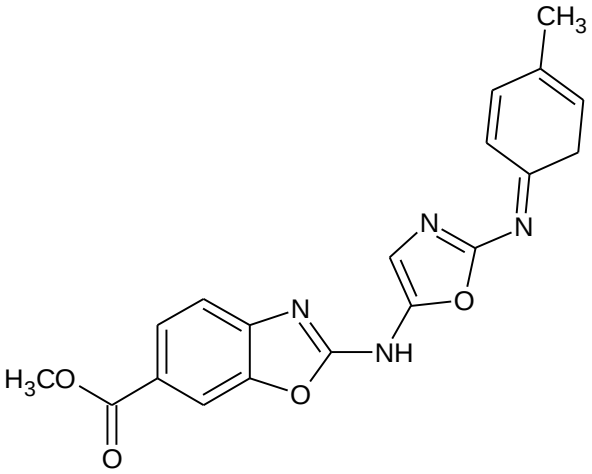
12

M.H. Potashman et al.,[60] have been synthesized a series of 2-aminobenzimidazole and benzoxazole **13** and evaluated for selective vascular endothelial growth factor-2 receptor kinase inhibitor activity. The compound 13 exhibited potent inhibitor activity.



13

R.C. Nageshwar et al.,[61] have been synthesized a series of methyl-2-(arylideneamino)*oxazol*-4ylamino)benzoxazole-5-carboxylate derivatives were synthesized. The Compounds were screened for antibacterial and antifungal activity, in which molecules **14 and 15** provided higher potency than the other tested compounds against both antibacterial and antifungal organisms.



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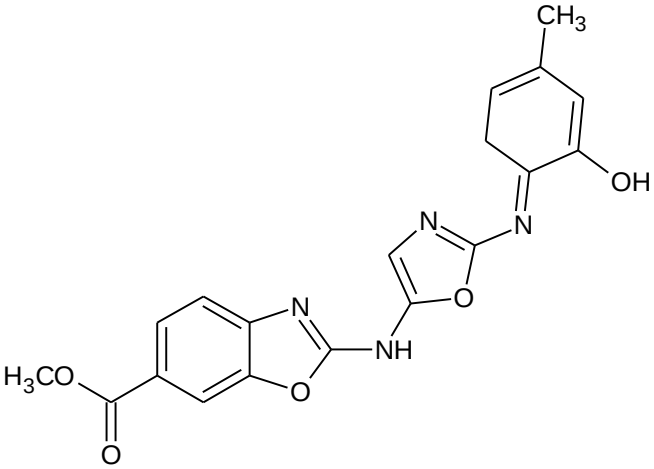
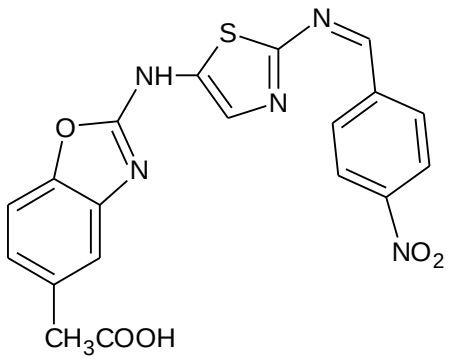


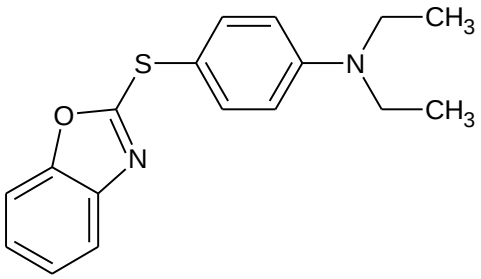
15

A. Srinivas et al.,[62] synthesized compound **16** and evaluated for anti-inflammatory activity. The molecule showed anti-inflammatory activity more than the standard (diclofenac sodium).



16

P. C. R. Stella et al.,[63] synthesized the novel compound 4-(1,3-benzoxazol-2-ylsulfanyl)-*N*,*N*-diethylaniline **17** and subjected to analgesic activity.



17

Observing the scope of benzoxazole derivatives in medicinal field and their importance in drug discovery, the present investigation is focused on the synthesis and biological investigation of target molecules to the best of our knowledge. In our laboratory few derivatives of benzoxazoles have been synthesized and the synthesized molecules were screened for selected biological activities.

The substituted benzoxazole derivatives have been synthesized in our laboratory in different protocols and displayed potent biological and pharmacological activities. Hence, the synthesis and biological evaluation of benzoxazoles attracted special attention.

In our research work, selected benzoxazole derivatives have been synthesized by new routes and the desired molecules were screened for biological activities like antibacterial, antifungal, antioxidant, cytotoxic and molecular docking studies.

For the systematic presentation, the thesis is divided into eight chapters as follows,

Chapter-1: Background and general introduction about benzoxazole derivatives.

Chapter-2: Synthesis of 2-{1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-ylidene}hydrazinecarbothioamide benzoxazole derivatives.

Chapter-3: Synthesis of novel 2-{[(1-ethyl-1*H*-benzimidazol-2-yl)methyl] sulfanyl}-6-nitro-1,3-benzoxazole derivatives.

Chapter-4: Synthesis of novel 2-{[2-hydrazinylidenepropyl]sulfanyl}-6-nitro-1,3-benzoxazole derivatives.

Chapter-5: Synthesis of novel N'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-1,3-dioxo-1,3-dihydro-2H-isoindole-2-carbothiohydrazide derivative

Chapter-6: Synthesis of novel 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one *N*-[1-(4-chlorophenyl)ethylidene]thiosemicarbazone derivatives.

Chapter-7: Biological and pharmacological evaluation of synthesized molecules.

Chapter-8: Conclusion

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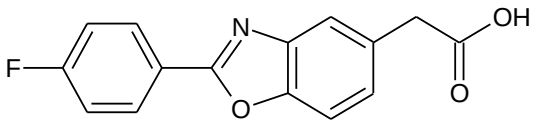
CHAPTER –II

SYNTHESIS OF NOVEL 1-[(6-NITRO-1,3-BENZOXAZOL-2-YL)SULFANYL]PROPAN-2 ONE THIOSEMICARBAZONE DERIVATIVES

2.1 Introduction

The rising prevalence of multi-drug resistant microbial infections in the past few decades had become serious health care problems. In fact, the more common and uncommon bacteria already susceptible to common antimicrobials are reported to have developed resistance to different antibiotics. As a consequence of this feature, it is necessary to prevent the emergence and distribution of resistant bacteria by developing a new effective antibacterial agents.

The benzoxazole has been the aim of many researchers for many years because they constitute an important class of heterocyclic compounds exhibiting various types of biological properties such as anticancer[1], antibacterial[2], anti HIV-1[3], antioxidant[4], cyclooxygenase inhibitory[5], antifungal[6], antibacterial[7], melatonin receptor agonist[8], antibiotic[9], antimycobacterial activities[10]. It is found in the chemical structures of pharmaceutical drugs such as Flunoxaprofen, Calcimycin, Zoxazolamine, Routiennocin, etc contains benzoxazole moiety. Considering these observations and in connection to previous publications in our research group we have planned to synthesis new class of biologically active derivatives of benzoxazole molecules.



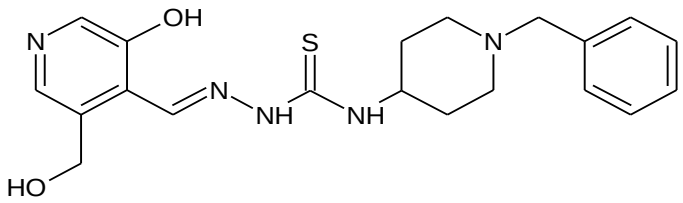
Flunoxaprofen

2.2 Introduction to Thiosemicarbazone

The literature survey raveled that, thiosemicarbazone derivatives of benzoxazole have find immense importance because of their versatile biological and pharmacological activities. On the other hand, thiosemicarbazides have showed potent intermediates for the synthesis of pharmaceutical and bioactive materials and

the derivatives of thiosemicarbazone have received considerable attention because of their anti-amoebic[11], trypanocidal[12], anticancer[13], anti HIV[14], anti-tumor[15], anti-inflammatory[16] CNS stimulant properties[17]. They also find applications as dyes, lubricants, analytical reagents[18] and antiviral agents[19].

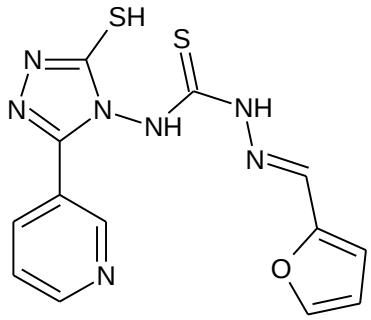
D. Palanimuthu et al.,[20] have been synthesized a novel series of thiosemicarbazones derived from 1-benzylpiperidine. The compound pyridoxal 4-(N-(1-benzylpiperidin-4-yl)thiosemicarbazone (PBPT) (**1)** emerged as the lead compound. This molecule demonstrated the most effective multi-functional agent for Alzheimer's (AD) disease treatment.



1

The series of compounds of N-(5-mercapto-3-pyridyl-3-yl-4H-1,2,4-triazol-4-yl)-thiosemicarbazone have been synthesized[21]. All the synthesized compounds showed antibacterial activity, in which the compounds 2-furyl (**2)** and 2-chlorophenyl (**3)** were reported to be the most significant antibacterial agents against *Staphylococcus aureus* and *Escherichia coli*.

The thiosemicarbazide linked with triazole derivatives were synthesized from N-(5-mercapto-3-pyridyl-3-yl-4H-1,2,4-triazol-4-yl)-thiosemicarbazone and determined their antifungal activity. Among synthesized molecules, 2-chlorophenyl **(3)**, phenyl **(4)** and 4-methoxyphenyl **(5)** showed potent antifungal activity against *Candida albicans* [21].



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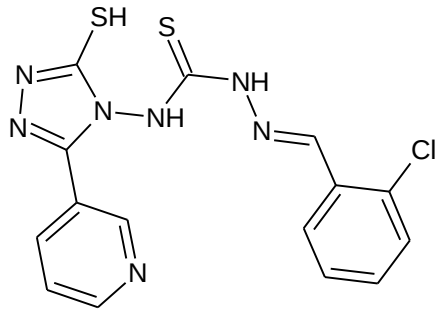

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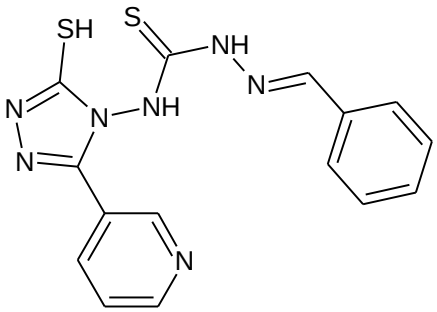

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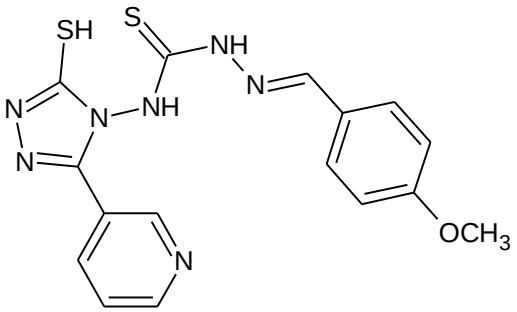
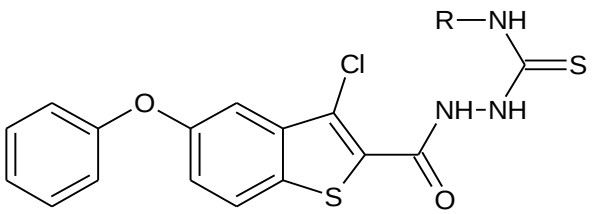


5

N-substituted arylthiosemicarbazide derivatives were prepared by the reaction of 2-hydrazinocarbonyl-3-chloro-5-phenoxybenzo[b]thiophene with substituted phenyl isothiocyanate. All the synthesized compounds were evaluated for antimicrobial properties by cup-plate agar fusion method[23] and it was observed that the compounds **(6-10)** were showed potent activities against *Escherichia coli* and *Bacillus megaterium*. This was reported by S.L. Vasoya et al., [22].



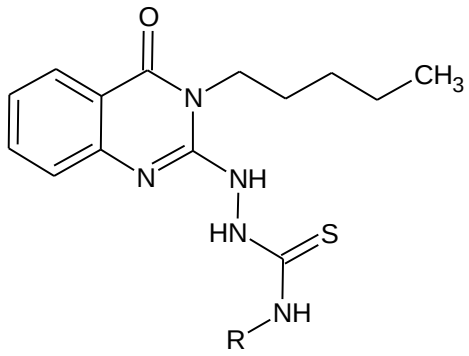
6-10

6: R = 3-ClC_6H_4 9: R = 4-$CH_3C_6H_4$

7: R = 2-$CH_3C_6H_4$ 10: R = 2-$OCH_3C_6H_4$

8: R = 4-$OCH_3C_6H_4$

The antitubercular activity of the series of compounds[23] was screened at 6.25µg/ml concentration against *Mycobacterium tuberculosis* H37RV in BACTEC 12B medium using the ALAMAR radiometric system. Among the tested compounds, **(11-13)** were found to be appreciably active against *M. tuberculosis* with MIC of 6 µg/ml.



11-13

R

11) 2-nitrotoluene **12**) 4-chlorotoluene **13**) 2- methyl pyridine

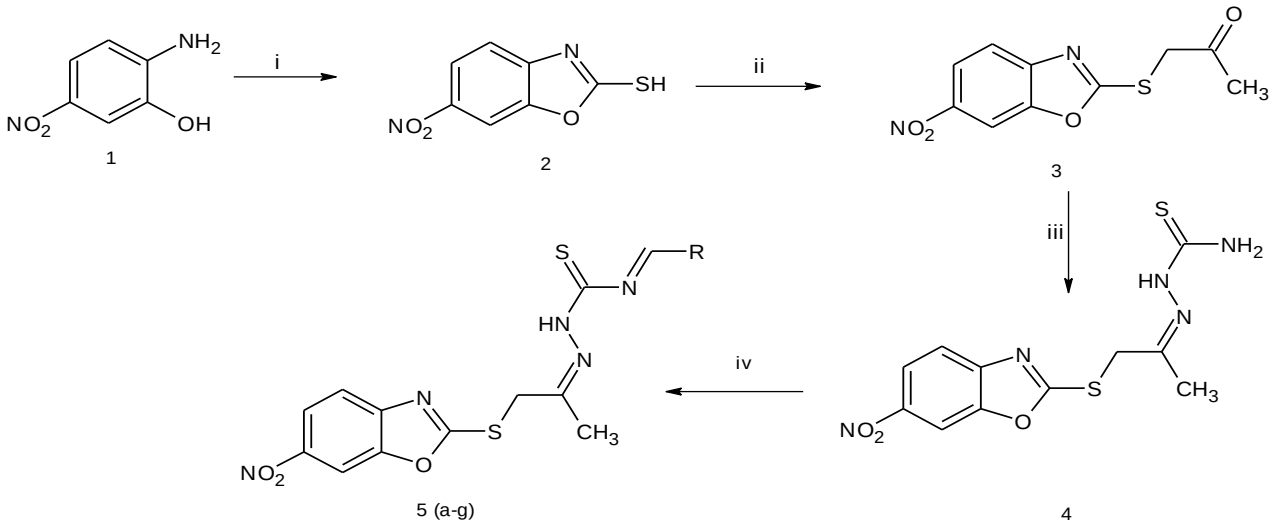
In view of these above biological and pharmacological activities of thiosemicarbazide with heterocyclic moieties, it was planned to synthesis novel benzoxazole derivatives associated with thiosemicarbazide nucleus and evaluated for antibacterial, antifungal, antioxidant and molecular docking studies.

2.3. Present Work

This chapter describes the synthesis of thiosemicarbazone benzoxazole derivatives.. The target benzoxazole derivatives were purified, characterized and confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral technique and they were screened for biological activities.

The present chapter describes the synthesis of the following benzoxazole derivatives **Scheme-2**

- 6-Nitro-1, 3-benzoxazole- 2- thiol **(2)**
- 1-[(6-Nitro-1, 3-benzoxazol-2-yl)sulfanyl] propan-2-one **(3)**
- 1-[(6-Nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one-thiosemicarbazone **(4)**
- 1-[(6-Nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one-thiosemicarbazone derivatives **5(a-g)**



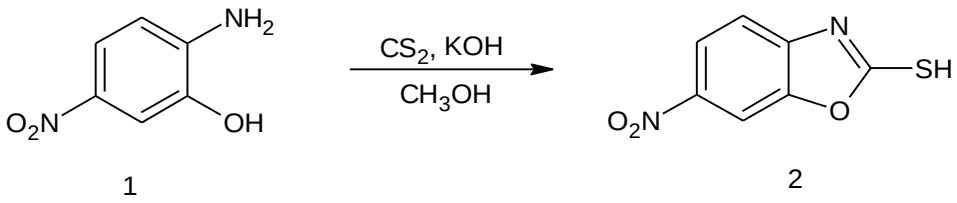
R		R	
5a	C_6H_5	5e	4-$NO_2C_6H_4$
5b	4-FC_6H_4	5f	4-$OCH_3C_6H_4$
5c	4-ClC_6H_4	5g	4-OHC_6H_4
5d	4-BrC_6H_4		

Scheme-2: Synthetic route for the synthesis of compounds **5(a-g)**

(i) CS_2, KOH, CH_3OH (ii) $ClCH_2COCH_3$, KOH, $CH_3COOC_2H_5$ (iii) $NH_2NHCSNH_2$, CH_3COOH, C_2H_5OH (iv) RCHO, DMF, CH_3COOH.

1 Synthesis of 6-nitro-1,3-benzoxazole- 2- thiol (2)

The literature survey raveled that, the -Cl, -Br, -NO_2 groups directly attached to the aromatic ring were found to exhibit diverse biological activities. Our research work has been focusing on the synthesis of nitro substituted derivatives of benzoxazoles. The compound 6-nitro-1, 3-benzoxazole- 2- thiol **(2)** was synthesized using 2-amino-5-nitrophenol **(1)** as a starting material. The starting material was purchased from Sigma Aldrich. It was refluxed in presence of potassium hydroxide and carbon disulfide in methanol as a solvent. The product was obtained in good yield **(Scheme-2.1).**



1 2

Scheme-2.1

The formation of compound **(2)** was confirmed by the spectral studies. The IR spectrum displayed the stretching frequency at 3077 cm^{-1} for –SH group, 1616 cm^{-1} for C=C bond of the aromatic ring and 1548 cm^{-1} for-C=N- bond **(fig. 2.1)**. The ^{1}H NMR Spectrum **(fig. 2.2)** exhibited a broad singlet at δ 14.20 is due to –SH proton. A singlet and two dublets appeared at δ 8.40, δ 8.21 and δ 7.38 corresponding to three protons of the aromatic ring. Further confirmation of the assigned structure of **(2)** was obtained by recording its mass spectrum **(fig. 2.3),** which displayed a molecular ion peak at m/z 196 confirmed the structure**.**

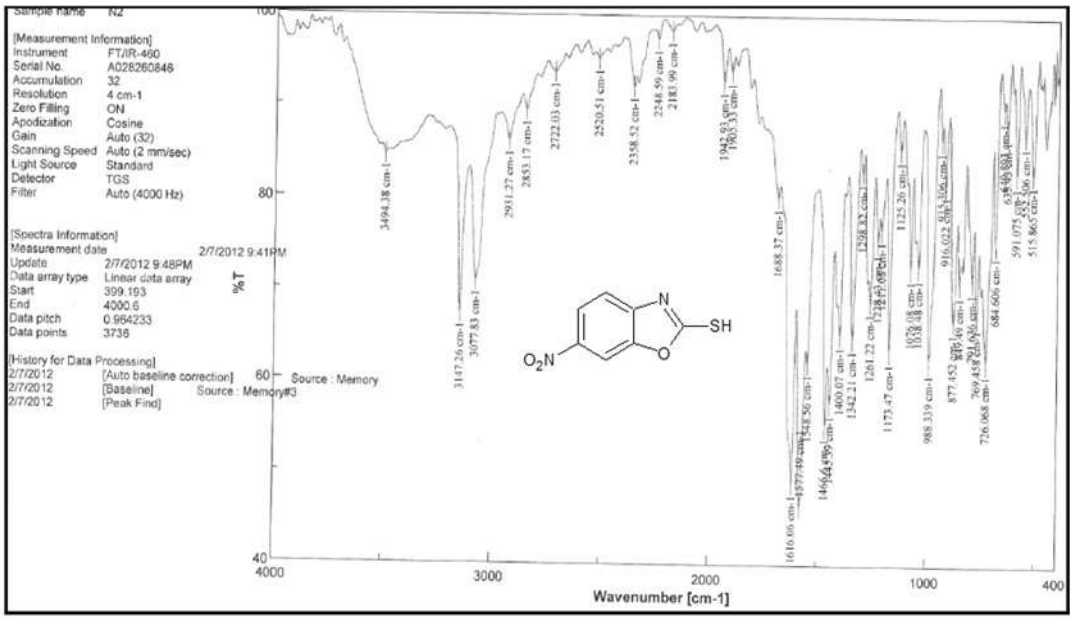


Fig.2.1 IR spectrum of compound 2

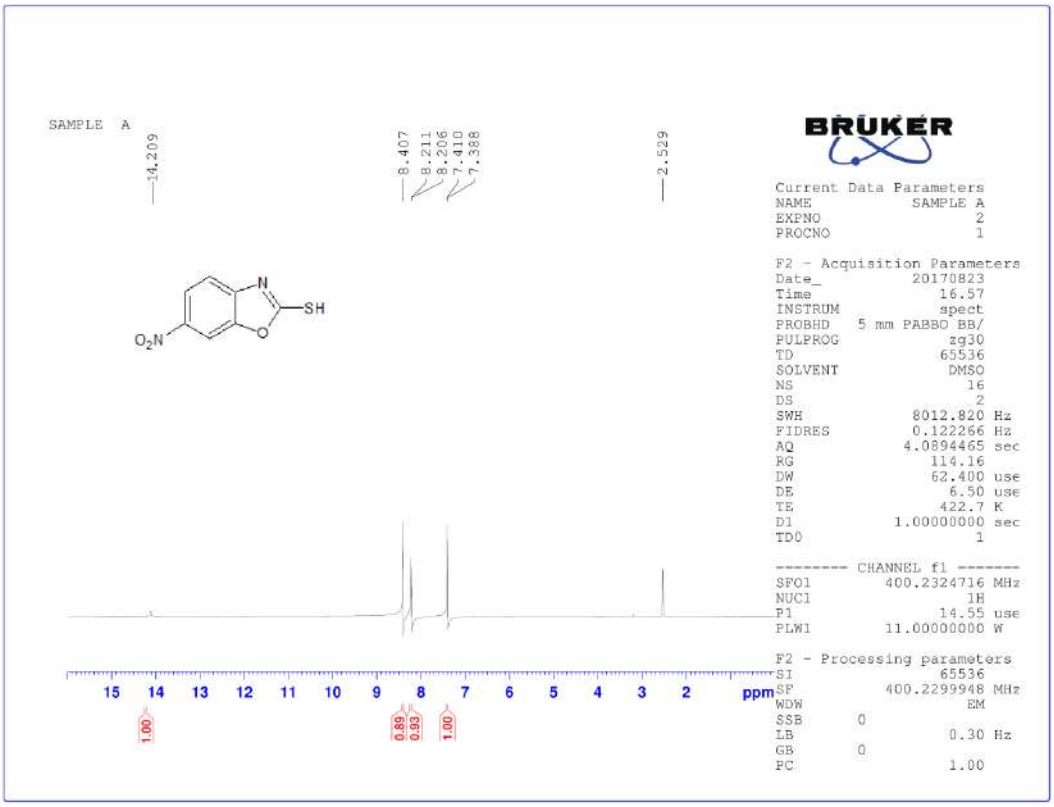


Fig.2.2 ^{1}H NMR spectrum of compound 2

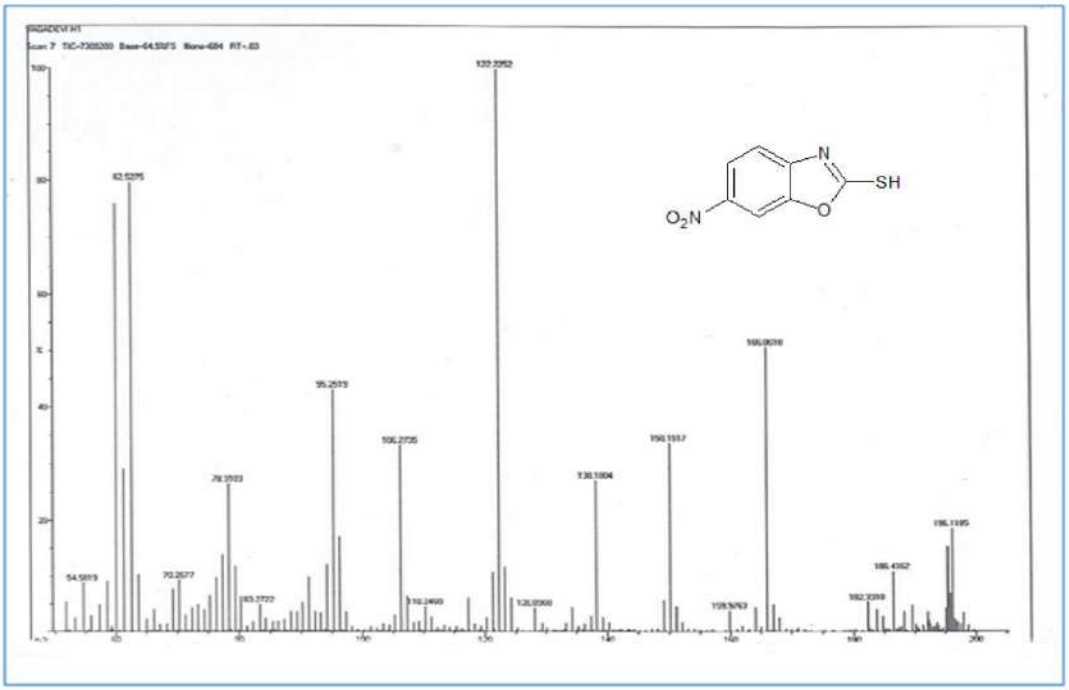
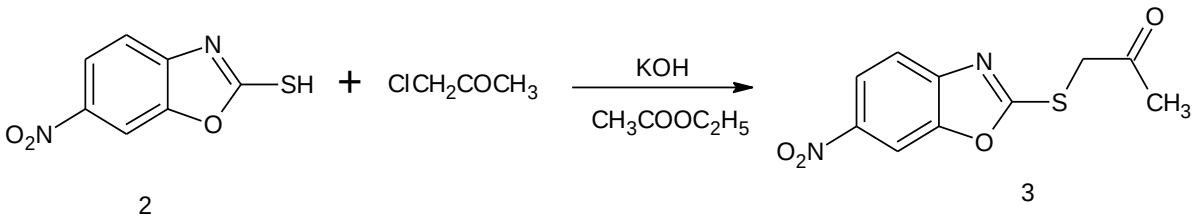


Fig.2.3 LCMS spectrum of compound 2

2 Synthesis of 1-[(6-nitro-1, 3-benzoxazol-2-yl)sulfanyl]propan-2-one (3)

To synthesis target molecule the –SH functionality is converted into ketone group by reacting with chloroacetone. The reaction happened in alkaline media with good yield. The structure was confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies **(Scheme-2.2).**



2 3

Scheme-2.2

The IR spectrum of **(3)** showed the disappearance of stretching frequency at 3377 cm^{-1} confirmed the absence of –SH group by appearing the stretching frequency at 1656 cm^{-1} for –C=O group **(fig. 2.4)**. The ^{1}H NMR spectrum showed two dublets and a singlet of aromatic protons appeared at δ 8.60 and 7.80. A new signal as a singlet at δ 4.56 for $–CH_2$ and followed by the signal at δ 2.34 for three protons of $–CH_3$ that confirmed the target compound (**3) (fig. 2.5)**. The ^{13}C NMR showed carbonyl carbon at δ 205.0 and C7 aromatic carbons are appeared at δ 105.99-170.42 correspondingly. The remaining signals at δ 43.33 for $–S-CH_2$, δ 15.02 for $–CH_3$, supported the formation of compound **(3) (fig. 2.6)**. The mass spectrum **(fig. 2.7)** showed a molecular ion peak at m/z 253.02 further confirmed the compound **(3).**

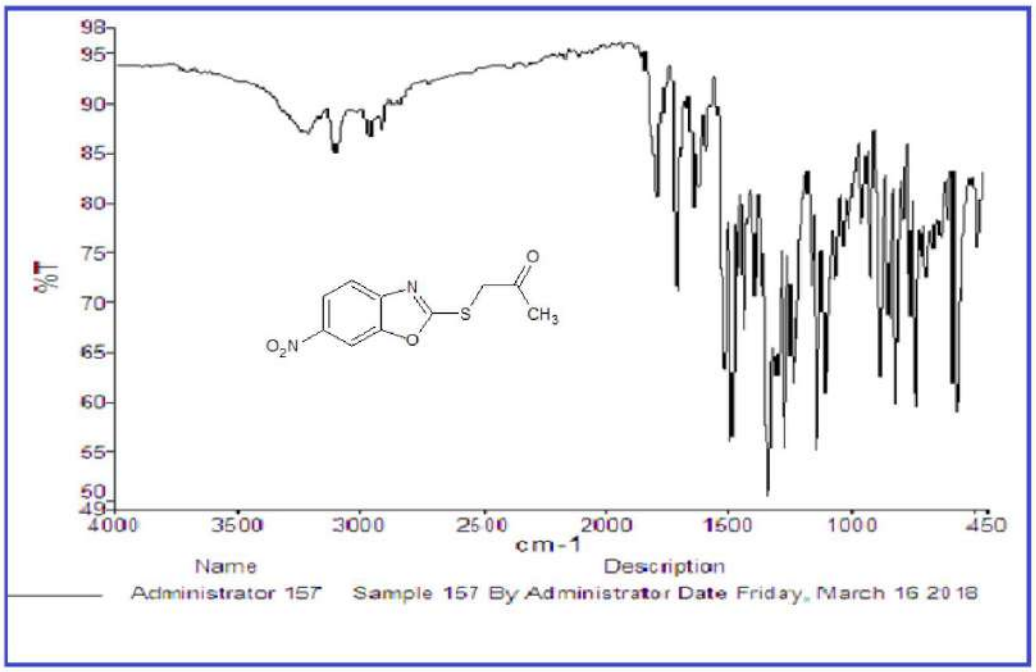


Fig.2.4 IR Spectrum of compound 3

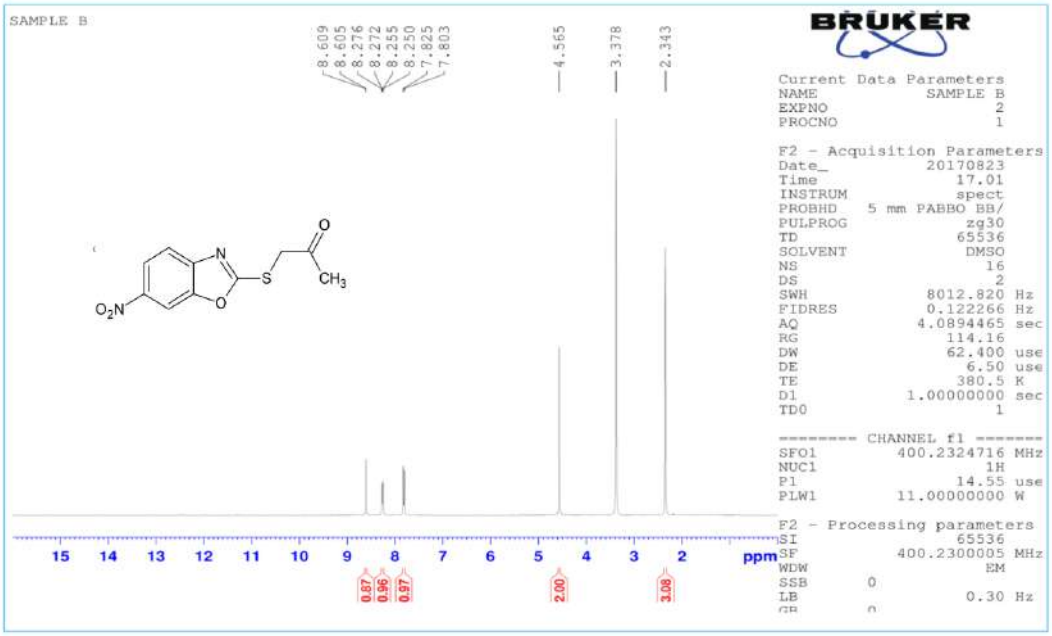


Fig.2.5 ^{1}H NMR Spectrum of compound 3

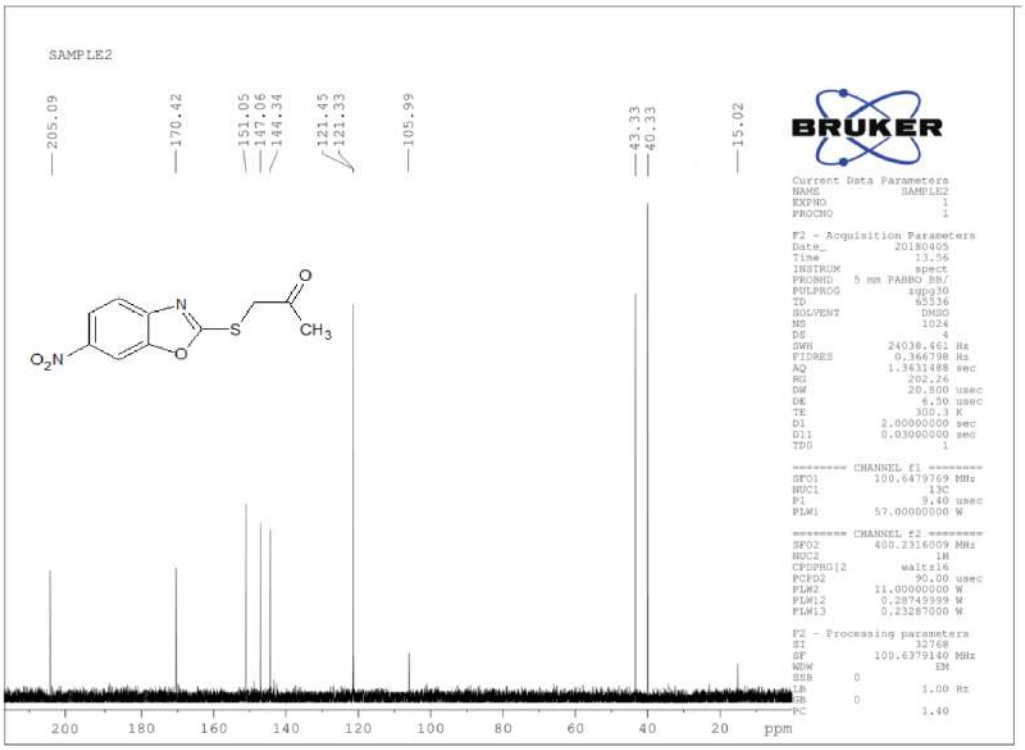


Fig.2.6 ^{13}C NMR Spectrum of compound 3

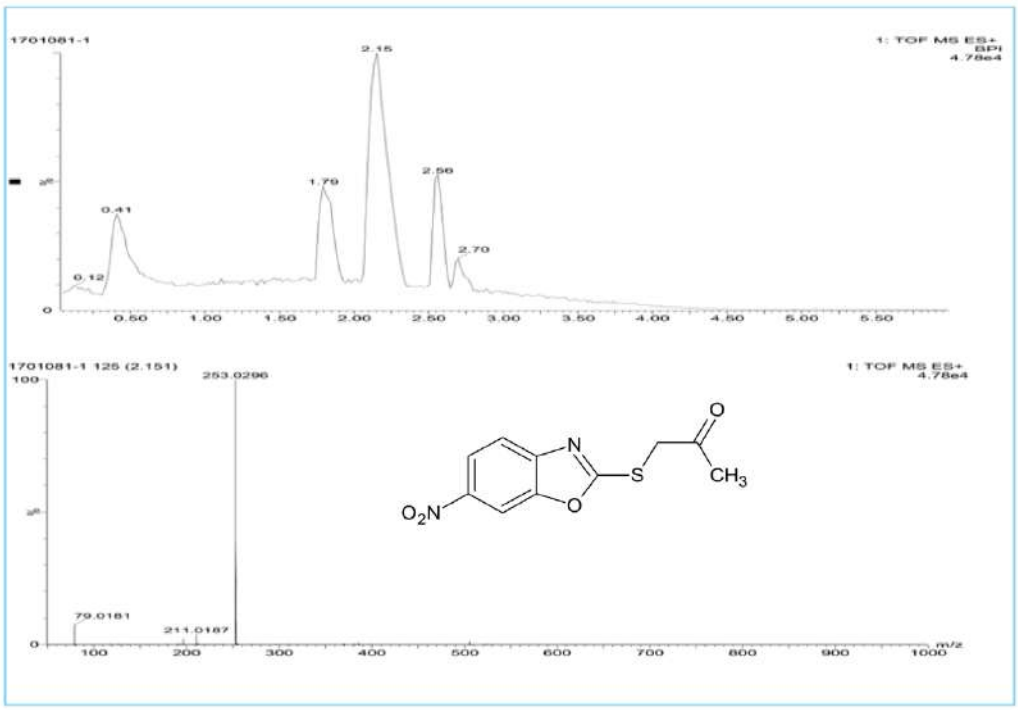
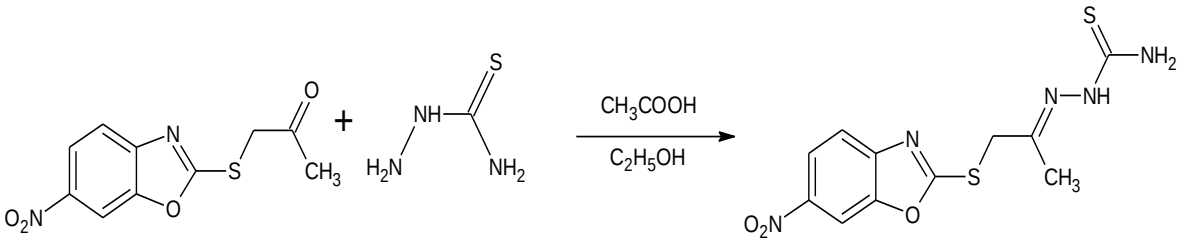


Fig.2.7 LCMS Spectrum of compound 3

3 Synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2 one thiosemicarbazone (4)

Literature survey raveled that thiosemicarbazide derivatives were important in medicinal chemistry due to their broad spectrum of biological and pharmacological activities. The compounds of Thiosemicarbazide derivatives were interesting targets molecules for drug design. During the past few decades, interest has been rapidly growing in gaining insight into the properties and transformations of thiosemicarbazides and their derivatives due to their appreciable pharmacological activities. In the present step, we have synthesized a series of benzoxazole derivatives encompassing thiosemicarbazide moiety.

The compound 4 was used as a starting molecule throughout the research work, to obtain the target molecule. When the compound 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one **(3)** was treated with thiosemicarbazide yielded the target molecule 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2 one-thiosemicarbazone. **(Scheme-2.3)**



3 4

Scheme-2.3

The IR spectrum of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2one thiosemicarbazone **(4)** showed stretching frequency at 3455 cm^{-1} for -NH_2 group, for -NH it is appeared at 3105 cm^{-1} and the disappearance of stretching frequency at 1656 cm^{-1} confirmed the absence –C=O group **(fig. 2.8)**. The ^1H NMR spectrum displayed a broad singlet at δ 10.36 for NH_2 (D_2O exchangeable) and The three

aromatic protons exhibited as multiplet at δ 8.62 -7.83 range and another two singlets appeared for –CH_2 and –CH_3 at δ 4.24 and δ 2.08 regions respectively. A signal at δ 1.28 as a singlet for –SH that confirmed the structure **(fig. 2.9)**. The signal in ^{13}C NMR at δ 179.43 for –C=S and six aromatic carbons appeared at the region δ 155.08-107.29, whereas signal at δ 40.54 for –S-CH_2, δ 16.39 for –CH_3, supported the confirmation of structure **(4) (fig. 2.10)**. The mass spectrum **(fig. 2.11)** exhibited a molecular ion peak at m/z 326.30, matches with the molecular weight of the compound **(4)**.

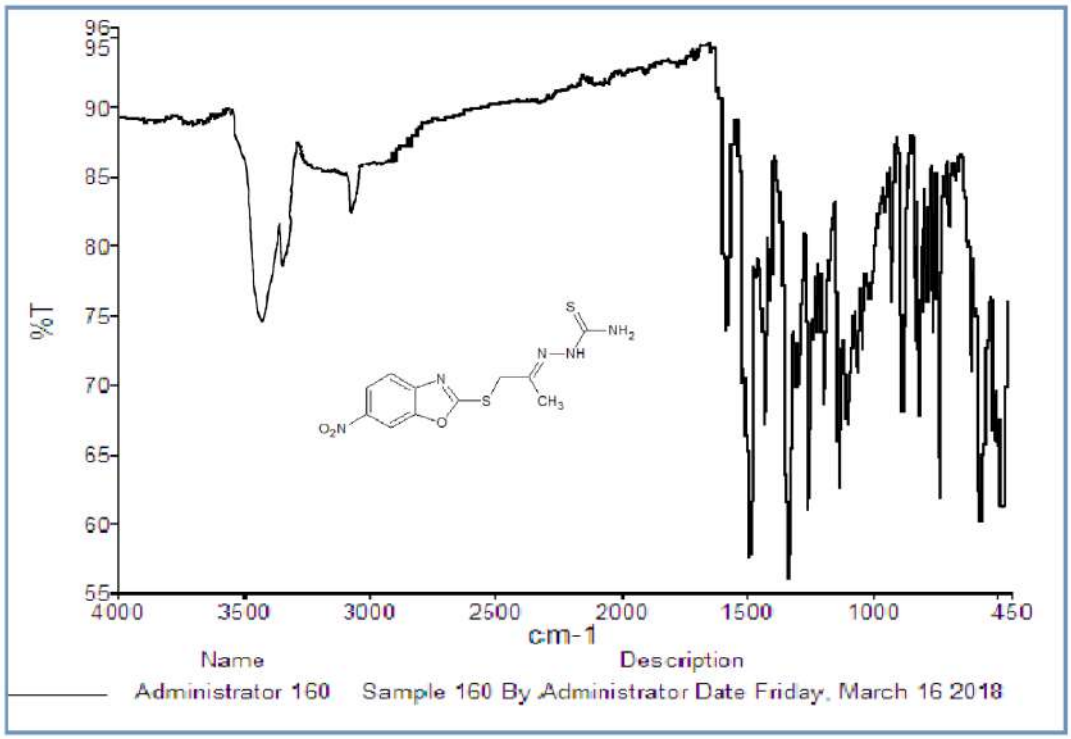


Fig.2.8 IR spectrum of compound 4

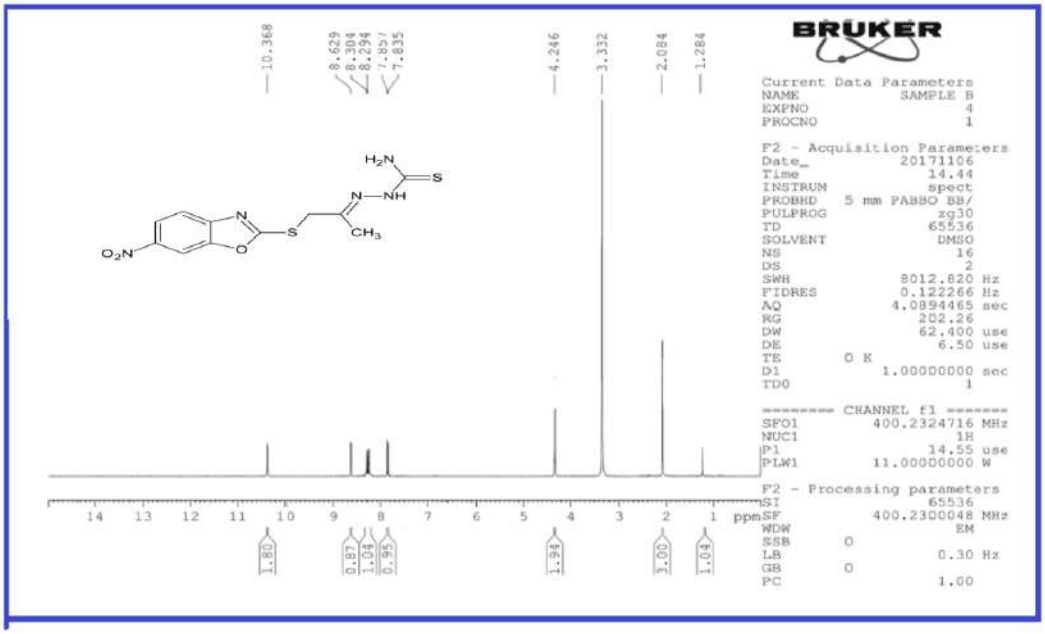


Fig.2.9 ^{1}H NMR Spectrum of compound 4

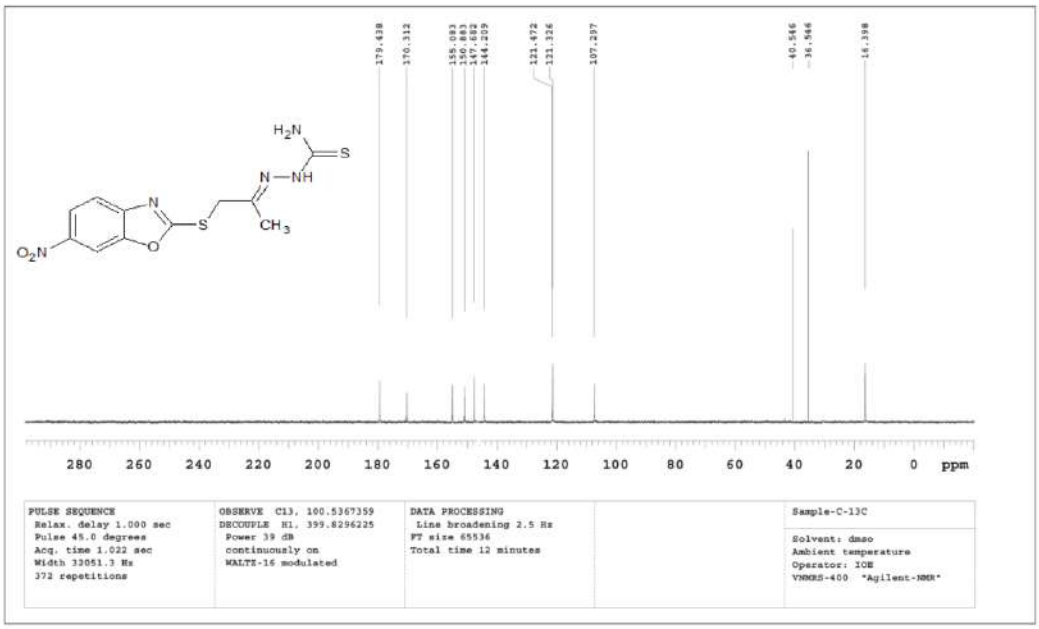


Fig.2.10 ^{13}C NMR Spectrum of compound 4

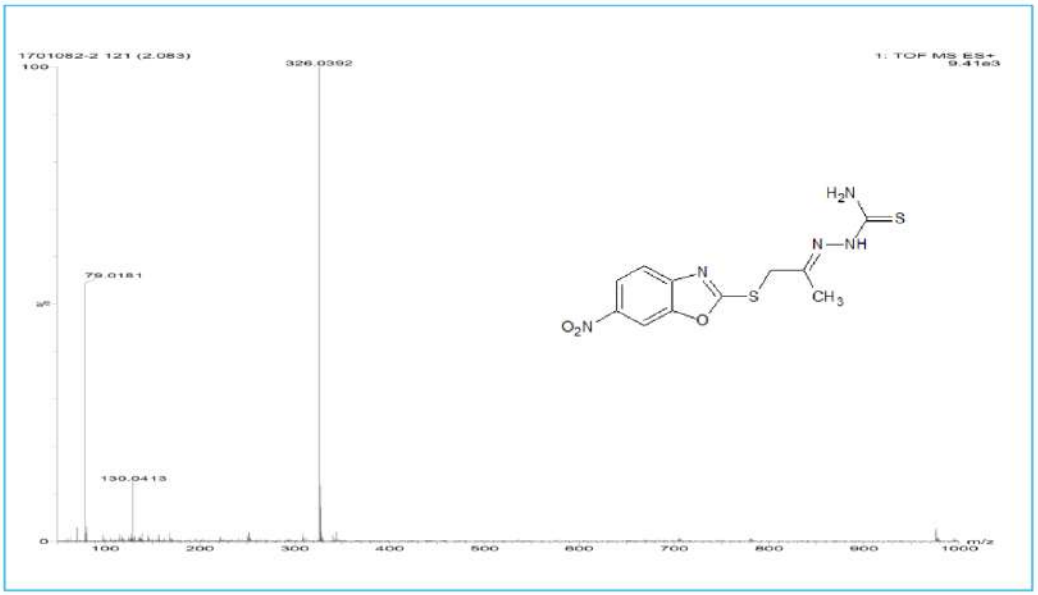


Fig.2.11 LCMS Spectrum of compound 4

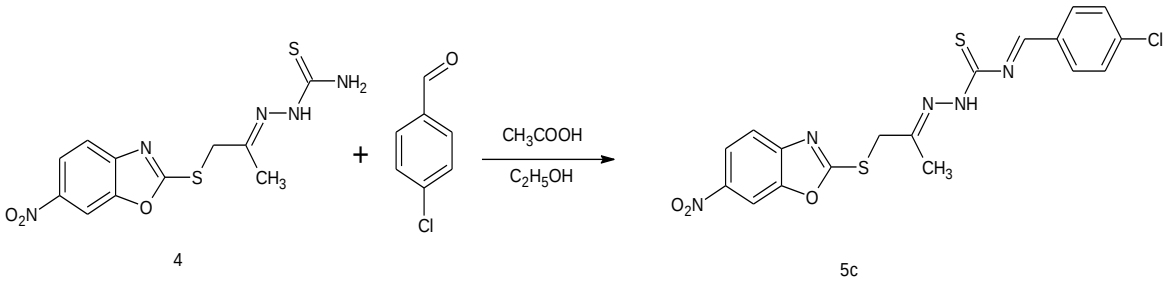
4 Synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one *N*-[(substitutedphenyl)methylidene]thiosemicarbazone 5(a-g)

Schiff bases are active against a wide range of organisms, since they play an important role in living organisms, such as decarboxylation, transamination and C-C bond cleavage. These were very important in the field of medicinal and analytical with versatile applications. Owing to their broad range of applications new class of schiff bases are synthesized. In the present work we have been synthesized a series of benzoxazole derivatives of schiff bases.

The Schiff bases **5(a-g)** were synthesized by the condensation reaction between the compound 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2 one thiosemicarbazone **4** and different substituted aromatic aldehyde in ethanol by using glacial acetic acid as catalyst.

The compound **4** was reacted with 4 chlorobenzaldehyde in presence of acetic acid in ethanol used as a solvent to get target molecule 1-[(6-nitro-1,3-

benzoxazol-2-yl)sulfanyl]propan-2-one*N*-[(4-chlorophenyl)methylidene] thiosemicarbazone **5c (Scheme-2.4)**



Scheme-2.4

The structure of the molecule **5(c)** was confirmed by spectral studies. The IR spectrum **(fig. 2.12)** of compound **5(c)** displayed the stretching frequency at 3230 cm^{-1} for –NH group and 1266 cm^{-1} for -C=N. The ^{1}H NMR spectrum exhibited a singlet at δ 11.84 for –NH proton and multiplet between δ 8.19-6.88 corresponding to eight aromatic protons. The two protons for $–CH_2$ group showed singlet at δ 4.20 and a singlet at δ 2.06 for three protons of $–CH_3$ **(fig. 2.13).** The ^{13}C NMR spectrum **(fig. 2.14)** displayed a peak at δ 190.55 for –C=S and 14c signals appeared between δ 167.92-106.30 correspondingly. The remaining signals exhibited at δ 43.43 δ 15.38 for $–CH_2$ and $–CH_3$ carbon respectively. The molecular mass peak at m/z 447 and M^{+2} at 449 exactly matched with its molecular weight **(fig. 2.15**) confirmed the structure of target molecule **5(c).**

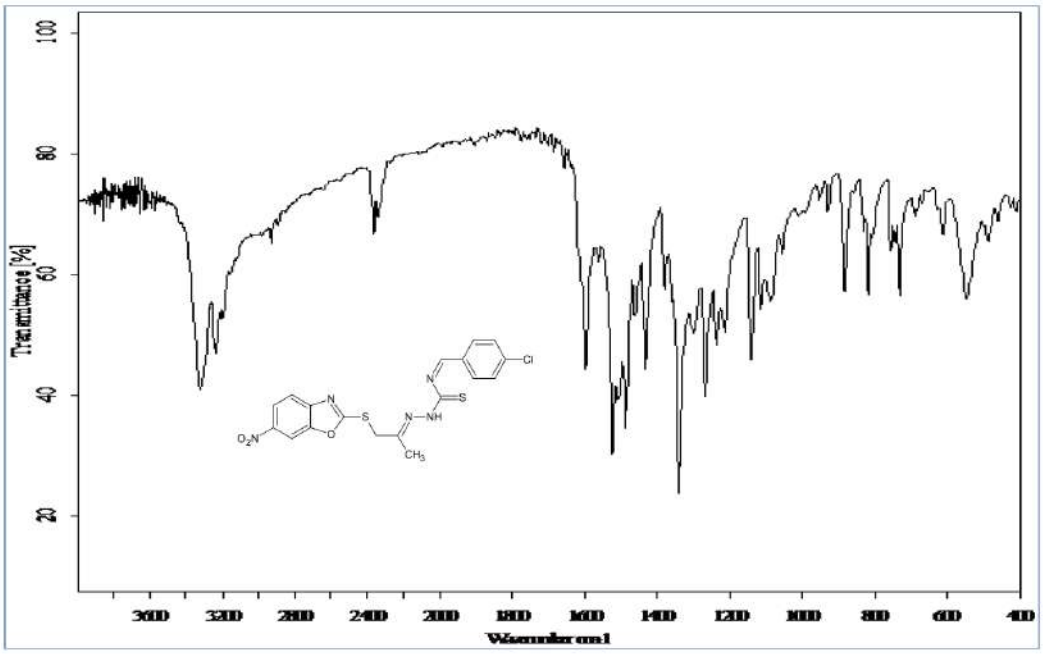


Fig.2.12 IR spectrum of compound 5c

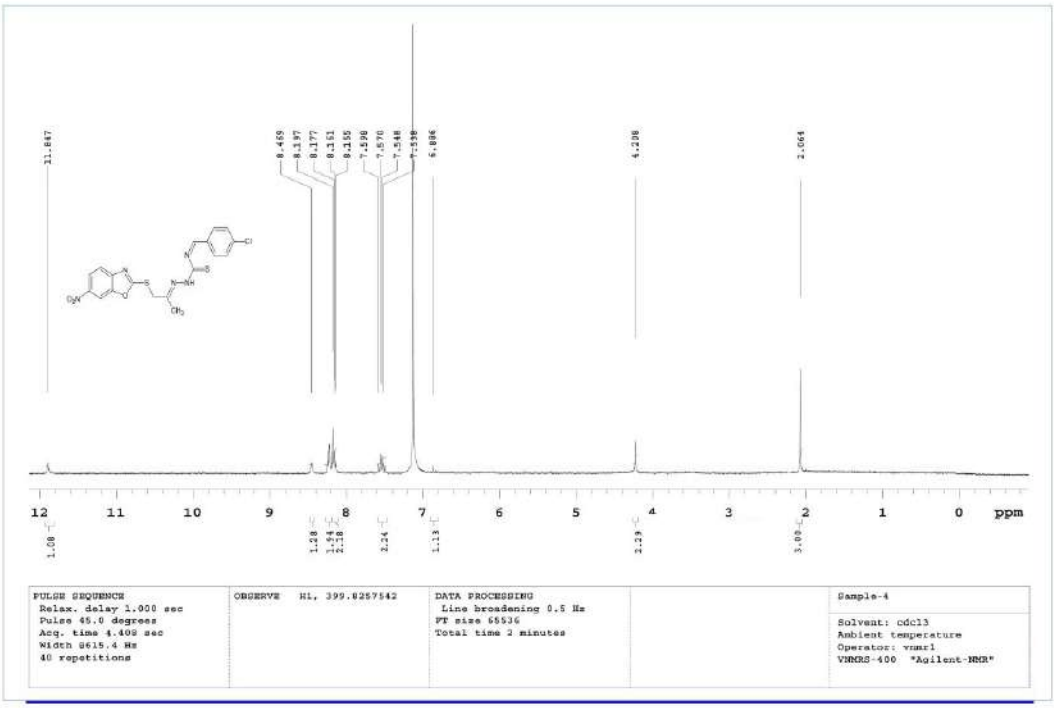


Fig.2.13 ^{1}H NMR Spectrum of compound 5c

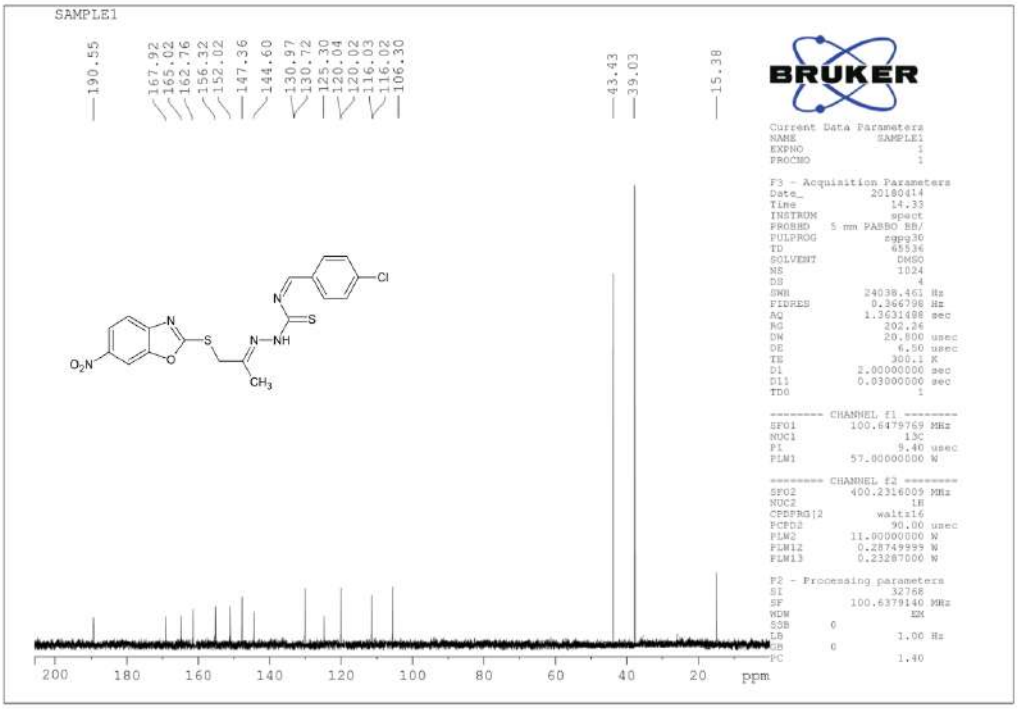


Fig.2.14 ^{13}C NMR Spectrum of compound 5c

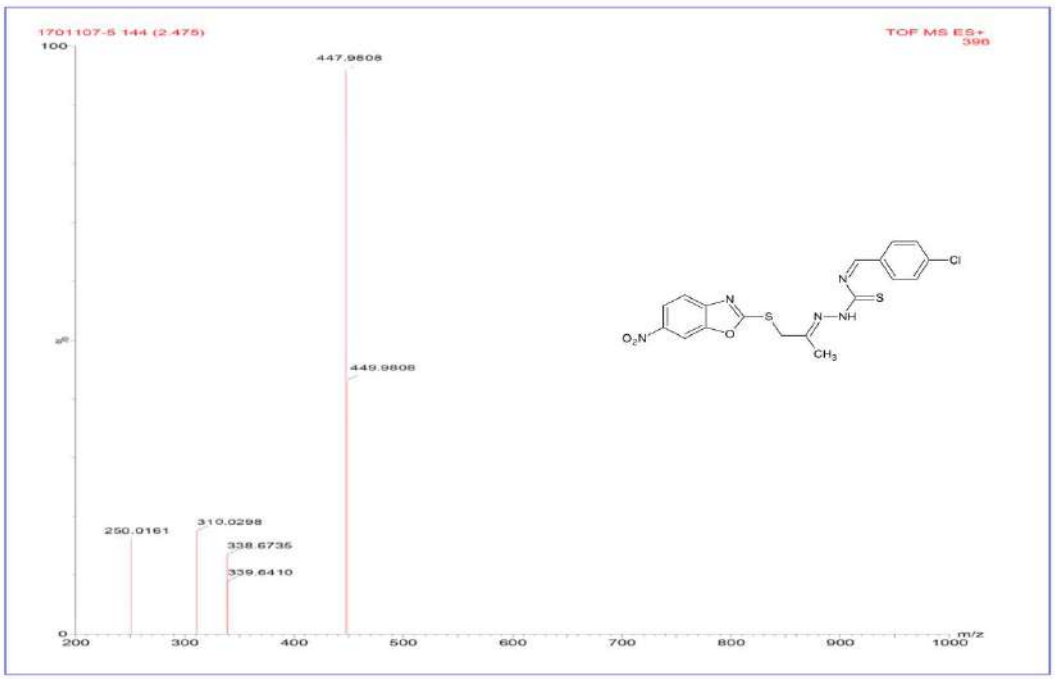
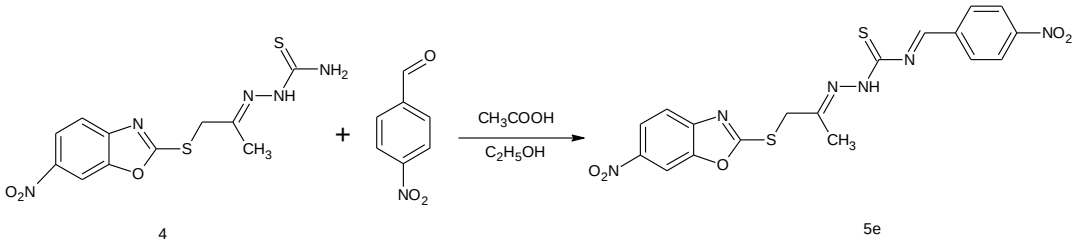


Fig.2.15 LCMS Spectrum of compound 5c

5 Synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one *N*-[-(4-methoxyphenyl)methylidene]thiosemicarbazone 5(e)

The compound **(4)** was treated with 4-Nitrobenzaldehyde in presence of acetic acid by using ethanol as a solvent. The product namely 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[-(4-methoxyphenyl)methylidene]thiosemicarbazone was obtained **(Scheme-2.5).**



4 5e

Scheme-2.5

In the compound **5(e)**, the IR spectrum exhibited stretching frequency at 3245 cm^{-1} for -NH group and whereas the stretching frequency at 1560 cm^{-1} for –C=N group **(fig. 2.16**). The ^{1}H NMR spectrum exhibited signal at δ 10.35 (D_2O exchangeable) as singlet for –NH group and δ 9.13 as singlet for –CH=N proton. The seven aromatic protons appeared as multiplet between δ 8.59-7.45 **(fig. 2.17**) confirmed the target molecule. It is also supported by ^{13}C NMR exhibited a peak at δ 190.59 for –C=S group and δ 42.53 for $–CH_2$ functionality **(fig. 2.18)**. The molecular mass peak at m/z 459.08 **(fig. 2.19)** evidenced the formation of product**.**

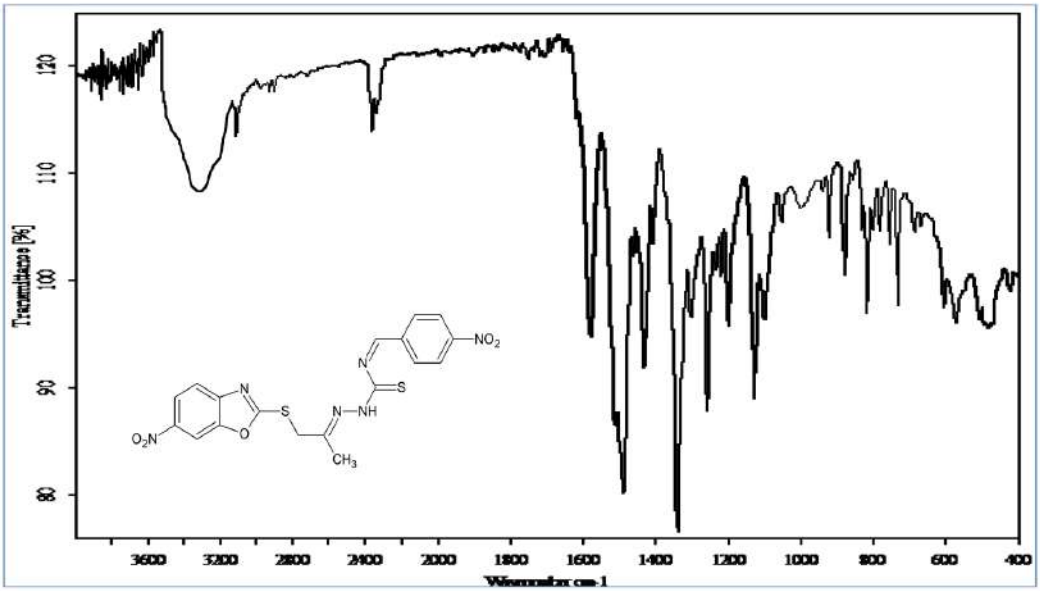


Fig.2.16 IR spectrum of compound 5e

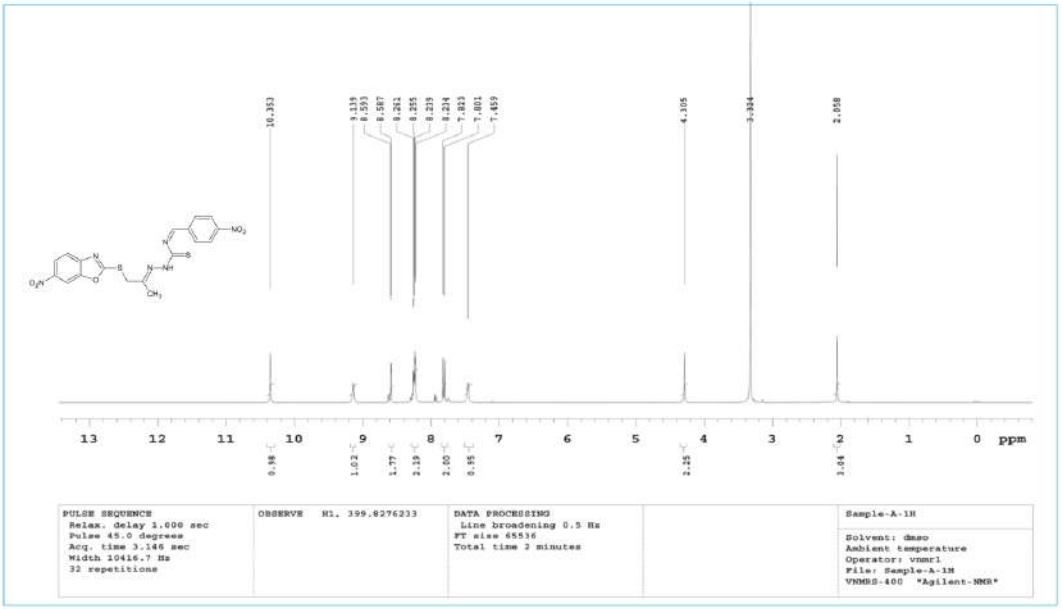


Fig.2.17 ^{1}H NMR Spectrum of compound 5e

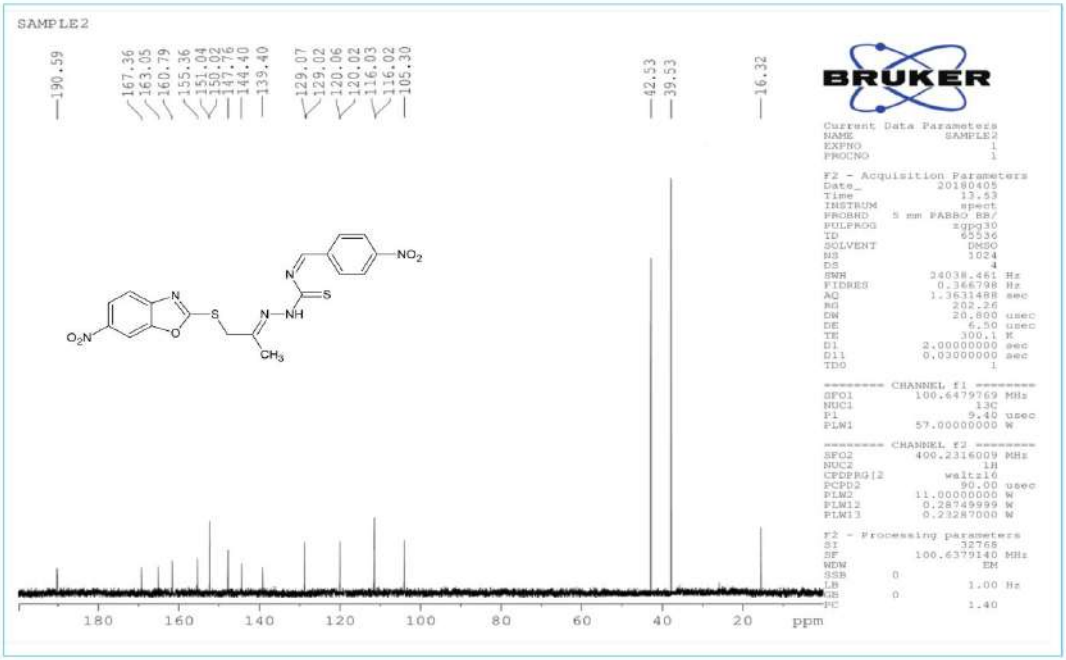


Fig.2.18 ^{13}C NMR Spectrum of compound 5e

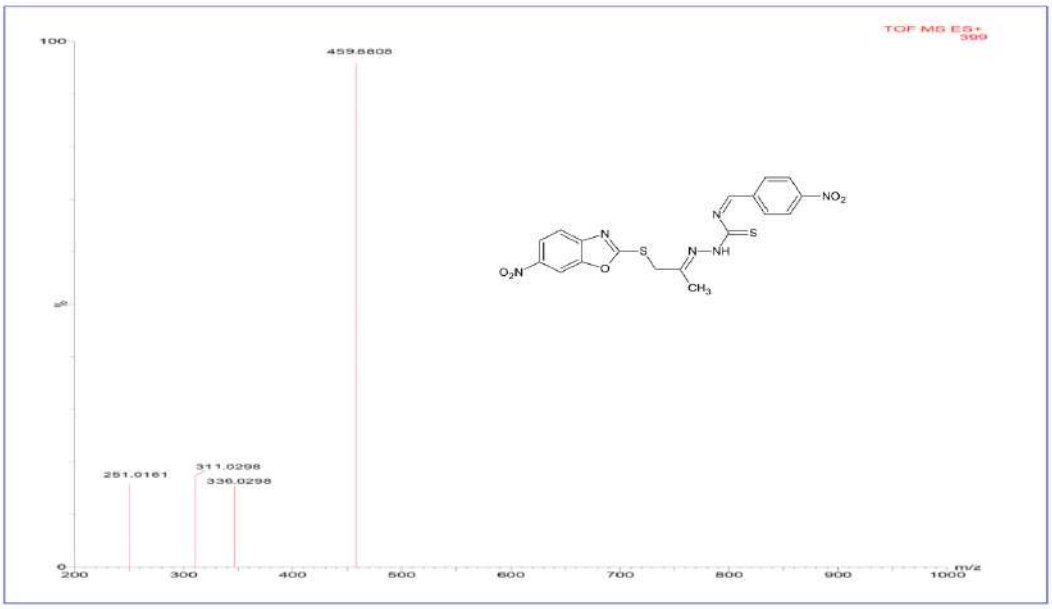
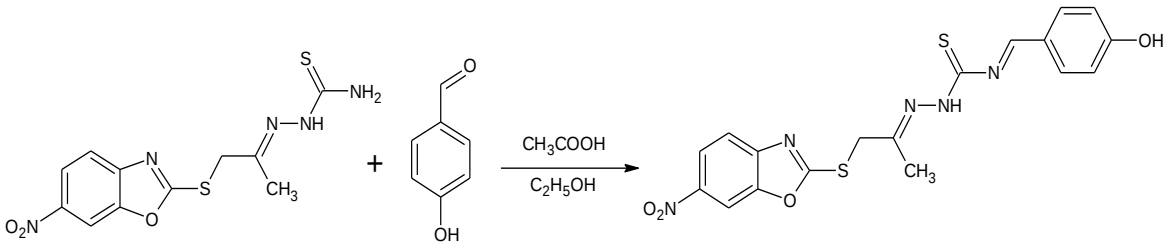


Fig.2.19 LCMS Spectrum of compound 5e

6 Synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one *N*-[(4-hydroxyphenyl) methylidene]thiosemicarbazone 5(g)

When the compound **4** was treated with 4 hydroxybenzaldehyde in presence of acetic acid in ethanol used as a solvent, 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one-*N*-[(4-hydroxyphenyl)methylidene]thiosemicarbazone was obtained **(Scheme-2.6).**



4 5g

Scheme-2.6

The compound **5(g)** exhibited singlets at δ 11.90 and δ 10.56 in ^{1}H NMR spectrum that confirmed the presence of –OH and –NH group. A singlet at δ 8.56 for –CH=N proton, the remaining aromatic protons appeared at the region of δ 8.29-6.98 in its ^{1}H NMR **(fig. 2.20)** confirmed the formation of the target compound. In the ^{13}C NMR spectrum, the peak at δ 190.25 for C=S, δ 42.33 regions for $–CH_2$ and methyl carbon was appeared at δ 16.38 this evidence strongly supported the formation of the title compound **(fig. 2.21).** The molecular weight of the compound m/z 429.08 exactly matched with molecular ion peak **(fig. 2.22),** this further confirmed the target molecule**.** The spectral data of the synthesized compounds are mentioned in the Table 2.1

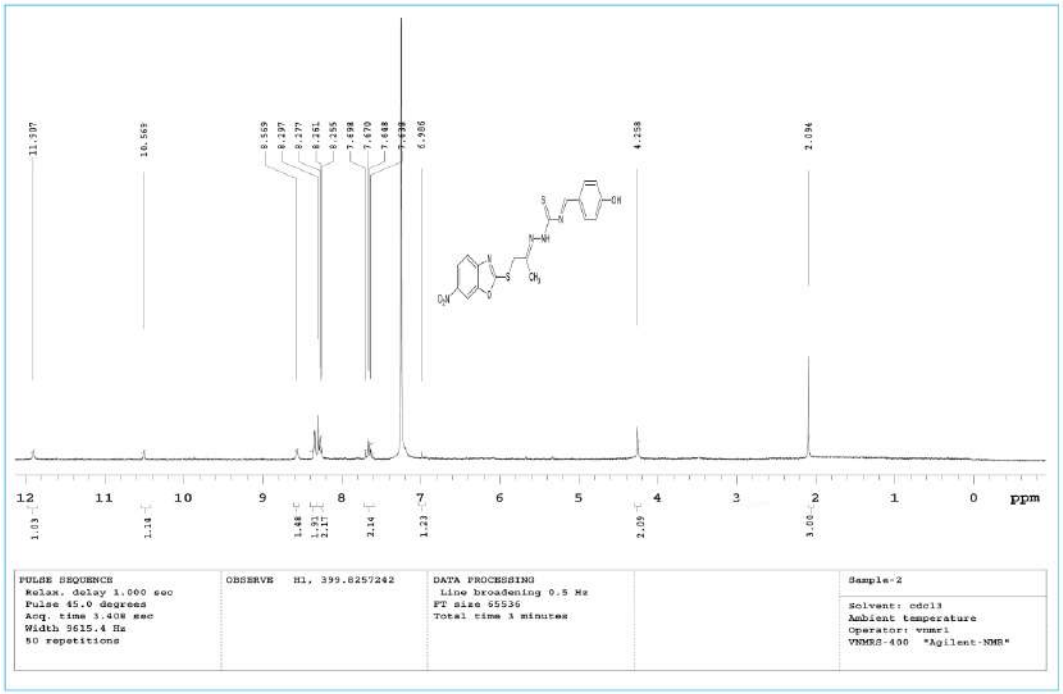


Fig.2.20 ^{1}H NMR Spectrum of compound 5g

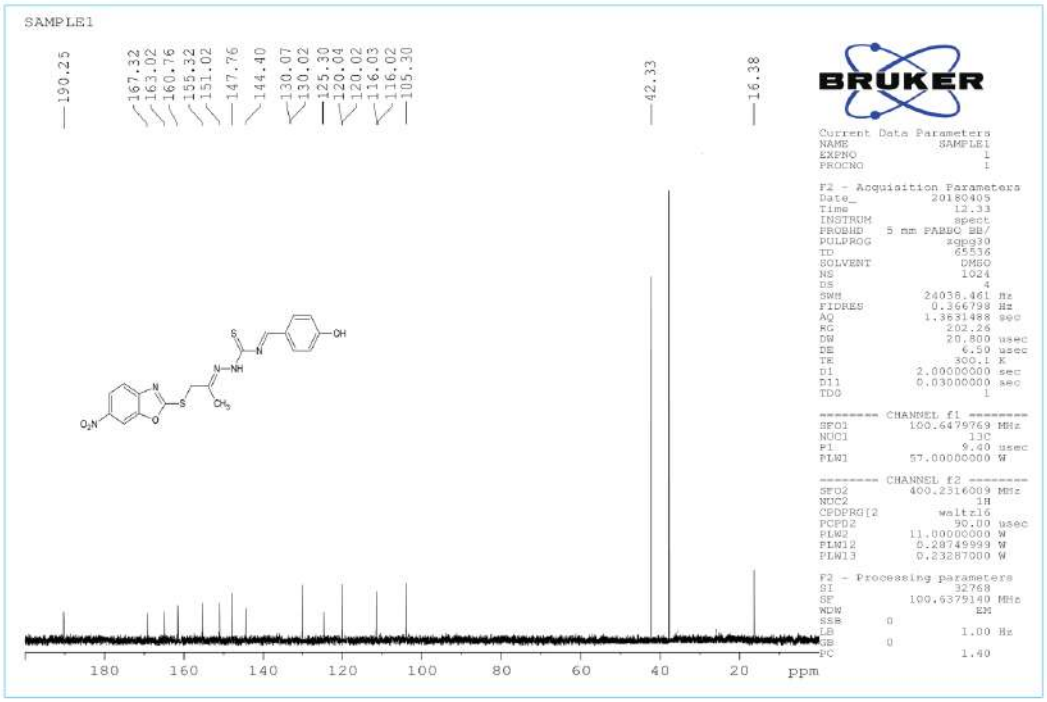


Fig.2.21 ^{13}C NMR Spectrum of compound 5g

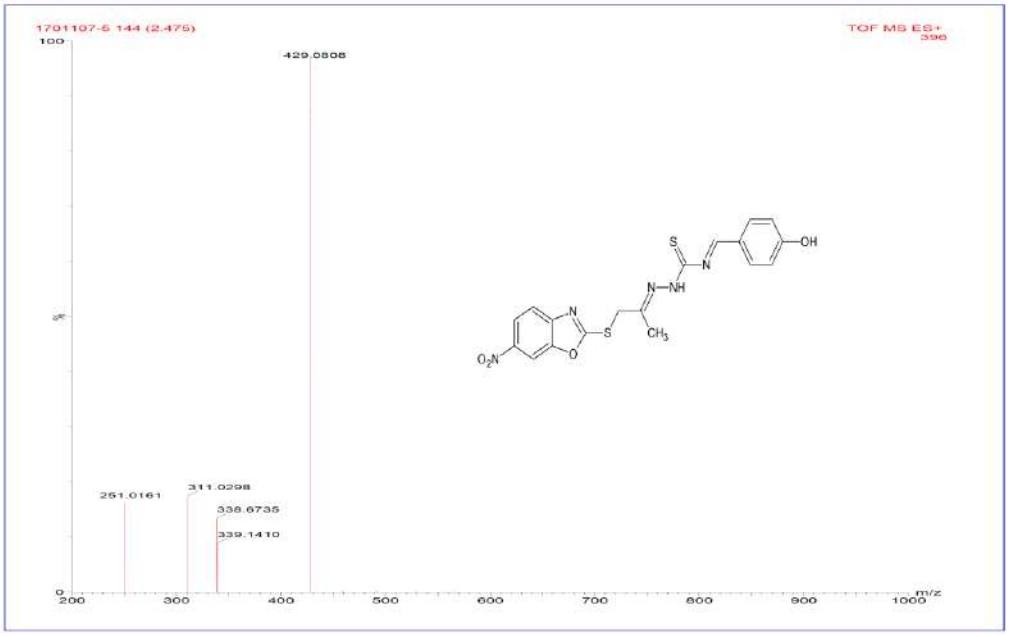


Fig.2.22 LCMS Spectrum of compound 5g

The spectral analysis data of remaining synthesized compounds are tabulated in **Table-2.1**

Compound	IR (KBr) in cm^{-1}	^{1} HNMR in δ PPM	^{13}C NMR in δ PPM	Mass M^{+} M^{+2}
5a	3316 (NH), 1653 (C=N) 1789 (C=S)	11.647 (s, H –NH), 8.469 (s, H -CN), 6.786-8.097 (m, 7H Ar-H), 4.108 (s, 2H $–CH_2$), 2.164 (s, 3H $–CH_3$)	194.42 (C=S), 165.27-106.25 (13C, Ar-C), 42.25 (CH_2), 16.27 (CH_3)	413.42
5b	3328 (NH), 1654 (C=N) 1714 (C=S)	10.506 (s, H –NH), 8.619 (s, H -CN), 6.859-8.093 (m, 7H Ar-H), 4.258 (s, 2H $–CH_2$), 2.094 (s, 3H $–CH_3$)	190.55 (C=S), 167.92-106.30 (13C, Ar-C), 43.43 (CH_2), 15.38 (CH_3)	431.48 433.23 (M^{+2}).
5d	3324 (NH), 1656 (C=N) 1752 (C=S)	10.503 (s, H –NH), 8.919 (s, H -CN), 7.159-8.197 (m, 7H Ar-H), 4.258 (s, 2H $–CH_2$), 2.094 (s, 3H $–CH_3$)	192.23 (C=S), 170.28-106.28 (13C, Ar-C), 40.28 (CH_2), 15.58 (CH_3)	493.42 494.12 (M^{+2}).
5f	3322 (NH), 1655 (C=N) 1713 (C=S)	10.536 (s, H –NH), 8.519 (s, H -CN), 6.879 -8.193 (m, 7H Ar-H),4.225 (s, 2H $–CH_2$), 3.068 (s, 3H $–OCH_3$), 2.268 (s, 3H $–CH_3$)	192.55 (C=S), 167.62-106.34 (13C, Ar-C), 55.40 (CH_3) 43.43 (CH_2), 15.38 (CH_3)	443.88

Experimental

1 Synthesis of 6-nitro-1,3-benzoxazole- 2- thiol (2)

The mixture of 2-amino-5-nitro phenol **(1)** (0.01 mol) and carbon disulfide (0.01 mol) in presence of potassium hydroxide were refluxed for 6h in 30 ml of methanol. The product was confirmed by TLC. The reaction mass was poured on to crushed ice and acidified with glacial acetic acid. Thus solid mass separated out, filtered and recrystallized from ethanol.

2 Synthesis of 1-[(6-nitro-1, 3-benzoxazol-2-yl)sulfanyl]propan-2-one (3)

The compound 6-nitro-1,3-benzoxazole-2- thiol (0.01 mol) and chloroacetone (0.01 mol) in presence of potassium hydroxide (0.01 mol) in 30 ml of ethyl acetate were refluxed for 5h. The product was confirmed by TLC. The reaction mixture was poured on to the crushed ice, acidified with glacial acetic acid. Thus solid separated was filtered and the obtained product was recrystallized using ethanol.

3 Synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-onethiosemicarbazone (4)

Mixture of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one (0.01mol) and thiosemicarbazide (0.01 mol) was heated under reflux in 30 ml of ethanol in presence of acetic acid. The resulting mixture was further allowed for 6h. The mixture was poured on to crushed ice, the solid separated out and it was filtered and recrystallized by methanol.

4 General procedure for the synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone derivatives 5(a-g)

Mixture of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone (0.01 m) and substituted aromatic aldehyde (0.01 m) in 50 ml of ethanol was refluxed for 4h in presence of catalytic amount of glacial acetic acid, the resulting mixture was poured onto crushed ice. The colored solid separated out was filtered and washed with cold water. The product was recrystallized from dry ethanol to get the derivatives **5(a-g)**. The physical data of compounds are tabulated in table Table 2.2

Table 2.2: physical data of compounds 5(a-g)

Compound	R	Molecular formula	Molecular weight	M.P. (^{0} c)	% of Yield	Found (Calculated) %		
						C	H	N
5a	C_6H_5	$C_{18}H_{15}N_5O_3S_2$	413.47	202	79	51.21 (52.29)	3.56 (3.66)	16.88 (16.94)
5b	4-F-C_6H_4	$C_{18}H_{14}FN_5O_3S_2$	431.46	212	74	50.11 (50.14)	3.27 (3.25)	16.23 (16.22)
5c	4-Cl-C_6H_4	$C_{18}H_{14}ClN_5O_3S_2$	447.91	218	84	48.27 (48.52)	3.15 (3.17)	15.64 (15.62)
5d	4-Br-C_6H_4	$C_{18}H_{14}BrN_5O_3S_2$	492.36	236	82	43.91 (43.93)	2.87 (2.85)	14.22 (14.24)
5e	4-NO_2-C_6H_4	$C_{18}H_{14}N_6O_5S_2$	458.47	216	76	47.16 (47.25)	3.08 (3.10)	18.33 (18.30)
5f	4-OCH_3-C_6H_4	$C_{19}H_{17}N_5O_4S_2$	443.49	244	75	51.46 (51.47)	3.86 (3.84)	15.79 (15.80)
5g	4-OH-C_6H_4	$C_{18}H_{15}N_5O_4S_2$	429.47	220	70	50.34 (50.36)	3.52 (3.51)	16.31 (16.34)

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CHAPTER III

SYNTHESIS OF NOVEL2-{[(1-ETHYL-1*H*-BENZIMIDAZOL-2-YL)METHYL] SULFANYL}-6-NITRO-1,3-BENZOXAZOLE

3.1 Introduction

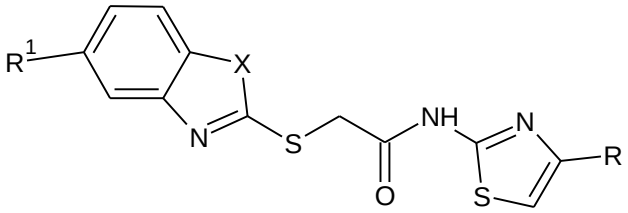
The practice of medicinal chemistry was devoted to the discovery and development of new agents for treating disease[1]. An important aspect of medicinal chemistry has been to establish a relationship between chemical structure and pharmacological activity. The chemistry of heterocyclic compounds was the most important in the discovery of new drugs. The study of these compounds was of great interest both in theoretical as well as practical aspects[2]. Benzoxazole finds important in research as a starting material for the synthesis of bioactive molecules. Benzoxazole was found within the chemical structures of pharmaceutical drugs such as Flunoxaprofen, Calcimycin, Zoxazolamine, Routiennocin, etc contains benzoxazole moiety. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites, which allow for functionalization. It was noticed that benzoxazole were used as antihistaminic[3], antifungal[4], cyclooxygenase inhibiting[5]. The main objective of synthetic chemistry and medicinal chemistry was to synthesize the compounds that give more yields with purity and show promising activity as therapeutic agents with no toxicity.

3.2 Introduction to Benzimidazole

Literature survey showed that Benzoxazole connected to benzimidazole derivatives have been found to be of great interest due to the broad spectrum of biological and pharmaceutical activities, such as Antimicrobial[6,7], antifungal[8], antiviral[9,10], anticancer[11,12], anti-tumor[13], anti-hepatitis-C-virus[14], kinase inhibitor[15,16], analgesic[17], antihypertensive[18], antiulcer[19] anti-inflammatory[20]. On the other hand, Schiff bases have an efficient antimicrobial and antifungal activities[21]. The benzimidazole nucleus has resulted in many drugs like albendazole, mebendazole, thiabendazole as antihelmintics, omeprazole, lansoprazole,

pantoprazole as proton pump inhibitors and many lead compounds in a wide range of other therapeutic areas.

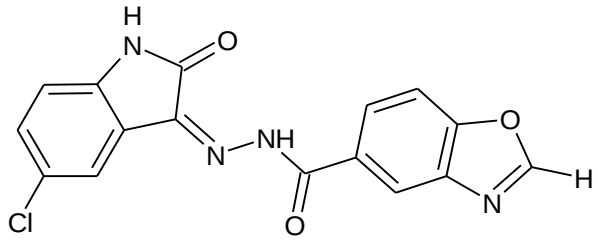
K. Zaferasim et al., Synthesized[22] the compound 2-[[(benzoxazole/benzimidazole-2-yl)sulfanyl]acetylamino]thiazoles derivatives (**1)** and evaluated for antimicrobial activities against *Micrococcus luteus* (NRLL B-4375), *Bacillus cereus* (NRRL B-3711), *Proteusvulgaris* (NRRL B-123), *Salmonella typhimurium* (NRRL B-4420), *Staphylococcus aureus*(NRRL B-767), *Escherichia coli* (NRRL B-3704), *Candida albicans* and *Candida globrata* strains, by using standard drugs chloramphenicol and ketoconazole. The compounds 1a, 1b, 1c, 1d and 1f showed potent antibacterial activity.



1

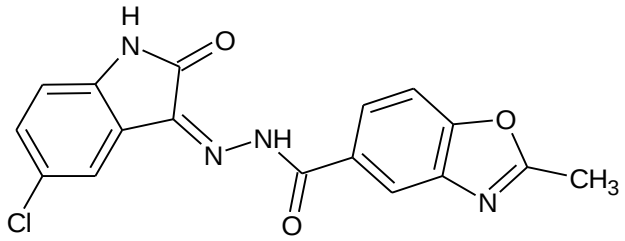
compound	R	R^1
1a	$COOC_2H_5$	H
1b	$COOC_2H_5$	Cl
1c	$COOC_2H_5$	NO_2
1d	$COOC_2H_5$	CH_3
1e	$COOC_2H_5$	H
1f	$COOC_2H_5$	Cl

M. Sarangapani et al., synthesized[23] the compounds (**2)** and (**3)** and the molecules were subjected to antibacterial activity against four bacterial strains *B.subtilis, S.aureus, E.coli* and *P.vulgaris* and two fungal strains *A.niger* and *C.verticulata*, all the compounds were showed moderate antibacterial activity.



2

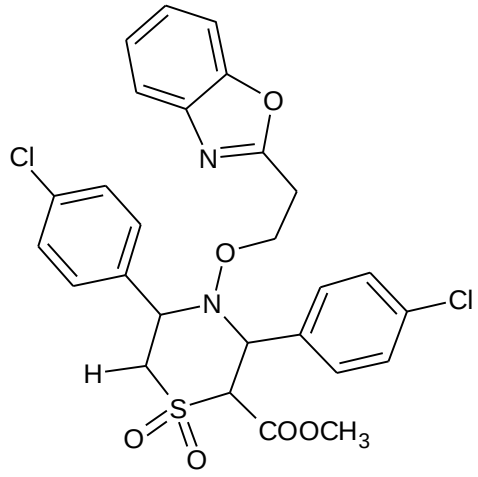
***N*'-[(3*Z*)-5-chloro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]-1,3-benzoxazole-5-carbohydrazide**



3

***N*'-[(3*Z*)-5-chloro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]-2-methyl-1,3-benzoxazole-5-carbohydrazide**

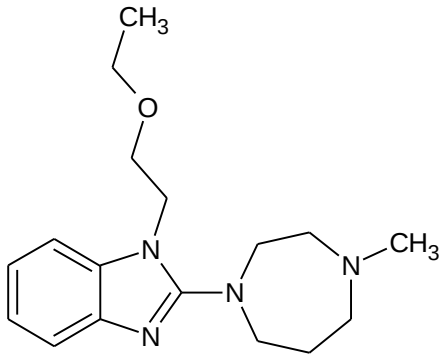
V. Sundari and Valliappan were synthesized[24] 3,5-diaryl-4-{2-ethoxybenzoxazol-2- yl}-tetrahydro-1,4-thiazine-1,1-dioxides and 2,6-dimethoxycarbonyl-3,5-diaryl-4-{2- ethoxybenzoxazol-2-yl}-tetrahydro-1,4-thiazine-1,1-dioxides (**4)** and they were evaluated for antibacterial and antifungal activities. The compound (**4)** exhibited the activity by inhibiting the growth of bacterial and fungal strains at a minute concentration of 25µg/ml when compared to standard drug Norfloxacin.



4

2.6-Dimethoxycarbonyl/3.5-diaryl-4-{2-ethoxybenoxazol-2-yl}-tetrahydro-1.4-thiazine-1,1-dioxides

L. Bielory et al., reported[25] the benzimidazole derivative 1-(2-ethoxyethyl)-2-(4-methyl-1,4-diazepan-1-yl)-1*H*-benzimidazole **(5)** acts as a second generation antihistamine, which is using in eye drops to treat allergic conjunctivitis. It behaves as a H1 receptor antagonist. It activates by blocking certain natural substances.



5

1-(2-ethoxyethyl)-2-(4-methyl-1,4-diazepan-1-yl)-1*H*-benzimidazole

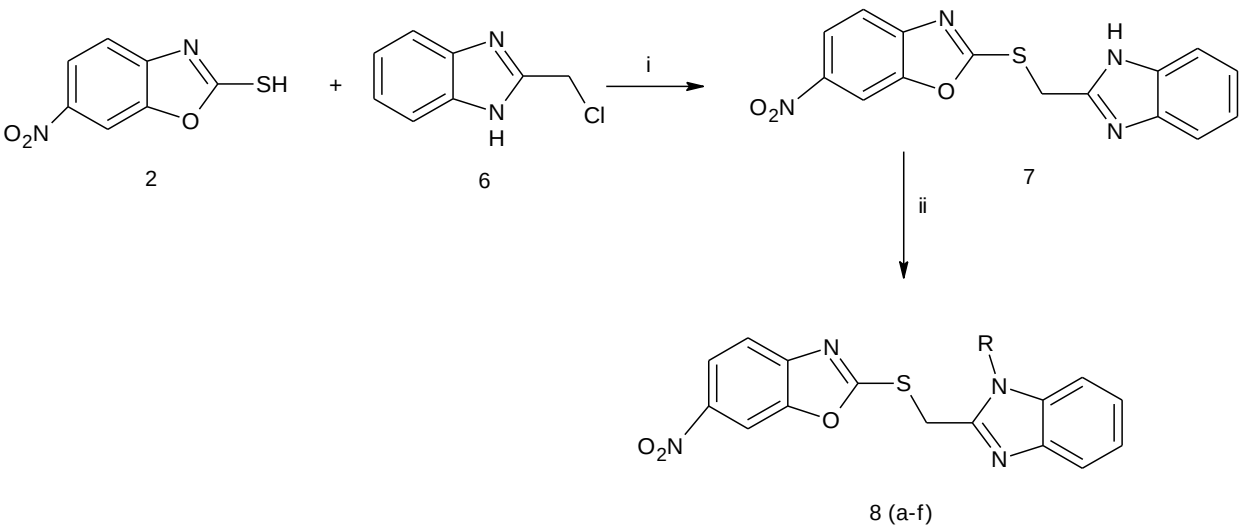
In view of these facts, the present work have been intended for the synthesis of a novel series of benzoxazole derivatives bearing a substituted benzimidazole nucleus and evaluated for antimicrobial and antioxidant activities.

3.3. Present Work

This chapter describes the synthesis of benzoxazole fused benzimidazole derivatives. The target benzoxazole derivatives were purified, characterized and confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral technique and they were screened for biological activities.

The present chapter describes the synthesis of the following benzoxazole derivatives

- Synthesis of 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-6-nitro-1,3-benzoxazole **(7)**
- Synthesis of 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-6-nitro-1,3-benzoxazole **8(a-f)**



Scheme-3: Synthetic route for the synthesis of compounds **8(a-f)**

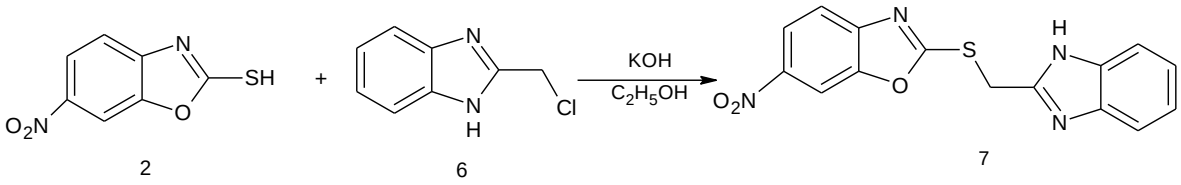
(i) KOH, C_2H_5OH (ii) R, CH_3COCH_3, DMF, K_2CO_3.

	R
8a	$-COCH_3$
8b	$-C_2H_5$
8c	$-CH_2COCl$
8d	$-CH_2COOH$
8e	$-CH_2COCH_3$
8f	$-C_2H_4Br$

3.4 Synthesis of 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-6-nitro-1,3-benzoxazole (7)

The benzimidazole nucleus is a crucial pharmacophore in drug discovery. Pharmaceutical properties such as antiviral, antitumor[28], antihistaminic, antimicrobial[29], and antihelminthic[30] activities are unique characteristics known for benzimidazole derivatives. Based on the above observations, we have planned to synthesize a novel series of benzoxazole derivatives derived from 2-chloromethyl-

1H-benzimidazole. A mixture of 6-nitro-1,3 benzoxazole- 2- thiol (**2)** and 2-(chloromethyl)-1*H*-benzimidazole (**6)** was refluxed on water bath by using potassium hydroxide as a catalyst in ethanol. The solid product separated and filtered off and recrystallized from ethanol **(Scheme-3.2)**.



Scheme-3.1

The structure of the compound **7** was confirmed by spectroscopic data. The IR spectrum **(fig. 3.1)** of the compound (**7)** exhibited a strong absorption band at 3310 cm^{-1} for –NH group. The ^{1}H NMR spectrum of compound (**7) (fig. 3.2)** exhibited a broad singlet at δ 11.50 for one proton -NH (D_2O exchangeable) and multiplet between δ 8.24-7.08 corresponds to seven protons of aromatic ring and singlet peak at δ 4.95 for two protons of $–CH_2$ protons confirmed the target compound. The ^{13}C NMR spectrum **(fig. 3.3)** showed twelve peaks in the region δ 170.22-105.44 for 14C aromatic carbon, the $–CH_2$ aliphatic carbon appeared at δ 42.53 this strongly supported the structure of (**7)**. Further the molecular ion peak at m/z 326.92 concluded the formation of compound (**7) (fig. 3.4)**.

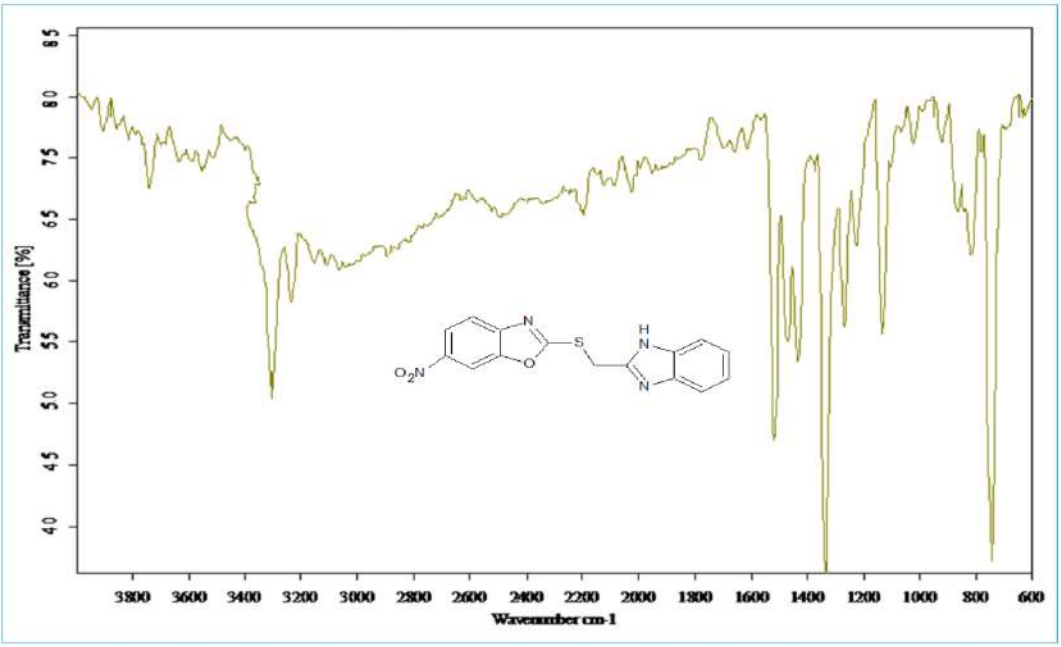


Fig.3.1 IR Spectrum of compound 7

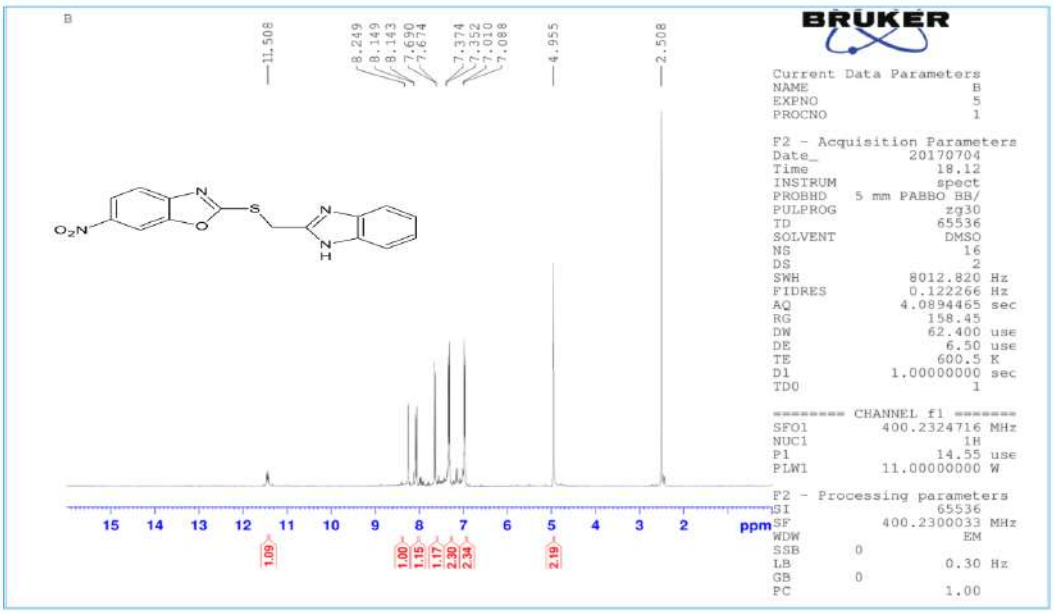


Fig.3.2 ^{1}H NMR Spectrum of compound 7

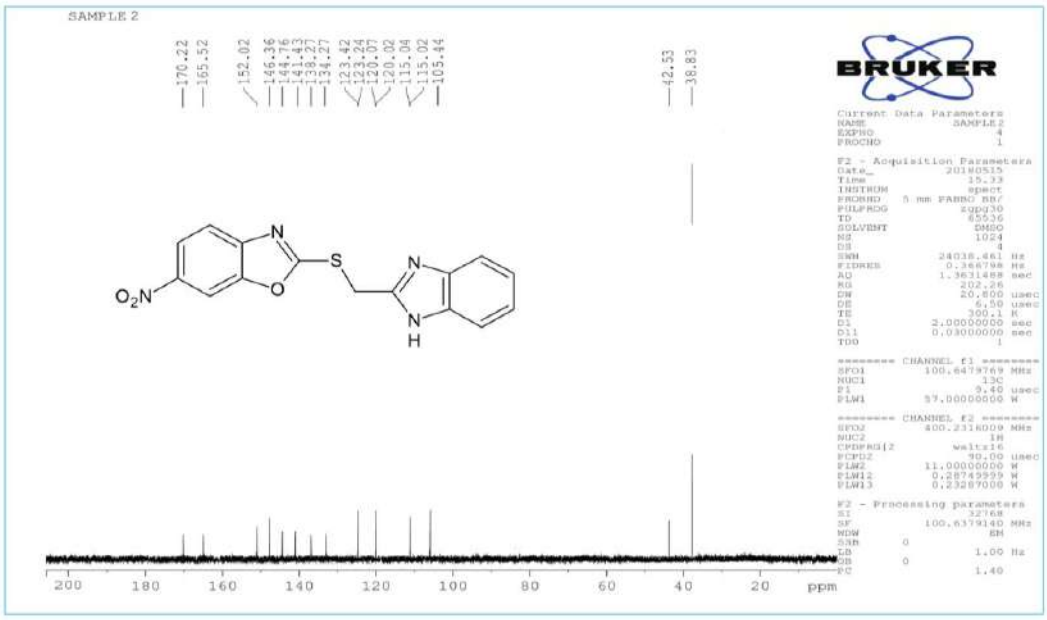


Fig.3.3 ^{13}C NMR Spectrum of compound 7

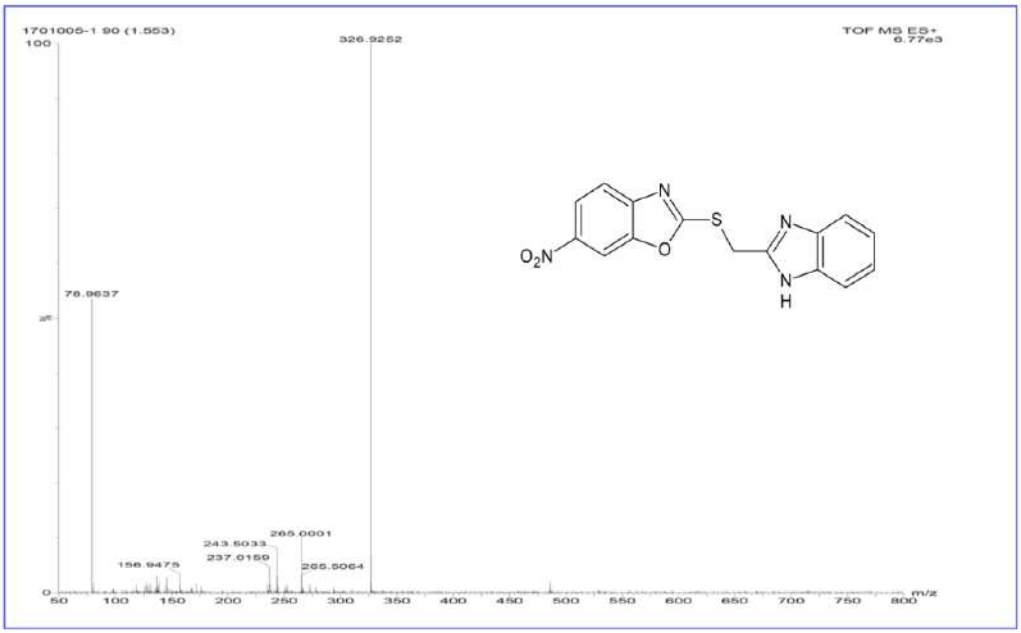
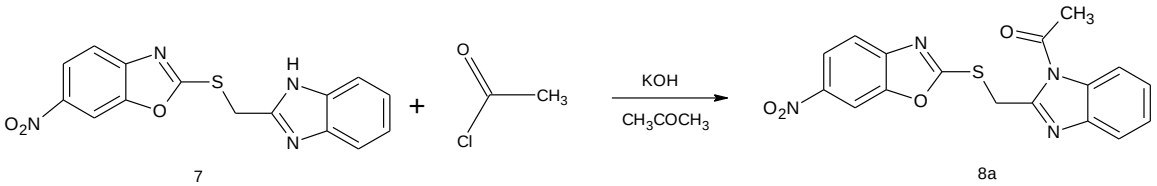


Fig.3.4 LCMS Spectrum of compound 7

3.5 Synthesis of 2-{[(1-acetyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole 8(a)

The chemistry and pharmacology of benzimidazoles have been of great interest in drug discovery. The present work has been focusing on the construction of benzoxazole linked with benzimidazole derivative **8(a)**. The compound 2-[(1H-benzimidazol-2-ylmethyl)sulfanyl]-6-nitro-1,3-benzoxazole (**7)** was treated with acetyl chloride in the presence of potassium hydroxide by using acetone as solvent, produced 2-{[(1-acetyl-1H-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole in good yield **(Scheme-3.2).**



7 8a

Scheme-3.2

The structure of the newly synthesized compounds was confirmed by spectral studies. The compound **8(a)** exhibited a strong stretching absorption at 1695.02 cm^{-1} in the IR spectrum **(fig. 3.5)** indicated the presence of –C=O group. In the ^{1}H NMR spectrum the seven aromatic protons appeared at δ 7.85-7.30 and the $–CH_2$ group showed a singlet at δ 4.26 and a new singlet at δ 1.37 for three protons of $–CH_3$ confirmed the formation of target molecule **(fig. 3.6)**. In ^{13}C NMR spectrum exhibited the peak at δ 170.22 for –C=O, $–CH_2$ aliphatic carbon appeared at δ 42.53 and $–CH_3$ carbon exhibited a peak at δ 24.53 **(fig. 3.7)** confirmed the formation of desired molecule. Appearance of the molecular ion peak at m/z 368.93 in the mass spectrum concluded the formation of the end product **(fig. 3.8).**

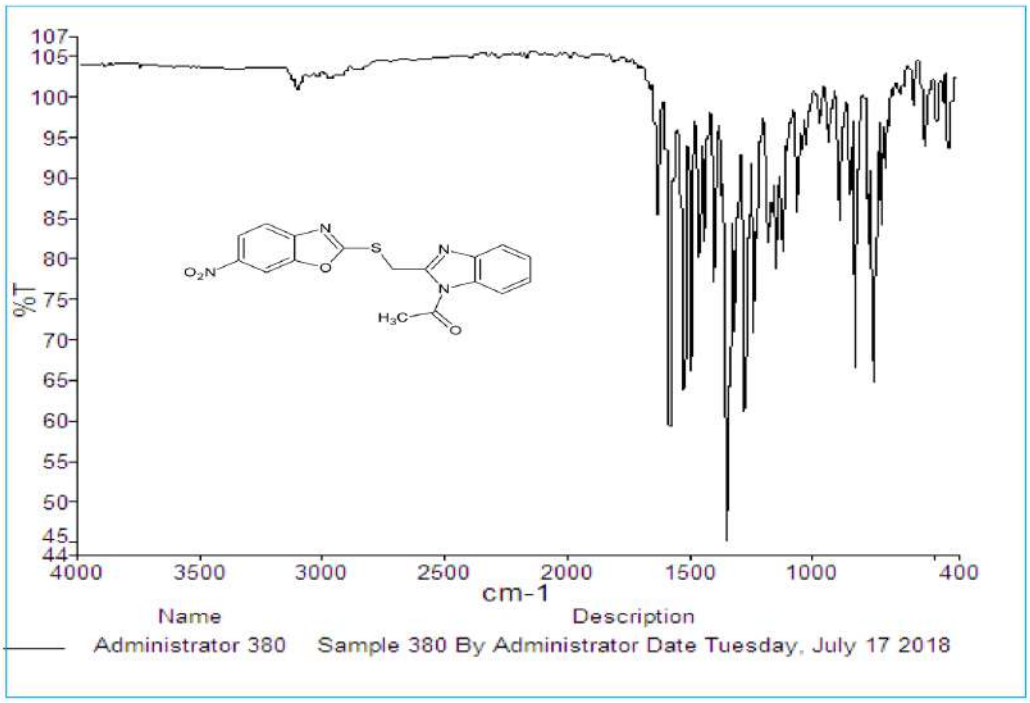


Fig.3.5 IR Spectrum of compound 8a

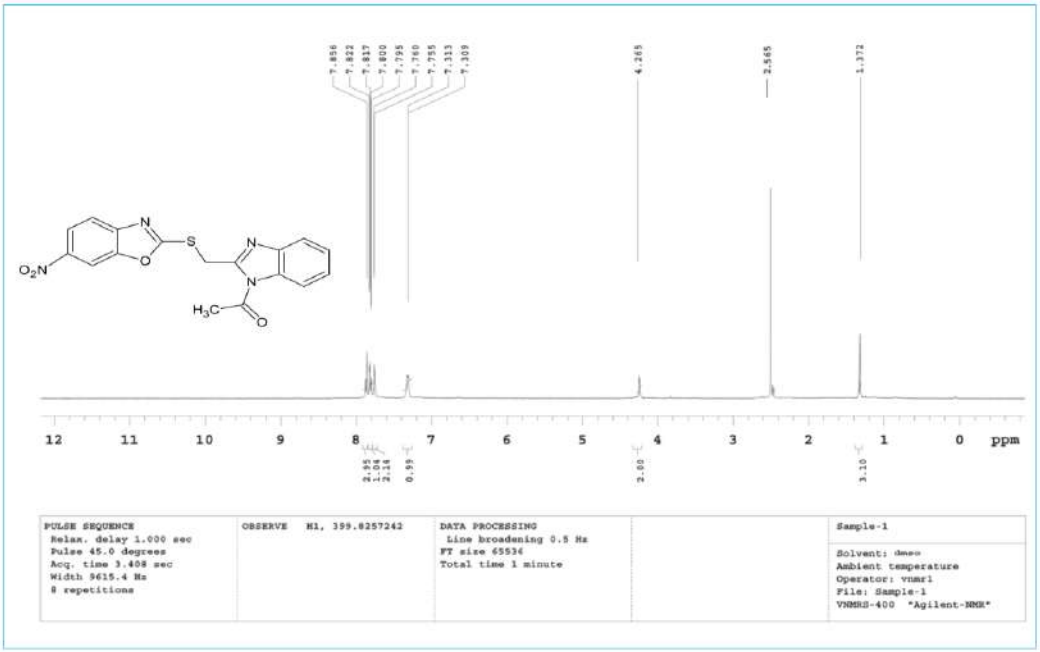


Fig.3.6 ^{1}H NMR Spectrum of compound 8a

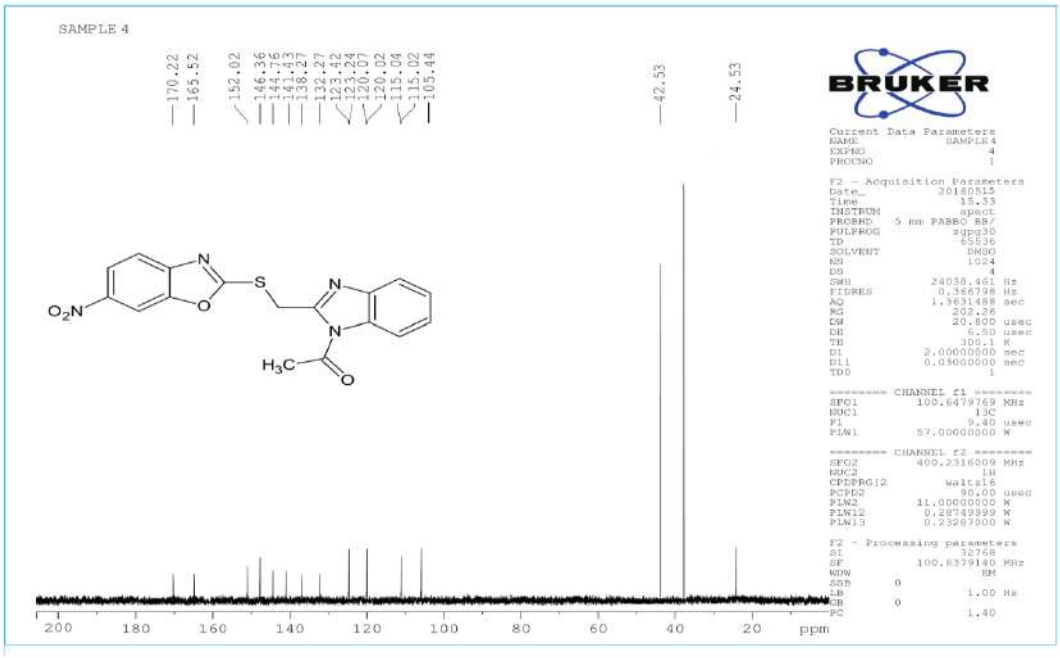


Fig.3.7 ^{13}C NMR Spectrum of compound 8a

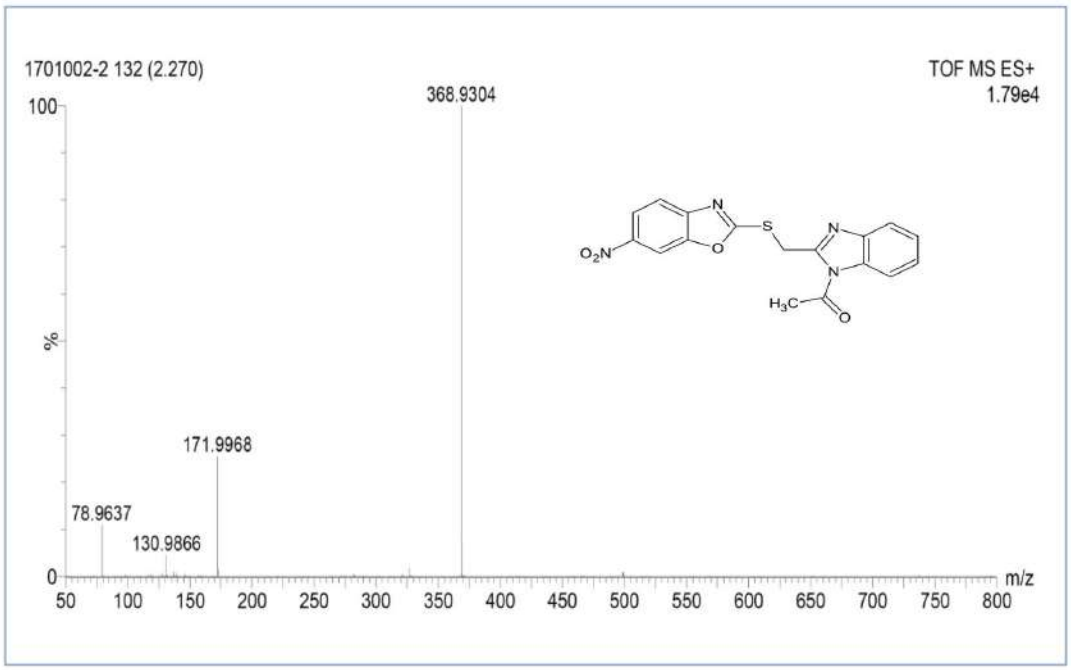
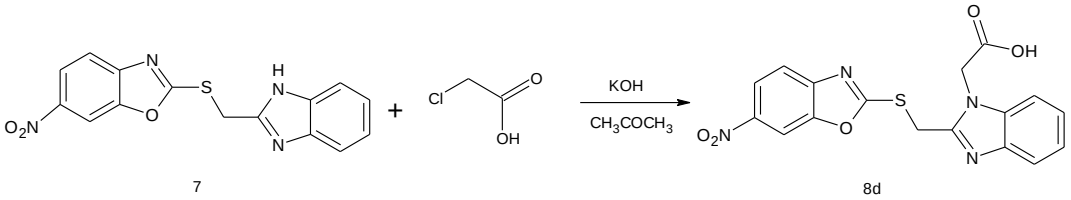


Fig.3.8 LCMS Spectrum of compound 8a

3.6 Synthesis of 2-{[(1-ethyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole 8(d)

It was well known that, the nitro and carboxylic substituted derivatives of benzoxazole exhibits promising pharmacological activities. Hence, the nitro and carboxylic derivatives of benzimidazole namely 2-{[(1-ethyl-1H-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole **8(d)** was prepared by the reaction between compound **7** and chloroacetic acid by using DMF as a solvent in basic media. The synthetic route of the reaction was mentioned below in **(Scheme-3.3).**



Scheme-3.3

The compound **8(d)** exhibited a broad peak at 3421.12 cm^{-1} **(fig. 3.9)** for -OH functionality and the strong stretching frequency at 1625.5 cm^{-1} for –C=O group. In the ^{1}H NMR spectrum, the –OH proton appeared as a broad singlet at δ 12.86 and Seven aromatic protons appeared as multiplet in the region of δ 7.95-7.30. The two $–CH_2$ groups appeared at δ 4.86 and δ 4.26 as singlet for N-CH_2 and S-CH_2 respectively. **(fig. 3.10).** The ^{13}C NMR showed signals for –C=O, S–CH_2 and N–CH_2 at δ 175.36, δ 55.30 and δ 43.53 respectively **(fig. 3.11)**. Further the mass spectrum of **8(d) (fig. 3.12)** confirmed the structure by appearing the molecular ion peak at m/z 384.43**.**

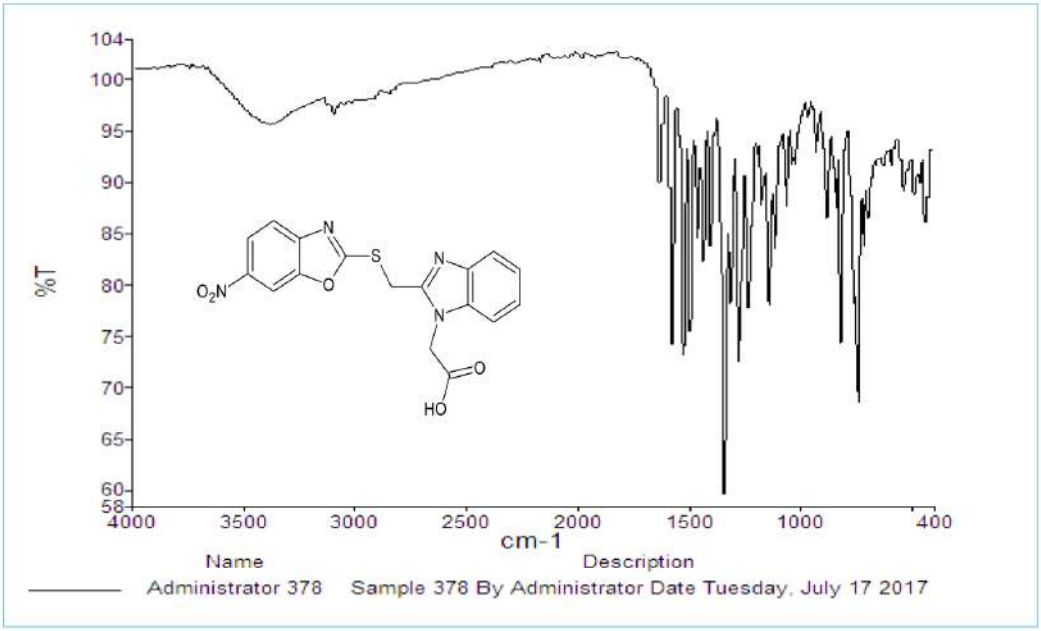


Fig.3.9 IR Spectrum of compound 8d

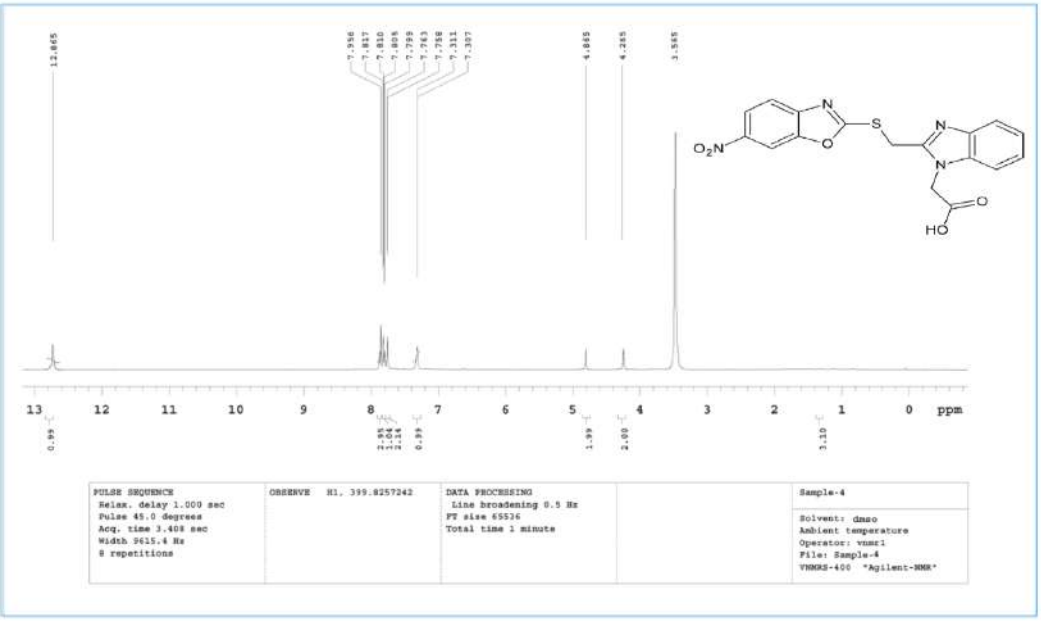


Fig.3.10 ^{1}H NMR spectrum of compound 8d

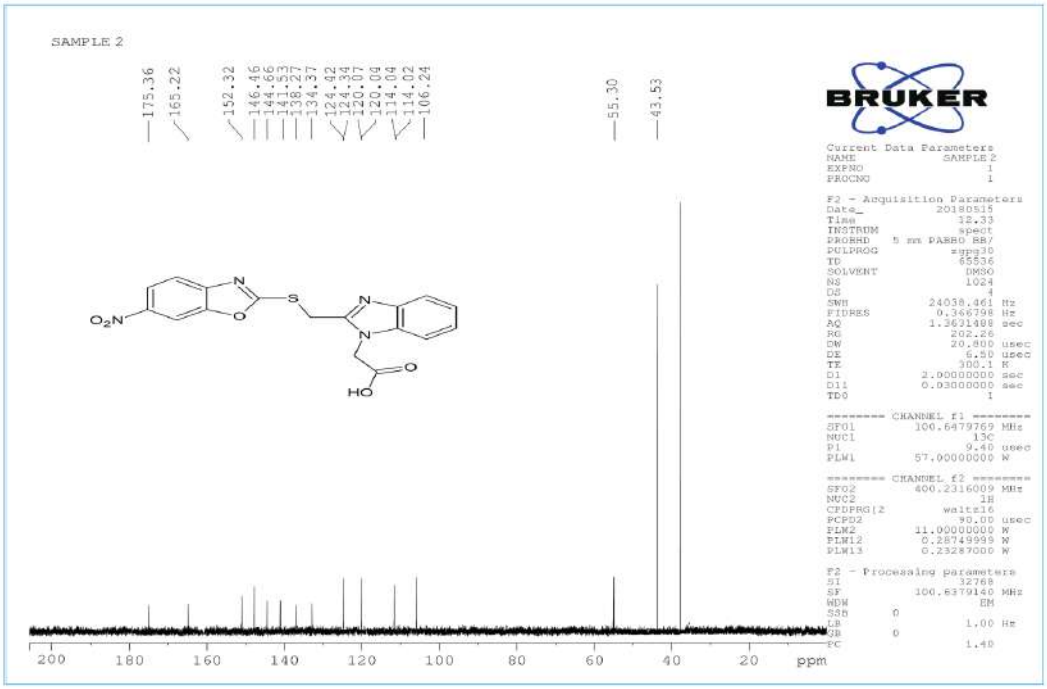


Fig.3.11 ^{13}C NMR spectrum of compound 8d

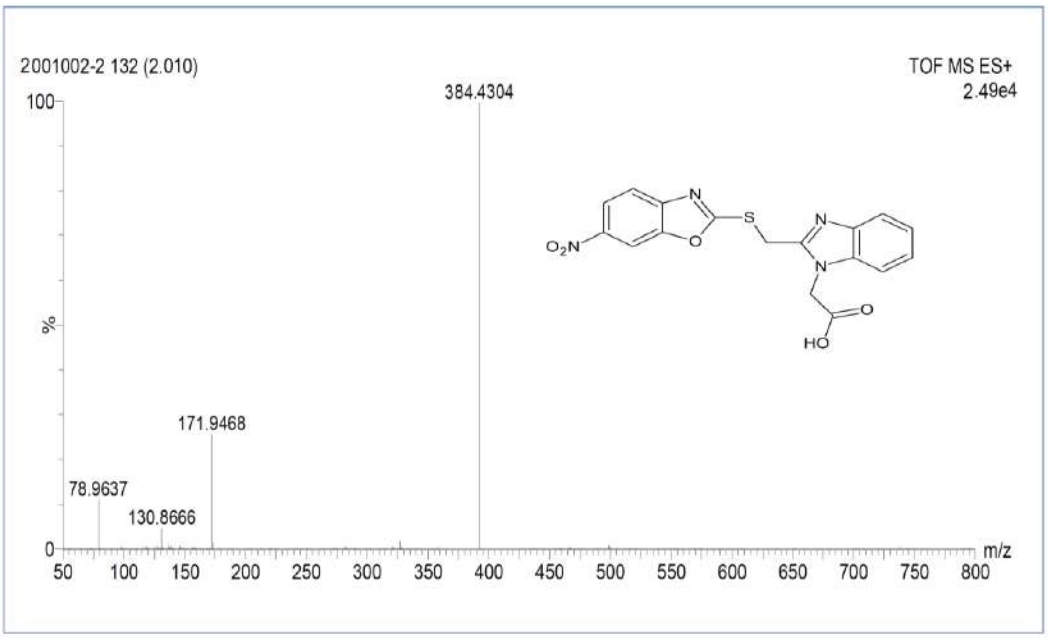
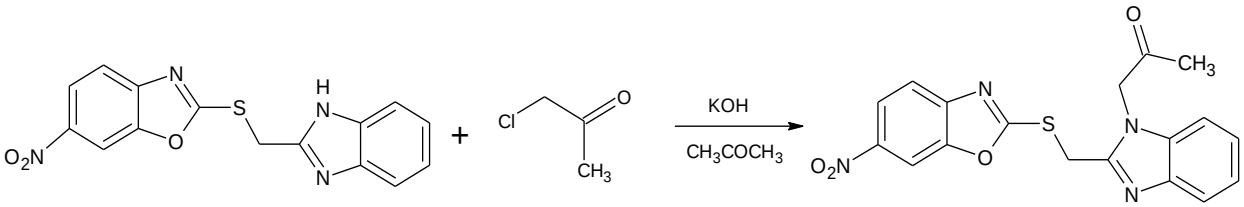


Fig.3.12 LCMS spectrum of compound 8d

3.7 Synthesis of 1-(2-{[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]methyl}-1*H*-benzimidazol-1-yl)propan-2-one 8(e)

1-(2-{[(6-nitro-1,3- benzoxazol-2-yl) sulfanyl] methyl}-1*H*-benzimidazol-1-yl)propan-2-one **8(e)** was prepared by refluxing the compound **7** and chloroacetone was stirred in the presence of acetone by using potassium carbonate as a catalyst **(Scheme 3.4)**.



7 8e

Scheme-3.4

In the ^{1}H NMR spectrum of the compound **8(e)**, the seven aromatic protons showed signals between δ 7.93-7.31 range correspondingly. The two $–CH_2$ groups showed signals at δ 4.76 and δ 4.25 as singlet for $N\text{-}CH_2$ and $S\text{-}CH_2$ respectively, the broad singlet appeared at 1.37 for $–CH_3$ **(fig. 3.13)** which confirmed the target molecule. ^{13}C NMR showed a signals at δ 206.36 for -C=O group, the 14C aromatic carbons exhibited signals at δ 166.22-105.44. For $N–CH_2$ and $S–CH_2$ showed signals at δ 53.30 and δ 42.03 respectively.**(fig. 3.14)** It was also supported by the mass spectrum. The molecular mass of the compound **8(e)** m/z 382.43 exactly matches with structure assigned to the compound **(fig. 3.15)** confirmed the synthesized title molecule **8(e).**

The spectral data and physical data of the synthesized compounds are tabulated in Table 3.1 and 3.2.

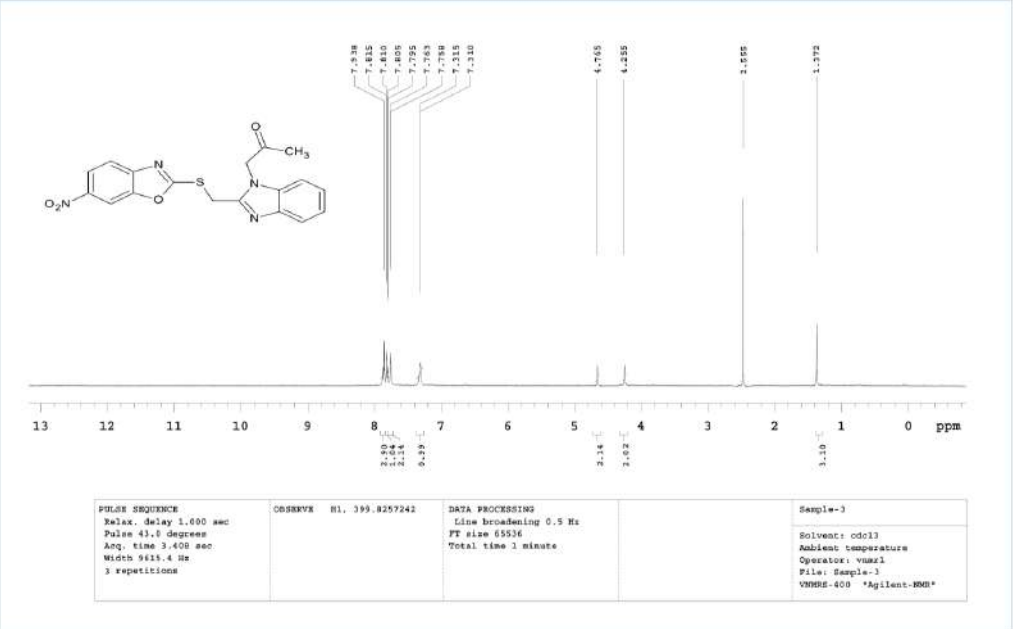


Fig.3.13 ^{1}H NMR spectrum of compound 8e

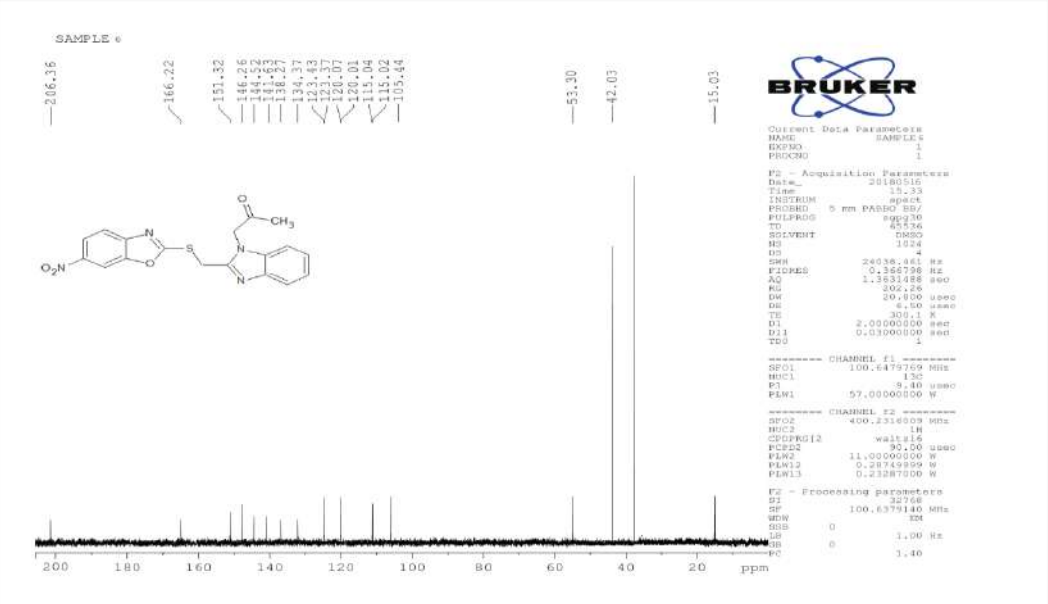


Fig.3.14 ^{13}C NMR spectrum of compound 8e

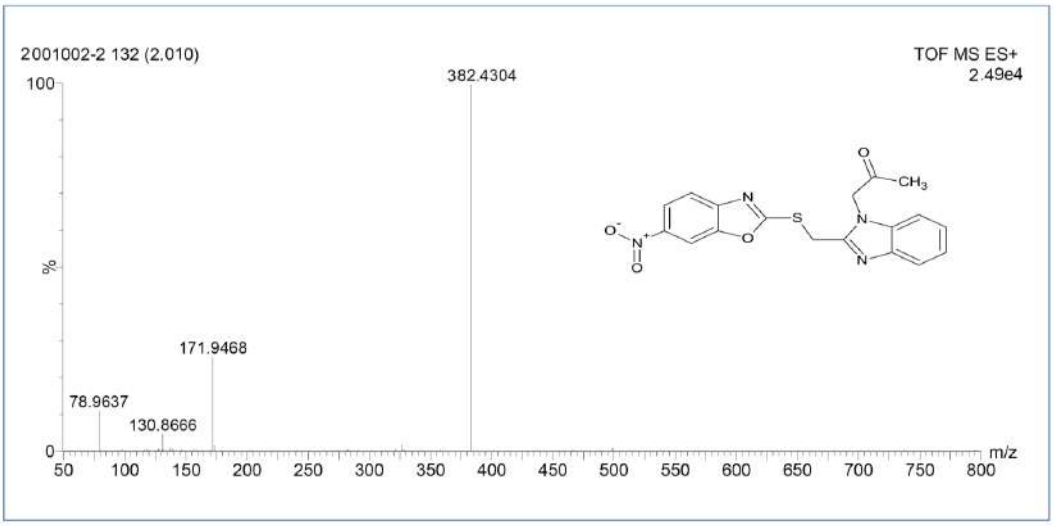


Fig.3.15 LCMS spectrum of compound 8e

Table 3.1 Spectral data of synthesized compounds

Compound	IR (KBr) in cm^{-1}	1 HNMR in δ PPM	^{13}C NMR in δ PPM	Mass M^+ M^{+2}
8b	2912 cm^{-1} (CH_3)	7.852-7.305 (m, 7H-Ar-H), 4.265 (q, 2H-CH_2), 1.372 (t,3 H-CH_3)	165.02-106.34 (14C, Ar-C), 55.40 (CH_2), 43.43 (CH_2), 15.38 (CH_3)	354.38
8c	1752 cm^{-1} (C=O) 2862 cm^{-1} (CH_2)	7.958-7.308 (m, 7H-Ar-H), 4.565 (s, 2H-CH_2), 4.265 (s, 2H-CH_2)	190.36 (C=O), 165.22-105.24 (14C, Ar-C), 54.30 (CH_2), 42.24 (CH_2)	403.08. (M+), 405.23 (M+2)
8f	787 cm^{-1} (Br)	8.449-7.652 (m, 7H-Ar-H), 4.355 (s, 2H-CH_2), 4.255 (t, 2H-CH_2) 3.553 (t, 2H-CH_2)	165.12-106.65 (14C, Ar-C), 55.24 (CH_2), 40.21 (CH_2), 32.56 (CH_2)	434.40 (M^+), 436.40 (M^{+2}).

Experimental

1 Synthesis of 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-6-nitro-1,3-benzoxazole (7)

A mixture of 6-nitro-1,3 benzoxazole-2-thiol **2** and 2-(chloromethyl)-1*H*-benzimidazole **4** in dry ethanol and catalytic amount of potassium hydroxide was refluxed on water bath for 8h. The reaction mixture was poured onto ice cold water to get solid product (**7)**.

2 Synthesis of 2-[(1*H*-benzimidazol-2-ylmethyl)sulfanyl]-6-nitro-1,3-benzoxazole derivatives 8 (a-f)

The compound **7** was treated with acetyl chloride, iodoethane, chloro acetyl chloride, chloroacetic acid, chloroacetone and dibromoethane in presence of acetone, DMSO, DMF, solvents to get target 2-[(1H-benzimidazol-2-ylmethyl)sulfanyl]-6-nitro-1,3-benzoxazole derivatives **8 (a-f)**

2.1 Synthesis of 2-{[(1-acetyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole 8(a)

The mixture of compound **7** (0.01 mol) and acetyl chloride (0.01 mol) were stirred with 40 ml of acetone in the presence of potassium hydroxide (0.01 mol) as a catalyst and reaction mass was refluxed for 8h. Then the reaction mixture was poured on to crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethanol.

2.2 Synthesis of 2-{[(1-ethyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole 8(b)

The compound **7** (0.01 mol) in 40 ml of DMSO mixed with iodoethane (1.2 eq) in presence of sodium hydroxide (0.01 mol) and reaction mixture was refluxed for 6h and cooled. The solid product separated was filtered, washed with water, dried and recrystallized from ethanol.

2.3 Synthesis of 2-({[1-(chloroacetyl)-1*H*-benzimidazol-2-yl]methyl} sulfanyl)-6-nitro-1,3-benzoxazole 8(c)

The compound **7** (0.01 mol) was refluxed for 6h in 30 ml of acetone with chloro acetyl chloride (1.2 eq) and pinch of base potassium carbonate as a catalyst. Then the reaction mass was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethanol.

2.4 Synthesis of (2-{[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]methyl}-1*H*-benzimidazol-1-yl)acetic acid 8(d)

The mixture of chloroacetic acid (1.1eq) and compound **7** (0.01 mol) were stirred with 20 ml of DMF in the presence of potassium carbonate (1 eq) as a catalyst for fifteen minute and refluxed for 8h. Then the reaction mixture was poured onto crushed ice. Solid product thus obtained was filtered, dried and recrystallized from ethanol.

2.5 Synthesis of 1-(2-{[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]methyl}-1*H*-benzimidazol-1-yl)propan-2-one 8(e)

The mixture of compound **7** (0.01 mol) and chloroacetone (0.01 mol) was stirred with 30 ml of Acetone in the presence of potassium carbonate (0.01 mol) as a catalyst and reaction mixture was refluxed for 6h. Then the reaction mass was poured onto crushed ice. Solid product separates out was filtered, dried and recrystallized from ethanol.

2.6 Synthesis of 2-({[1-(2-bromoethyl)-1*H*-benzimidazol-2-yl]methyl} sulfanyl)-6-nitro-1,3-benzoxazole 8(f)

The compound **7** (0.01 mol) in 30 ml of DMSO mixed with dibromoethane (1.2 eq) in presence of sodium hydroxide (0.01 mol) as catalyst and reaction mixture was refluxed for 6h and cooled. The solid separated was filtered, washed with water, dried and recrystallized from methanol. The physical data of the compounds **8 (a-f)** was mention in the Table 3.2

Table 3.2: physical data of compounds 8 (a-f)

Compound	R	Molecular formula	Molecular weight	M.P. (^{0} c)	% of Yield	Found (Calculated) %		
						C	H	N
8a	$COCH_3$	$C_{17}H_{12}N_4O_4S$	368.36	200	82	55.43 (50.44)	3.28 (3.25)	15.12 (15.14)
8b	C_2H_5	$C_{17}H_{14}N_4O_3S$	354.38	168	80	57.62 (57.56)	3.98 (3.94)	15.81 (15.84)
8c	CH_2COCl	$C_{17}H_{11}ClN_4O_4S$	402.81	204	84	50.69 (50.64)	2.75 (2.72)	13.91 (13.90)
8d	CH_2COOH	$C_{17}H_{12}N_4O_5S$	384.36	194	78	53.12 (53.10)	3.15 (3.16)	14.12 (14.08)
8e	CH_2COCH_3	$C_{18}H_{14}N_4O_4S$	382.39	212	80	56.54 (56.50)	3.69 (3.70)	14.65 (14.63)
8f	C_2H_4Br	$C_{17}H_{13}BrN_4O_3S$	433.27	198	80	47.12 (47.10)	3.02 (3.01)	12.93 (12.94)

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CHAPTER IV

SYNTHESIS OF NOVEL 2{[2HYDRAZINYLIDENEPROPYL]SULFANYL}-6-NITRO-1,3-BENZOXAZOLE DERIVATIVES

4.1 Introduction

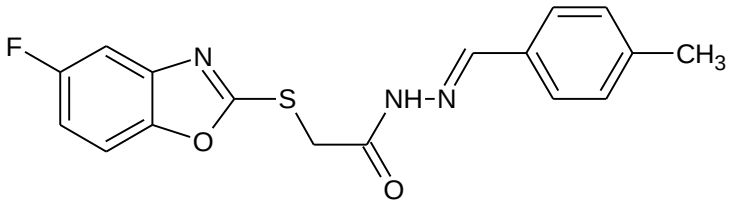
Human beings have been in constant discloser to pathogens for many millennia. Microbial infections are a growing complication in contemporary medicine, yet only a few antimicrobial agents were used in clinical practice. Even if several antimycobacterial agents are currently available, there is a demanding necessary for the development of new and specific antimycobacterial agents. Among the heterocycles, benzoxazole derivatives constitute much importance in medicinal field due to their broad range of biological activity. As like benzoxazole hydrazones exhibits a great interest due to their pharmaceutical activities.

4.2 Introduction to Hydrazones

Hydrazones contain azomethine –NHN=CH group and were considered as derivatives of aldehydes and ketones in which the oxygen atom has been supplanted by the -NH_2 functional group. These molecules possess diverse pharmacological potential, hence these are widely studied owing to their preparation in useful heterocyclic drugs. This has driven researchers to synthesize different heterocyclic compounds bearing hydrazones. Medicinal chemists across the globe have done immense work on hydrazones and developed various drug from hydrzone derivatives, which possess potent biological activities and low toxicity profiles. Various synthetic protocols and through SAR studies showed substituted hydrazones have been developed and found to be active against toxic pathogens. They are known to occupy remarkable biological activities such as cancer chemotherapy[1-9]. Hydrazide–hydrazone compounds play a very important role due to their potentially high antibacterial, antifungal, antimicrobial and anticonvulsant agents.[10-17] Moreover, many of them showed analgesic and antiplatelet properties.[18-22] Therapeutic prominence of the hydrazide–hydrazone derivatives has been well established. Compounds containing hydrazide–hydrazone moiety were reported to

exhibit anticancer[22-29] and anti-HIV properties[30] and hence, they have acquired an important place in medicinal chemistry. Recently, hydrazide–hydrazones have gained great importance due to their diverse biological activity, including anti-inflammatory, antimalarial and anti-tuberculosis activities.[19, 31-35] with the aim of obtaining novel keto hydrazide–hydrazones, we report here in the synthesis of a series of keto hydrazide hydrazones and followed by antitumor evaluations on human cancer cell lines[36, 37].

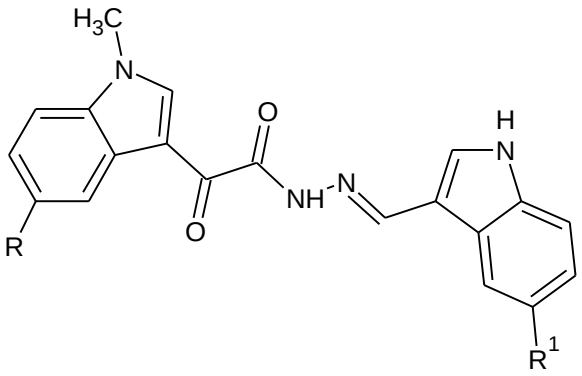
Novel benzoxazole-based hydrazone derivatives were synthesized and investigated for their cytotoxic activity this was reported by M.D. Altintop[38]. Biphenyl-substituted compound **1g** was identified as the most promising anticancer agent activity.



1(a-g)

compound	R
1a	H
1b	NO_2
1c	CN
1d	F
1e	Cl
1f	Br
1g	phenyl

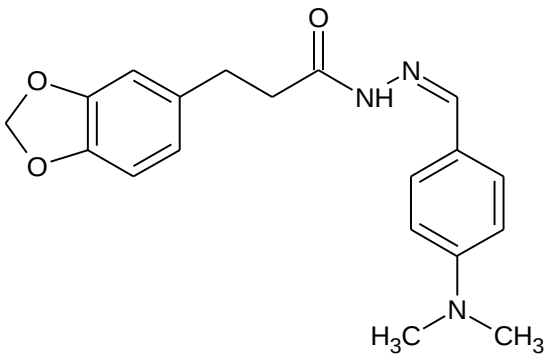
R. Durgesh et al.,[39] synthesized hydrazide–hydrazone derivatives and investigated for their cytotoxicity against human cancer cell lines. The compounds **2** and **3** were showed potent anticancer activity and identified as promising drug.



2-3

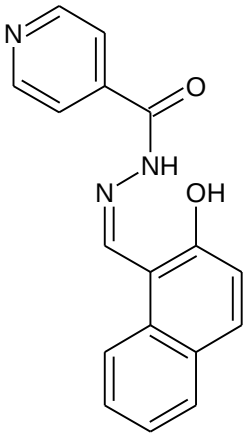
	R	R^1
2	Br	Br
3	H	H

A new series of compounds that belong to the N-acylarylhydrazone class were synthesized from natural safrole. [(4-N,N-Dimethylaminobenzylidene-3-(3,4-methylenedioxyphenyl)propionylhydrazine] **4** was found to be a more potent anti-inflammatory drugs[40].



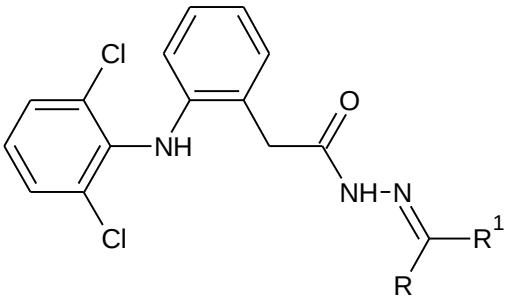
4

The novel aroylhydrazone chelator 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone **5** has been synthesized by W. Asikiya et al., and screened for anti-malarial activity. The results were showed that compound **5** exhibited significant ant-malarial activity [41].

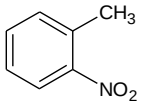
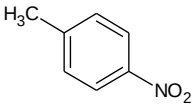
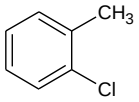
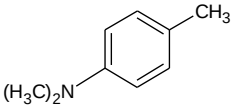
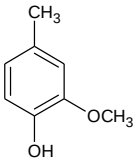
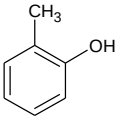
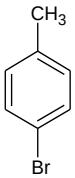
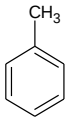


5

A series of diclofenac acid hydrazones **6** were synthesized and evaluated for their antimycobacterial activities. The results indicated that the compound **6g** showed potent *in vitro* antimycobacterial activity [42].



6

Compound	R	R^1
6a		H
6b		H
6c		H
6d		H
6e		H
6f		H
6g		

By considering these properties hydrazones are widely used in organic synthesis. The present investigation is focusing on the synthesis of hydrazones derivatives of benzoxazoles and screened for biological and pharmacological studies.

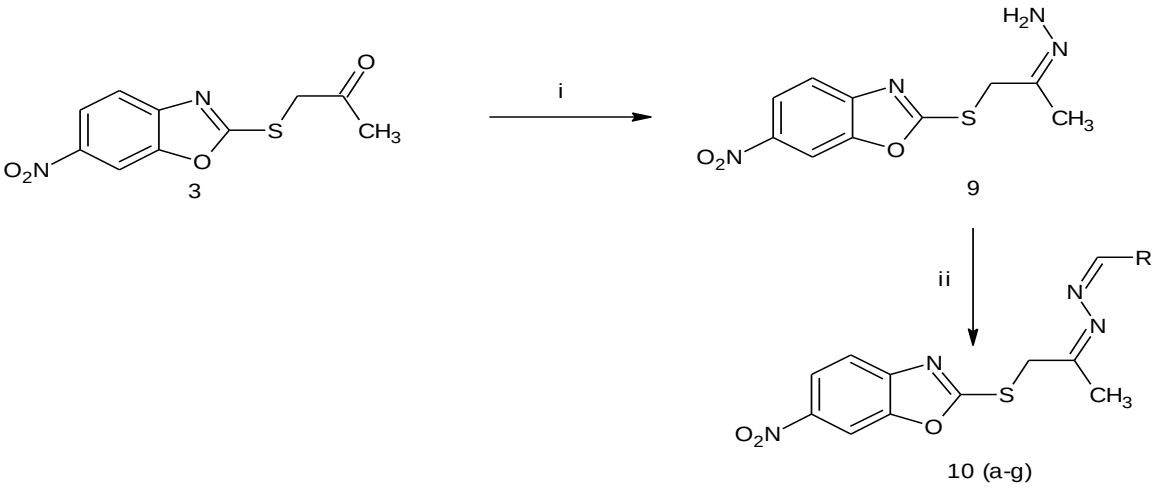
4.3. Present Work

This current chapter describes the synthesis of hydrazones linked with benzoxazole derivatives through -S- bridge which includes hydrazine. The target benzoxazole derivatives were purified, characterized and confirmed by IR, ^{1}H NMR,

^{13}C NMR and mass spectral technique and they were screened for biological activities.

The present chapter describes the synthesis of the following benzoxazole derivatives

- 1-[(6-Nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one **(3)**
- 2-{[-2-Hydrazonopropyl]sulfanyl}-6-nitro-1,3-benzoxazole **(9)**
- 2-{[-2-Hydrazonopropyl]sulfanyl}-6-nitro-1,3-benzoxazole derivatives **10(a-g)**



Scheme-4: Synthetic route for the synthesis of Compounds **10(a-g)**

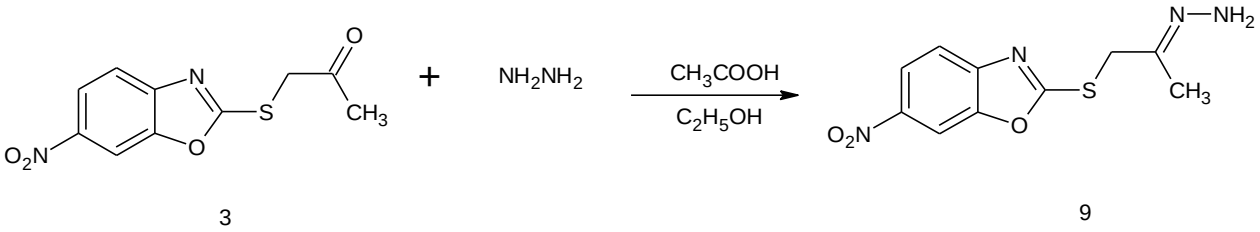
(i) NH_2NH_2. CH_3COOH, C_2H_5OH (ii) RCHO, DMF, CH_3COOH.

	R		**R**
10a	C_6H_5	10e	$4\text{-}NO_2C_6H_4$
10b	$4\text{-}FC_6H_4$	10f	$4\text{-}OCH_3C_6H_4$
10c	$4\text{-}ClC_6H_4$	10g	$4\text{-}OHC_6H_4$
10d	$4\text{-}BrC_6H_4$		

1 Synthesis of 2-{[-2-hydrazonopropyl]sulfanyl}-6-nitro-1,3-benzoxazole (9)

Hydrazones have attracted a great deal of interest as an important class of lead compounds for the development of new chemical entities to treat numerous diseases due to their unique structural features and diverse biological and pharmacological activities.

The nitro-substituted 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl] propan-2-one **3** was reacted with hydrazine hydrate in presence of acetic acid in ethanol to produce 2-{[2-hydrazonopropyl]sulfanyl}-6-nitro-1,3-benzoxazole **(Scheme-4.1).**



3 9

Scheme-4.1

In the IR spectrum of the compound **9** exhibited the stretching frequency of –NH group at 3324.5 cm^{-1} (**fig. 4.1**). The ^{1}H NMR showed a broad singlet for -NH_2 at δ 10.98, for three aromatic protons appeared as a multiplet at δ 7.94-7.75respectively. A singlet peaks for two protons of -S-CH_2 at δ 4.52 and signals at δ 2.15 for three protons of –CH_3 (**fig. 4.2**). The ^{13}C NMR showed peak for -C=N signal at δ 165.21 and aromatic carbons in the range of δ 155.31-108.67 for seven carbons (**fig. 4.3**). The molecular ion peak appeared at m/z 266.94 (**fig. 4.4**) confirmed the desired molecule **9.**

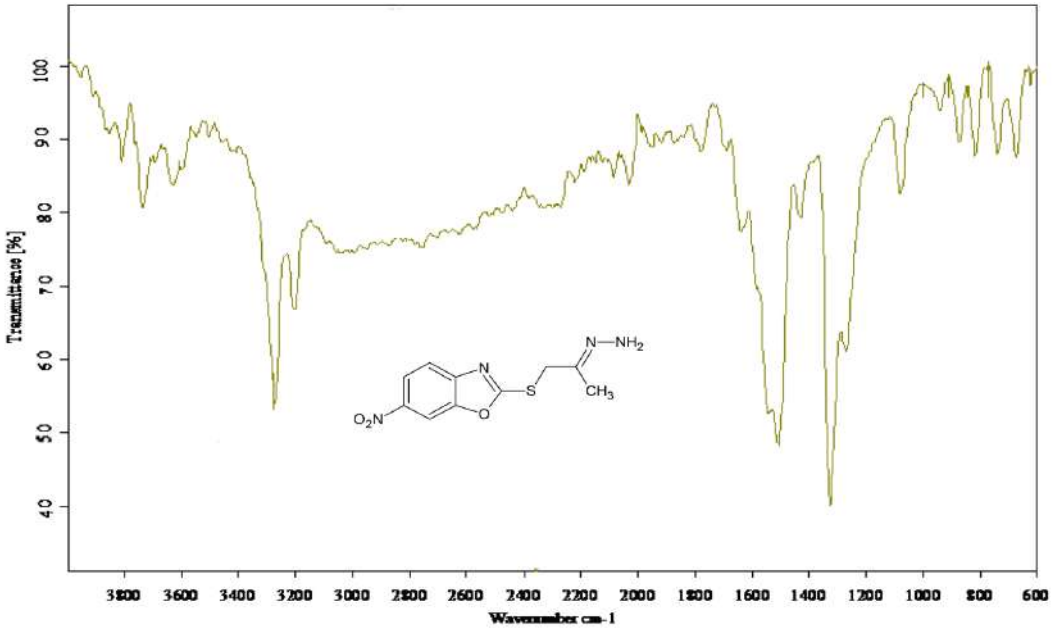


Fig.4.1 IR spectrum of compound 9

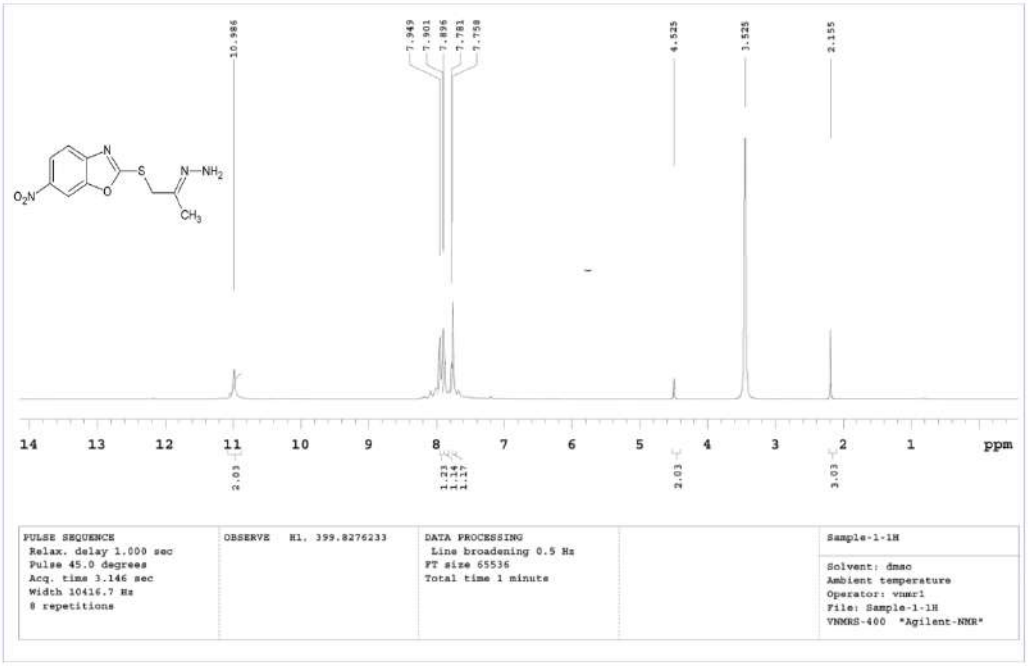


Fig.4.2 ^{1}H NMR spectrum of compound 9

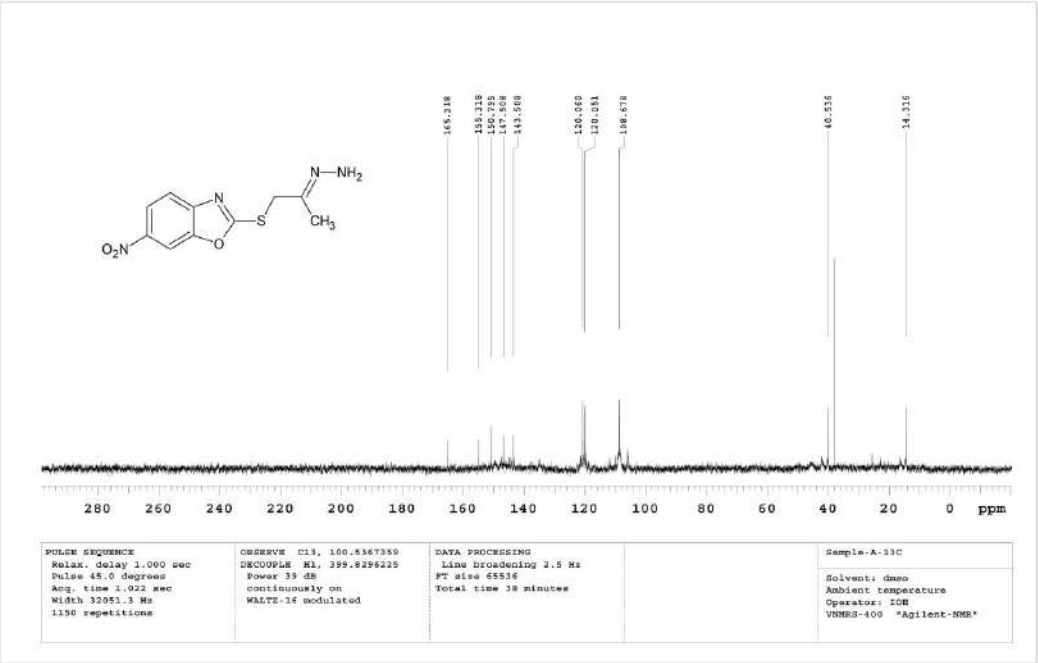


Fig.4.3 ^{13}C NMR Spectrum of compound 9

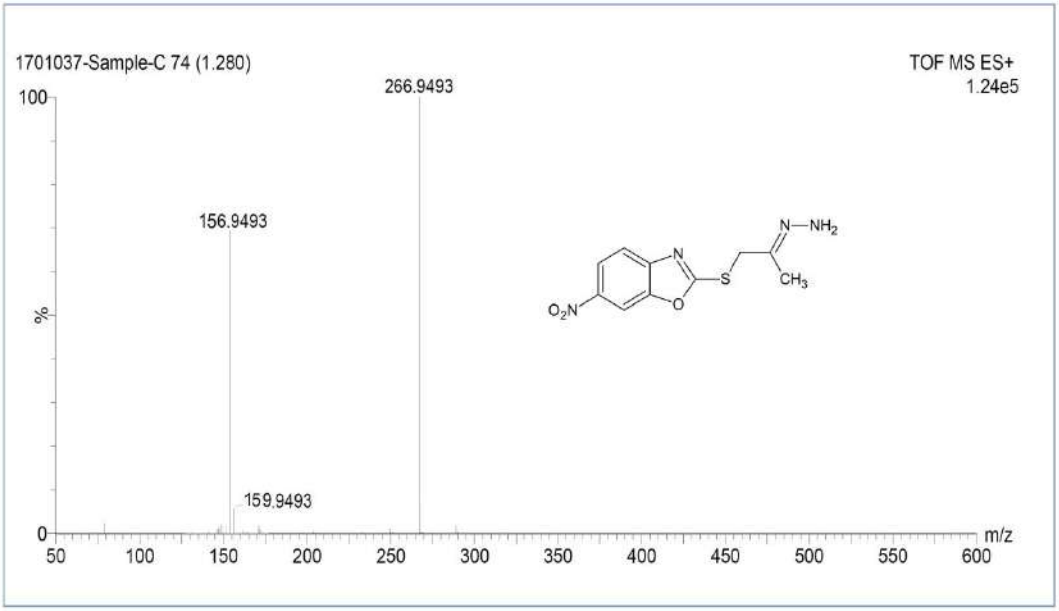
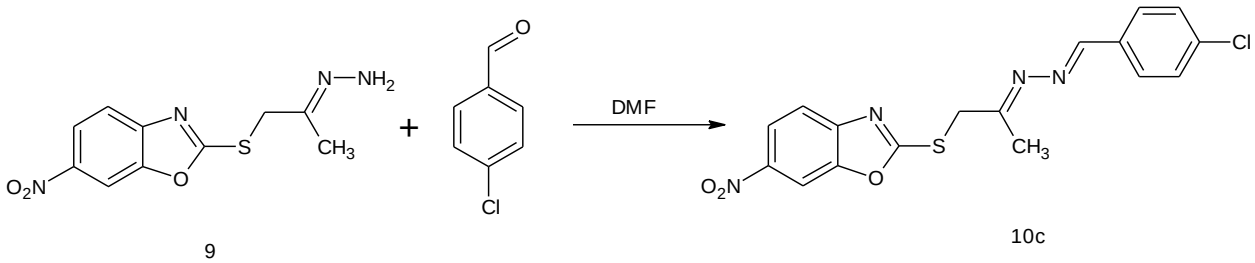


Fig.4.4 LCMS Spectrum of compound 9

2 Synthesis of 2-{[2-{-[(4-chlorophenyl)methylidene] hydrazono} propyl]sulfanyl}-6-nitro-1,3-benzoxazole (10c)

Schiff bases were the compounds carrying imine or azomethane (–C=N–) functional group. These are the condensation products of primary amines with carbonyl compounds. Schiff bases form a very important class of the most widely used organic compounds and has a broad range of applications in many fields such as analytical, biological and in organic chemistry. Schiff bases have gained importance in medicinal and pharmaceutical fields due to a broad spectrum of biological activities. Hence, we have showed interest in the synthesis of Schiff base-bearing benzoxazole derivatives.

The compound **9** was treated with substituted aromatic aldehydes in DMF to get 2-{[2-{[(4-chlorophenyl)methylidene]hydrazono}propyl]sulfanyl}-6-nitro-1,3-benzoxazole **10c (Scheme 4.2).**



Scheme-4.2

The molecule **10c** displayed IR stretching frequency at 1580.06 cm^{-1} for –C=N group **(fig. 4.5)**. The ^{1}H NMR spectrum exhibited a singlet for –CH=N proton at δ 8.935 and the seven aromatic protons exhibited as multiplet at δ 7.95-7.59, a signal for –S-CH_2 at δ 4.52 as singlet and for –CH_3 at δ 2.15 as singlet **(fig. 4.6)** respectively. It was supported by ^{13}C NMR spectrum, 13 aromatic carbon displayed signals at the region δ 166.62-120.5 and the peak at δ 105.69 δ 40.40 δ 17.47 for –

C=N-, –S-CH_2 for –CH_3, (**fig. 4.7).** The molecular ion peak of the compound **10c** was appeared at m/z 387.87 and M^{+2} 389.07 **(fig. 4.8).** It was a strong proof for the formation of desired compound. The presence of one -Cl atom is confirmed by this spectrum as the M^+ and M^{+2} values is of 3:1 ratio.

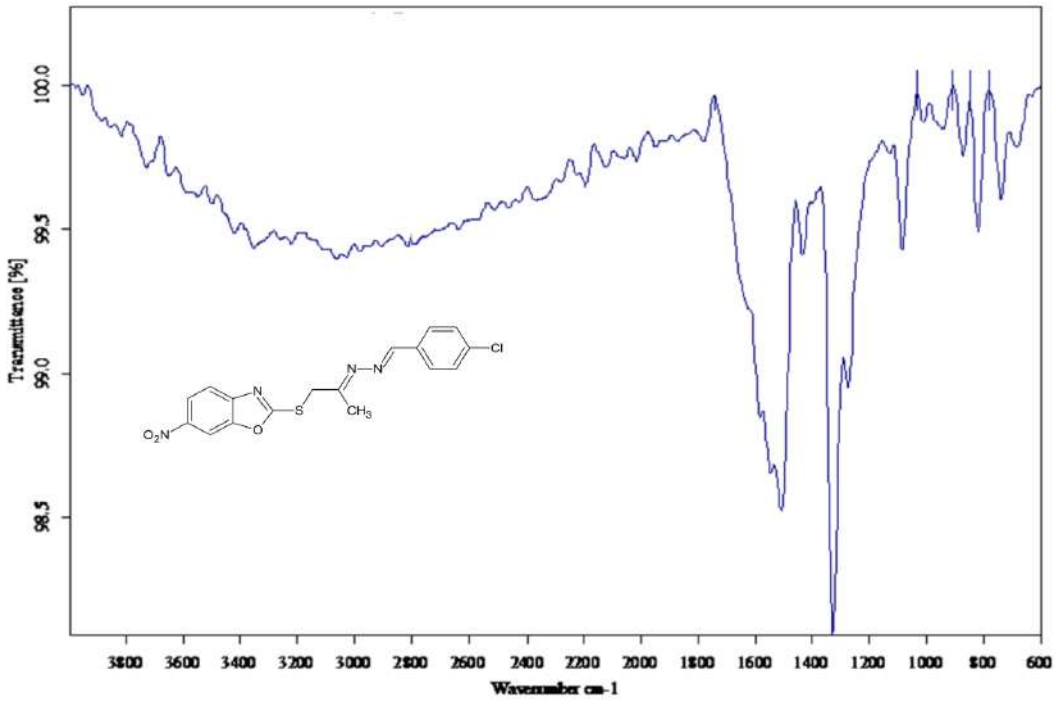


Fig.4.5 IR Spectrum of compound 10c

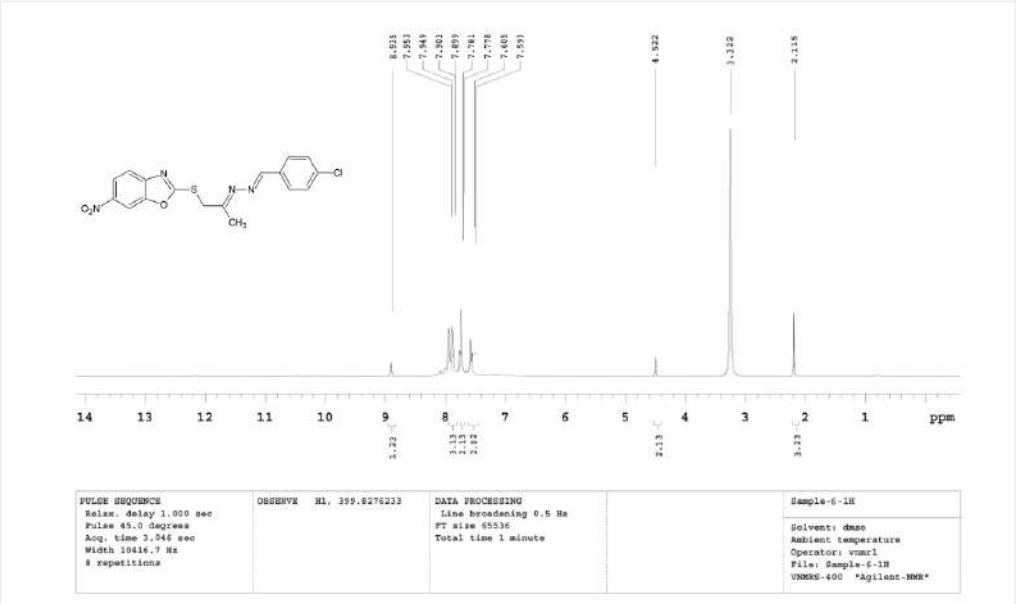


Fig.4.6 ^{1}H NMR Spectrum of compound 10c

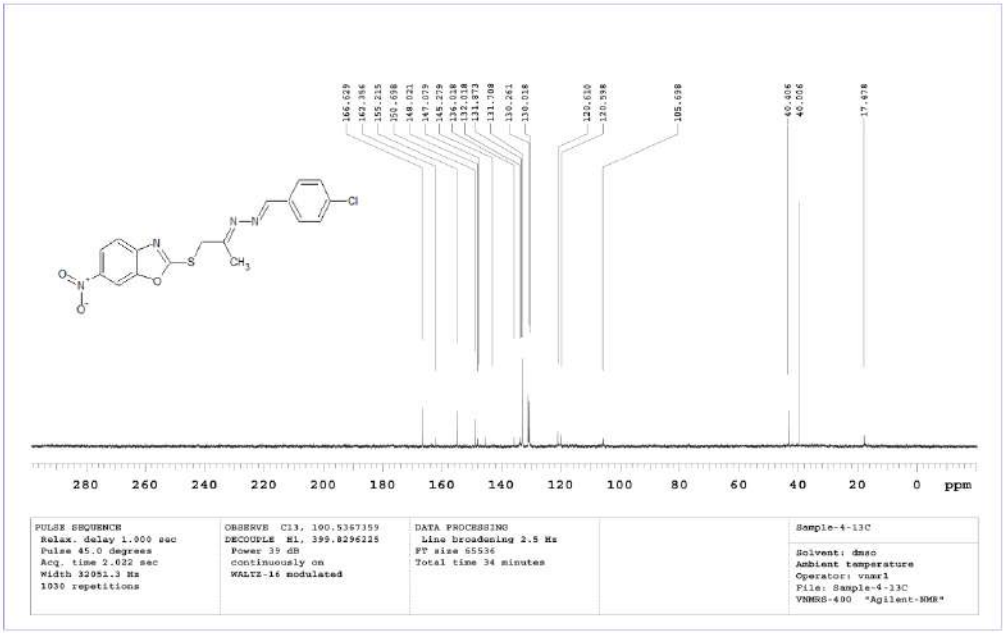


Fig.4.7 ^{13}C NMR Spectrum of compound 10c

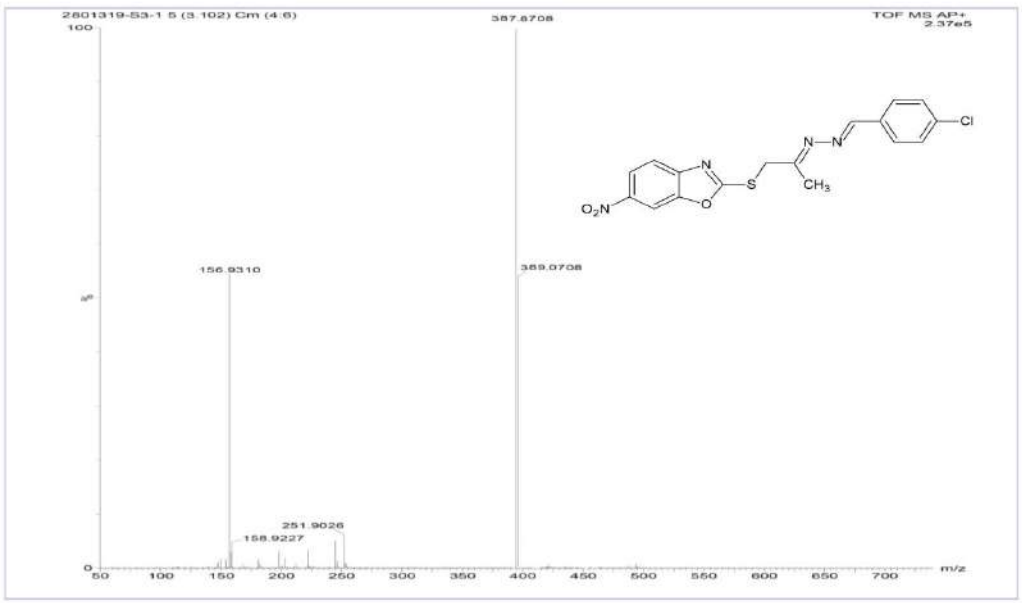
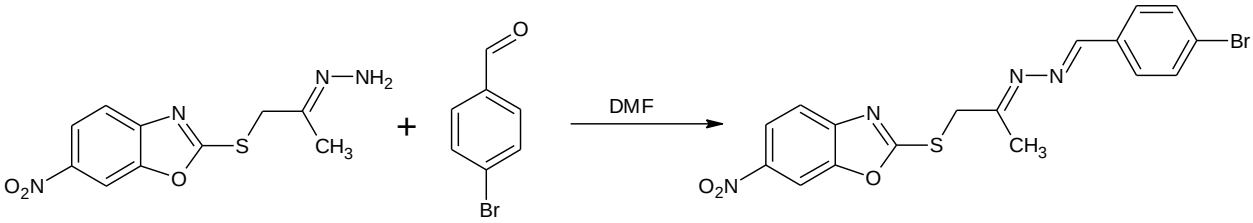


Fig.4.8 LCMS Spectrum of compound 10c

3 Synthesis of 2-{[-2-{-[(4-bromophenyl) methylidene] hydrazono} propyl]sulfanyl}-6-nitro-1,3-benzoxazole (10d)

The desired molecule namely, 2-{[2-{[(4-chlorophenyl) methylidene] hydrazono}propyl]sulfanyl}-6-nitro-1,3-benzoxazole was obtained by the condensation of the compound **9** with 4-bromobenzaldehyde in DMF, the product was obtained in good yield, **10d (Scheme-4.3).**



9 10d

Scheme-4.3

The compound **10d** showed a strong stretching frequency at 1610 cm^{-1} for –C=N group (**fig. 4.9**). In the ^{1}H NMR spectrum a singlet at δ 8.93 for –CH=N proton and the seven aromatic protons appeared between δ 7.95-7.58 as multiplet (**fig. 4.10**), a signal for –S-CH_2 at δ 4.52 as singlet and for –CH_3 at δ 2.15 as singlet. The ^{13}C NMR spectrum has showed 14 aromatic carbons were observed at δ 105.8-166.8, the signals at δ 17.87 for –CH_3, δ 40.44 for –S-CH_2 (**fig. 4.11**).

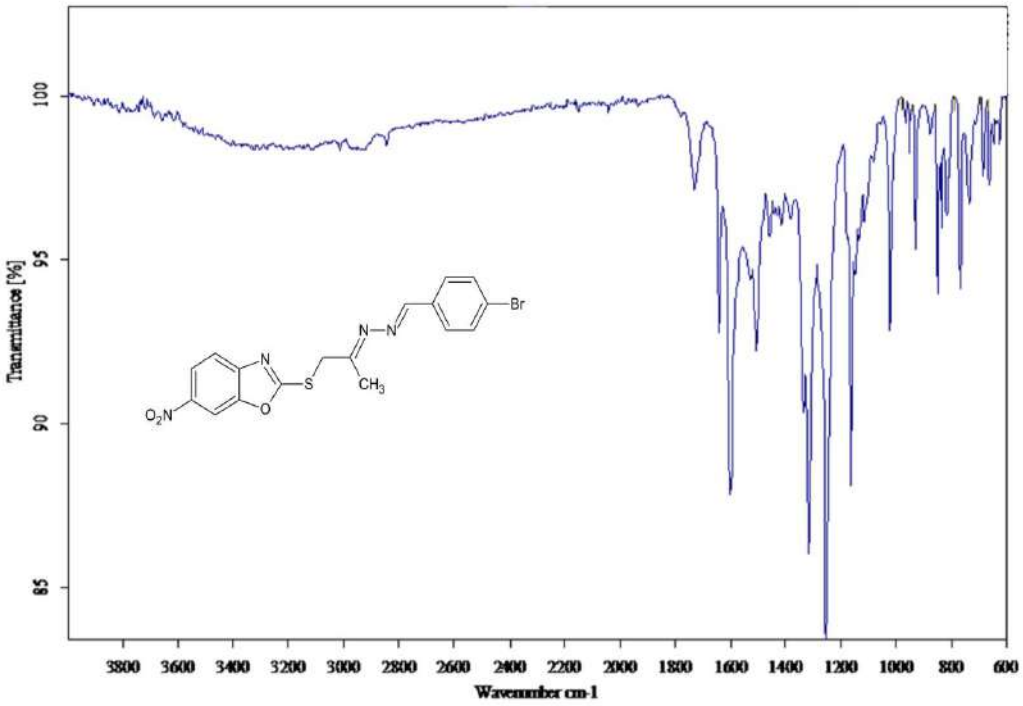


Fig.4.9 IR Spectrum of compound 10d

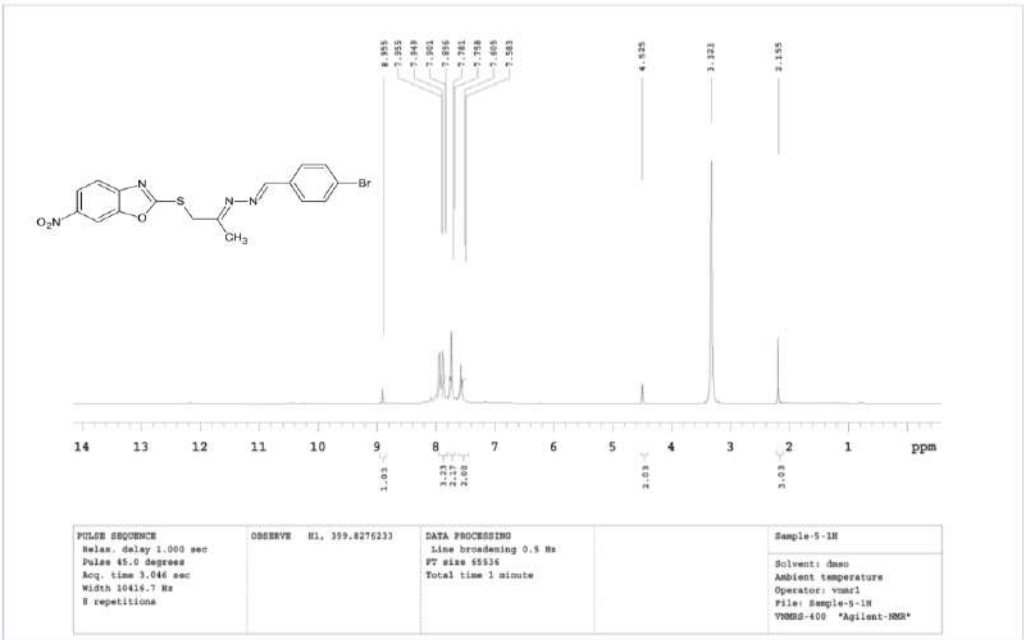


Fig.4.10 ^{1}H NMR Spectrum of compound 10d

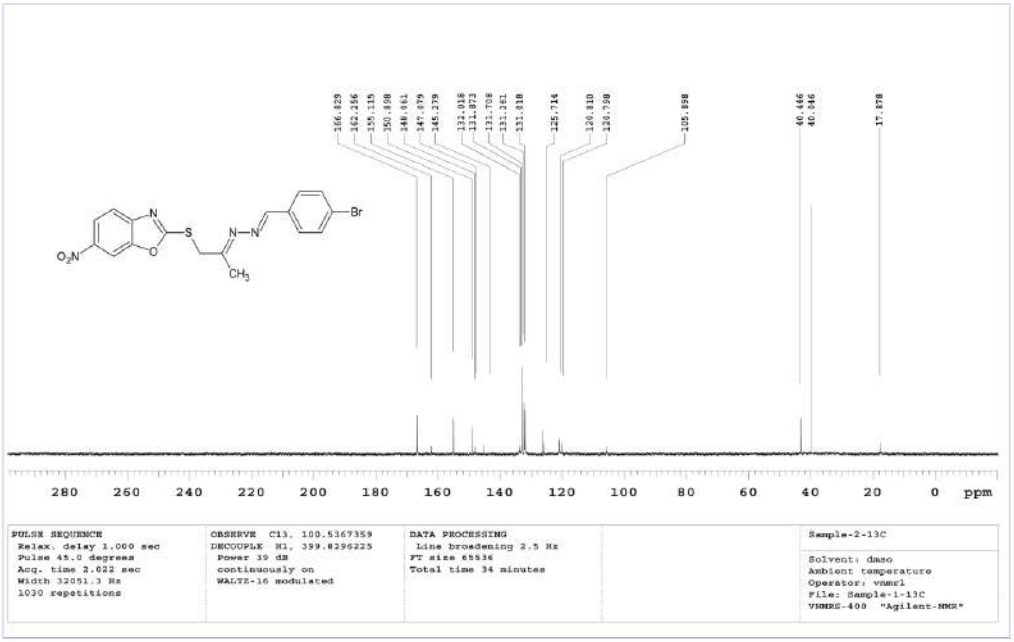


Fig.4.11 ^{13}C NMR Spectrum of compound 10d

Table 4.1 Spectral data of synthesized compounds

Compound	IR (KBr) in cm^{-1}	^{1} HNMR in δ PPM	^{13}C NMR in δ PPM	Mass
10a	1585 (C=N),	8.86 (s, H -CN), 7.48-8.06 (m, 8H Ar-H), 4.20 (s, 2H $–CH_2$), 2.26 (s, 3H $–CH_3$)	166.27-105.25 (13C, Ar-C), 41.25 (CH_2), 17.27 (CH_3)	354.42
10b	1604 (C=N)	8.71 (s, H -CN), 7.05-8.19 (m, 7H Ar-H), 4.05 (s, 2H $–CH_2$), 2.29 (s, 3H $–CH_3$)	166.92-106.20 (13C, Ar-C), 41.43 (CH_2), 16.38 (CH_3)	372.33
10e	1626 (C=N)	9.11 (s, H -CN), 7.559-8.29 (m, 7H Ar-H), 4.18 (s, 2H $–CH_2$), 2.12 (s, 3H $–CH_3$)	167.28-106.18 (13C, Ar-C), 42.28 (CH_2), 17.58 (CH_3)	399.38
10f	1645 (C=N)	8.41 (s, H –CN), 6.97 -8.29 (m, 7H Ar-H), 4.13 (s, 2H $–CH_2$), 3.26 (s, 3H $–OCH_3$), 2.16 (s, 3H $–CH_3$)	165.62-105.34 (13C, Ar-C), 52.40 (OCH_3) 42.43 (CH_2), 16.38 (CH_3)	384.88
10g	1665 (C=N)	10.82 (s, H –OH),8.41 (s, H –CN), 6.87 -8.19 (m, 7H Ar-H), 4.23 (s, 2H $–CH_2$), 2.16 (s, 3H $–CH_3$)	167.12-106.14 (13C, Ar-C), 41.43 (CH_2), 17.48 (CH_3)	370.38

4.4 Experimental

1 Synthesis of 2-{[-2-hydrazonopropyl]sulfanyl}-6-nitro-1,3-benzoxazole (9)

Mixture of 1-[(5-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one (0.01mol) and hydrazine hydrate (0.01 mol) was refluxed in ethanol in presence of acetic acid. The product was monitored by TLC. The resulting mixture was further allowed for 6h. The mixture was poured on to crushed ice, thus the solid separated out was filtered and recrystallized by methanol.

2 General procedure for the synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one-thiosemicarbazone derivatives 10(a-g)

Mixture of 2-{[-2-hydrazonopropyl]sulfanyl}-6-nitro-1,3-benzoxazole **(9)** (0.01 m) and substituted aromatic aldehyde (0.01 m) in 50 ml of DMF was refluxed for 4h. The resulting mixture was poured onto crushed ice. The solid separated out, was filtered and washed with cold water. The product was recrystallized from ethanol.

The compounds **10(a-g)** were prepared by following similar procedure as mentioned above.

Table 4.2: physical data of compounds 10 (a-g)

Compound	R	Molecular formula	Molecular weight	M.P. (^{0} c)	% of Yield	Found (Calculated) %		
						C	H	N
10a	C_6H_5	$C_{17}H_{14}N_4O_3S$	354.38	196	78	57.61 (57.69)	3.98 (3.94)	15.81 (15.88)
10b	4-F-C_6H_4	$C_{17}H_{13}FN_4O_3S$	372.37	220	80	54.83 (54.84)	3.52 (3.50)	15.50 (15.48)
10c	4-Cl-C_6H_4	$C_{17}H_{13}ClN_4O_3S$	388.82	208	84	52.51 (52.53)	3.37 (3.40)	14.41 (14.48)
10d	4-Br-C_6H_4	$C_{17}H_{13}BrN_4O_3S$	433.27	226	76	47.12 (47.10)	3.02 (3.04)	12.93 (12.90)
10e	4-NO_2-C_6H_4	$C_{17}H_{13}N_5O_5S$	399.38	242	80	51.12 (51.08)	3.28 (3.20)	17.54 (17.50)
10f	4-OCH_3-C_6H_4	$C_{18}H_{16}N_4O_4S$	384.40	228	78	56.24 (56.30)	4.20 (4.24)	14.57 (14.55)
10g	4-OH-C_6H_4	$C_{17}H_{14}N_4O_4S$	370.38	248	84	55.13 (55.26)	3.81 (3.75)	15.13 (15.16)

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CHAPTER V

SYNTHESIS OF NOVEL N' {1-METHYL-2[(6NITRO-1,3-BENZOXAZOL-2-YL)SULFANYL]ETHYLIDENE}-1,3-DIOXO-1,3-DIHYDRO-2H-ISOINDOLE-2-CARBOTHIOHYDRAZIDE

5.1 Introduction

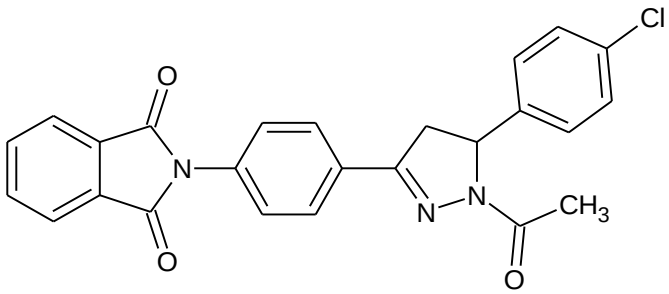
Exploration of novel chemical entities for biological applications has become an important area of research in the field of drug discovery[1-2]. Heterocyclic molecules are present in majority of the clinically accepted drug molecules. An important feature of heterocycles is their ability to sustain enormous structural diversity which is absolutely necessary to establish lead compounds for a variety of pharmacological activities[3]. Nitrogen containing heterocycles have been recognized as privileged structures to bind the receptor sites of macromolecules, leading to the perturbation of their metabolic functions, which was a characteristic feature of biologically active molecules[4]. Benzoxazoles were reported to show a broad spectrum of biological activities. Such as antihistaminic[5], antifungal[6], cyclooxygenase Inhibiting[7], antitumor[8], antiulcer[9], anticonvulsant[10], hypoglycemic[11], anti-inflammatory[12-14] and antitubercular activity[15], antibacterial activity[16]. In the present work we have focusing on the synthesis of different Benzoxazole derivatives containing phthalimide moiety.

5.2 Introduction to phthaimide derivatives

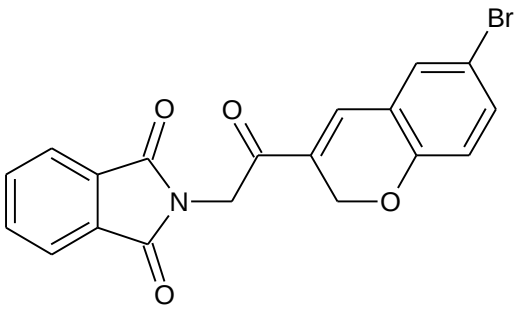
Imides are important in organic chemistry, especially aromatic heterocyclic imides played an vital role in the biological activities. N-Aryl phthalimides has pronounced plant growth regulator[17]. Many cyclic imide and their derivatives have analgesic properties[18] and showed activity against some species of bacteria, some imides similar to phthalimide showed activity against yeast, fungus and microorganisms[19-20], anti-cancer[21], anti-microbial[22-23], anti-oxidant[24] and anti-inflammatory[25]. According to the World Health Organization (WHO), infectious and parasitic diseases are still the second cause of death worldwide. This is assumed to be due to resistance to the anti-microbial agents used. There are a number of studies showing that compounds bearing a phthalimide core may be a

scaffold for designing new anti-microbial agents[26]. By considering the importance of phthalimides in biological and pharmacological field, we have showed interest towards the synthesis of novel benzoxazole derivatives containing phthalimide nucleus.

The new series of phthalimide derivatives **1-2** were synthesized by F. Lamie et al.,[27] and screened for *in vitro* anti-microbial, anti-inflammatory and cytotoxic activity. The compounds **1** and **2** exhibited potent cytotoxic activity.

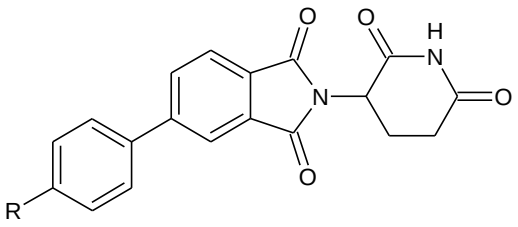


1



2

S. G. Stewart et al.,[28] have been synthesized phthalidomide derivatives **3** and **4** All the phthalidomide analogues were investigated for their proinflammator cytokine Tumor Necrosis Factor (TNF). The results were found that molecules 3 and 4 displayed potent activity.



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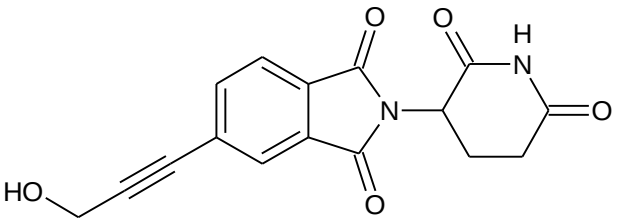
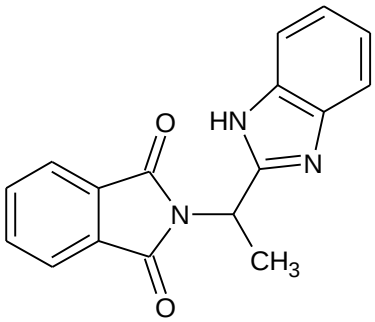


4

N-[1(1*H*-benzimidazol-2-yl)ethyl]phthalimide and its derivatives **5** were synthesized by A. Homsi et al.,[29]and screened for their biological activity. The molecule **5** exhibited potent anti-mycobacterial activity.



5

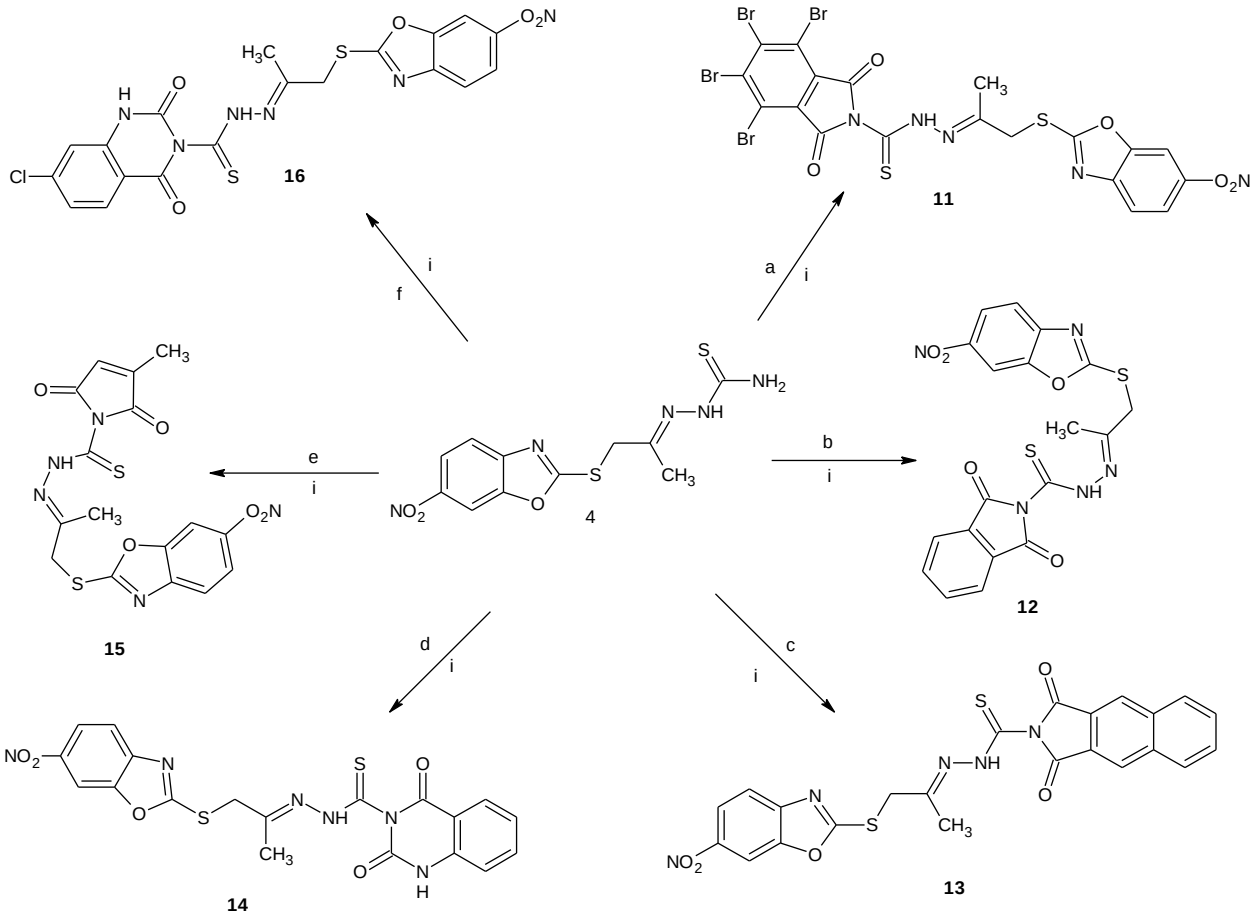
By considering the above observations, we have planned to synthesize some novel heterocyclic compounds. Our work is focusing on the synthesis of nitrobenzoxazole fused with phthalimide derivatives and screened for antibacterial, antioxidant, cytotoxic activity and molecular docking studies.

5.3 Present work

This chapter describes the synthesis of thiosemicarbazone benzoxazole derivatives, which includes phthalimide moiety. The target benzoxazole molecules were purified, characterized and confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral technique and they were screened for above selected biological activities.

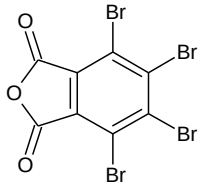
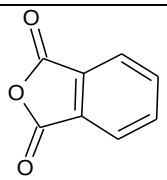
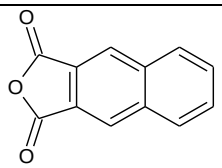
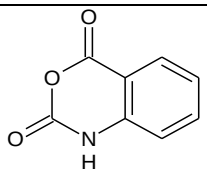
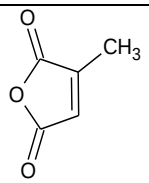
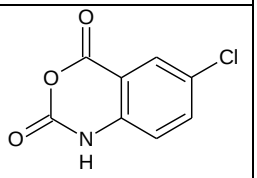
The present chapter describes the synthesis of the following benzoxazole derivatives

- 1-[(6-Nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone **4**
- 4,5,6,7-Tetrabromo-*N*'-{(1*E*)-1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] ethylidene} -1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-carbothiohydrazide **11**
- N'-{1-Methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-carbothiohydrazide **12**
- N'-{1-Methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-1,3-dioxo-1,3-dihydro-2*H*-benzo[*f*]isoindole-2-carbothiohydrazide **13**
- N'-{1-Methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-2,4-dioxo-4,4a-dihydroquinazoline-3(2*H*)-carbothiohydrazide **14**
- 3-Methyl-*N*'-{1-methyl-2- [(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] ethylidene}-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-1-carbothiohydrazide **15**
- 7-Chloro-*N*'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl] ethylidene}-2,4-dioxo-1,4-dihydroquinazoline-3(2*H*)-carbothiohydrazide **16**



Scheme-5: Synthetic route of Compounds **11-16**

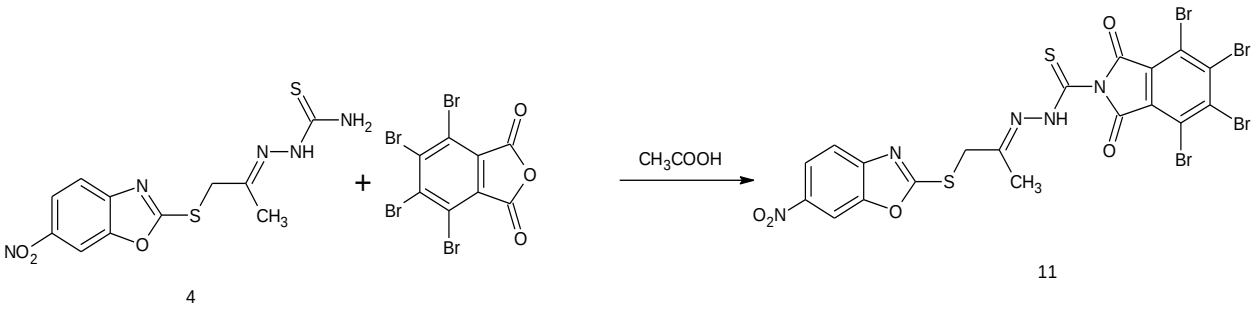
(i) CH_3COOH

Code	Compound	Code	compound	Code	Compound
a		**b**		**c**	
d		**e**		**f**	

1 Synthesis of 4,5,6,7-tetrabromo-*N*'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-carbothiohydrazide

Phthalimides having a structural feature –CO-N(R)CO- and an imide ring, which plays a vital role in biological and pharmaceutical field. Phthalimides have been used as a starting materials and intermediates for the synthesis of several alkaloids and pharmacophores.

When the compound **4** was treated with the tetra bromo phallic anhydrides in presence of acetic acid, the compound 4,5,6,7-tetrabromo-*N*'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-carbothiohydrazide **11** was obtained **(Scheme-5.1).**



4 11

Scheme-5.1

The newly synthesized molecules were confirmed by spectral studies. The compound **11** exhibited a strong stretching absorption peak at 3356 cm^{-1} in IR spectrum for NH group (**fig. 5.1**). In ^{1}H NMR showed a singlet at the region δ 10.46 confirmed the –NH group. The aromatic protons exhibited at δ 8.62- 7.83 and followed by a two singlets of –S-CH_2 and –CH_3 appeared at δ 4.24 and δ 2.08 (**fig. 5.2**) confirmed the formation of target compound. The ^{13}C NMR spectrum displayed a peak at δ 191.52 for –C=S carbon and the signals appeared at δ 178.62 for C=O, 13C aromatic carbons showed at δ 165.82-105.79-region respectively.

For S-CH_2 carbon displayed at δ 42.54, the methyl carbon showed a peak at δ 17.52 (**fig. 5.3**) confirmed the synthesized molecule.

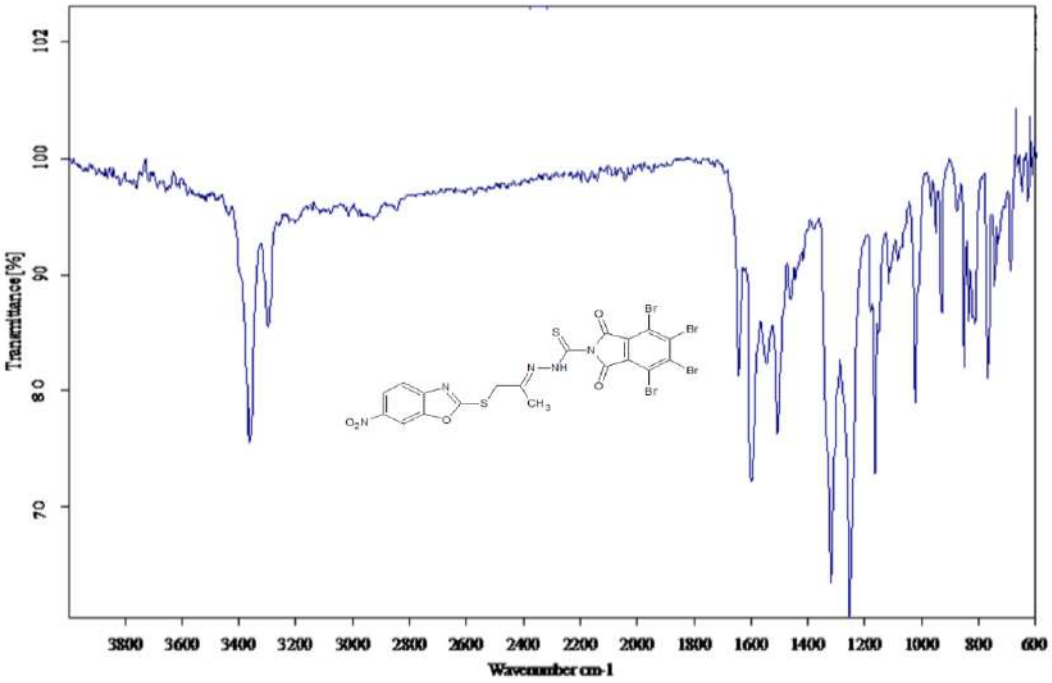


Fig.5.1 IR Spectrum of compound 11

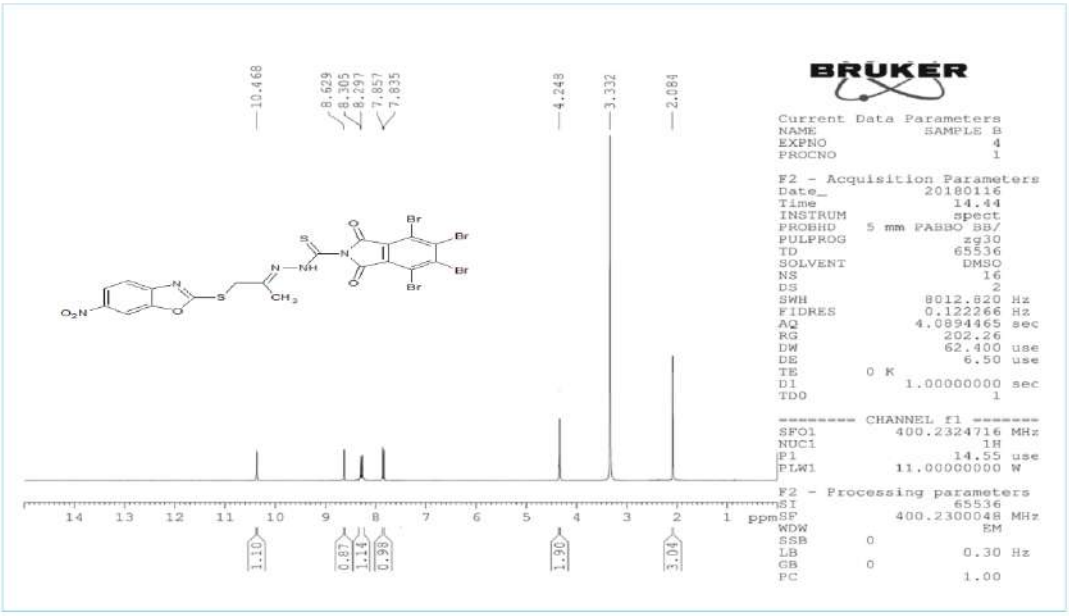


Fig.5.2 ^{1}H NMR Spectrum of compound 11

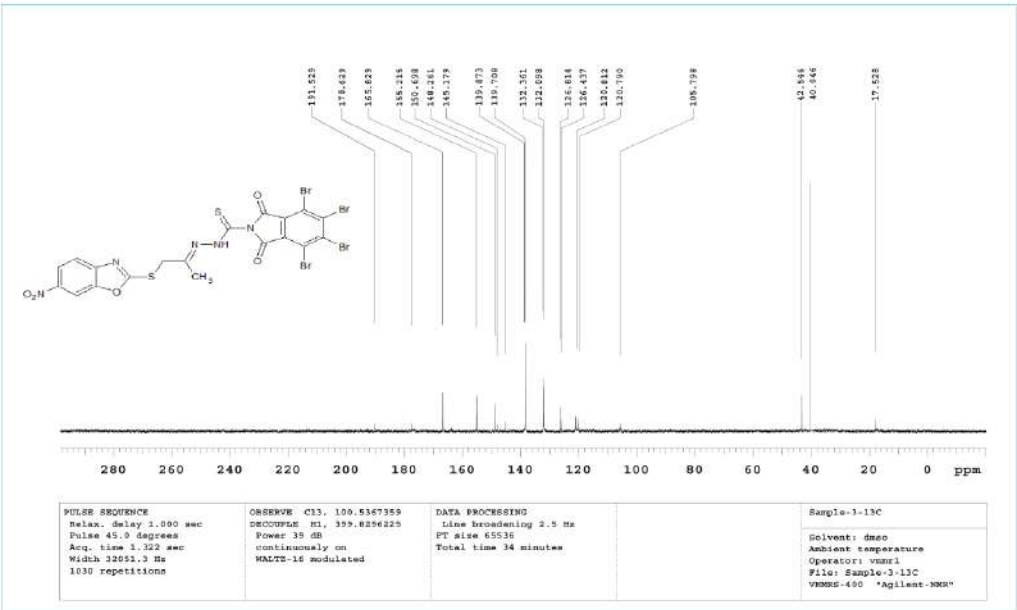
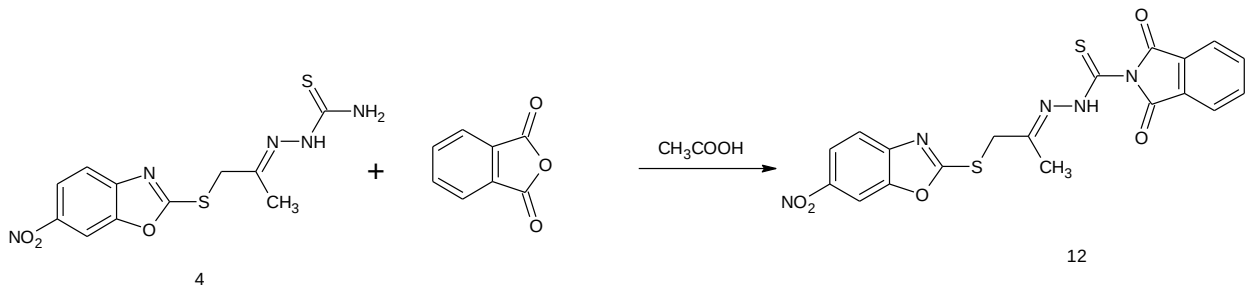


Fig.5.3 ^{13}C NMR Spectrum of compound 11

2 Synthesis of *N*'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl] ethylidene}-1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-carbothiohydrazide

The molecule having phthalimides moiety(-CO-N(R)-CO-) exhibited hydrophobic properties, this nature increases their potential cross biological membranes[30]. There are a number of studies showing that compounds bearing a phthalimide core may be a scaffold for designing new anti-microbial agents[31].

A new class of benzoxazole derivatives were synthesized by using the compound 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone **4.** When the compound **4** was treated with phthalic anhydride in presence of acetic acid, N'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-carbothiohydrazide **12** was obtained in good yield. (**Scheme 5.2).**



Scheme 5.2

The molecule **12** exhibited IR stretching frequency at 1625.06 cm^{-1} for –C=O group and -NH group appeared at 3340 cm^{-1} (**fig. 5.4)**. The ^{1}H NMR spectrum displayed a singlet for -NH proton at δ 11.13 and the 3 aromatic protons were appeared as multiplet at δ 7.95-7.16 **(fig. 5.5)** respectively. A signal for –S-CH_2 at δ 4.52 as a singlet and a singlet exhibited at δ 2.15 for –CH_3. It was supported by ^{13}C NMR spectrum, the peak at δ 190.8 exhibited for –C=S group and aromatic carbon displayed signals at the region δ 166.8-105.7 for aromatic carbons (**fig. 5.6).** For S-CH_2 carbon signal displayed at δ 40.54, the methyl carbon showed a peak at δ 17.82. The molecular ion peak of the compound **12** was appeared at m/z 456.07 **(fig. 5.7)** further confirmed the molecules**.**

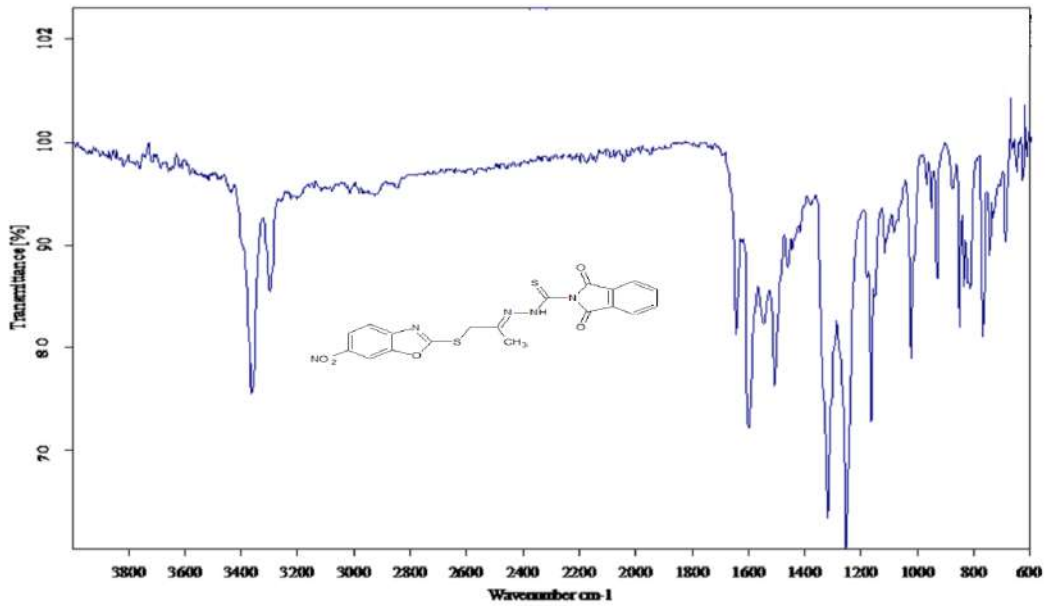


Fig.5.4 IR spectrum of compound 12

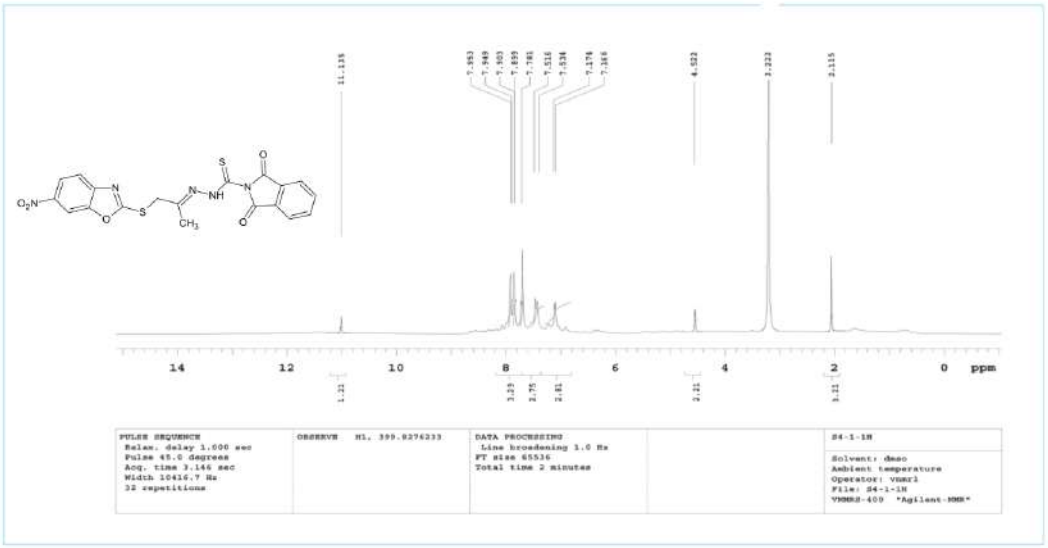


Fig.5.5 ^{1}H NMR spectrum of compound 12

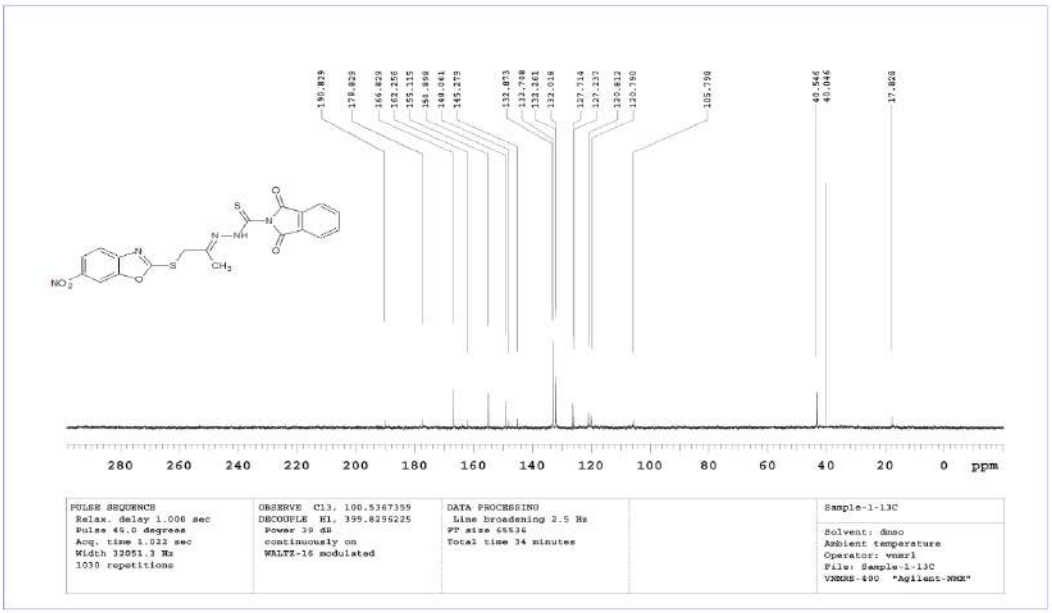


Fig.5.6 ^{13}C NMR Spectrum of compound 12

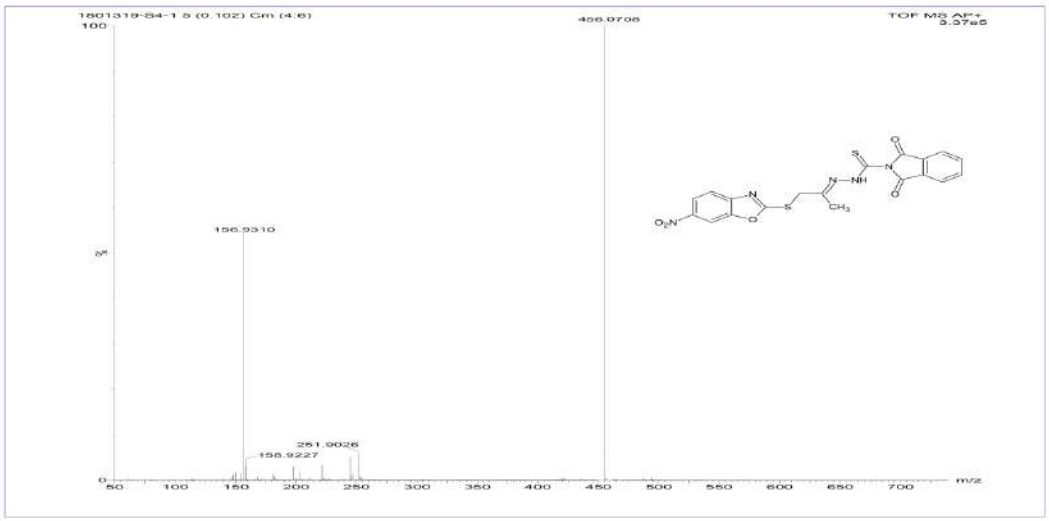


Fig.5.7 LCMS Spectrum of compound 12

Table 5.1 Spectral data of synthesized compounds 13-16

Compound	IR (KBr) in cm^{-1}	^{1} HNMR in δ PPM	^{13}C NMR in δ PPM	Mass M^{+} M^{+2} M^{+3}
13	3306 (NH), 1643 (C=N) 1769 (C=S) 1692 (C=O)	11.34 (s, H –NH), 7.38-8.29 (m, 9H Ar-H), 4.10 (s, 2H $–CH_2$), 2.25 (s, 3H $–CH_3$),	192.42 (C=S), 165.27-106.25 (18C, Ar-C), 41.25 (CH_2), 16.27 (CH_3)	505.52
14	3358 (NH), 1664 (C=N) 1754 (C=S) 1682 (C=O)	11.20 (s, H –NH), 10.96 (s, H, C-NH-C), 7.15-8.13 (m, 7H Ar-H), 4.45 (s, 2H $–CH_2$), 2.29 (s, 3H $–CH_3$	191.55 (C=S), 166.92-106.30 (14C, Ar-C), 41.43 (CH_2), 16.38 (CH_3)	470.48

15	3324 (NH), 1656 (C=N) 1752 (C=S) 1662 (C=O)	11.10 (s, H –NH), 6.75-8.257 (m, 7H Ar-H), 4.15 (s, 2H $–CH_2$), 2.19 (s, 3H $–CH_3$) 2.11 (s, H $–CH_3$)	195.23 (C=S), 170.28-106.28 (10C, Ar-C), 41.28 (CH_2), 15.58 (CH_3)	419.43
16	3382 (NH), 1650 (C=N) 1703 (C=S) 1692 (C=O)	11.63 (s, H –NH), 11.06 (s, H, C-NH-C)7.07 -8.19 (m, 7H Ar-H), 4.22 (s, 2H $–CH_2$), 2.06 (s, 3H $–CH_3$),	190.45 (C=S), 167.62-106.34 (13C, Ar-C), 42.43 (CH_2), 16.38 (CH_3)	504.92 506.23

Experimental

1 Preparation of 4,5,6,7-tetrabromo-*N*'-{(1*E*)-1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] ethylidene} -1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-carbothiohydrazide (11)

The compound 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone **(4)** was refluxed with 4,5,6,7-tetrabromo-2-benzofuran-1,3-dione (0.55 gm) in acetic acid (30 ml) for 10h on oil bath. The completion of the reaction was monitored by TLC. Then the reaction mixture was cooled, poured to ice cold water and filtered to get solid product **(11).**

2 Preparation of *N*'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-1,3-dioxo-1,3-dihydro-2*H*-isoindole-2-carbothiohydrazide (12)

Equimolar mixture of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone (0.5 gm) **(4)** and phallic anhydride (0.6 gm) in acetic acid

was refluxed for 7h on oil bath. The progress of the reaction mixture was monitored by TLC. Then the reaction mass was poured onto crushed ice. The obtained solid product was filtered off, washed, dried and recrystallized from ethanol to get the compound **(12)**.

The similar method was used for the synthesis of compounds **13-16**

The physical data of newly synthesized compounds **11-16** are tabulated in **Table-5.2**

Table-5.2: The physical data of the compounds 11-16

Compound	Molecular formula	Molecular weight	M.P. (^{0} c)	% of Yield	Found (Calculated) %		
					C	H	N
11	$C_{19}H_9Br_4N_5O_5S_2$	771.05	281	78	29.54 (29.60)	1.10 (1.18)	9.02 (9.08)
12	$C_{19}H_{13}N_5O_5S_2$	455.46	272	80	50.15 (50.10)	2.75 (2.88)	15.33 (15.38)
13	$C_{23}H_{15}N_5O_5S_2$	505.52	235	82	54.60 (54.65)	2.95 (2.99)	13.80 (13.85)
14	$C_{19}H_{14}N_6O_5S_2$	470.48	244	78	48.48 (48.50)	3.01 (3.00)	17.82 (17.86)
15	$C_{16}H_{13}N_5O_5S_2$	419.43	256	81	51.36 (45.82)	3.10 (3.12)	16.64 (16.70)
16	$C_{19}H_{13}ClN_6O_5S_2$	504.92	226	84	45.18 (45.20)	2.55 (2.60)	16.60 (16.64)

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CHAPTER VI

SYNTHESIS OF NOVEL 1-[(6-NITRO-1,3-BENZOXAZOL-2-YL)SULFANYL]PROPAN-2-ONE *N*-[1-(4-CHLOROPHENYL)ETHYLIDENE]THIOSEMICARBAZONE DERIVATIVES

6.1 Introduction

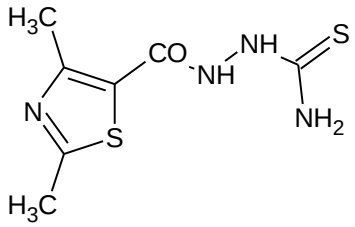
Traditionally, small molecules have been a reliable source for discovering novel biologically, pharmacologically active compounds. Nitrogen and sulfur containing compounds dominated much of synthetic, analytical and medicinal chemistry. In the last few decades numerous of 2-substituted benzoxazole derivatives were studied extensively for antitumor[1–7], antiviral[8–14] and antimicrobial activities[15–23] as nonnucleoside topoisomerase 1 poison, HIV-1 reverse transcriptase and DNA gyrase inhibitors[12–14]. An essential component of the search for novel leads in a drug designing program is the synthesis of molecules, which are novel yet resemble known biologically active molecules by the virtue of the presence of some different functionality features. Certain small heterocyclic compounds act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally effective molecules. Thiosemicarbazide was a useful structural moiety that has the potential to exhibit chemical functionality in biologically potent molecules and in the optimization of this molecule can result in ground breaking discovery of novel class of therapeutic agents.

6.2 Introduction to Thiosemicarbazone

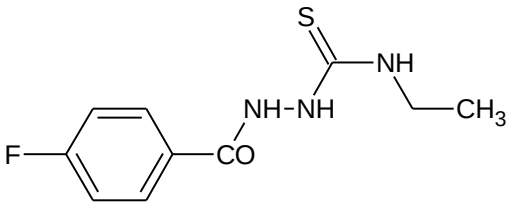
Thiosemicarbazides has occupied a very important place in drug industry. Use of these compounds in organic synthesis has become a classical strategy for the synthesis of various heterocycles. Their reactions with compounds containing C=O and C=N groups was an improtant method for the synthesis of biologically potent triazoles and thiazoles. It was widely accepted that, the prerequisite for thio compounds to express their physiological effects was through S-oxygenation[24]. Oxidation of organo-sulfur compounds appears to be involved in many cellular functions[25], including the reductive degradation of polypeptide hormones and proteins, regulation of protein synthesis, maintenance of intracellular redox

potential, protection of cell from oxidative damage, etc. The chemistry of hydrazine derivatives, such as thiosemicarbazide and its hydrazones were of immense interest owing to their wide range of synthetic and analytical applications and biological activities[26]. Thiosemicarbazides and their derivatives showed interesting biological activities, including anticancer[27], antiHIV[28], antibacterial[29], antiviral[30] and antifungal[31] owing to their ability to diffuse through the semipermeable membrane of cell lines[32-35]. They play an vital role in the regulation of plant growth[36].

M. Sheikhy et al.,[37] has been reported that the Derivatives of Thiosemicarbazides and Thiosemicarbazone, 1-(2,4-dimethylthiazole-5-carboxyl)-N-4-ethylthiosemicarbazide **1**, 1-(4-fluorobenzoyl)-N-4-ethylthiosemicarbazide **2**, 2-pyridinealdehyde-4-N-methylthiosemicarbazone (**3**) and 2-acetyl pyridine-4-N,N'-dimethylthiosemicarbazone **4** were found to be potent antibacterial agents.



1



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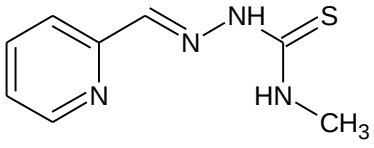

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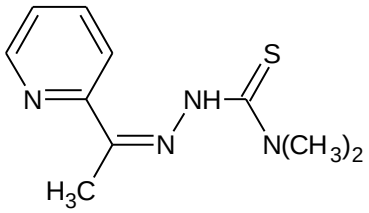
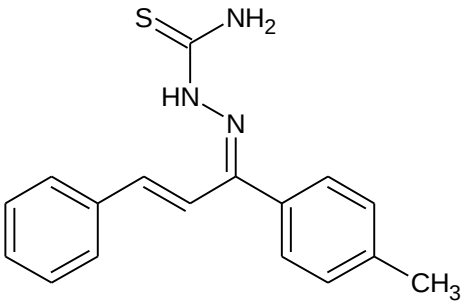


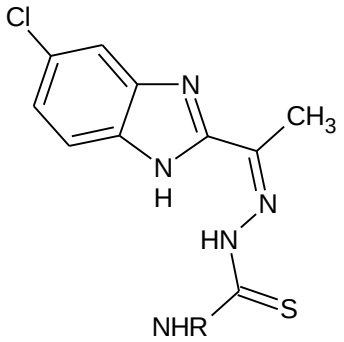
4

A series of novel thiosemicarbazide derivatives of chalcone were synthesized and screened for biological activities. Among the synthesized compounds, compound **5** exhibited the potent antiproliferative activity[38].



5

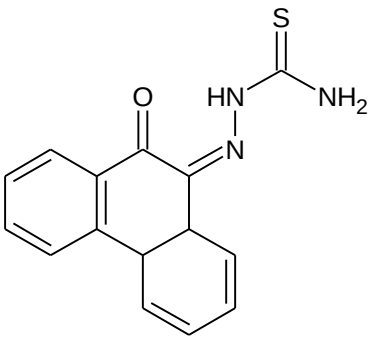
H.D. Patel et al., synthesized[39] a novel series of thiosemicarbazones of 1-(5-chloro-1H-benzimidazol-2-yl)ethanone **6(a-e)** and screened for their in *vitro* antitumor activity. The result found that compound **6b** a phenyl substituted agent showed remarkable activity against most of all the cancer cell lines.



6(a-e)

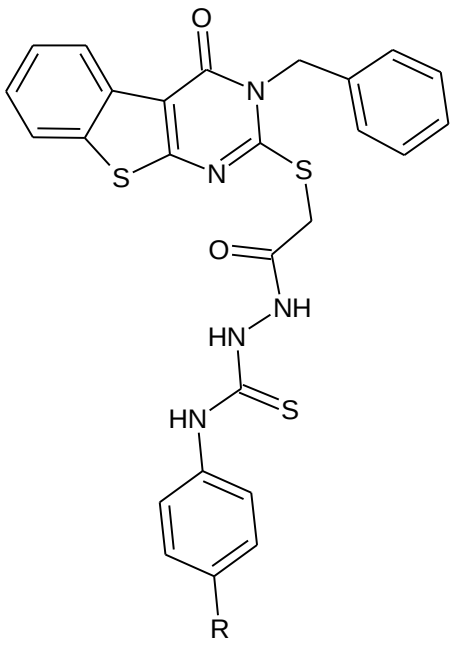
Compounds	R
a	H
b	C_6H_5
c	CH_2CH_3
d	C_4H_9
e	C_6H_{11}

S. Padhye et al.,[40] have been synthesized the palladium complex of phenanthrenequinone thiosemicarbazone **7** and evaluated for antitumor activity**.** The compound 7 exhibited remarkable activity against drug-sensitive.



7

A series of novel derivatives of 4-aryl-1-[2-(3-benzyl-4-oxo(3*H*)-5,6,7,8-tetrahydro[1]benzothieno[2,3-*d*] pyrimidin-2-ylsulphanyl) acetyl] thiosemicarbazide **8(a-d)** have been synthesized by S.B. Salib et al.,[41] and these molecules were evaluated for their antitumor activity. The compound **8b** displayed considerable antitumor activity.



8

	R		**R**
8a:	H	8c:	Cl
8b:	Br	8d:	OCH_3

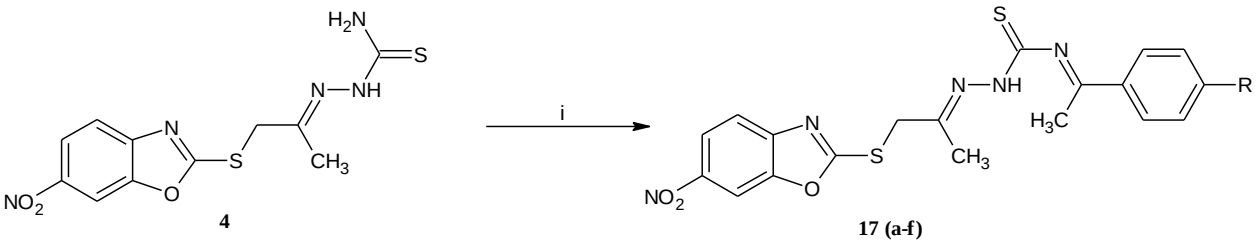
In view of these above biological and pharmacological importance of thiosemicarbazones with heterocyclic moieties, it was planned to synthesis of novel benzoxazole derivatives associated with thiosemicarbazones moiety and investigated for antibacterial, antioxidant, cytotoxic activity and molecular docking studies.

6.3 Present Work

This chapter describes the synthesis of thiosemicarbazone benzoxazole derivatives encompassing thiosemicarbazide. Thiosemicarbazides played an important role in drug discovery. Use of these compounds in organic synthesis has been developed a classical approach for the synthesis of numerous heterocycles. The target benzoxazole derivatives were purified, characterized and confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral technique and they were screened for biological activities.

The present chapter describes the synthesis of the following benzoxazole derivatives

- 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one-thiosemicarbazone **(4)**
- 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one-thiosemicarbazone **17(a-f)**



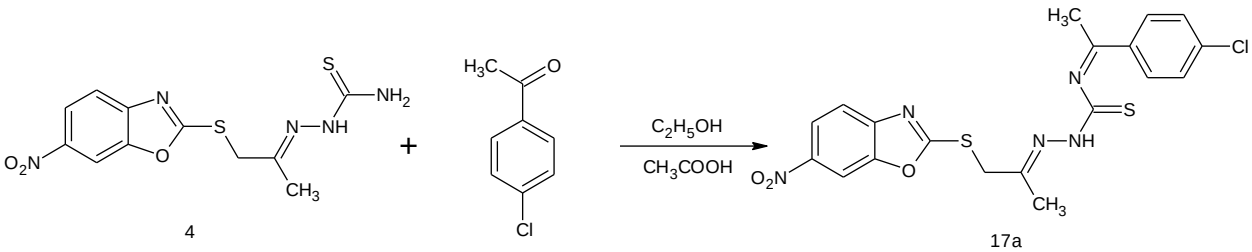
Scheme-6: The Synthetic route of Compounds **17(a-f)** (i) RCOR` CH_3COOH, C_2H_5OH.

	R		**R**
17a	4-Cl	17d	4-NO_2
17b	4-OCH_3	17e	4-OH
17c	4-Br	17f	4-F

1 Synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[1-(4- chlorophenyl) ethylidene]thiosemicarbazone (17a)

Thiosemicarbazide derivatives exhibit significant biological activities, such as antitumor, fungicidal, bactericidal, anti-inflammatory and antiviral[42-44] and also the thiosemicarbazide is a highly efficient pharmacophores in drug molecular design.

The molecule 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone **4** was treated with chloro acetophenone in acedic media by using ethanol as a solvent, the compound 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one*N*-[1-(4-chlorophenyl)ethylidene]thiosemicarbazone **17a** was obtained in good yield. **(Scheme-6.1)**



Scheme-6.1

In the IR spectrum of the compound **17a**, the stretching frequency of –NH group was exhibited at 3303.5 cm^{-1} (**fig. 6.1**). The ^{1}H NMR spectrum displayed a singlet for –NH, (D_2O exchangeable) S–CH_2, –CH_3 and –CH_3 protons at δ 11.25, δ 4.54, δ 2.25 and δ 2.12 respectively and followed by seven aromatic protons appeared in the region of δ 7.94-7.16 (**fig. 6.2**), which confirmed the target compound. The ^{13}C NMR showed peak for –C=S, –CH_2, –S-CH_2-CH_3 and –CH_3 signal at 191.5, 42.54, 21.52 and 17.52 respectively and aromatic carbon in the range of δ 155.25-105.79 for 13 aromatic carbons respectively (**fig. 6.3**). It was also supported by mass spectrum. The molecular ion peak exhibited at m/z 461.22, 463.22 M^{+2} (**fig. 6.4**) confirmed the formation of target molecule **17a** and also the presence of one –Cl atom.

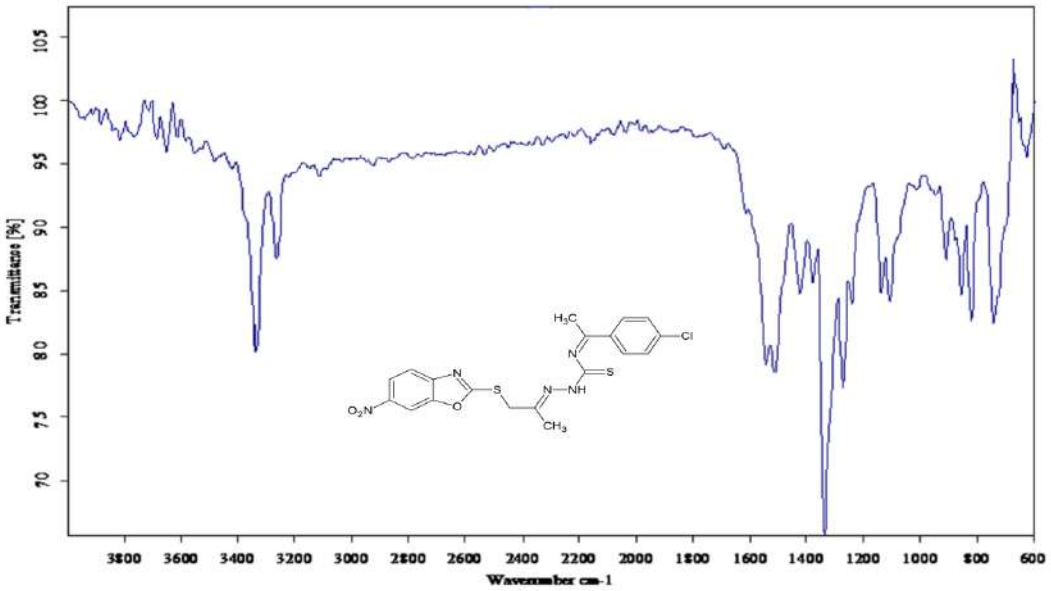


Fig.6.1 IR spectrum of compound 17a

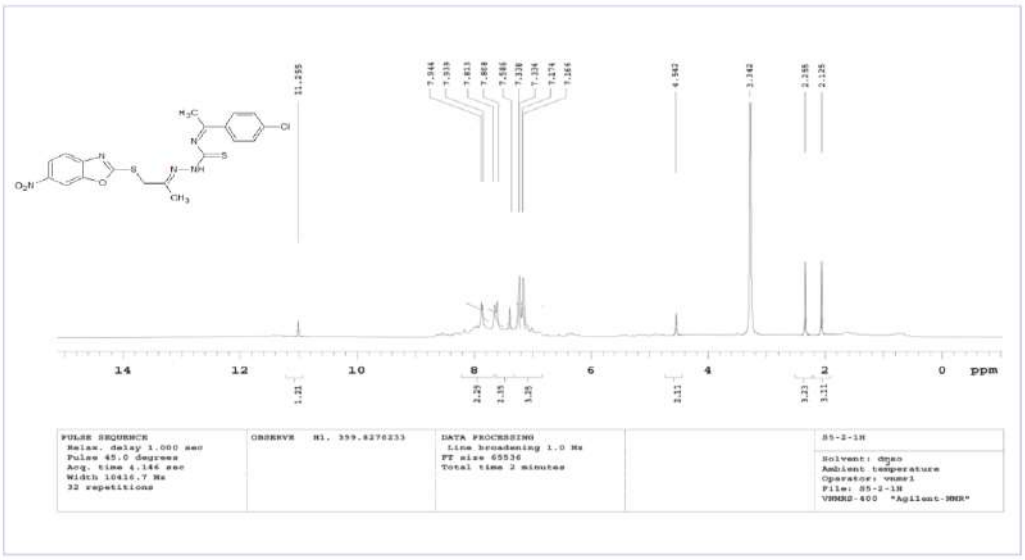


Fig.6.2 ^{1}H NMR spectrum of compound 17a

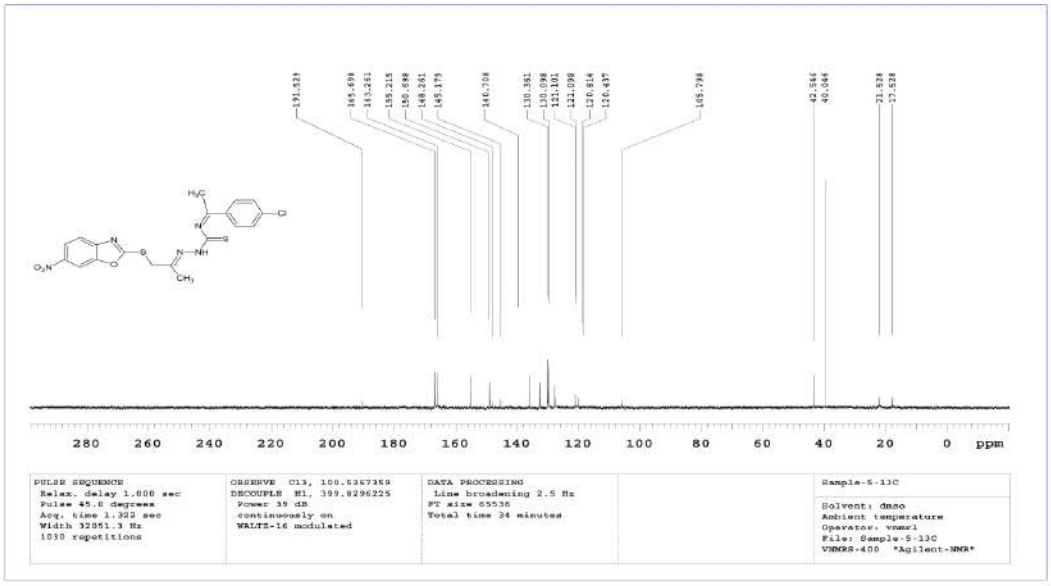


Fig.6.3 ^{13}C NMR Spectrum of compound 17a

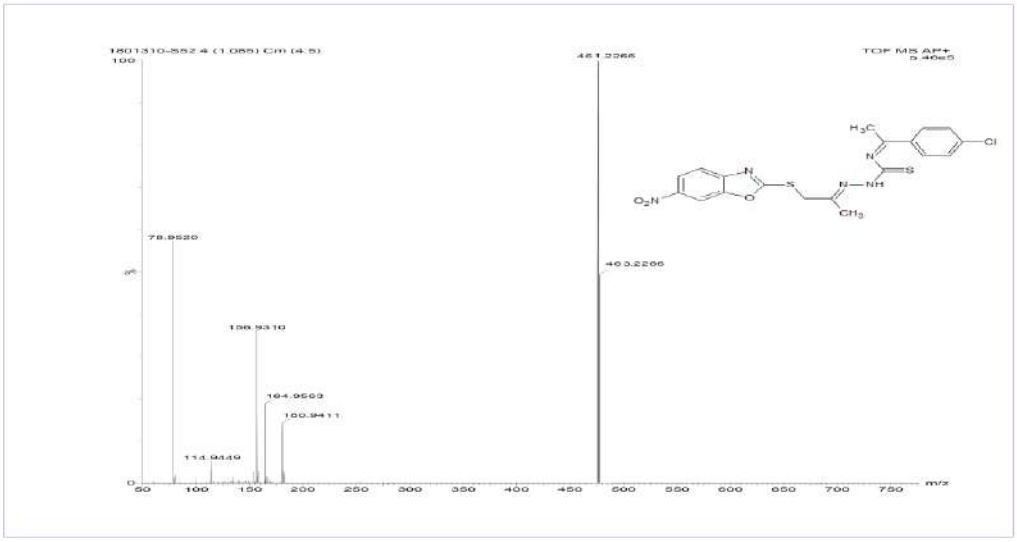
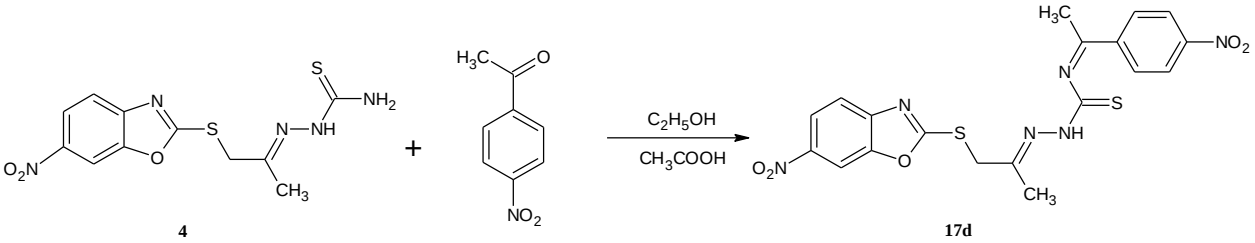


Fig.6.4 LCMS Spectrum of compound 17a

2 Synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[1-(4-nitrophenyl) ethylidene]thiosemicarbazone (17d)

The compound 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone **4** was reacted with nitro acetophenone to obtain 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-oneN-[1-(4-nitrophenyl)ethylidene] thiosemicarbazone (**17d**) **(Scheme- 6.2).**



Scheme-6.2

The compound **17d** exhibited strong stretching frequency at 3363.5 cm^{-1} for –NH group (**fig. 6.5**). In the ^1H NMR spectrum, the –NH proton displayed as a broad singlet at δ 11.23 and seven aromatic proton exhibited as multiplet at δ 7.95-7.16 respectively, for $–CH_2$ proton appeared as singlet at δ 4.52 and the three protons of

$-CH_3$ displayed as singlet at δ 2.15 another $-CH_3$ appeared as singlet at δ 2.21 (**fig. 6.6**). The ^{13}C NMR showed signals for –C=S, $-CH_2$, $-CH_3$ and $-CH_3$ at δ 191.5, δ 42.54, δ 21.52 and δ 17.52 respectively (**fig. 6.7**). Further the mass spectrum of **17d** (**fig. 6.8**) was confirmed the structure by appearing the molecular ion peak at 473.22 m/z confirmed the synthesized molecule **(Scheme-6.2).** The spectral data and physical data are depicted in the Table 6.1 and Table 6.2

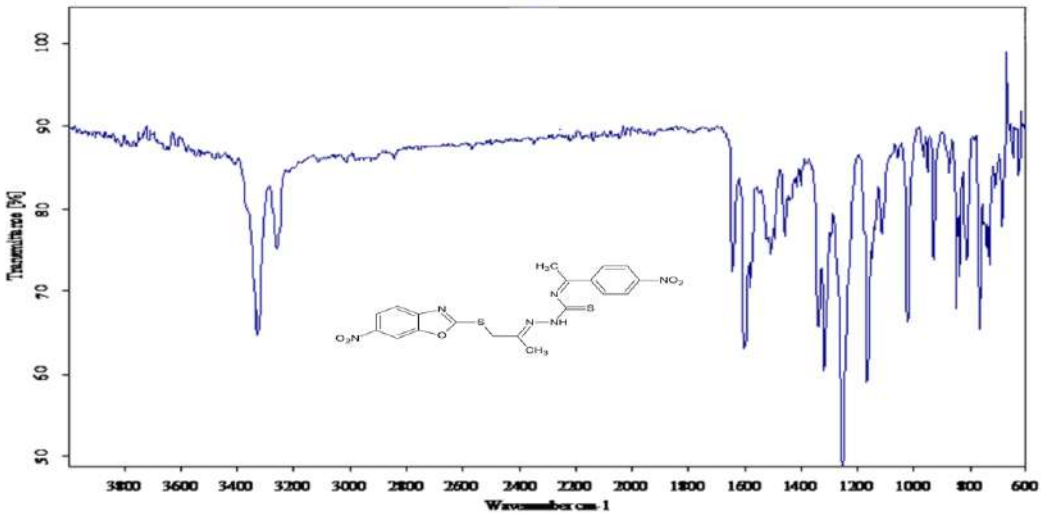


Fig.6.5 IR spectrum of compound 17d

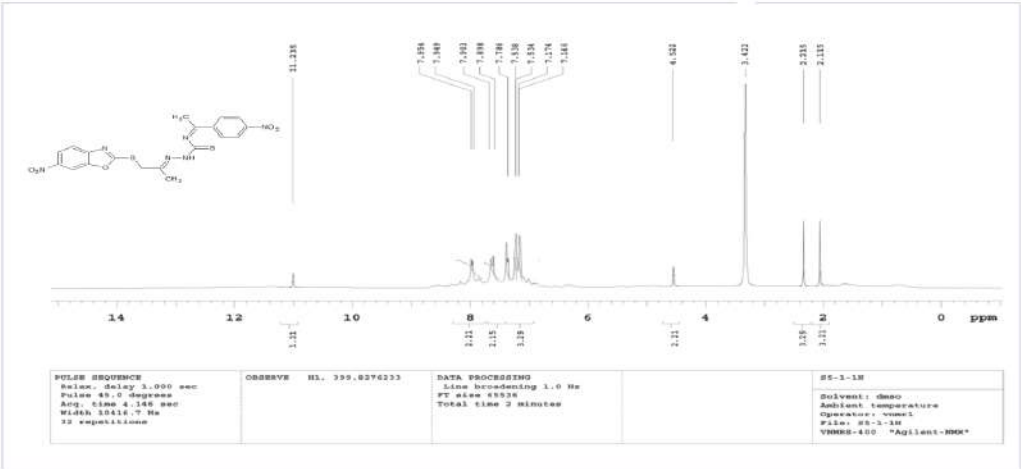


Fig.6.6 ^{1}H NMR Spectrum of compound 17d

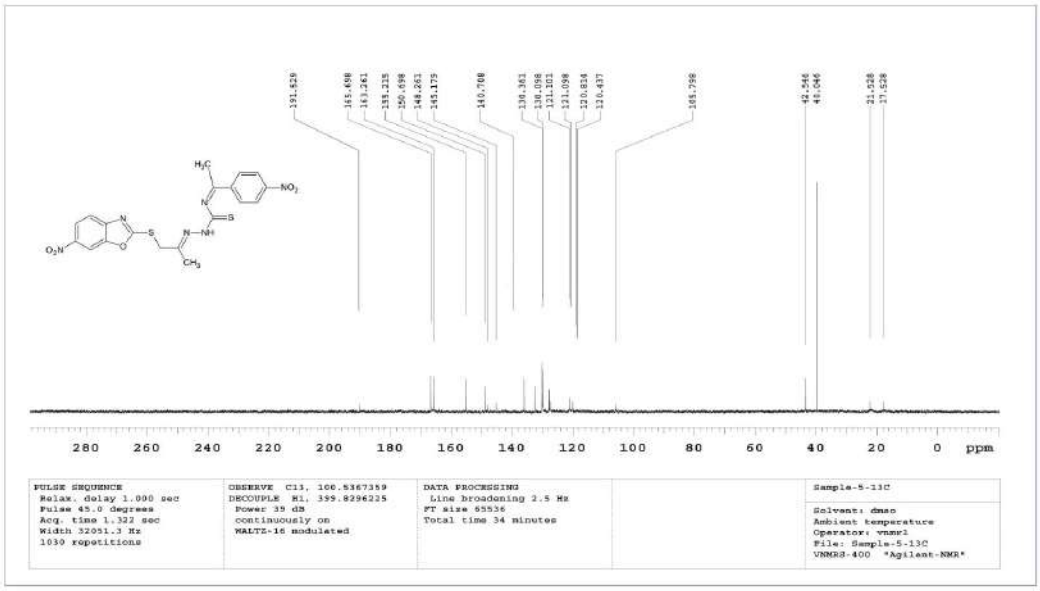


Fig.6.7 ^{13}C NMR Spectrum of compound 17d

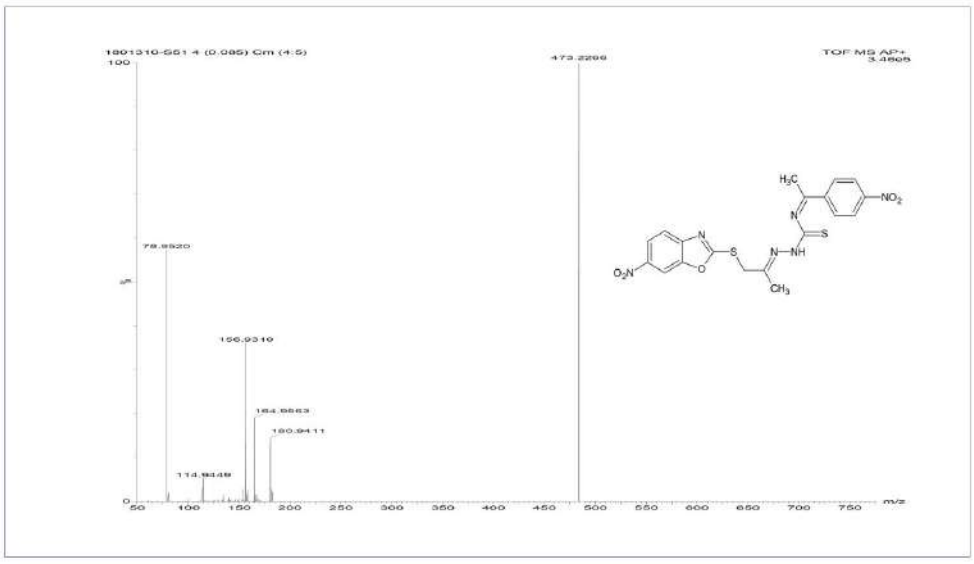


Fig.6.8 LCMS Spectrum of compound 17d

Table 6.1 Spectral data of synthesized compounds

Compound	IR (KBr) in cm^{-1}	^{1} HNMR in δ PPM	^{13}C NMR in δ PPM	Mass
17b	3306 (NH), 1643 (C=N) 1769 (C=S)	11.347 (s, H –NH), 8.669 (s, H -CN), 6.886-8.197 (m, 7H Ar-H), 4.208 (s, 2H $–CH_2$), 2.1564 (s, 3H $–CH_3$), 2.3564 (s, 3H $–CH_3$)	190.42 (C=S), 165.27-106.25 (13C, Ar-C), 40.25 (CH_2), 15.27 (CH_3)	457.52
17c	3358 (NH), 1664 (C=N) 1754 (C=S)	11.206 (s, H –NH), 8.419 (s, H -CN), 7.559-8.23 (m, 7H Ar-H), 4.158 (s, 2H $–CH_2$), 2.194 (s, 3H $–CH_3$) 2.2564 (s, 3H $–CH_3$)	194.55 (C=S), 166.92-106.30 (13C, Ar-C), 42.43 (CH_2), 14.38 (CH_3)	506.39 508.12 (M+ 2).
17e	3324 (NH), 1656 (C=N) 1752 (C=S)	10.503 (s, H –NH), 8.819 (s, H -CN), 6.859-8.197 (m, 7H Ar-H), 4.158 (s, 2H $–CH_2$), 2.194 (s, 3H $–CH_3$) 2.3064 (s, 3H $–CH_3$)	191.23 (C=S), 170.28-106.28 (13C, Ar-C), 42.28 (CH_2), 14.58 (CH_3)	443.49
17f	3382 (NH), 1650 (C=N) 1703 (C=S)	10.936 (s, H –NH), 8.619 (s, H -CN), 7.079 -8.193 (m, 7H Ar-H), 4.225 (s, 2H $–CH_2$), 2.068 (s, 3H $–CH_3$), 2.268 (s, 3H $–CH_3$)	194.55 (C=S), 167.62-106.34 (13C, Ar-C), 56.40 (CH_3) 40.43 (CH_2), 14.38 (CH_3)	445.49

Experimental

1 synthesis of 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone derivatives 17(a-f)

The compound 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one thiosemicarbazone **(4)** and substituted aromatic amines were refluxed on for 5 h in ethanol in presence of catalytic amount of acetic acid. The formation of the product was confirmed by TLC. The reaction mixture was poured on to the crushed ice, thus solid separated and it was filtered, the obtained product was recrystallized using ethanol.

Table 6.2: physical data of compounds 17 (a-f)

Compound	R	Molecular formula	Molecular weight	M.P. (^{0} c)	% of Yield	Found (Calculated) %		
						C	H	N
17a	4-Cl	$C_{19}H_{16}ClN_5O_3S_2$	461.94	210	81	49.34 (49.40)	3.45 (3.49)	15.08 (15.16)
17b	4-OCH_3	$C_{20}H_{19}N_5O_4S_2$	457.52	210	80	52.11 (52.50)	4.20 (4.19)	15.23 (15.31)
17c	4-Br	$C_{19}H_{16}BrN_5O_3S_2$	506.39	224	80	45.10 (45.06)	3.10 (3.18)	15.70 (15.78)
17d	4-NO_2	$C_{19}H_{16}N_6O_5S_2$	472.49	238	84	48.31 (48.30)	2.87 (3.41)	17.45 (17.49)
17e	4-OH	$C_{19}H_{17}N_5O_4S_2$	443.49	202	78	51.36 (51.46)	3.88 (3.86)	15.74 (15.79)
17f	4-F	$C_{19}H_{16}FN_5O_3S_2$	445.49	260	80	51.20 (51.23)	3.60 (3.62)	15.79 (15.72)

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CHAPTER VII

BIOLOGICAL AND PHARMACOLOGICAL EVALUATION OF SYNTHESIZED MOLECULES.

7 General Introduction

Literature survey revealed that benzoxazole derivatives played a vital role in the medicinal field. This is one of the leading cause for the development of new benzoxazole moieties in chemistry. This fact stimulated many researchers in designing, synthesizing and screening for the biological activities of benzoxazole derivatives.

The major objectives of the present research work are to explore biologically pharmacologically potent synthetic benzoxazole derivatives. Thus, a few series of benzoxazole derivatives have been designed, synthesized and selected compounds are evaluated for the following activities.

7.1 Antibacterial Activity

7.2 Antifungal Activity

7.3 Minimum Inhibition Concentration

7.4 Antioxidant

7.5 Cytotoxic activity

7.6 Anti-TB Activity

7.7 DNA Cleavage Studies

7.8 Molecular Docking Studies

7.1 Antibacterial Activity

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously rising. This increase has been attributed to indiscriminate use of wide range of antibiotics, immunosuppressive, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection[1-4]. In addition, in developing countries, synthetic drugs

are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is necessary to search new infection-fighting strategies to control microbial infections[5]. The search for compounds with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistance microorganisms[6-7].

In view of the above findings, we have planned to screen newly synthesized derivatives of benzoxazole [compounds from scheme 2, scheme 3, scheme 4, scheme 5, scheme 6] were screened for antibacterial activity and the results were tabulated in the **Table-7.1-7.5** and the values were represented in bar diagram in **Fig.1-5.**

Methods and materials

The newly synthesized compounds were evaluated for *in vitro* growth inhibitory activities against a panel of standard strains of pathogenic microorganisms including three Gram-positive bacteria, three Gram-negative bacteria namely *staphylococcus aureus, staphylococcus epidemidis* and *bacillus cereus* and gram negative bacteria namely *pseudomonas aeruginosa*, *vibrio cholera* and *Escherichia coli* respectively by agar well diffusion method. By using Tetracycline as a standard, 100 μg/mL of sterile distilled water, three different concentrations (100, 50 and 25 μg /mL in 10% DMSO) and control (10% DMSO) were introduced to respective labeled wells. The plates were allowed to stand for 30 min. and were incubated at 37ºC for 24h in upright position and the zone of inhibition was recorded.

Table 7.1: Antibacterial activity data of synthesized compounds 5(a-g)

Compound	Concentration in *µg/ml*	Growth inhibition against *bacteria in mm*					
		S.aureus	*S.epidermidis*	*B.cereus*	*P.aeruginosa*	*V.cholerae*	*E.coli*
5a	25	12.63±0.15	13.52±0.02	12.56±0.04	10.56±0.15	12.92±0.06	13.42±0.11
	50	14.22±0.56	14.96±0.04	15.90±0.06	13.20±0.15	13.06±0.04	14.23±0.20
	100	18.12±0.15	18.22±0.07	22.11±0.19	16.20±0.09	16.23±0.27	18.21±0.02
5b	25	14.25±0.05	15.25±0.15	12.11±0.17	13.03±0.21	13.32±0.20	14.26±0.17
	50	17.54±0.07	17.35±0.30	14.00±0.00	17.10±0.20	18.29±0.03	16.27±0.16
	100	19.85±0.32	21.47±0.33	20.14±0..00	19.07±0.21	21.45±0.21	20.78±0.07
5c	25	15.18±0.13	16.82±0.34	18.64±0.24	16.42±0.20	16.26±0.06	12.14±0.12
	50	19.45±0.18	18.34±0.14	21.45±0.17	18.54±0.13	18.24±0.16	15.24±0.23
	100	23.51±0.57	23.45±0.23	26.84±0.14	23.12±0.10	25.89±0.16	23.81±0.18
5d	25	18.23±0.24	16.03±0.25	17.26±0.20	17.52±0.12	16.25±0.26	15.27±0.45
	50	22.56±0.07	18.10±0.26	20.28±0.05	20.12±0.12	18.42±0.31	21.54±0.03
	100	25.52±0.11	23450±0.20	27.27±0.00	23.12±0.12	26.45±0.31	24.56±0.25
5e	25	16.70±0.20	13.30±0.19	13.28±0.05	12.08±0.20	15.28±0.05	15.21±0.33
	50	19.43±0.21	15.41±0.06	15.28±0.21	16.06±0.15	17.56±0.02	18.21±0.04
	100	21.45±0.09	19.45±0.04	21.32±0.02	20.57±0.12	20.49±0.08	20.17±0.03
5f	25	18.47±0.21	17.54±0.11	14.23±0.12	12.20±0.01	16.13±0.02	13.89±0.01
	50	22.15±0.26	19.35±0.21	18.65±0.09	15.30±0.22	18.14±0.01	19.02±0.31
	100	26.45±0.21	24.26±0.27	23.34±0.05	20.10±0.02	22.14±0.02	22.50±0.25
5g	25	13.53±0.05	15.25±0.15	10.54±0.17	12.25±0.21	11.82±0.20	13.56±0.17
	50	17.54±0.07	17.35±0.30	14.00±0.00	16.22±0.20	18.29±0.03	16.27±0.16
	100	19.85±0.32	21.47±0.33	20.14±0..00	18.52±0.21	20.59±0.21	20.78±0.07
Control	100	-	-	-	-	-	-
Std	100	27.12±0.01	25.03±0.31	30.05±0.45	24.00±0.01	28.25±0.14	26.11±0.20

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Tetracycline**

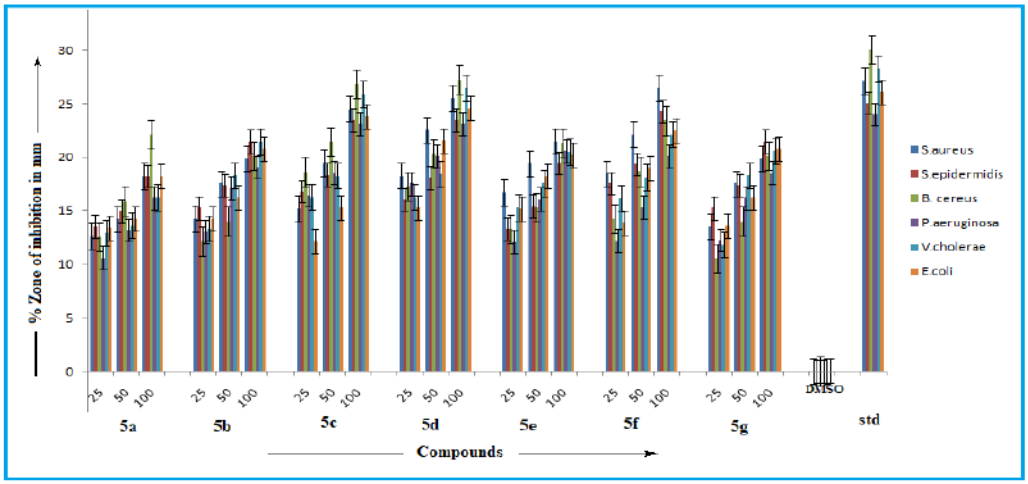


Fig. 1

Table 7.2: *In vitro* antibacterial activities of the target compounds of series 8 (a-f)

	Zone of inhibition in mm					
Compounds	***S. aureus***	***S. epidemidis***	***B. cereus***	***P.aeruginosa***	***V.cholerae***	***E. coli***
8a	**16±0.01**	**21±0.02**	**14±0.14**	**22±0.10**	**18±0.14**	**16±0.10**
8b	**18±0.12**	**20±0.06**	**18±0.02**	**14±0.08**	**20±0.04**	**18±0.04**
8c	**23±0.05**	**27±0.04**	**22±0.06**	**23±0.05**	**25±0.02**	**22±0.02**
8d	**16±0.03**	**18±0.10**	**16±0.01**	**17±0.03**	**14±0.08**	**16±0.08**
8e	**23±0.11**	**27±0.04**	**22±0.04**	**23±0.09**	**24±0.06**	**22±0.04**
8f	**16±0.02**	**18±0.14**	**14±0.06**	**15±0.02**	**18±0.10**	**16±0.05**
DMSO	**-**	**-**	**-**	**-**	**-**	**-**
Std	**25**	**30**	**24**	**25**	**27**	**25**

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Tetracycline**

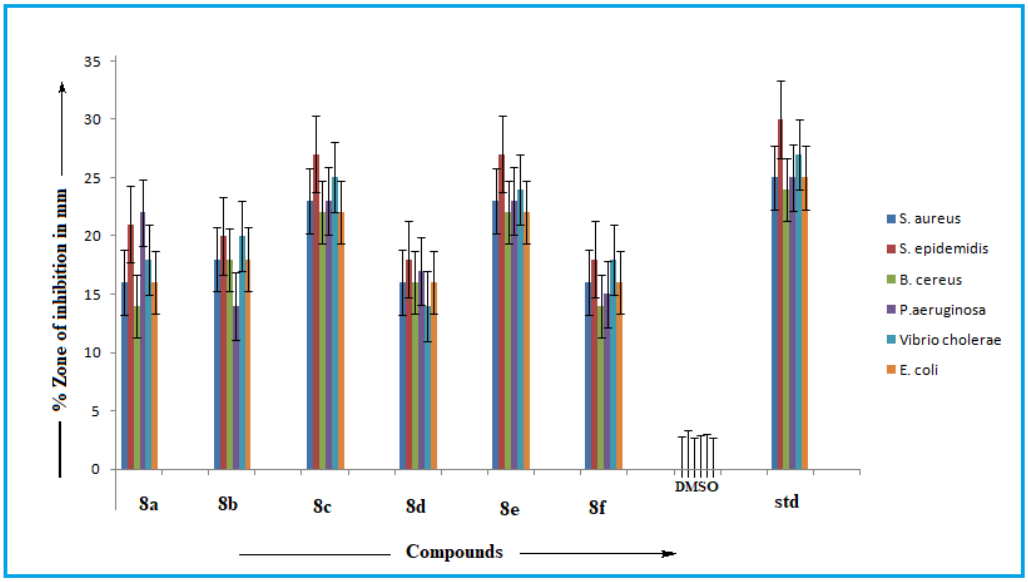

% Zone of inhibition in mm
35
30
25
20
15
10
5
0
8a
8b
8c
8d
8e
8f
DMSO
std
Compounds
S. aureus
S. epidemidis
B. cereus
P.aeruginosa
Vibrio cholerae
E. coli

Fig. 2

Table 7.3: Antibacterial activity data of synthesized compounds 10(a-g)

Compound	Concentration in *µg/ml*	Growth inhibition against *bacteria in mm*					
		S.aureus	*S.epidermidis*	*B.cereus*	*P.aeruginosa*	*V.cholerae*	*E.coli*
10a	25	14.89±0.15	11.23±0.02	14.28±0.04	12.36±0.15	15.48±0.06	13.99±0.11
	50	18.43±0.56	16.41±0.04	16.28±0.06	17.06±0.15	19.56±0.04	16.21±0.20
	100	21.53±0.15	19.45±0.07	20.74±0.19	20.22±0.09	21.63±0.27	20.96±0.02
10b	25	15.25±0.05	16.25±0.15	13.11±0.17	12.03±0.21	14.32±0.20	12.26±0.17
	50	16.54±0.07	18.35±0.30	15.00±0.00	16.10±0.20	16.29±0.03	15.27±0.16
	100	18.85±0.32	22.47±0.33	21.14±0.00	18.07±0.21	20.45±0.21	22.78±0.07
10c	25	16.18±0.13	17.82±0.34	19.64±0.24	15.42±0.20	15.26±0.06	13.14±0.12
	50	20.45±0.18	20.34±0.14	22.45±0.17	17.54±0.13	18.24±0.16	18.24±0.23
	100	24.51±0.57	24.45±0.23	25.84±0.14	24.12±0.10	24.89±0.16	24.81±0.18
10d	25	15.63±0.24	15.33±0.25	16.26±0.20	14.22±0.12	16.25±0.26	17.27±0.45
	50	19.36±0.07	17.20±0.26	21.28±0.05	18.15±0.14	19.42±0.31	20.54±0.03
	100	24.54±0.11	23.20±0.20	26.27±0.00	22.12±0.12	25.55±0.31	23.56±0.25
10e	25	15.70±0.20	12.30±0.19	14.28±0.05	13.08±0.20	16.28±0.05	14.21±0.33
	50	18.43±0.21	16.41±0.06	16.28±0.21	17.06±0.15	19.56±0.02	16.21±0.04
	100	20.45±0.09	20.45±0.04	22.32±0.02	21.57±0.12	21.49±0.08	21.17±0.03
10f	25	19.47±0.21	16.54±0.11	15.23±0.12	13.20±0.01	14.13±0.02	14.89±0.01
	50	21.15±0.26	18.35±0.21	17.65±0.09	16.30±0.22	16.14±0.01	18.02±0.31
	100	25.45±0.21	25.26±0.27	23.34±0.05	19.10±0.02	23.14±0.02	24.50±0.25
10g	25	16.22±0.24	17.25±0.25	12.33±0.20	14.22±0.12	14.13±0.26	15.69±0.45
	50	18.30±0.07	18.35±0.26	17.65±0.05	16.30±0.14	16.14±0.31	18.02±0.03
	100	22.33±0.11	25.26±0.20	23.34±0.00	20.05±0.12	23.14±0.31	23.14±0.25
Control	100	-	-	-	-	-	-
Std	100	27.12±0.01	25.03±0.31	30.05±0.45	24.00±0.01	28.25±0.14	26.11±0.20

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Tetracycline**

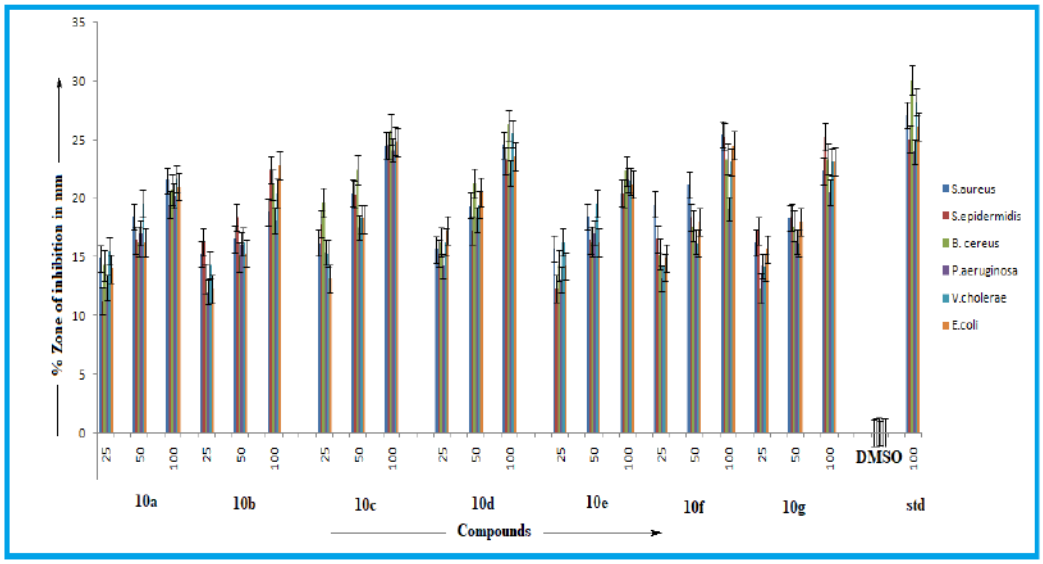

% Zone of inhibition in mm
35
30
25
20
15
10
5
0
25
50
100
DMSO
10a
10b
10c
10d
10e
10f
10g
std
Compounds
S.aureus
S.epidermidis
B. cereus
P.aeruginosa
V.cholerae
E.coli

Fig. 3

Table 7.4: Antibacterial activity data of synthesized compounds 11-16

Compound	Concentration in *µg/ml*	Growth inhibition against *bacteria in mm*					
		S.aureus	*S.epidermidis*	*B.cereus*	*P.aeruginosa*	*V.cholerae*	*E.coli*
11	25	14.43±0.24	16.63±0.25	15.86±0.20	15.02±0.12	16.25±0.26	18.87±0.45
	50	18.16±0.07	18.20±0.26	22.68±0.05	19.23±0.14	18.62±0.31	22.64±0.03
	100	23.44±0.11	24.20±0.20	25.97±0.00	23.42±0.12	24.53±0.31	24.46±0.25
12	25	14.95±0.05	15.65±0.15	12.61±0.17	13.23±0.21	13.52±0.20	11.56±0.17
	50	16.44±0.07	17.95±0.30	14.20±0.00	15.50±0.20	15.39±0.03	14.37±0.16
	100	18.65±0.32	21.67±0.33	20.64±0..00	17.37±0.21	19.25±0.21	20.48±0.07
13	25	17.98±0.13	16.92±0.34	18.64±0.24	16.82±0.20	16.26±0.06	14.14±0.12
	50	21.85±0.18	19.55±0.14	19.56±0.17	18.34±0.13	19.24±0.16	19.24±0.23
	100	21.24±0.57	20.15±0.23	20.22±0.14	20.22±0.10	20.45±0.16	21.54±0.18
14	25	12.73±0.15	11.52±0.02	12.56±0.04	12.56±0.15	12.32±0.06	13.42±0.11
	50	14.62±0.56	14.96±0.04	15.90±0.06	15.20±0.15	15.86±0.04	16.23±0.20
	100	18.32±0.15	18.22±0.07	22.11±0.19	18.20±0.09	18.33±0.27	18.61±0.02
15	25	14.70±0.20	13.30±0.19	12.28±0.05	12.08±0.20	14.68±0.05	13.21±0.33
	50	17.43±0.21	17.41±0.06	17.28±0.21	16.06±0.15	18.56±0.02	15.21±0.04
	100	19.45±0.09	21.45±0.04	21.32±0.02	20.57±0.12	20.49±0.08	20.17±0.03
16	25	18.47±0.21	14.54±0.11	16.23±0.12	14.20±0.01	15.13±0.02	15.89±0.01
	50	20.15±0.26	19.35±0.21	19.65±0.09	17.30±0.22	18.14±0.01	19.02±0.31
	100	26.45±0.21	24.26±0.27	26.34±0.05	20.10±0.02	25.14±0.02	25.50±0.25
Control	100	-	-	-	-	-	-
Std	100	27.12±0.01	25.03±0.31	30.05±0.45	24.00±0.01	28.25±0.14	26.11±0.20

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Tetracycline**

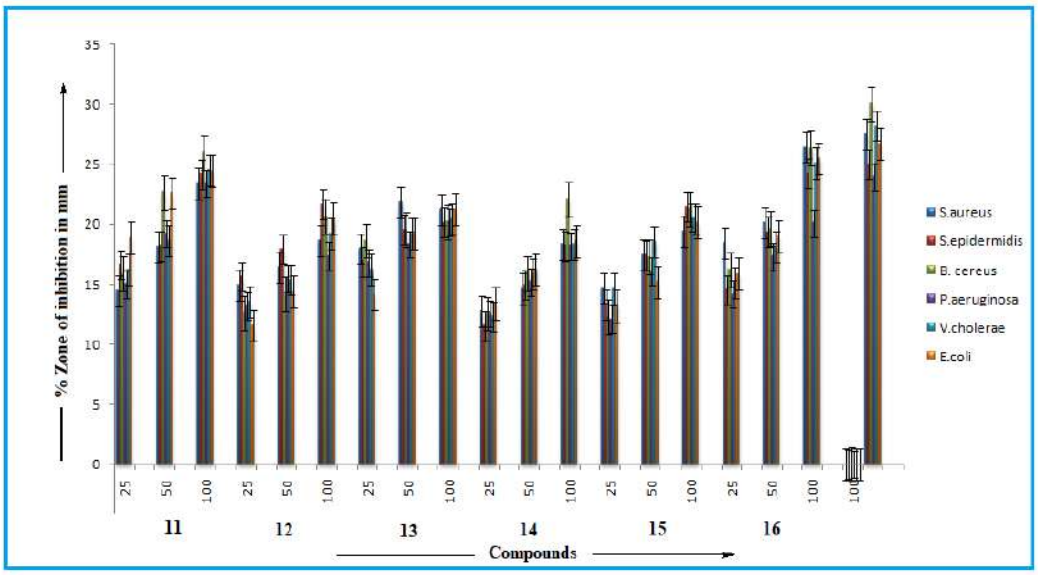

% Zone of inhibition in mm
35
30
25
20
15
10
5
0
25
50
100
11
12
13
14
15
16
Compounds
S.aureus
S.epidermidis
B. cereus
P.aeruginosa
V.cholerae
E.coli

Fig. 4

Table 7.5: Antibacterial activity data of synthesized compounds 17(a-f)

Compound	Concentration in *µg/ml*	Growth inhibition against *bacteria in mm*					
		S.aureus	***S.epidermidis***	***B.cereus***	***P.aeruginosa***	***V.cholerae***	***E.coli***
17a	**25**	**15.43±0.24**	**17.63±0.25**	**14.86±0.20**	**13.12±0.12**	**15.25±0.26**	**17.87±0.45**
	50	**19.16±0.07**	**18.20±0.26**	**21.68±0.05**	**16.43±0.14**	**17.62±0.31**	**21.64±0.03**
	100	**22.44±0.11**	**23.20±0.20**	**24.97±0.00**	**22.42±0.12**	**23.53±0.31**	**24.46±0.25**
17b	**25**	**13.98±0.13**	**12.92±0.34**	**14.64±0.24**	**14.82±0.20**	**15.26±0.06**	**15.14±0.12**
	50	**16.52±0.18**	**15.54±0.14**	**19.45±0.17**	**17.34±0.13**	**16.56±0.16**	**20.24±0.23**
	100	**20.89±0.57**	**20.33±0.23**	**21.27±0.14**	**20.55±0.10**	**19.25±0.16**	**21.47±0.18**
17c	**25**	**16.42±0.05**	**15.45±0.15**	**16.77±0.17**	**15.55±0.21**	**16.10±0.20**	**15.49±0.17**
	50	**20.54±0.07**	**18.36±0.30**	**20.49±0.00**	**20.14±0.20**	**21.14±0.03**	**19.48±0.16**
	100	**26.22±0.32**	**22.45±0.33**	**26.89±0.00**	**23.22±0.21**	**25.78±0.21**	**24.85±0.07**
17d	**25**	**14.73±0.15**	**12.52±0.02**	**11.56±0.04**	**13.56±0.15**	**13.32±0.06**	**12.42±0.11**
	50	**16.62±0.56**	**16.96±0.04**	**14.90±0.06**	**16.20±0.15**	**17.86±0.04**	**15.23±0.20**
	100	**19.32±0.15**	**20.22±0.07**	**21.11±0.19**	**19.20±0.09**	**19.33±0.27**	**17.61±0.02**
17e	**25**	**12.70±0.20**	**12.30±0.19**	**14.28±0.05**	**11.08±0.20**	**15.68±0.05**	**15.21±0.33**
	50	**15.43±0.21**	**18.41±0.06**	**18.28±0.21**	**14.06±0.15**	**19.56±0.02**	**17.21±0.04**
	100	**18.45±0.09**	**20.45±0.04**	**22.32±0.02**	**18.57±0.12**	**22.49±0.08**	**22.17±0.03**
17f	**25**	**15.47±0.21**	**12.54±0.11**	**15.23±0.12**	**16.20±0.01**	**13.13±0.02**	**14.89±0.01**
	50	**21.15±0.26**	**18.35±0.21**	**18.65±0.09**	**18.30±0.22**	**17.14±0.01**	**18.02±0.31**
	100	**22.10±0.21**	**21.45±0.27**	**21.86±0.05**	**20.44±0.02**	**20.48±0.02**	**20.10±0.25**
Control	**100**	**-**	**-**	**-**	**-**	**-**	**-**
Std	**100**	**27.12±0.01**	**25.03±0.31**	**30.05±0.45**	**24.00±0.01**	**28.25±0.14**	**26.11±0.20**

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Tetracycline**

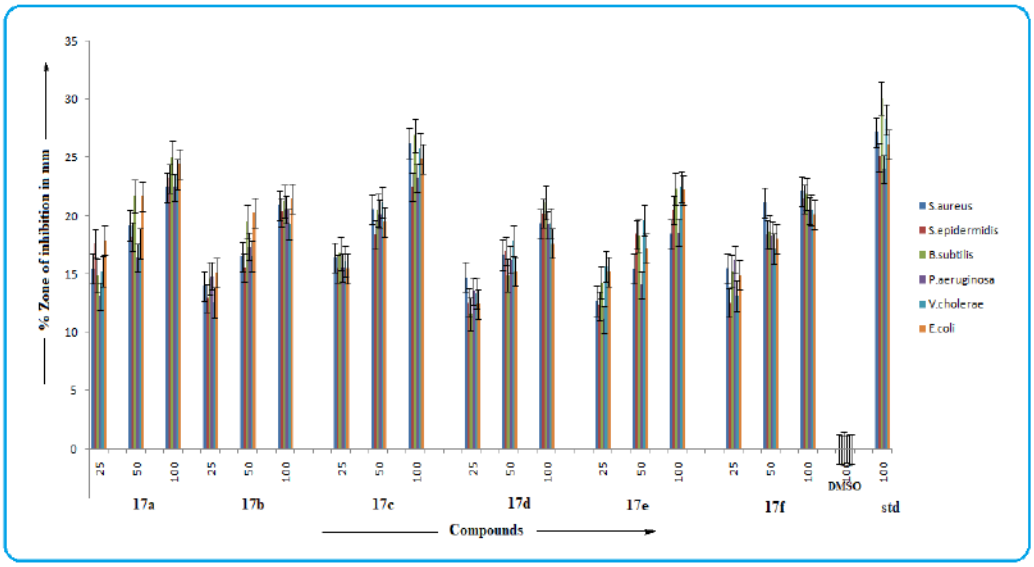


Fig. 5

7.1.1 Result and discussion

All the screened compounds were exhibited considerable antibacterial activity. The compounds **5c** and **5d (scheme -II)** showed potent antibacterial activity against *E. coli*, *S.aureus*, *P. aeruginosa*, *B. cereus*, *S. epidermidis* and the compound **5c** was most potent against *B. cereus* **(table-7.1) (fig.1)**. In **scheme-III**, the compounds **8c and 8e** showed considerable activity against *V. Cholera*, *S. epidermidis* **(table-7.2) (fig.2)**. In **scheme IV**, the compounds **10c** and **10d** displayed evident activity **(table-7.3) (fig.3)**. In **scheme V** the molecules **11** and **16** exhibited noticeable activity **(table-7.4) (fig.4)**. In **scheme-VI** the compounds **17a** and **17c** **(table-7.5) (fig.5)** exhibited equipotent activity when compared with standard Tetracycline against *S. aureus B. cereus* strains.

7.2 Antifungal Activity

Serious infections caused by microorganisms resistant to commonly used antimicrobials have become a major healthcare problem worldwide in the 21st century. This is responsible for the significant raising in morbidity and mortality, longer hospitalization and increased health care costs. In recent years, the number of

availability of new antimicrobial agents has been decreased for human use across the globe. The development of new antimicrobial agents is very expensive and time consuming, leading to diminishing interest of pharmaceutical industries. The cost of bringing a new product to the market is increasing at a rate of 10% per annum. There are perhaps over 10,000 species of fungi, but less than 100 cause diseases in human.[9] Moreover, fungal pathogens started to develop resistance to commonly used antifungal chemotherapeutic agents. So there is urgent need for the development of new antifungal agents. Hence, the antifungal activity of the compounds were tested against two fungal strains *Aspergillus aureus* and *Aspergillus fumigates* by sabouraud dextrose agar diffusion method by using Fluconazole as a standard.

Methods and materials

The newly synthesized compounds were evaluated against *Aspergillus aureus* and *Aspergillus fumigates* fungus, using the sabouraud dextrose agar diffusion method. Wells were made (6mm diameter) with a sterile cork borer. The standard drug namely Fluconazole used (100µg/mL of sterile distilled water) and control was added to respectively labeled wells. To these wells 140 µL to each (100, 50 and 25µg/mL in 10% DMSO) of the test stock solution compounds were introduced and the plates were allowed to cool for an hour to facilitate the diffusion. The plates were incubated at 37ºC for 72h. At the end of the incubation period, the diameter of the zone of inhibition around the wells was measured using vernier caliper.

Table 7.6: Antifungal activity data of synthesized compounds 5(a-g)

Compound	Concentration in *µg/ml*	Growth inhibition against *fungicides in mm*	
		A.aureus	*A.fumigatus*
5a	25	12.52±0.25	11.56±0.20
	50	16.23±0.31	14.66±0.20
	100	18.66±0.21	17.15±0.23
5b	25	14.11±0.17	15.14±0.15
	50	16.44±0.20	18.36±0.15
	100	19.59±0.33	20.35±0.10
5c	25	16.58±0.19	14.25±0.09
	50	18.62±0.25	17.28±0.05
	100	21.36±0.10	21.06±0.01
5d	25	19.42±0.17	19.21±0.14
	50	23.56±0.20	22.36±0.25
	100	26.22±0.23	23.15±0.15
5e	25	18.17±0.16	16.25±0.10
	50	22.13±0.14	20.20±0.07
	100	25.65±0.02	23.42±0.09
5f	25	14.23±0.01	16.23±0.18
	50	15.92±0.22	18.65±0.10
	100	19.23±0.11	20.23±0.01
5g	25	12.33±0.01	13.56±0.18
	50	18.62±0.22	17.28±0.10
	100	20.89±0.11	20.49±0.01
Control	100	-	-
Std	100	28.05±0.11	24.52±0.15

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Fluconazole**

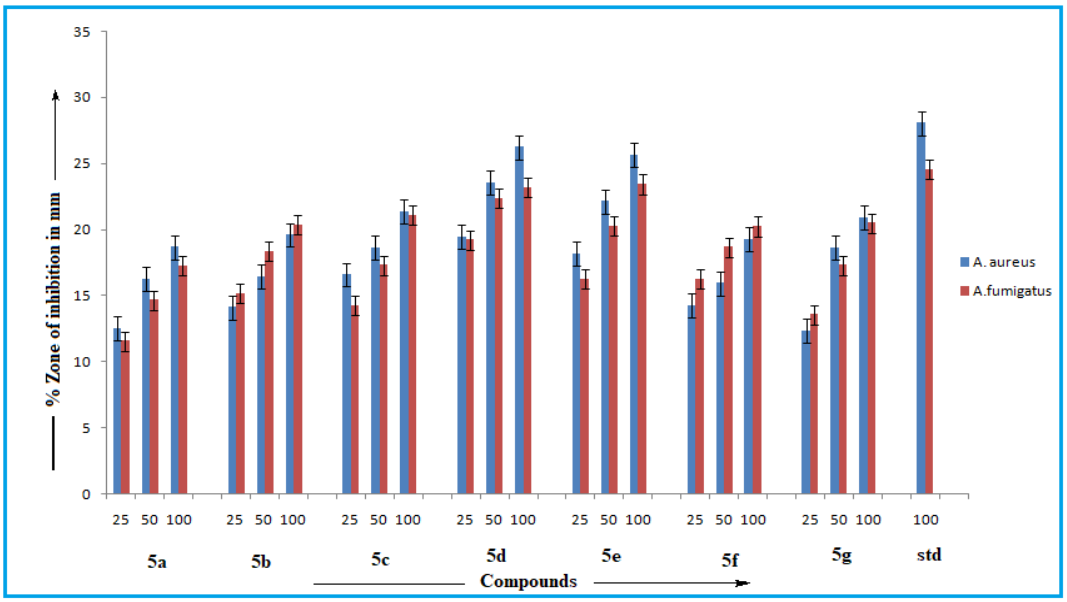


Fig. 6

Table 7.7: *In vitro* Antifungal Activity of compounds 8(a-f)

Compounds	Zone of inhibition in mm	
	A. aureus	*A.fumigatus*
8a	17±0.02	20±0.02
8b	16±0.04	19±0.04
8c	23±0.10	26±0.06
8d	16±0.08	19±0.09
8e	23±0.03	26±0.10
8f	18±0.05	19±0.02
DMSO	-	-
Std	25	30

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Fluconazole**

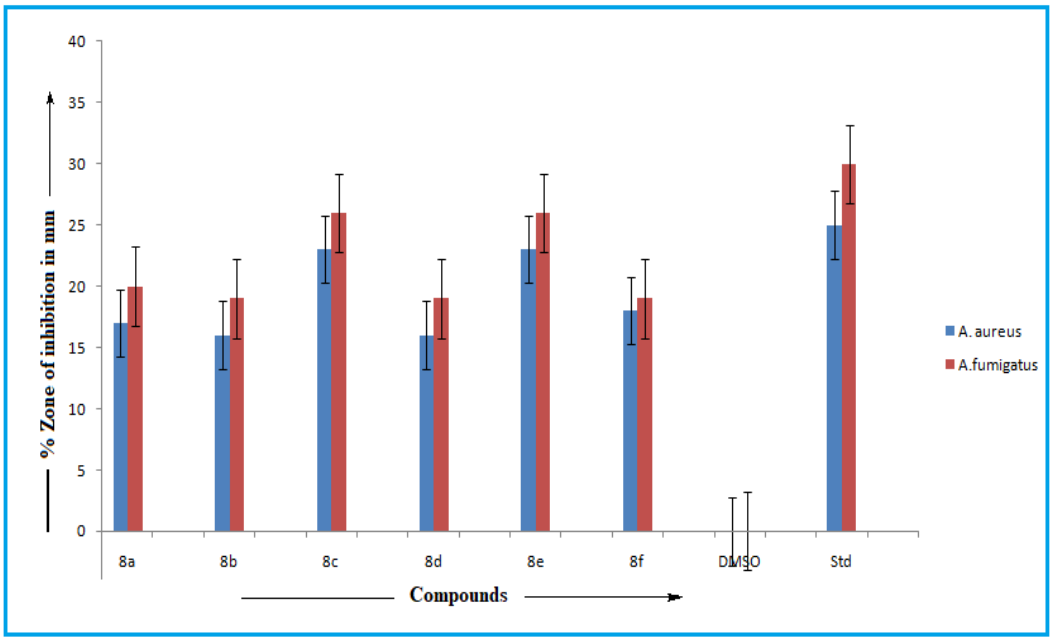

40
35
30
25
20
15
10
5
0
% Zone of inhibition in mm
8a
8b
8c
8d
8e
8f
DMSO
Std
Compounds
A. aureus
A.fumigatus

Fig. 7

Table 7.8: Antifungal activity data of synthesized compounds 10(a-f)

Compound	Concentration in *µg/ml*	Growth inhibition against *fungicides in mm*	
		A.aureus	*A.fumigatus*
10a	25	16.23±0.25	15.56±0.20
	50	20.50±0.31	20.45±0.20
	100	20.22±0.21	21.22±0.23
10b	25	15.11±0.17	16.14±0.15
	50	17.44±0.20	19.36±0.15
	100	20.59±0.33	21.35±0.10
10c	25	16.58±0.19	15.25±0.09
	50	17.62±0.25	18.28±0.05
	100	23.36±0.10	22.06±0.01
10d	25	18.42±0.17	17.21±0.14
	50	22.56±0.20	21.36±0.25
	100	25.22±0.23	24.15±0.15
10e	25	17.27±0.16	14.25±0.10
	50	21.13±0.14	20.20±0.07
	100	22.65±0.02	24.42±0.09
10f	25	15.23±0.01	17.23±0.18
	50	16.92±0.22	19.65±0.10
	100	20.23±0.11	21.23±0.01
10g	25	15.11±0.17	16.14±0.15
	50	17.44±0.20	19.36±0.15
	100	20.59±0.33	21.35±0.10
Control	100	-	-
Std	100	28.05±0.11	24.52±0.15

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Fluconazole**

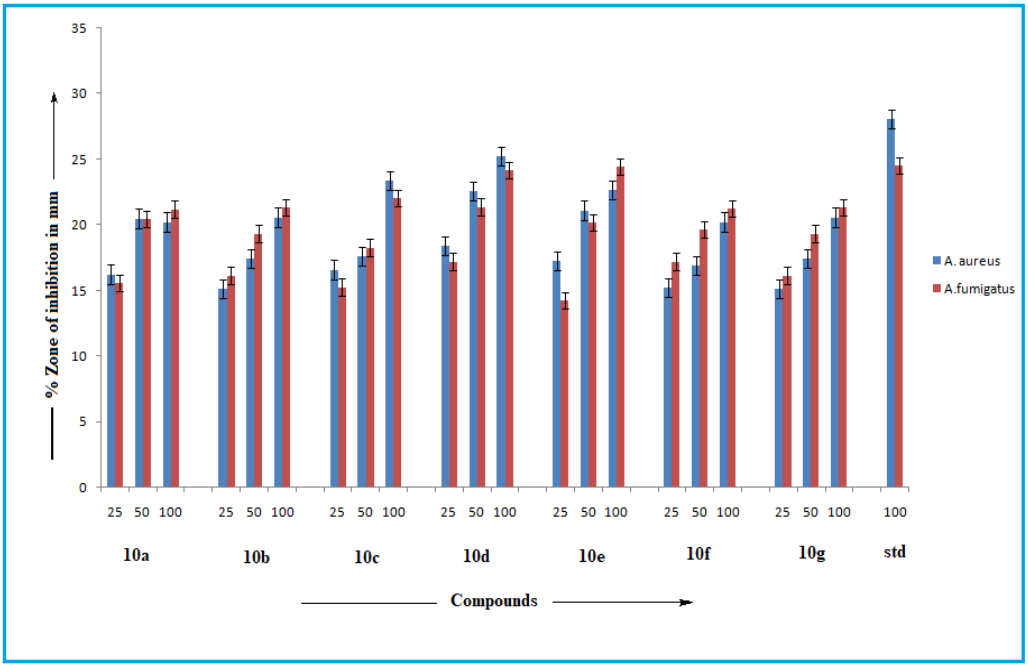

% Zone of inhibition in mm
35
30
25
20
15
10
5
0
25 50 100 25 50 100 25 50 100 25 50 100 25 50 100 25 50 100 25 50 100 100
10a
10b
10c
10d
10e
10f
10g
std
Compounds
A. aureus
A.fumigatus

Fig. 8

Table 7.9: Antifungal activity data of synthesized compounds 11-16

Compound	Concentration in *µg/ml*	Growth inhibition against *fungicides in mm*	
		A.aureus	***A.fumigatus***
11	**25**	**14.93±0.25**	**16.56±0.20**
	50	**20.14±0.31**	**20.45±0.20**
	100	**25.23±0.21**	**23.45±0.23**
12	**25**	**14.11±0.17**	**13.56±0.15**
	50	**16.44±0.20**	**15.42±0.15**
	100	**18.10±0.33**	**20.23±0.10**
13	**25**	**12.86±0.16**	**13.25±0.10**
	50	**16.23±0.14**	**18.42±0.07**
	100	**28.44±0.02**	**19.33±0.09**
14	**25**	**11.45±0.17**	**13.22±0.14**
	50	**15.77±0.20**	**16.88±0.25**
	100	**18.25±0.23**	**18.41±0.15**
15	**25**	**13.78±0.19**	**12.44±0.09**
	50	**16.48±0.25**	**15.44±0.05**
	100	**18.11±0.10**	**18.45±0.01**
16	**25**	**16.45±0.01**	**17.45±0.18**
	50	**20.55±0.22**	**21.47±0.10**
	100	**24.89±0.11**	**23.23±0.01**
Control	**100**	**-**	**-**
Std	**100**	**28.05±0.11**	**24.52±0.15**

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Fluconazole**

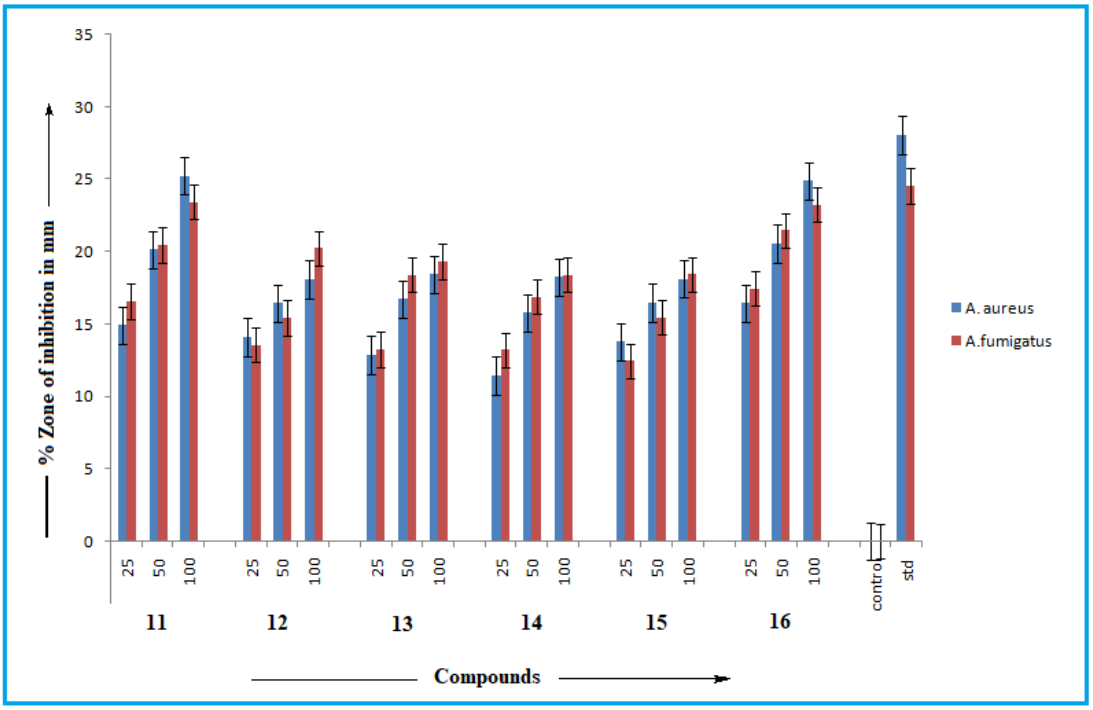

% Zone of inhibition in mm
35
30
25
20
15
10
5
0
25
50
100
11
12
13
14
15
16
control
std
Compounds
A. aureus
A.fumigatus

Fig. 9

Table -7.10: Antifungal activity data of synthesized compounds 17(a-f)

Compound	Concentration in *µg/ml*	Growth inhibition against *fungicides in mm*	
		A.aureus	***A.fumigatus***
17a	**25**	**12.93±0.25**	**14.56±0.20**
	50	**17.50±0.31**	**18.45±0.20**
	100	**25.95±0.21**	**23.56±0.23**
17b	**25**	**13.11±0.17**	**14.14±0.15**
	50	**15.44±0.20**	**16.36±0.15**
	100	**18.59±0.33**	**19.35±0.10**
17c	**25**	**11.23±0.16**	**15.25±0.10**
	50	**15.53±0.14**	**18.45±0.07**
	100	**18.55±0.02**	**20.11±0.09**
17d	**25**	**16.55±0.19**	**15.99±0.09**
	50	**18.99±0.25**	**17.56±0.05**
	100	**26.55±0.10**	**23.56±0.01**
17e	**25**	**12.42±0.17**	**13.21±0.14**
	50	**18.56±0.20**	**16.56±0.25**
	100	**20.15±0.23**	**19.26±0.15**
17f	**25**	**15.33±0.01**	**15.23±0.18**
	50	**18.42±0.22**	**21.65±0.10**
	100	**20.45±0.11**	**20.12±0.01**
Control	**100**	**0**	**0**
Std	**100**	**28.05±0.11**	**24.52±0.15**

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Fluconazole**

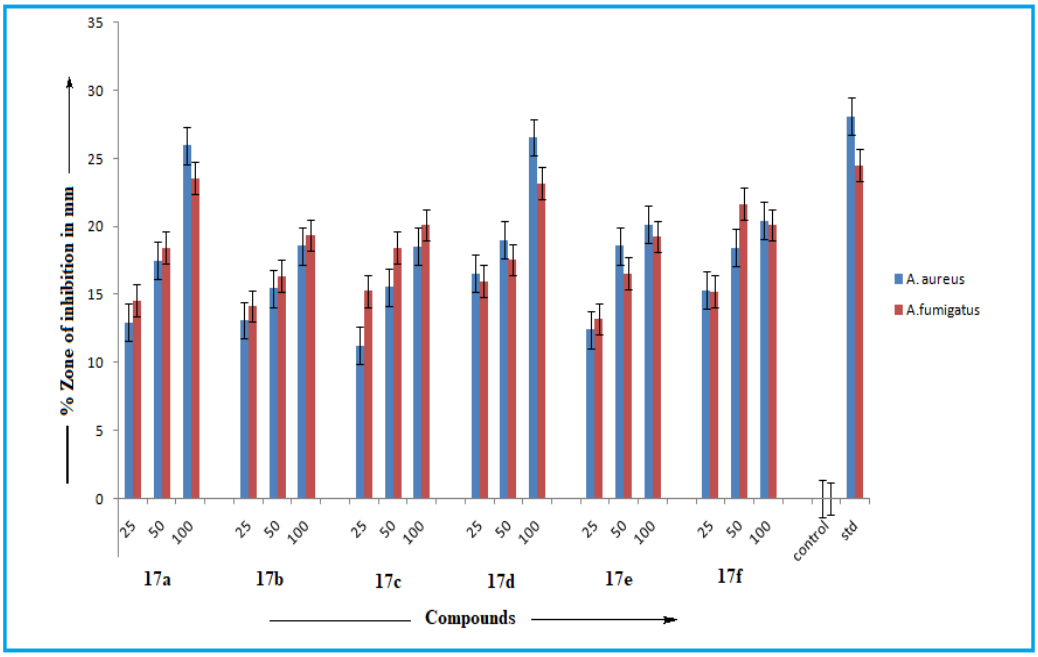


Fig. 10

7.2.1 Result and discussion

Among all the synthesized compounds**5d** and **5e Scheme-II** showed potent antifungal activity against both the strains and the compound **5d** is most potent against *Aspergillus aureus* **(table-7.6) (fig.6)**. In **scheme-III**, the compounds **8c and 8e** showed considerable activity **(table-7.7) (fig.7)**. In **Scheme-IV**, the compounds **10d** and **10e** displayed evident activity **(table-7.8) (fig.8)**. In **Scheme-V** the molecules **11** and **16** exhibited noticeable activity **(table-7.9) (fig.9)**. In **scheme-VI** the compounds **17a** and **17d (table-7.10) (fig.10)** exhibited equipotent activity as compared with standard Fluconazole against *Aspergillus aureus* and *Aspergillus fumigates* strains.

7.3 Minimum Inhibitory Concentration (MIC)

Antibiotics are used to tread wide range of infection to prevent infection[8] dilution methods are used to determine the minimum inhibition concentration of antibacterial agents. MIC methods were widely used in the comparative testing of new biologically active agents. In clinical laboratories they are used to create the susceptibility of organisms that give equivocal results in disk tests. In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates of broth containing dilutions of the antimicrobial agent. The lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism; known as the MIC. The synthesized compounds which showed potent antimicrobial activity was again taken and screening for Minimum Inhibitory Concentration activity.

Methods and materials

The MIC of all the synthesized compounds were determined by micro dilution method. The respective clinical antimicrobial strain was spread separately on the medium. The Wells were made (6mm diameter) with a sterile cork borerunder safe aseptic conditions. The synthesized compounds at different concentrations (25, 50, and 100 μg/mL), were loaded into respective labeled wells. The drugs Tetracycline and Fluconazole were used as standard for the comparison of antibacterial activities, respectively.

Table 7.11: MIC data of antibacterial activity of the synthesized compounds 5(a-g)

Compound	Growth inhibition against *bacteria in µg/ml*					
	S.aureus	*S.epidermidis*	*B. cereus*	*P.aeruginosa*	*V.cholerae*	*E.coli*
5a	250	250	250	250	250	250
5b	250	250	NT	250	500	250
5c	500	500	700	500	500	250
5d	700	500	700	700	700	500
5e	500	250	250	250	500	500
5g	250	250	NT	250	500	250
Std	250	500	500	250	250	250

***Std: Tetracycline**

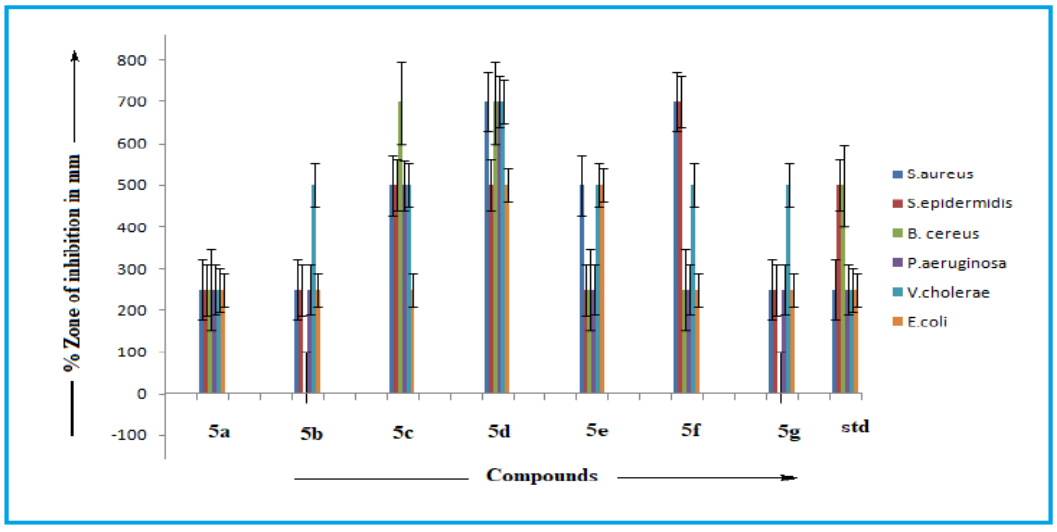


Fig. 11

Table 7.12: MIC data of Antibacterial activity of synthesized compounds 8(a-f)

Compound	Concentration in *µg/ml*	Growth inhibition against *bacteria in mm*					
		S.aureus	*S.epidermidis*	*B. cereus*	*P.aeruginosa*	*V.cholerae*	*E.coli*
8a	25	15.25±0.24	15.22±0.25	13.47±0.20	14.25±0.12	16.47±0.26	14.89±0.45
	50	18.20±0.07	17.28±0.26	16.56±0.05	18.25±0.12	19.24±0.31	17.24±0.03
	100	23.25±0.11	19.25±0.20	21.25±0.00	21.25±0.12	22.56±0.31	19.51±0.25
8b	25	13.45±0.24	13.15±0.25	14.48±0.20	14.10±0.12	14.41±0.26	13.78±0.45
	50	15.98±0.07	16.24±0.26	15.85±0.05	16.25±0.12	17.45±0.31	14.88±0.03
	100	18.55±0.11	20.15±0.20	19.56±0.00	20.21±0.12	19.23±0.31	19.47±0.25
8c	25	15.42±0.05	16.85±0.15	17.45±0.17	16.56±0.21	17.01±0.20	15.66±0.17
	50	19.45±0.07	18.34±0.30	21.45±0.00	19.25±0.20	18.24±0.03	15.24±0.16
	100	25.45±0.32	23.98±0.33	27.45±0.00	23.14±0.21	26.28±0.21	24.56±0.07
8d	25	10.25±0.13	10.89±0.34	11.89±0.24	11.25±0.20	13.24±0.06	12.36±0.12
	50	15.24±0.18	13.49±0.14	14.85±0.17	14.25±0.13	14.85±0.16	13.44±0.23
	100	20.12±0.57	20.78±0.23	23.47±0.14	18.47±0.10	21.56±0.16	21.85±0.18
8e	25	15.89±0.15	15.89±0.02	14.76±0.04	14.35±0.15	16.24±0.06	16.42±0.11
	50	19.56±0.56	19.23±0.04	18.55±0.06	18.25±0.15	18.44±0.04	18.36±0.20
	100	26.23±0.15	23.14±0.07	28.26±0.19	22.26±0.09	26.87±0.27	24.89±0.02
8f	25	16.70±0.20	14.99±0.19	13.28±0.05	13.25±0.20	15478±0.05	12.98±0.33
	50	19.43±0.21	18.56±0.06	16.23±0.21	16.21±0.15	17.52±0.02	18.24±0.04
	100	21.56±0.09	20.56±0.04	22.89±0.02	19.23±0.12	22.12±0.08	20.65±0.03
Control	100	-	-	-	-	-	-
Std	100	27.12±0.01	25.03±0.31	30.05±0.45	24.00±0.01	28.25±0.14	26.11±0.20

***Std: Tetracycline**

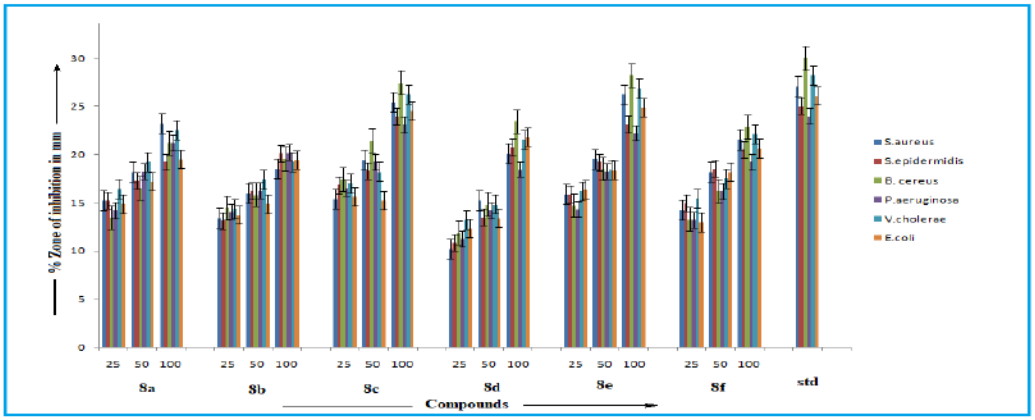


Fig. 12

Table 7.13: MIC data of antibacterial activity of the synthesized compounds 10 (a-g)

Compound	Growth inhibition against *bacteria in µg/ml*					
	S.aureus	***S.epidermidis***	***B. cereus***	***P.aeruginosa***	***V.cholerae***	***E.coli***
10a	**500**	**500**	**700**	**700**	**700**	**500**
10b	**500**	**250**	**NT**	**250**	**500**	**500**
10c	**700**	**500**	**700**	**500**	**250**	**250**
10d	**500**	**250**	**250**	**250**	**250**	**250**
10e	**700**	**250**	**250**	**250**	**500**	**500**
10f	**700**	**700**	**250**	**250**	**250**	**250**
10g	**500**	**250**	**NT**	**250**	**500**	**500**
Std	**250**	**NT**	**NT**	**250**	**250**	**250**

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Std: Tetracycline**

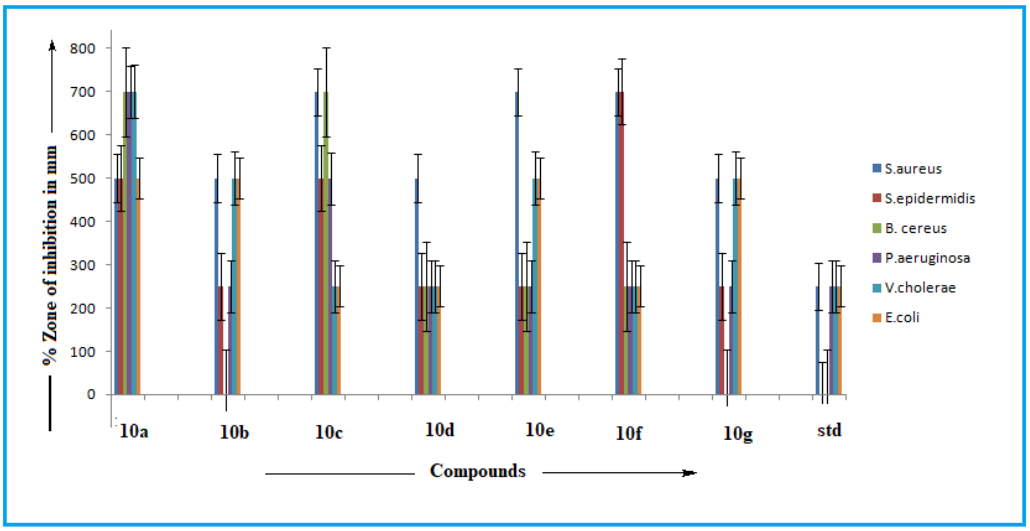


Fig. 13

Table 7.14: MIC data of antibacterial activity of the synthesized compounds 11-16

Compound	Growth inhibition against *bacteria in μg/ml*					
	S.aureus	*S.epidermidis*	*B. cereus*	*P.aeruginosa*	*V.cholerae*	*E.coli*
11	700	250	500	500	500	250
12	700	500	NT	500	250	700
13	500	250	700	250	700	500
14	500	250	250	700	700	500
15	500	250	250	250	700	500
16	700	700	250	250	250	250
Std	250	NT	NT	500	700	250

***Std: Tetracycline**

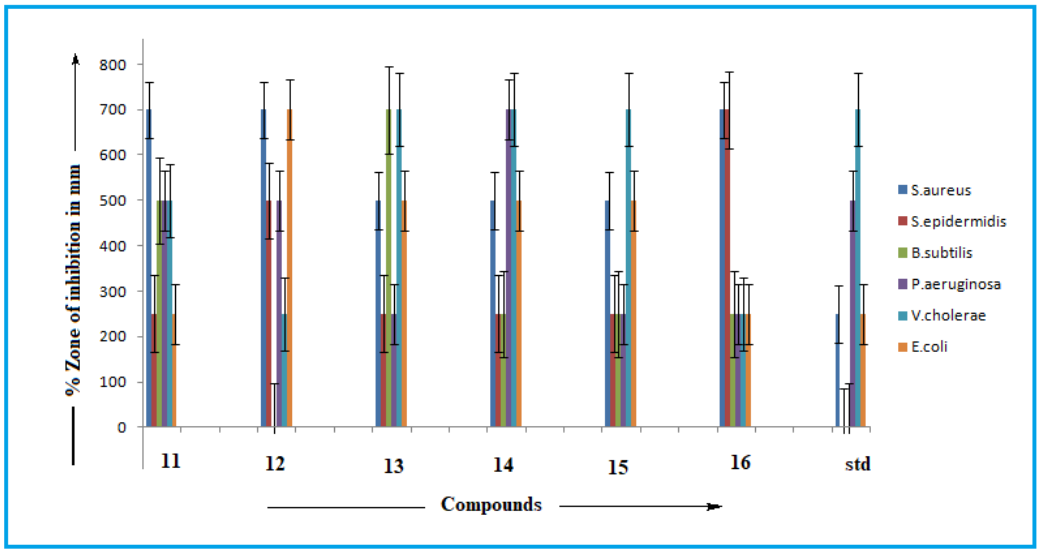


Fig. 14

Table 7.15: MIC data of antibacterial activity of the synthesized compounds 17 (a-f)

Compound	Growth inhibition against *bacteria in µg/ml*					
	S.aureus	*S.epidermidis*	*B. cereus*	*P.aeruginosa*	*V.cholerae*	*E.coli*
17a	700	250	700	500	700	250
17b	500	700	NT	500	250	500
17c	700	250	700	250	500	500
17d	500	250	250	700	500	500
17e	700	250	250	250	700	500
17f	700	500	250	500	500	250
Std	250	NT	NT	500	700	250

***Std: Tetracycline**

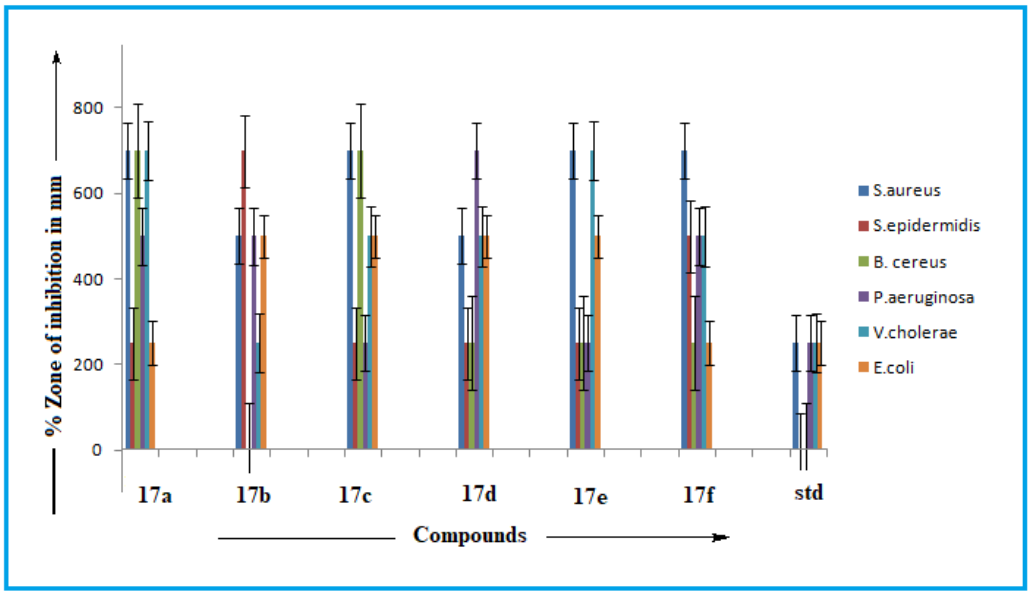


Fig. 15

Table 7.16: MIC data of antifungal activity of the synthesized compounds 5 (a-g)

Compound	Growth inhibition against *fungicides in µg/ml*	
	A.aureus	*A.fumigatus*
5a	700	700
5b	250	500
5c	700	500
5d	700	700
5e	700	500
5f	250	500
5g	250	500
Stnd	250	250

***Std: Fluconazole**

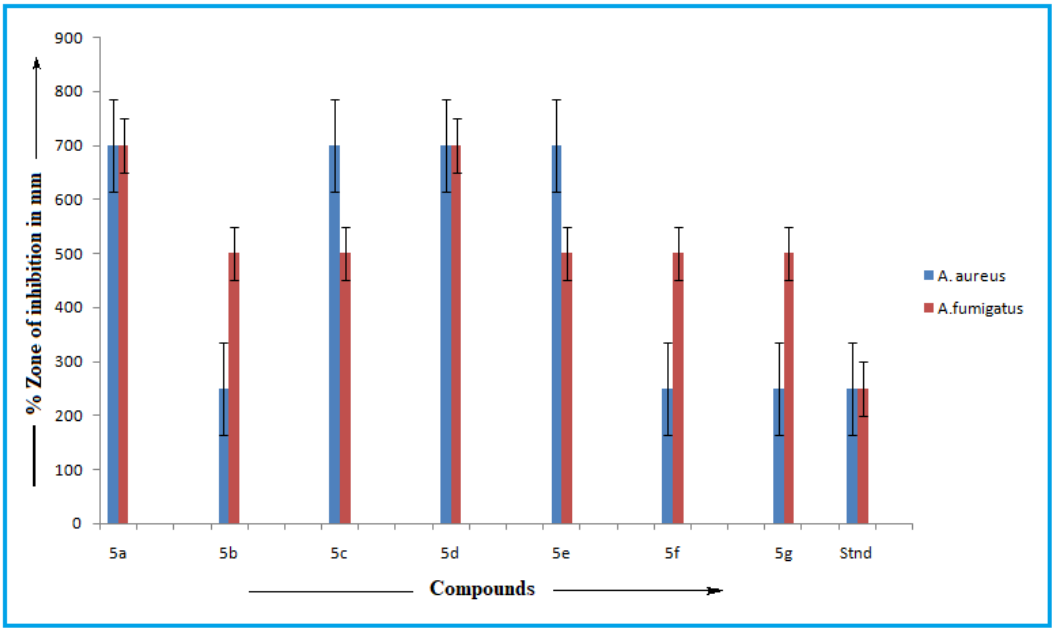

900
800
700
600
500
400
300
200
100
0
% Zone of inhibition in mm
5a
5b
5c
5d
5e
5f
5g
Stnd
Compounds
A. aureus
A.fumigatus

Fig. 16

Table 7.17: MIC data of Antifungal activity of synthesized compounds 8(a-f)

Compound	Concentration in *µg/ml*	Growth inhibition against *fungicides in mm*	
		A.aureus	*A.fumigatus*
8a	25	14.25±0.25	15.24±0.20
	50	18.23±0.31	18.56±0.20
	100	20.27±0.21	29.92±0.23
8b	25	15.41±0.17	14.29±0.15
	50	17.24±0.20	16.27±0.15
	100	20.22±0.33	18.51±0.10
8c	25	16.22±0.16	15.29±0.10
	50	18.44±0.14	17.89±0.07
	100	26.49±0.02	22.89±0.09
8d	25	14.25±0.17	15.19±0.14
	50	16.82±0.20	17.33±0.25
	100	19.72±0.23	19.77±0.15
8e	25	16.42±0.19	16.27±0.09
	50	19.24±0.25	18.24±0.05
	100	25.49±0.10	23.14±0.01
8f	25	13.54±0.01	14.25±0.18
	50	16.78±0.22	17.22±0.10
	100	18.45±0.11	19.27±0.01
Control	100	-	-
Std	100	28.05±0.11	24.52±0.15

***Each value is expressed as mean ±SD of three replicates for the zone of inhibition**

***Stnd: Fluconazole**

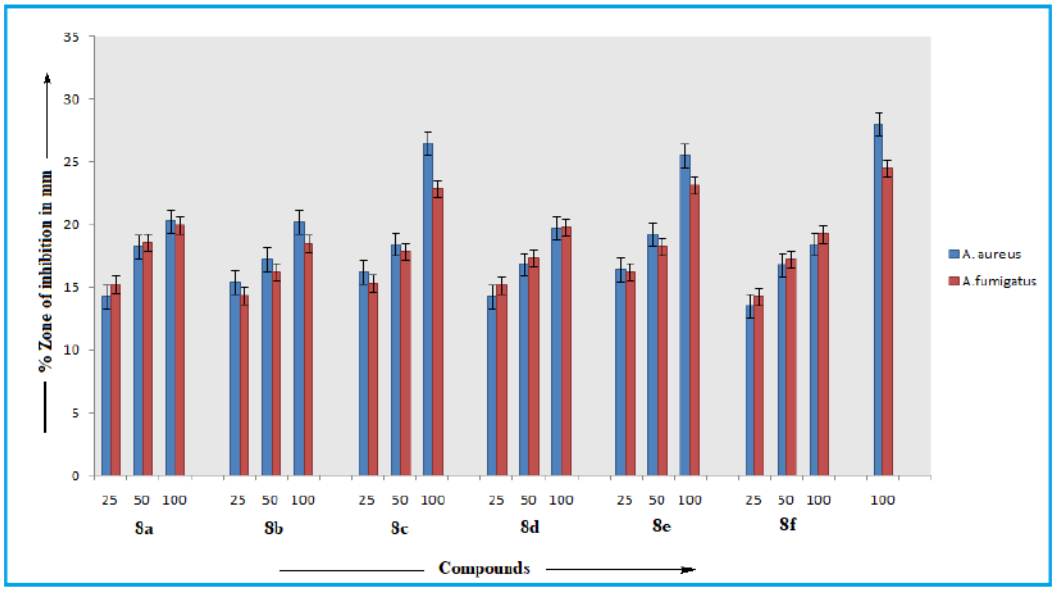


Fig. 17

Table 7.18: MIC data of antifungal activity of the synthesized compounds 10(a-g)

Compound	Growth inhibition against *fungicides in µg/ml*	
	A.aureus	*A.fumigatus*
10a	700	500
10b	250	700
10c	250	500
10d	500	700
10e	700	500
10f	250	700
10g	250	700
Stnd	250	250

***Std:Fluconazole**

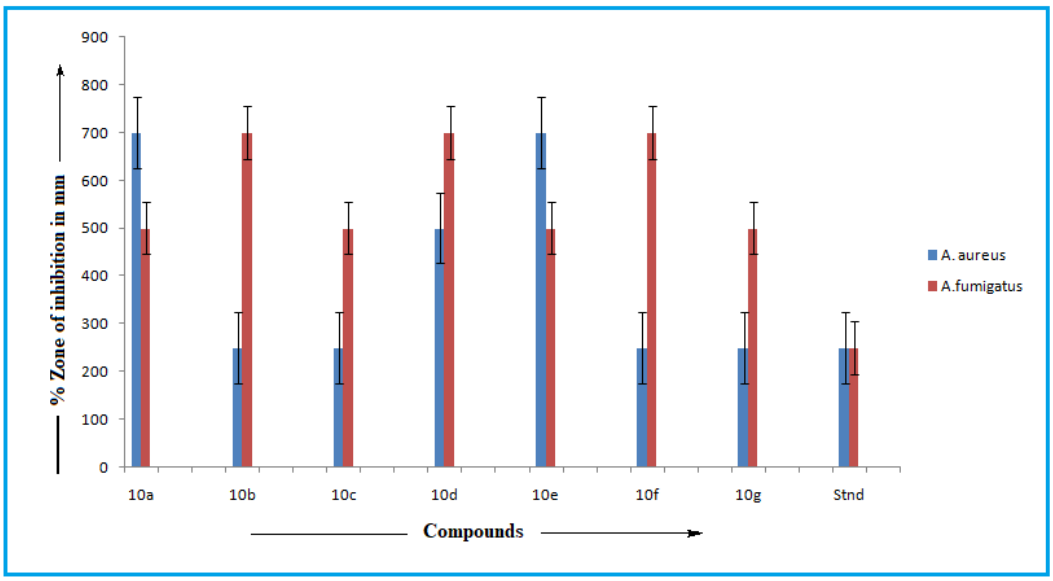


Fig. 18

Table 7.19: MIC data of antifungal activity of the synthesized compounds 11-16

Compound	Growth inhibition against *fungicides in µg/ml*	
	A.aureus	*A.fumigatus*
11	**700**	**500**
12	**250**	**700**
13	**250**	**500**
14	**250**	**500**
15	**500**	**250**
16	**250**	**500**
Stnd	**700**	**500**

***Std:Fluconazole**

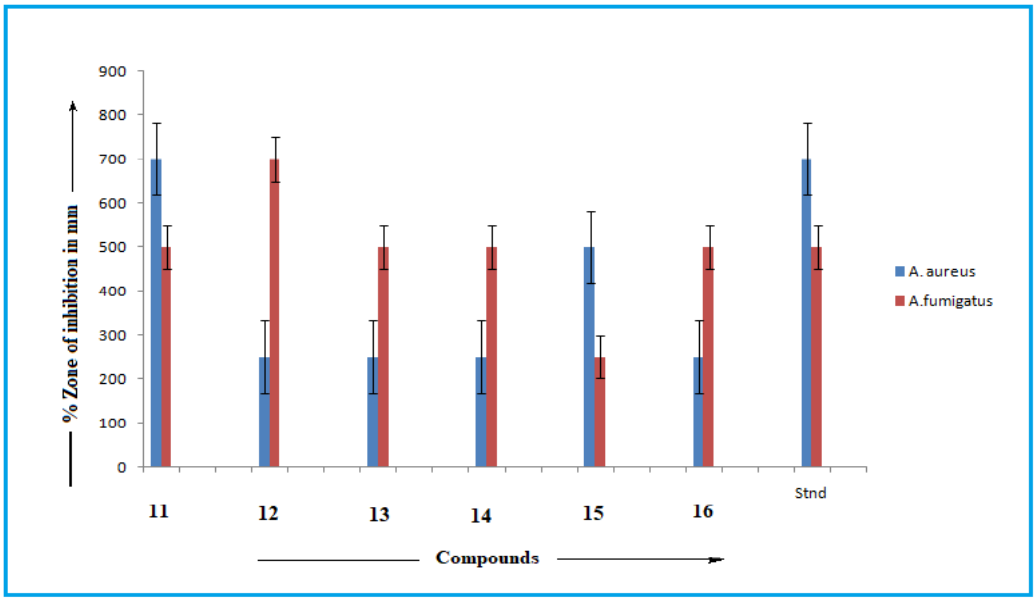


Fig. 19

Table 7.20: MIC data of antifungal activity of the synthesized compounds 17 (a-f)

Compound	Growth inhibition against *fungicides in µg/ml*	
	A.aureus	*A.fumigatus*
17a	500	500
17b	250	500
17c	500	700
17d	250	700
17e	500	500
17f	500	500
Stnd	700	500

***Std:Fluconazole**

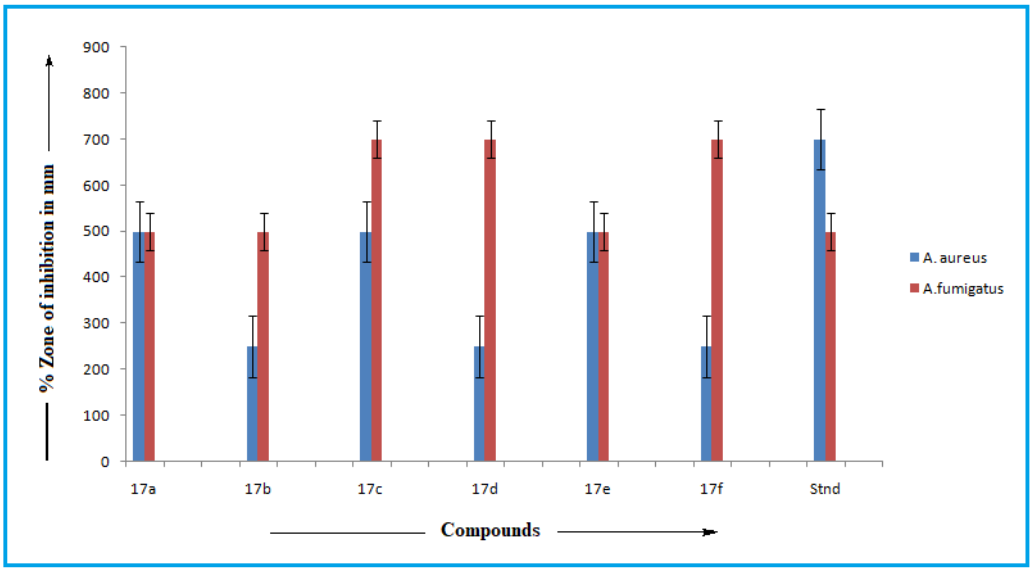


Fig. 20

7.3.1 Result and Discussion

The MIC of the compounds was tested against Gram positive, Gram negative bacteria and two fungal strains by agar well diffusion method. The compounds **5c, 8e, 10e, 11,** and **17a** showed potent inhibition at lowest concentration.

7.4 Antioxidant Activity

Oxidation processes are intrinsic to the energy management of all living organisms and are therefore kept under strict control by several cellular mechanisms[9]. Free radicals are molecules, ions or atoms with unpaired electrons in their outermost shell of electrons[10]. These species, which are constantly formed in human body, can become toxic when generated in excess or in the presence of a deficiency in the naturally occurring antioxidant defenses. High levels of free radicals can cause damage to biomolecules such as lipids, proteins, enzymes and DNA in cells and tissues. This may result in many diseases such as: cancer, diabetes, cardiovascular and autoimmune diseases and neurodegenerative disorders, aging, and other diseases through the violent reactivity of the free radicals[11–13].

Antioxidants were important compounds that reduce or neutralize the free radicals, thus protecting the cells from oxidative injury[14]. Therefore, considerable research has been directed towards the identification of new antioxidants to prevent radical-induced damage.

Free-radical-scavenging activity using the DPPH method

The synthesized compounds were evaluated for antioxidant scavenging activity by DPPH assay method. The compounds of different concentrations were dissolved in methanol and were added to each vial of 5mL. To this vials 3 mL of 0.004% DPPH in methanol was added and the mixtures have been incubated in dark condition at room temperature for 30 min. Ascorbic acid was used as the standard. The absorbance reduced while the DPPH is scavenged by way of an antioxidant. DPPH scavenging activity was calculated by the use of the following equation and absorbance measured at 517 nm.

$$\textbf{Scavenging ratio (\%)} = \frac{(\mathbf{Ai}-\mathbf{Ao})}{(\mathbf{Ac}-\mathbf{Ao})} \times 100\%$$

Where

Ai is the absorbance within the presence of the check compound.

A_o is absorbance of the clean inside the absence of the check compound.

Ac is the absorbance within the absence of the test compound.

Table 7.21: Scavenging activity of the benzoxazole derivatives 5(a-g)

Concentration in *µg/ml*	5a	5b	5c	5d	5e	5f	5g
0	-	-	-	-	-	-	-
5	22±0.01	15±0.01	19±0.05	16±0.04	20±0.01	21±0.21	16±0.04
10	25±0.05	16±0.08	20±0.21	19±0.02	24±0.04	24±0.24	18±0.02
15	30±0.07	18±0.04	24±0.21	21±0.04	27±0.03	28±0.20	22±0.04
20	32±0.06	21±0.07	28±0.22	22±0.07	32±0.07	31±0.09	24±0.07
25	36±0.01	22±0.01	29±0.07	26±0.21	33±0.04	35±0.01	26±0.21
Ascarbic acid	40±0.08	24±0.02	34±0.04	30±0.25	36±0.08	38±0.21	38±0.25

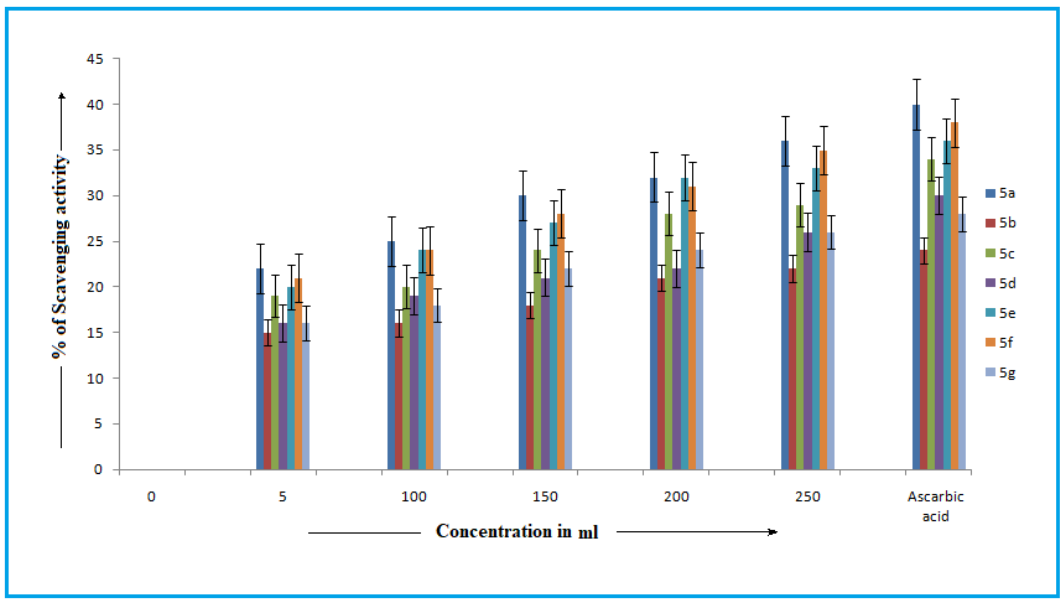


Fig. 21

Table 7.22: Scavenging activity of Benzoxazole derivatives 8 (a-f)

Concentration in *µg/ml*	8a	8b	8c	8d	8e	8f
0	-	-	-	-	-	-
5	20±0.01	22±0.21	21±0.05	14±0.04	18±0.01	16±0.01
10	23±0.05	26±0.24	22±0.21	16±0.02	22±0.04	18±0.08
15	28±0.07	30±0.20	26±0.21	20±0.04	24±0.03	20±0.04
20	31±0.06	33±0.09	32±0.22	24±0.07	28±0.07	23±0.07
25	34±0.01	36±0.01	34±0.07	26±0.21	32±0.04	26±0.01
Ascarbic acid	38±0.08	40±0.21	39±0.04	30±0.25	34±0.08	28±0.02

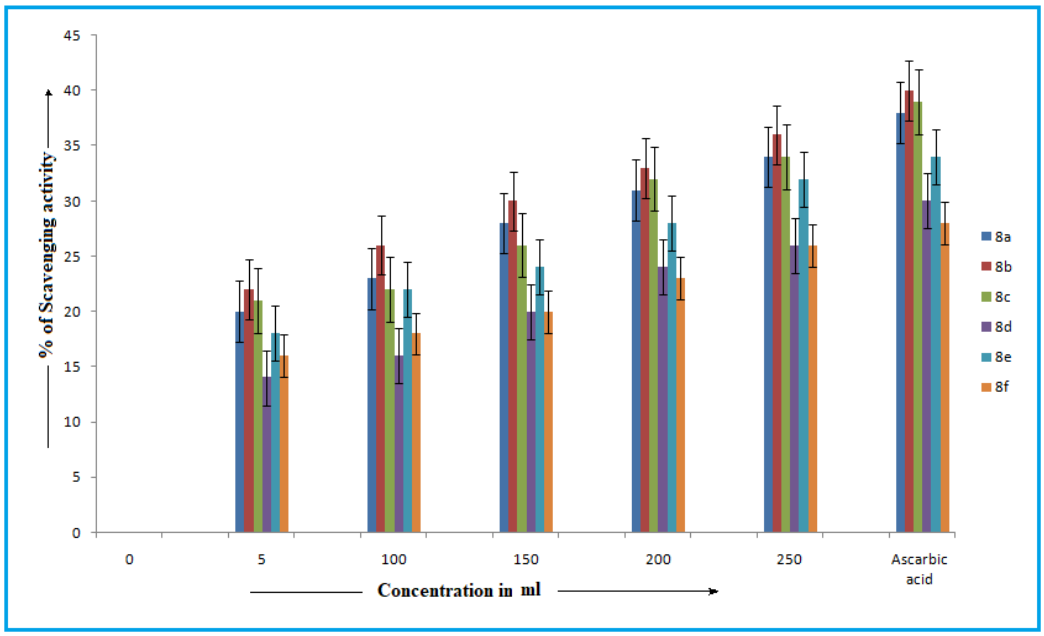


Fig. 22

Table 7.23: Scavenging activity of Benzoxazole derivatives 10 (a-g)

Concentration in *µg/ml*	10a	10b	10c	10d	10e	10f	10g
0	-	-	-	-	-	-	-
5	23±0.01	22±0.21	18±0.05	16±0.04	19±0.01	14±0.01	18±0.05
10	26±0.05	26±0.24	21±0.21	20±0.02	24±0.04	16±0.08	21±0.21
15	31±0.07	29±0.20	25±0.21	22±0.04	27±0.03	19±0.04	25±0.21
20	33±0.06	32±0.09	27±0.22	23±0.07	30±0.07	22±0.07	27±0.22
25	37±0.01	34±0.01	28±0.07	25±0.21	32±0.04	24±0.01	28±0.07
Ascarbic acid	41±0.08	37±0.21	35±0.04	31±0.25	35±0.08	26±0.02	35±0.04

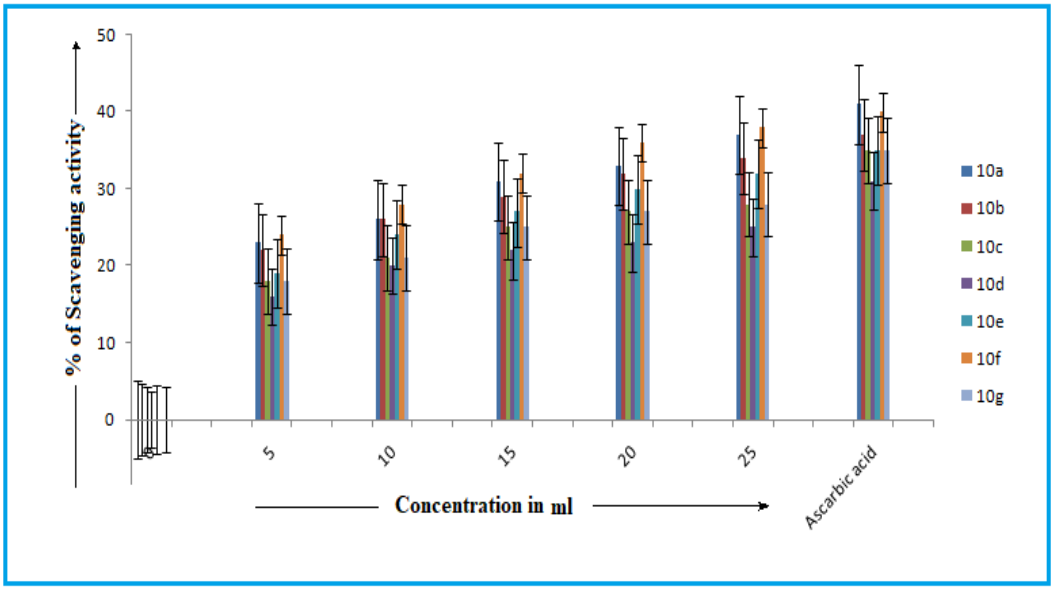


Fig. 23

Table 7.24: Scavenging activity of Benzoxazole derivatives 11-16

Concentration in *µg/ml*	11	12	13	14	15	16
0	-	-	-	-	-	-
5	32±0.01	30±0.21	17±0.05	15±0.04	18±0.01	13±0.01
10	36±0.05	34±0.24	20±0.21	21±0.02	23±0.04	16±0.08
15	38±0.07	30±0.20	24±0.21	23±0.04	25±0.03	18±0.04
20	42±0.06	40±0.09	26±0.22	24±0.07	28±0.07	23±0.07
25	45±0.01	44±0.01	29±0.07	26±0.21	30±0.04	25±0.01
Ascarbic acid	47±0.08	48±0.21	35±0.04	30±0.25	35±0.08	26±0.02

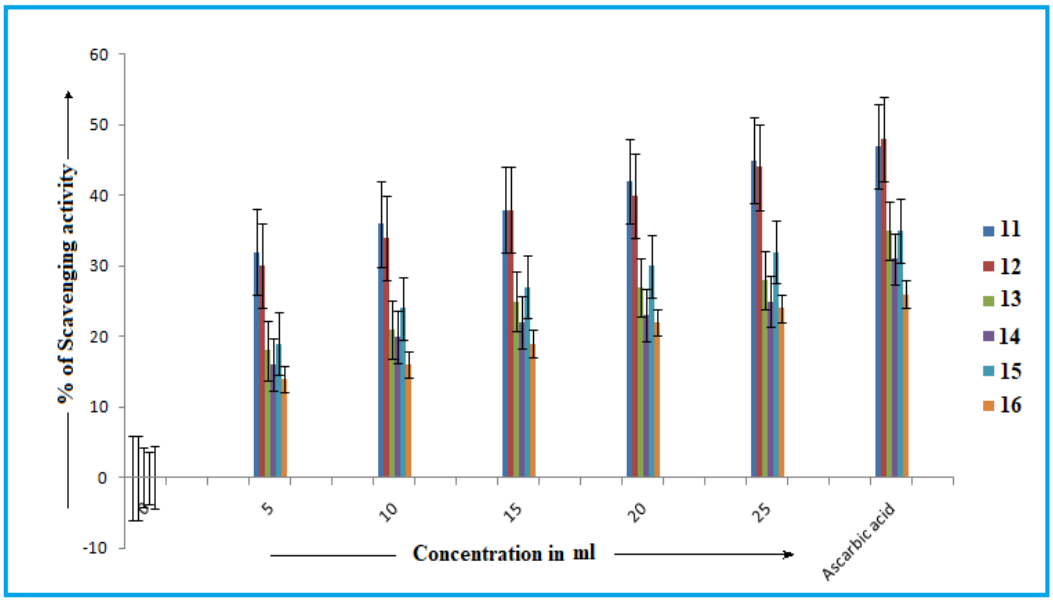


Fig. 24

Table 7.25: Scavenging activity of Benzoxazole derivatives 17 (a-f)

Concentration in *µg/ml*	17a	17b	17c	17d	17e	17f
0	-	-	-	-	-	-
5	30±0.01	20±0.21	18±0.05	16±0.04	12±0.01	28±0.01
10	34±0.05	24±0.24	21±0.21	20±0.02	16±0.08	32±0.04
15	38±0.07	29±0.20	25±0.21	24±0.04	19±0.04	35±0.03
20	42±0.06	34±0.09	28±0.22	26±0.07	23±0.07	41±0.07
25	44±0.01	36±0.01	30±0.07	28±0.21	25±0.01	46±0.04
Ascarbic acid	48±0.08	37±0.21	35±0.04	30±0.25	26±0.02	48±0.08

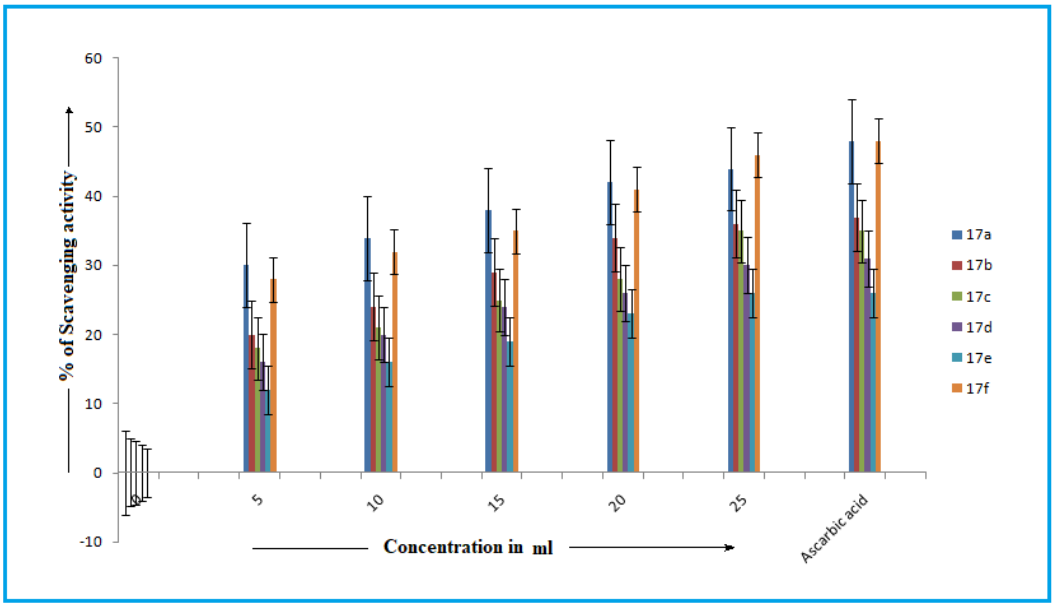


Fig. 25

7.4.1 Result and discussion

The synthesized compounds **5(a-g)**, **8(a-f), 10(a-g), 11-16** and **17(a-f)** were screened for antioxidant activity at different concentration in methanol and ascorbic acid was used as a standard

The compound **5c, 5f, 8a, 8b, 10c, 10f, 11, 12, 17a, and 17f** were found to be more potent free radical scavenging activity as compared with other synthesized compounds.

7.5 Cytotoxic Activity

Cancer is one of the major diseases in the world causing mortality around the world. Cancer is the uncontrolled proliferation of the cells in any part of the body causes bulge of organ or tumor of the cells. The habitually affecting parts of the body are mainly lungs, liver, cervical, breast, stomach, oral[15]. Lung cancer and skin cancer are two main cancers affecting the humans on their habitual conditions. Lung cancer is mainly because of smoking, exposure to toxins[16,17], Skin cancer is due to carcinogens, smoking, chronic and subchronic wounds, use of immunosuppressive drugs[18,19]. Now a days, chemotherapy is usually employed for the treatment of cancer includes alkylating agents, antimetabolites, antitumor antibiotics, platinum analogs. So, there is a necessary to identify the new molecules for the treatment of cancer with low economy, high potency curing, lesser side effects. In this point of view, we have aimed our present study to evaluate the *in vitro* cytotoxicity activity of benzoxazole derivatives onA549 lung cancer cell lines. The values are tabulated in the table **7.26-7.28**

Methods and materials

The cells were seeded at a density of approximately 5×103cells/well in a 96-well flat-bottom micro plate and maintained at 37^0C in 95% humidity and 5% CO_2 for overnight. Different concentration (500, 250, 125, 62.5, 31.250, 15.125µg/mL) of samples was treated. The cells were incubated for another 48h.The cells in well were washed twice with phosphate buffer solution and 20 µL of the MTT staining solution (5mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 37^0C. After 4h, 100 µL of di- methyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals and absorbance was recorded with a 570 nm using micro plate reader (1, 2).

Formula :

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of Negative control}} \times 100$$

Using graph Pad Prism Version5.1, we calculate the IC 50 of compounds

Note – DMSO Concentration is less 1.5% in experiments

Concentrations are in duplicates

Table 7.26: Cytotoxic Activity Data of Synthesized Compounds

IC 50 value of compounds in µg/ml

Compound	A549
10a	311
10b	224.3
10c	222.8
10d	105.5
10e	361.7
10f	431.2
10g	356.2
Paclitaxel(µg)	273.25
Paclitaxel(µM)	0.32

Conc µl/mL	10a	10b	10c	10d	10e	10f	10g
500	26.41	55.26	60	35.66	43.87	48.89	24.12
250	36.24	63.65	64.23	44.21	57.63	60.01	38.22
125	58.95	72.82	68.23	68.48	71.01	70.51	60.35
62.5	66.54	78.24	76.23	75.41	78.65	82.36	72.14
31.25	68.23	80.23	90.23	84.65	82.14	84.12	79.23
15.625	70.25	82.36	99.25	86.35	84.65	94.11	84.23
C	100						

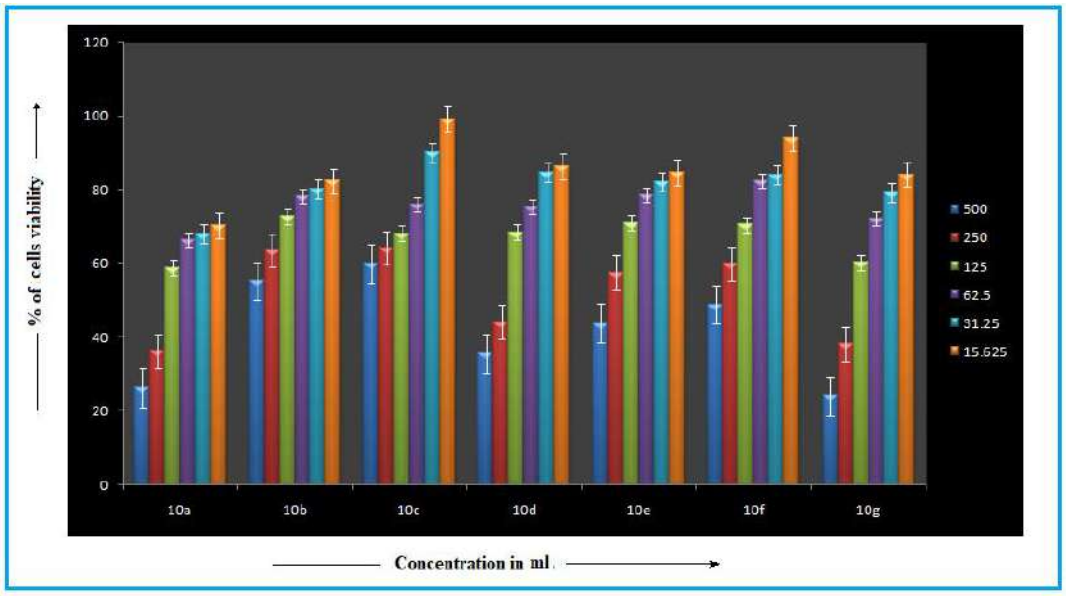


Fig. 26

Table 7.27: Cytotoxic Activity Data of Synthesized Compounds

IC 50 value of compounds in µg/ml

Compound	A549
11	253
12	232.3
13	210.8
14	102.5
15	267.7
16	324.2
Paclitaxel(µg)	236.25
Paclitaxel(µM)	0.33

Conc µl/mL	11	12	13	14	15	16
500.000	42.71	32.88	24.41	62.57	42.87	46.89
250.000	53.33	44.16	42.42	71.06	52.63	62.01
125.000	68.85	64.65	74.13	83.83	72.01	71.51
62.500	83.20	74.87	80.62	90.26	80.75	80.36
31.250	86.57	85.68	91.66	92.00	85.56	91.78
15.625	96.52	95.12	96.40	96.06	93.14	95.48
C	100					

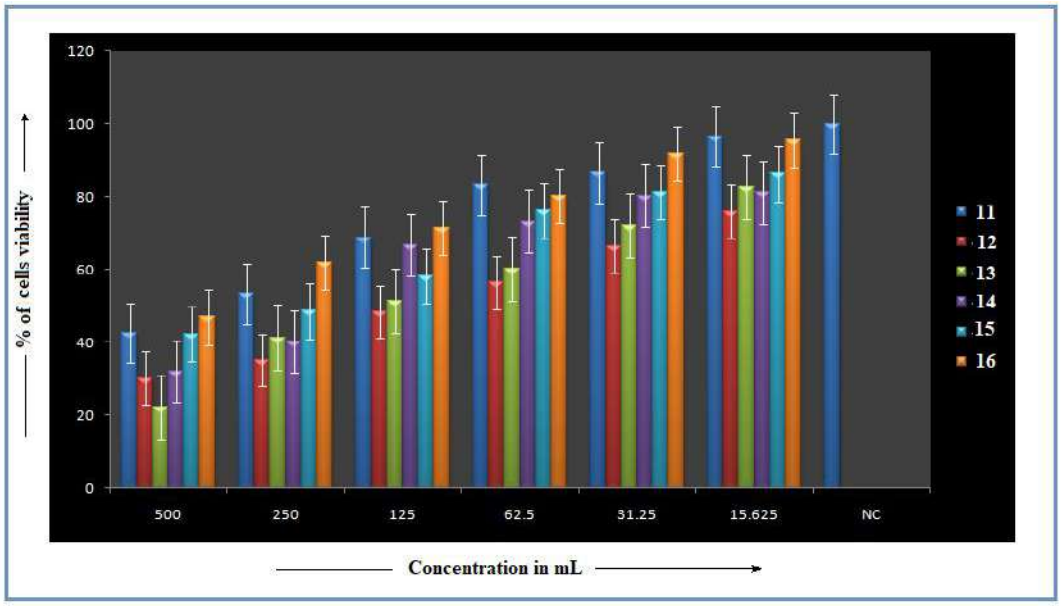


Fig. 27

Table 7.28: Cytotoxic Activity Data of Synthesized Compounds

IC 50 value of compounds in µg/ml

Compound	A549
17a	212
17b	216.3
17c	192.8
17d	113.5
17e	237.7
17f	303.2
Paclitaxel(µg)	224.25
Paclitaxel(µM)	0.36

Conc µl/mL	17a	17b	17c	17d	17e	17f
500	48.28	28.15	28.83	32.11	52.26	30.12
250	56.23	33.19	44.92	42.18	60.49	42.11
125	72.15	46.59	55.19	63.19	72.16	54.92
62.5	84.29	54.27	60.33	76.28	78.23	66.92
31.25	88.11	69.14	74.59	81.49	84.35	78.15
15.625	97.45	78.36	80.62	83.16	95.65	80.15
C	100					

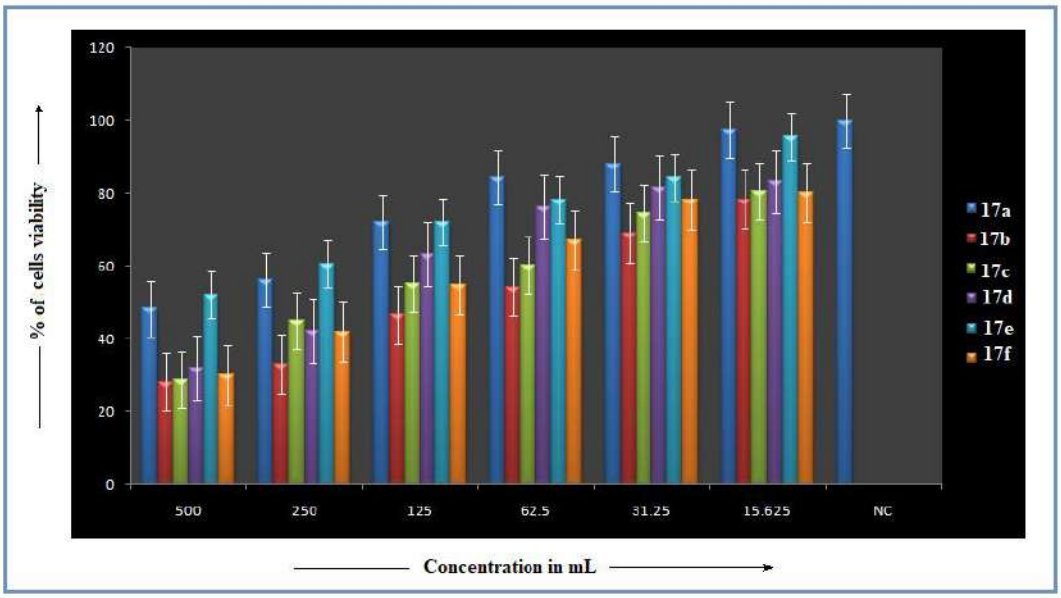


Fig. 28

7.5.1 Result and discussion

The cytotoxicity of the synthesized benzoxazole derivativeswas determined by using A549 (Lung Cancer) cell lines. Only few **10c** and **10f** in **Scheme IV**, **11** and **16** showed potent cytotoxic activity in **scheme V** and in **Scheme VI** the compounds **17a** and **17f** (**table 7.26-7.28 and fig 26-28**) displayed considerable cytotoxic activity**.**

7.6 Anti tuberculosis activity

In the past few decades, the dramatically increasing prevalence of multidrug-resistant microbial infections have caused a serious healthcare problem.Tuberculosis (TB) is a disease of antiquity caused by infection with members of the *Mycobacterium tuberculosis* complex, which includes *M. tuberculosis* (Mtb), *M. africanum, M. bovis, M. caprae, M. microti, M. pinnipedii*and *M. canetti* but the Mtb organism is the main pathogen infecting more than one-third ofthe world's human population[20]. It is an airborne diseaseaffecting millions of people each year being the second leadingcause of death worldwide, after the human immunodeficiencyvirus (HIV). In 1993, World Health Organization (WHO) declared TB 'a global health emergency'[21]. Several benzoxazole derivatives were reported to possess antitubercular activity. In view of these facts in the present work we have synthesized new benzoxazole derivatives and screened for Anti- tubercular activity. The anti-tubercular activity values were given in fig **29-31**

Methods and materials

The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA). This methodology wasnon-toxic, thermally stable reagent and show good correlation with propotional and BACTEC radiometric method. *Mycobacteria tuberculosis* (Vaccine strain, H37

RV strain): ATCC No- 27294 has used as Standard Strain. Standard drugs for the Anti-Tb test were Pyrazinamide, Ciprofloxacin and Streptomycin.

200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

Graphical representation of Anti-tubercular activity of synthesized compounds 10(a-g)

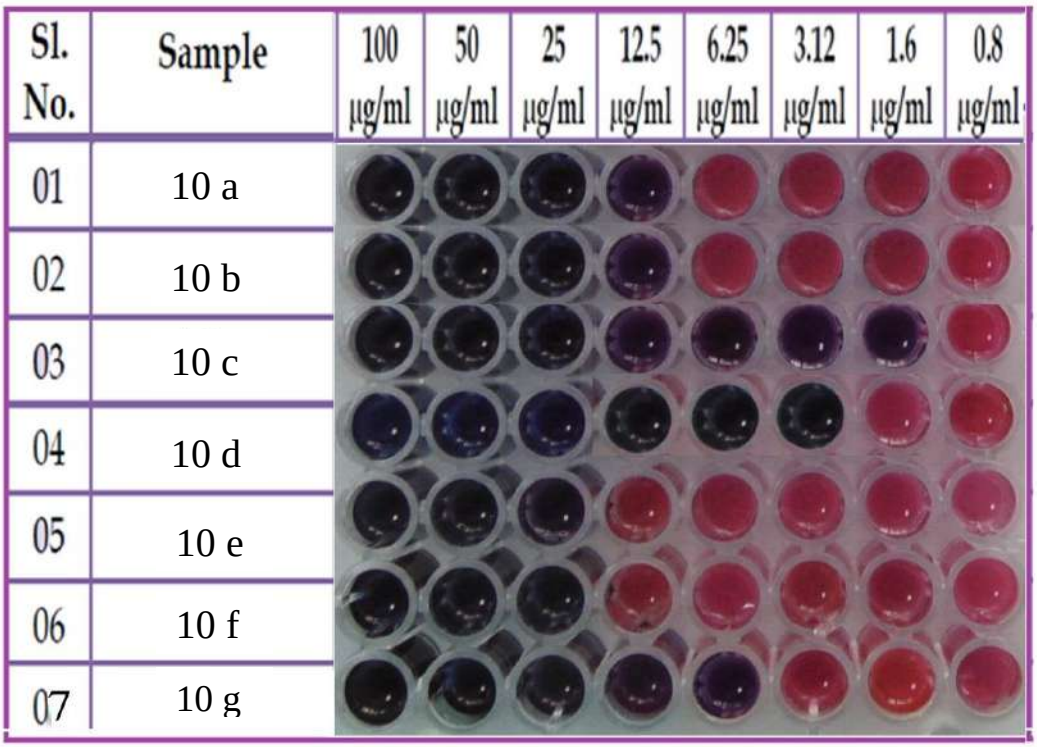


Fig. 29

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	10 a	S	S	S	S	R	R	R	R
02	10 b	S	S	S	S	R	R	R	R
03	10 c	S	S	S	S	S	S	S	R
04	10 d	S	S	S	S	S	S	R	R
05	10 e	S	S	S	R	R	R	R	R
06	10 f	S	S	S	R	R	R	R	R
07	10 g	S	S	S	S	S	R	R	R

Note: S- Sensitive, R-Resistant

Graphical representation of Anti-tubercular activity of synthesized compounds 11-16

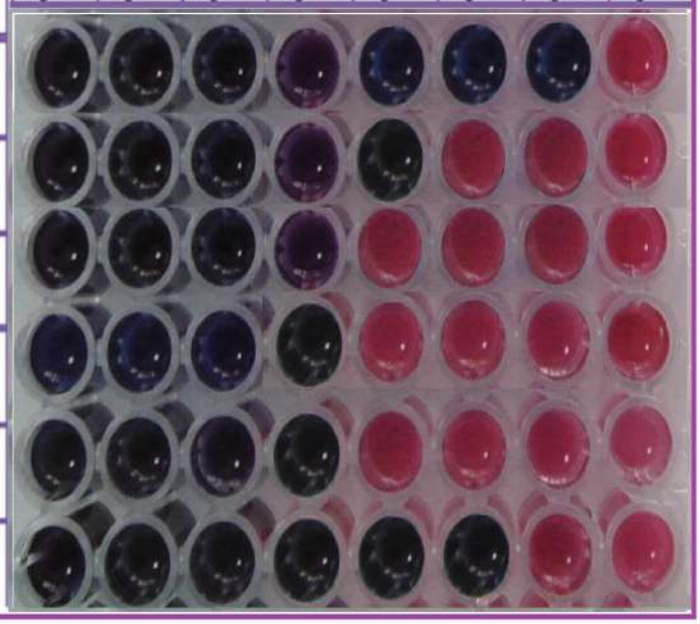
Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	11								
02	12								
03	13								
04	14								
05	15								
06	16								

Fig. 30

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	11	S	S	S	S	S	S	S	R
02	12	S	S	S	R	R	R	R	R
03	13	S	S	S	R	R	R	R	R
04	14	S	S	S	R	R	R	R	R
05	15	S	S	S	R	R	R	R	R
06	16	S	S	S	S	S	R	R	R

Note: S- Sensitive, R-Resistant

Graphical representation of Anti-tubercular activity of synthesized compounds 17 (a-f)

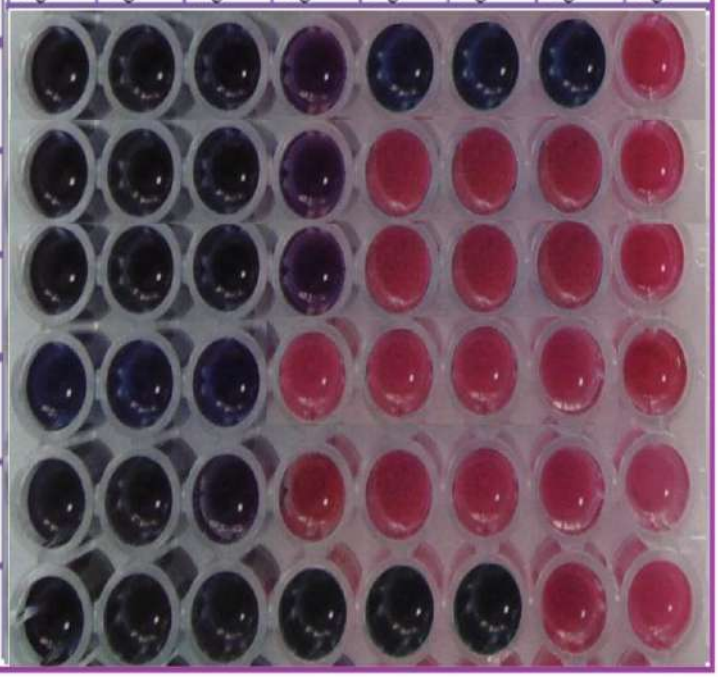
Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	17a								
02	17b								
03	17c								
04	17d								
05	17e								
06	17f								

Fig. 31

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	17a	S	S	S	S	S	S	S	R
02	17b	S	S	S	S	R	R	R	R
03	17c	S	S	S	S	R	R	R	R
04	17d	S	S	S	R	R	R	R	R
05	17e	S	S	S	R	R	R	R	R
06	17f	S	S	S	S	S	S	R	R

Note: S- Sensitive, R-Resistant

7.6.1 Results and Discussion

The Anti-tubercular activity of compounds was tested against *Mycobacteria tuberculosis* (Vaccine strain, H37 RV strain). The molecules in **Scheme IV** the compounds 10c and 10d **exhibited potent Anti-**tubercularactivity.**11** and **16** in **Scheme V** where as in **Scheme VI** the compounds **17a** and **17f** exhibited significant Anti-tubercular activity when compared with (Pyrazinamide, Ciprofloxacin and Streptomycin.) Standard drugs. The results were tabulated in table as well in **figure.29-31.**

7.7 DNA Cleavage Studies

In the pharmacological drug research the deoxyribonucleic acid (DNA) has become intracellular target sites for wide range of anti-cancer agents[22]. The interaction between drug molecule and DNA has become the active research now a day. As they facilitate molecular interaction studies that can lead to the development of novel therapeutic agents with different mechanisms and action models. The recent

advances in chemotherapy for cancer, high toxicity and lower specificity of current drugs motivates the researchers to develop safe and more promising anti-cancer agents.[23] by observing these facts the newly synthesized benzoxazole derivatives were screened for DNA cleavage Studies. The methodology and the test results are given below.

Methods and materials

The synthesized molecules were screened for DNA cleavage Studies against Calf-thymus DNA (50 μg/test). It was carried out by Agarose gel electrophoresis method. The test compounds were introduced accordingly to the DNA samples and these samples were incubated for 2h at 37ºC. Weigh 200mg of agarose and dissolve it in 25 ml of TAE buffer solution (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 ltr) by boiling. When the gel attains ~55ºC, pour it into the gel cassette fitted with comb. After the gel solidifies carefully remove the comb, place the gel in the electrophoresis chamber flooded with TAE buffer. Add DNA sample (mixed with bromophenol blue dye (1X) @ 1:1 ratio), into the wells, along with standard DNA marker and pass the constant electricity of 50 V. for around 45 min Remove the gel and carefully stain with ETBR solution (10 μg/ml) for 10-15 min and observe the bands under UV transilluminator.

The test results of DNA cleavage studies are given below in graphical representation in the **Fig. 32-34**

DNA Cleavage Studies of synthesized benzoxazole derivatives 10 (a-g)

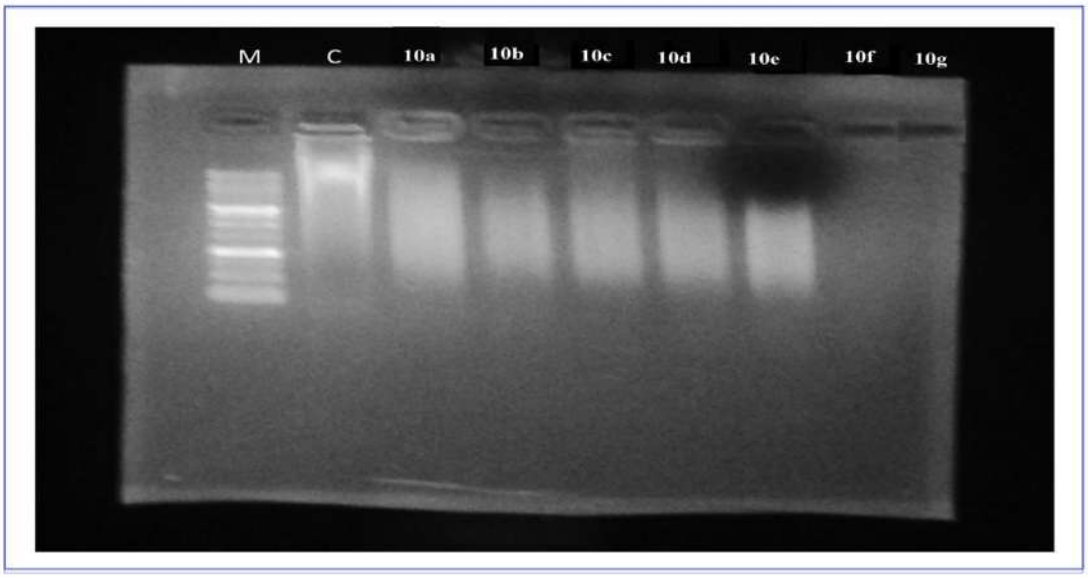


Fig. 32

DNA Cleavage Studies of synthesized benzoxazole derivatives 11-16

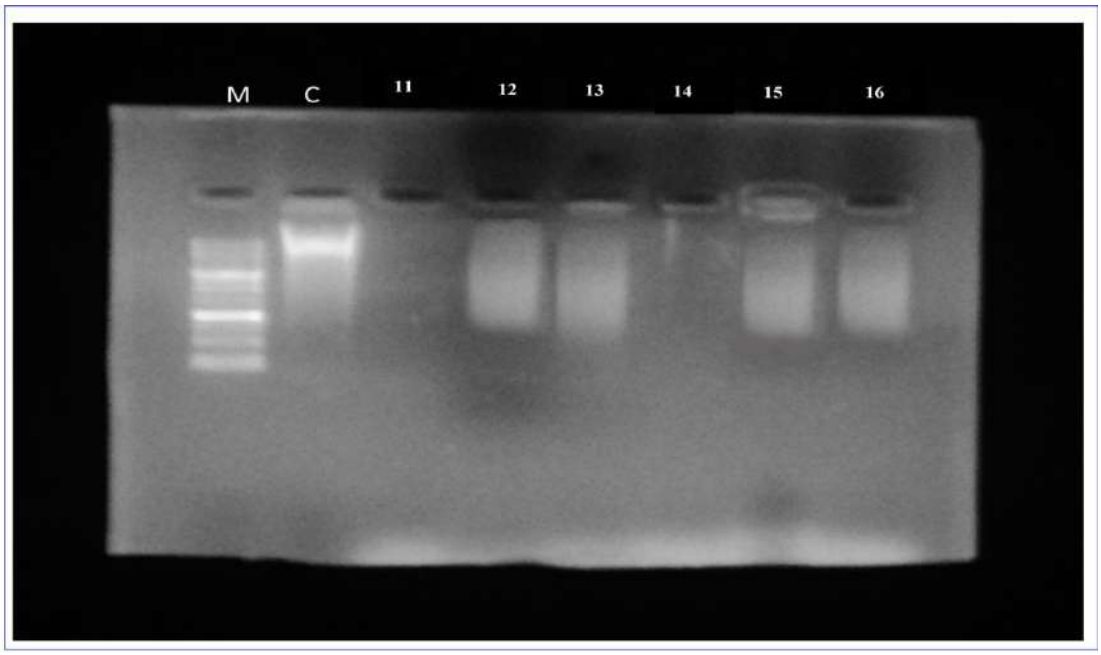


Fig. 33

DNA Cleavage Studies of synthesized benzoxazole derivatives 17 (a-f)

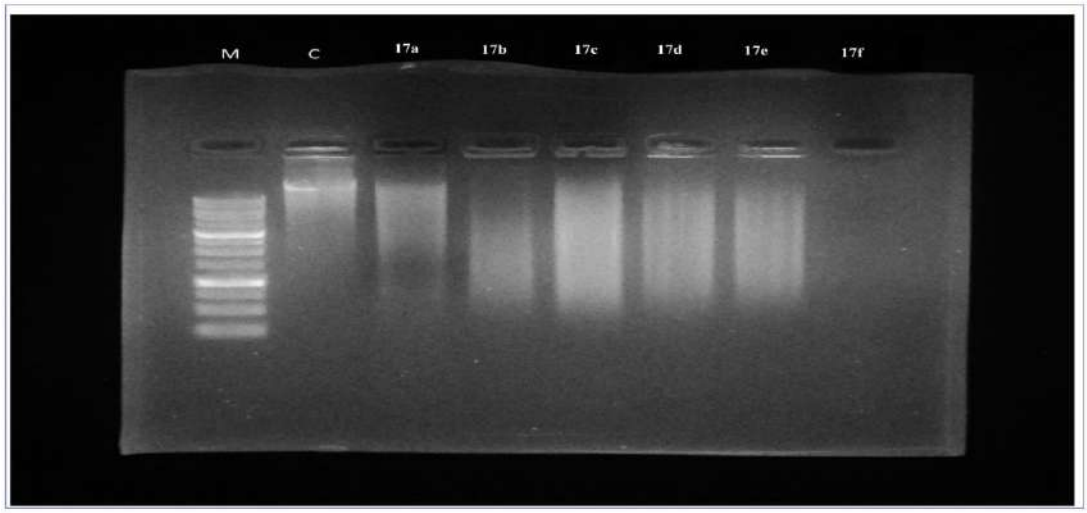


Fig. 34

7.7.1 Results and discussion

The DNA cleavage Studies of synthesized various benzoxazole derivatives were evaluated against Calf-thymus DNA. Only few compounds **10d** and **10f** in **Scheme 4**, **11** and **14** showed potent DNA cleavage activity in **scheme 6 fig 32-34** and the compounds **17a** and **17f** displayed significant cleavage activity in **scheme 6.**

7.8 Molecular Docking Studies

This chapter is focusing on the molecular docking studies of the synthesized benzoxazole derivatives with different enzyme target. Docking studies were performed on commercial software using HEX 8.0 and in supporting with discovery studio visualizer (DSV). Structures of different protein crystal structure of appropriate protein crystal structure were taken from the Protein Data Bank**.**

Background

The pharmaceutical research based industries has increasingly employed modern medicinal chemistry methods, which includes molecular modeling, as powerful tools for the investigation of structure-activity relationships (SAR)[24]. In

addition to pharmacodynamics properties (e.g., affinity, efficacy, potency, selectivity) and pharmacokinetic data (absorption, distribution, metabolism, and excretion) also studied through the application of these protocols[25]. The field has progressed with advances in biomolecular spectroscopic methods such as nuclear magnetic resonance (NMR), X-ray crystallography and which have enabled rapid progress in molecular and structural biology. These techniques is been used for the resolution of more than 100,000 3D protein structures, providing significant structural information about key macromolecular drug targets[26]. Efforts in storing, organizing and exploring such data have generated a growing demand for robust and sophisticated computational tools. Based on this perspective, the accurate integration of *in silico* and experimental protocols has given the up-to-date better understanding of the intricate aspects of intermolecular recognition[27]. Within this framework, structure-based drug design (SBDD) methods are a widely used component of modern medicinal chemistry[28]. Molecular docking, structure-based virtual screening (SBVS) and molecular dynamics (MD) are among the most frequently used SBDD strategies due to their broad range of applications in the analysis of molecular recognition aspects such as binding energetics, molecular interactions and induced conformational changes[29]. The integration of these approaches has been successfully used in a various of investigations of structural, chemical and biological data[30,31].

Molecular docking is a most frequently used method in SBDD. Because of its ability to predict, with a substantial degree of accuracy, the conformation of small-molecule ligands within the accurate target binding site[32]. Following the development of the first algorithms in the 1980s, molecular docking has become an essential tool in pharmacological drug discovery[33]. For example, studies of molecular events, including ligand binding modes and ligand-receptor complex that stabilize the corresponding intermolecular interactions can be performed[34].

Procedure

Molecular modeling studies were performed using HEX 8.0 and in supporting with discovery studio visualizer (DSV). Synthesis of biologically active compounds, which exhibited promising antioxidant activity *in vitro* to find the preferred binding conformations in the receptor. Three dimensional structures of the ligand and metal complexes were outlined by Chem draw ultra-software. We selected protein receptor SEC2 (PDB code: 1STE) in *Staphylococcus aureus*, and human pancreatic ribonuclease with high cytotoxic and antitumor activities (PDB: 3F8G). The docking study was carried out by the protein loaded in HEX 8.0 and the errors of the protein were corrected by the structure preparation processes in DSV. The selected ligands were docked against the lead competitive inhibitor ligand DTT at the crystal enzyme structure of the target protein and the best energy conformations of receptor ligand were studied, and the energy of binding was calculated as the difference between the energy of the complex and the individual energies of enzyme and ligand.

Molecular docking studies of synthesized molecules in **chapter II (5a, 5c, 5d, 5g)** , **chapter III (8a, 8b, 8c) Chapter IV (10c, 10d, 10g) chapter V (13, 14, 15,)** and **Chapter IV (17a, 17d)** were evaluated. In the present study, an attempt was made to evaluate their antimicrobial and cytotoxic properties, so we selected protein receptor SEC2 (PDB code: 1STE) in *Staphylococcus aureus*, and human pancreatic ribonuclease with high cytotoxic and antitumor activities (PDB:3F8G) which is involved in causing cytotoxic and antimicrobial infections.

The prepared molecules interacts with human pancreatic ribonuclease with high cytotoxic and antitumor activities (PDB:3F8G) and protein receptor SEC2 (PDB code: 1STE) in *Staphylococcus aureus* exhibit docking score with comparable binding interaction energy. The standard receptor was compared with

the lowest docking score. Selective compounds have been docked and compared with the standard receptor drug (E-total value) and observed the significantly good inhibiting potency towards the receptor of the novel compounds.

Ligands of compounds **(5a, 5c, 5d, 5g)** in **Table-7.29** and **(8a, 8b, 8c)** in **Table-7.30,** which are present in the active site protein receptor SEC2 (PDB code: 1STE) in Staphylococcus aureus. Thereby potentially inhibiting the antimicrobial of the receptor **(Fig. 35-41)** respectively. The obtained results provide a sufficient explanation and better compromise between docking scores and *in vitro* activity of cytotoxic study.

Table-7.29 Docking result of synthesized compounds in the binding site of antimicrobial protein receptor SEC2

Compounds	**Binding energy (kcal/mol)**	**Docking receptor**
5a	-319.08	SEC2 Staphylococcus aureus
5g	-298.25	
5c	-280.11	
5d	-260.11	

Two and three dimensional interactions of synthesized molecules with the active sites of antimicrobial protein receptor SEC2 in *Staphylococcus aureus* and cytotoxic activities (PDB: 3F8G) are displayed in picture in the following pages.

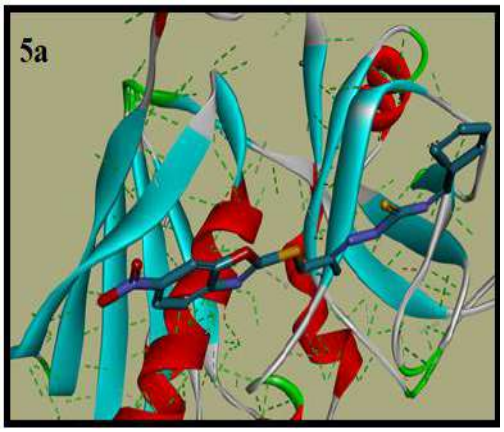


Fig. 35

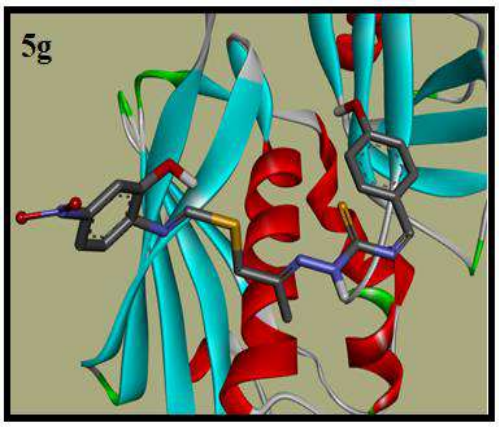


Fig. 36

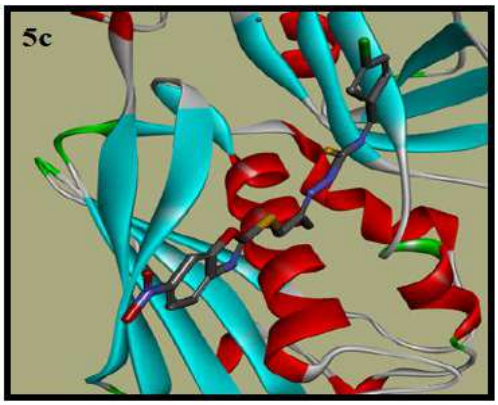


Fig. 37

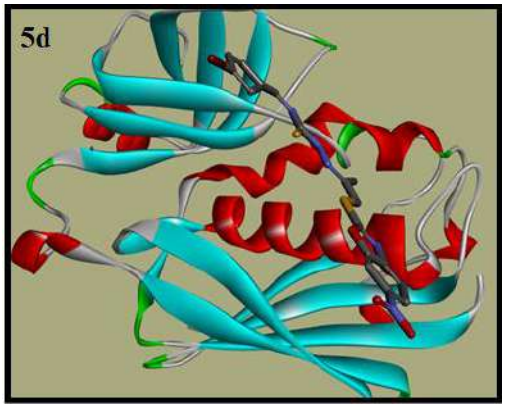


Fig.38

Table-7.30 Docking result of synthesized compounds in the binding site of receptor

Compounds	Binding energy (kcal/mol)	Docking receptor
8a	-227.14	SEC2 Staphylococcus aureus
8b	-230.11	
8c	-234.44	

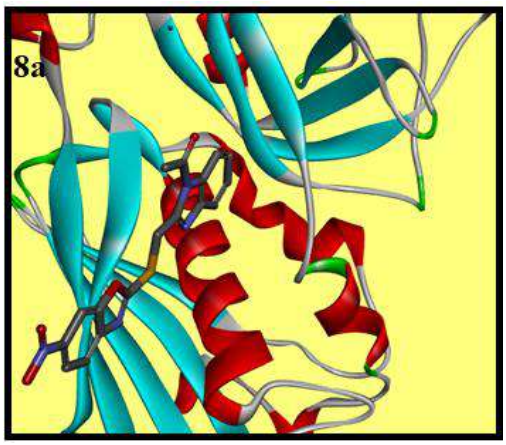


Fig.39

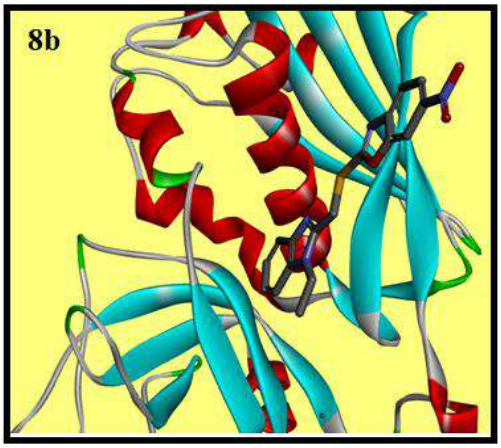


Fig.40

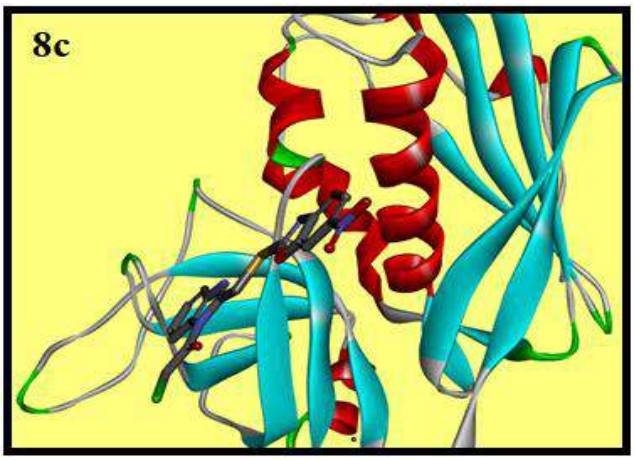


Fig.41

Table-7.31 Docking result of synthesized compounds in the binding site of receptor

Compounds	Binding energy (kcal/mol)	Docking receptor
10c	-254.33	3F8G Human pancreatic ribonuclease
10d	-266.54	
10g	-289.22	

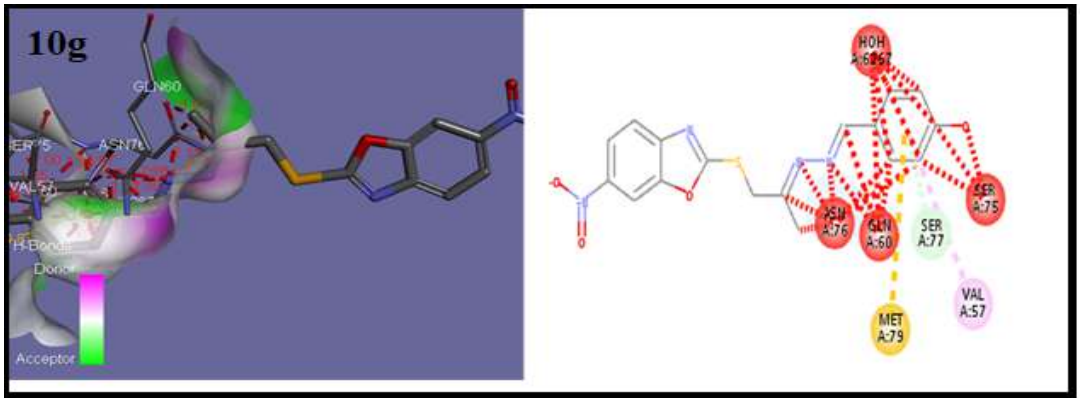


Fig. 42

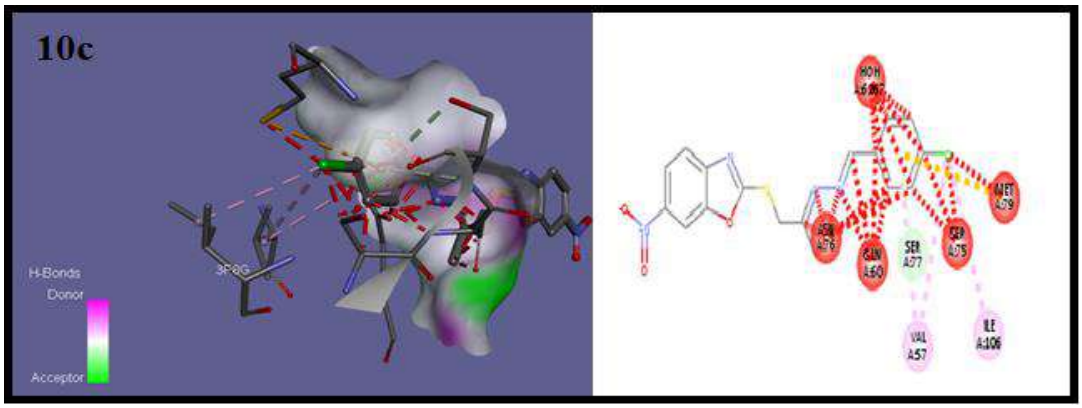


Fig. 43

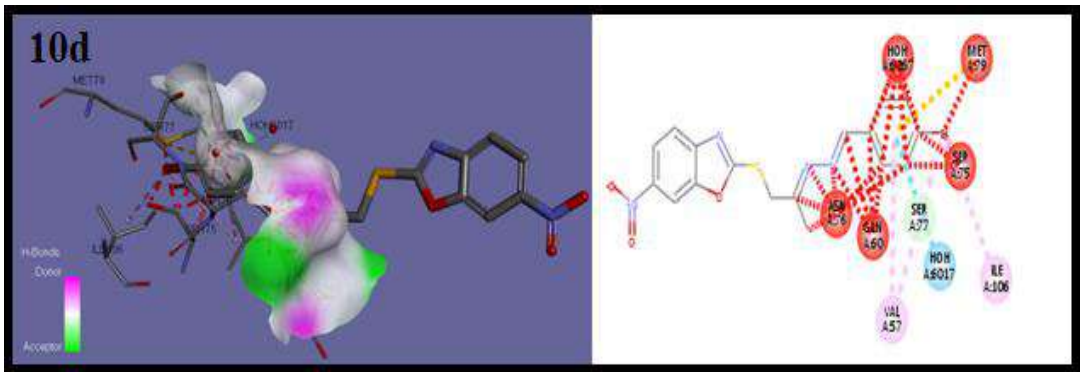


Fig. 44

Table-7.32 Docking result of synthesized compounds in the binding site of receptor

Compounds	Binding energy (kcal/mol)	Docking receptor
13	-360.71	3F8G Human pancreatic ribonuclease
14	-352.11	
15	-347.87	

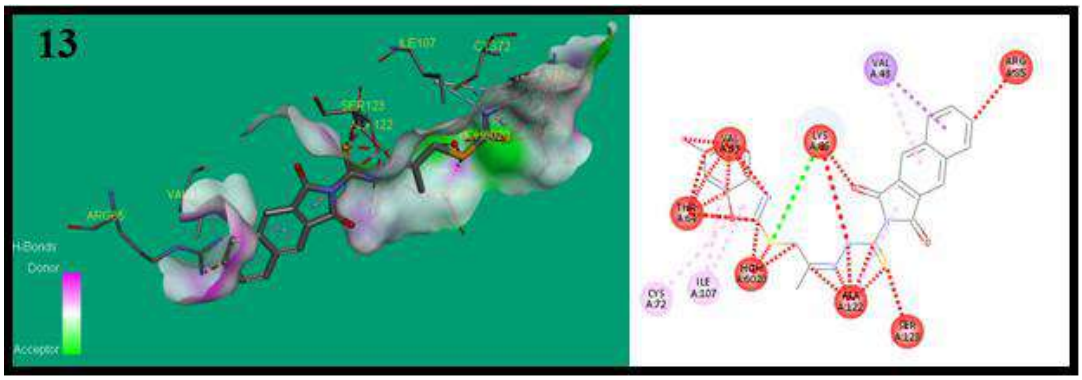


Fig. 45

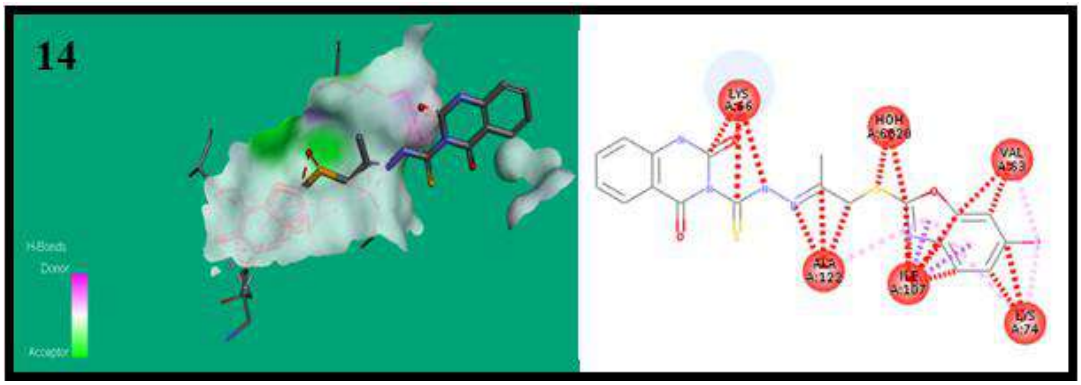


Fig. 46

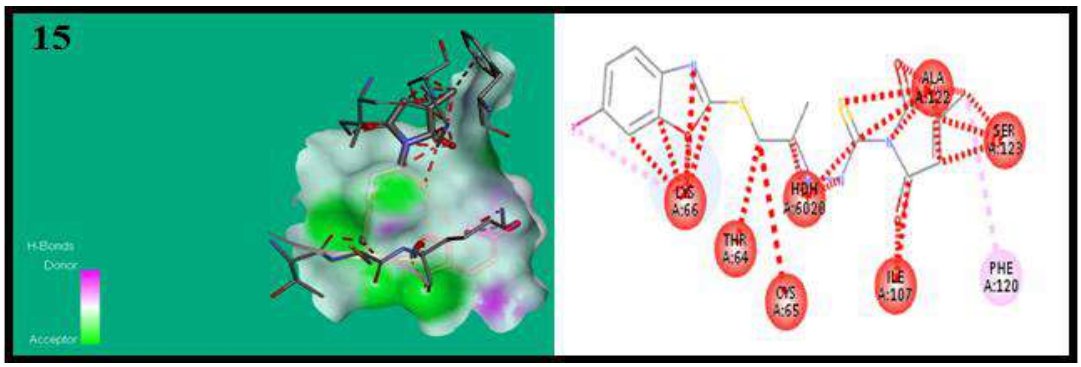


Fig. 47

Table-7.33 Docking result of synthesized compounds in the binding site of receptor

Compounds	Binding energy (kcal/mol)	Docking receptor
17b	-344.63	3F8G Human pancreatic ribonuclease
17d	-349.52	

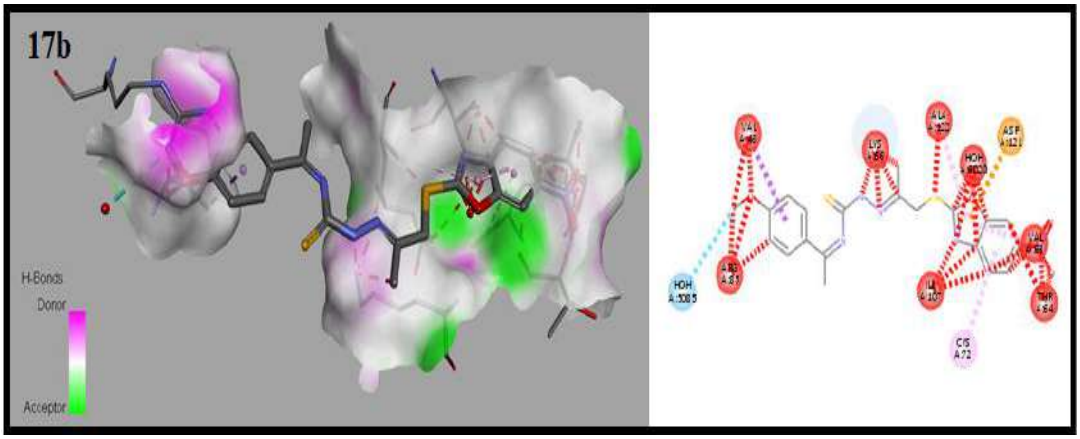


Fig. 48

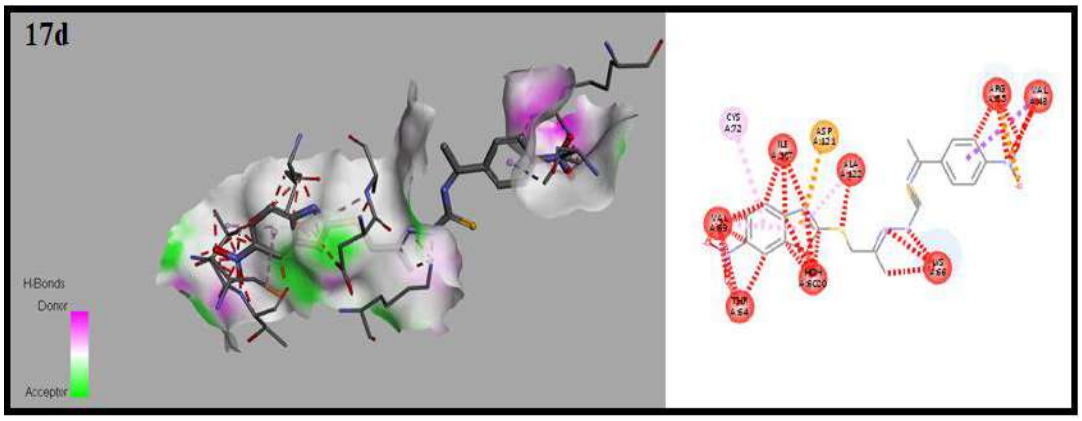


Fig. 49

7.8.1 Result and discussion

All the synthesized molecules are screened for *in silico* molecular docking studies, since docking scores of the compounds were very close to each other. The synthesized molecules **chapter II** (**5a, 5c, 5d, 5g)**, **chapter III (8a, 8b, 8c) Chapter IV (10c, 10d, 10g) chapter V (13, 14, 15)** and **Chapter IV (17a, 17d)** were evaluated. In the present study, an attempt was made to evaluate their antimicrobial and cytotoxic properties, so we selected protein receptor SEC2 (PDB code: 1STE) in *Staphylococcus aureus*, and human pancreatic ribonuclease with high cytotoxic and antitumor activities (PDB: 3F8G) which is involved in causing cytotoxic and antimicrobial infections. The compounds **5a**, **5g, 8b and 8c** showed high docking score against the antimicrobial protein receptor, it gives a molecular the binding energy (E-total value) at **-230.11 to -319.08** Kcal/mol. The results are depicted in the table and graphical representation **(table-7.29-7.30) (Fig.35-41)**.

The compounds **10d, 10g, 13, 14,** and **17a, 17d** showed higher docking score and least binding interaction with receptor with various amino acids to the receptor (PDB: 3F8G). the active pocket sites and given a molecular interaction energy (E-total value) in the range of **-266.54, -289.22, -360.71,-352.11,** and **-344.63,-349.52,** respectively **(table-7.21-7.33) (Fig.42-49)**.

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CONCLUSION

Literature survey reveals that benzoxazole derivatives played a vital role in the medicinal field in view of this, the present research work focused on synthesis and biological and pharmacological investigation. We subjected synthesized compounds for various biological and pharmacological activities. New routes were adopted for the synthesis of benzoxazole derivatives and the target molecules were screened for selected biological activates.

Few nitro substituted benzoxazole derivatives containing thiosemicarbazone, hydrazones, phthaimide molecules. The structures of the synthesized molecules were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. Most of the synthesized molecules showed considerable biological and pharmacological activities when compared with the standard drugs.

8.1 Antibacterial

The antibacterial efficacy of the compounds were tested against Gram positive and Gram negative bacteria namely *Staphylococcus aureus, Staphylococcus epidemidis, Bacillus subtilis, P.aeruginosa, Vibrio cholerae and E. coli* by agar well diffusion method[18]. The synthesized molecules (**5c)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-one *N*-[(4-chlorophenyl) methylidene]thiosemicarbazone, **(5d)-** (1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl] propan-2-oneN- [(4-bromophenyl) methylidene] thiosemicarbazone, **(8c)-** 2(-{[1-(chloroacetyl)- 1*H*-benzimidazol-2-yl] methyl} sulfanyl) - 6-nitro -1,3-benzoxazole, **(8e)-** 1- (2- { [(6-nitro- 1,3- benzoxazol- 2-yl) sulfanyl] methyl } -1*H*-benzimidazol-1-yl) propan-2-one**, (10c)-** 2-{[2-{ [(4-chlorophenyl) methylidene] hydrazono} propyl] sulfanyl}-6-nitro-1,3-benzoxazole, **(10d)-** 2-{[2-{[(4-bromophenyl) methylidene] hydrazono} propyl] sulfanyl}-6-nitro-1,3-benzoxazole, **(11)-** 4,5,6,7-tetrabromo-N'- {1-methyl-2- [(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] ethylidene}-1,3-dioxo-1,3-dihydro-2H-isoindole-2-carbothiohydrazide, **(16)-** 6 -chloro-N'-{1-methyl-2- [(6-nitro-1,3-benzoxazol-2-yl)sulfanyl] ethylidene}-2,4-dioxo-4,4a -dihydroquinazoline -3(2H)-

carbothiohydrazide, **(17a)-** 1- [(6-nitro-1,3-benzoxazol -2-yl) sulfanyl] propan-2-one N-[1-(4 -chlorophenyl) ethylidene] thiosemicarbazone and **(17c)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-one N-[1-(4-bromophenyl) ethylidene] thiosemicarbazone were showed potent antibacterial activity when compared with standard drug *Tetracycline*. The presence of the groups namely chloro, bromo, nitro, may be responsible for the potent antibacterial activities of the synthesized compounds.

8.2 Antifungal

The antifungal activity of the compounds was tested against two fungal strains *Aspergillus aureus* and *Aspergillus fumigates* by using the sabouraud dextrose agar diffusion method[19] by using Fluconazole as standard. Among all the synthesized compounds (**5d)-** (1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-oneN-[(4-bromophenyl) methylidene] thiosemicarbazone, (**5e)-** 1-[(6-nitro- 1,3-benzoxazol -2-yl)sulfanyl] propan- 2-one *N*-[(4-nitrophenyl) methylidene] thiosemicarbazone, (**8c)-** 2-({[1-(chloroacetyl) -1*H*-benzimidazol -2-yl]methyl} sulfanyl) -6-nitro-1,3-benzoxazole, **(8e)-** 1- (2-{[(6-nitro-1,3- benzoxazol-2-yl) sulfanyl] methyl} -1*H*- benzimidazol-1-yl) propan-2-one, **(10d)-** 2- {[2-{[(4-bromophenyl) methylidene] hydrazono} propyl] sulfanyl} -6-nitro-1,3-benzoxazole, (**10e)-** 6-nitro-2-{[2-{[(4-nitrophenyl) methylidene] hydrazono} propyl] sulfanyl}-1,3-benzoxazole, (**11)-** 4,5,6,7-tetrabromo-N'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene} -1,3-dioxo-1,3-dihydro- 2H-isoindole-2-carbothiohydrazide, (**16)-** 7-chloro-N'-{1-methyl-2- [(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-2,4-dioxo-4,4a- dihydroquinazoline-3(2H)-carbothiohydrazide, (**17a)-** 1 -[(6-nitro -1,3-benzoxazol -2-yl) sulfanyl] propan-2-one N-[1-(4-chlorophenyl) ethylidene] thiosemicarbazone and **(17d)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-one N- [1- (4-nitrophenyl) ethylidene] thiosemicarbazone showed considerable antifungal activities when compared with the standard drug *Fluconazole*. The significant antifungal activities may be due to

the presence of the substituents namely chloro, bromo groups present in the synthesized molecules.

8.3 Minimum inhibition concentration

The MIC of the compounds was tested against Gram positive, Gram negative bacteria and two fungal strains by agar well diffusion method[20]. The compounds (**5d)-** (1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-oneN- [(4-bromophenyl) methylidene]thiosemicarbazone, **(8c)-** 2-({[1-(chloroacetyl) -1*H*-benzimidazol-2-yl] methyl} sulfanyl) -6-nitro-1,3-benzoxazole, **(10e)-** 6-nitro-2-{[2-{[(4-nitrophenyl) methylidene] hydrazono} propyl] sulfanyl} -1,3-benzoxazole, **(11)-** 4,5,6,7-tetrabromo -N'-{1-methyl-2- [(6-nitro- 1,3-benzoxazol-2-yl) sulfanyl] ethylidene} -1,3-dioxo-1,3-dihydro-2H-isoindole-2-carbothiohydrazide and (**17a)-** 1-[(6-nitro-1,3-benzoxazol -2-yl) sulfanyl] propan -2-one N-[1-(4-chlorophenyl) ethylidene] thiosemicarbazone which showed potential MIC values against bacterial and fungal strains when compared with standard drug *Tetracycline* and *Fluconazole* . The maximum activity showed by the above mentioned synthesized compounds may be due to the presence of the groups like methoxy, nitro and chloro.

8.4 Antioxidant

Antioxidant activity of benzoxazole derivatives were evaluated by using DPPH free radical assay[21] by using Ascarbic acid as standard. Few of the compounds (**5c)-** 1-[(6-nitro -1,3-benzoxazol-2-yl) sulfanyl] propan-2-one *N*-[(4-chlorophenyl) methylidene] thiosemicarbazone, **(5f)-** 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[(4-methoxyphenyl)methylidene]thiosemicarbazone, (**8a)-** 2-{[(1-acetyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-

benzoxazole, (**8b)-** 2-{[(1-ethyl-1*H*-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole, (**10c)-** 2-{[2-{[(4-chlorophenyl) methylidene] hydrazono} propyl] sulfanyl}-6-nitro-1,3-benzoxazole, (**10f)-** 2-{2-{[(4-methoxyphenyl)

methylidene] hydrazono} propyl] sulfanyl} -6-nitro -1,3- benzoxazole, (**11)-** 4,5,6,7- tetrabromo- N'-{1-methyl -2-[(6-nitro-1,3 -benzoxazol-2-yl) sulfanyl] ethylidene}-1,3-dioxo-1,3-dihydro-2H-isoindole-2-carbothiohydrazide, (**12)-** N'-{1-methyl-2- [(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] ethylidene}-1,3-dioxo-1,3-dihydro- 2H-isoindole- 2-carbothiohydrazide, (**17a)-** 1-[(6-nitro- 1,3-benzoxazol -2-yl)sulfanyl] propan-2-one N-[1-(4-chlorophenyl)ethylidene] thiosemicarbazone and **(17f)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-one N-[1-(4-nitrophenyl)ethylidene]thiosemicarbazone showed potent antioxidant compounds. The above mentioned few compounds has shown potent antioxidant activity, may be due to the presence of substituents like nitro, chloro groups.

8.5 Cytotoxic activity

The cytotoxicity of the synthesized benzoxazole derivatives was determined by using A549 (Lung Cancer) cell lines. Only few compounds (**10c)-** 2-{[2-{[(4-chlorophenyl) methylidene] hydrazono}propyl] sulfanyl}- 6-nitro- 1,3-benzoxazole, (**10f)-** 2-{2-{ [(4-methoxyphenyl) methylidene] hydrazono} propyl] sulfanyl} -6-nitro- 1,3- benzoxazole, (**11)-** 4,5,6,7 -tetrabromo- N'- {1-methyl-2-[(6-nitro- 1,3-benzoxazol-2-yl) sulfanyl]ethylidene}-1,3-dioxo-1,3-dihydro-2H-isoindole-2-carbothiohydrazide, **(16)-** 7-chloro- N'-{1-methyl-2- [(6-nitro- 1,3-benzoxazol-2-yl) sulfanyl] ethylidene}-2,4-dioxo-4,4a-dihydroquinazoline-3(2H)-carbothiohydrazide, **(17a)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-one N-[1-(4-chlorophenyl) ethylidene] thiosemicarbazone and **(17f)-** 1-[(6-nitro- 1,3-benzoxazol-2-yl)sulfanyl] propan-2-one N-[1-(4-nitrophenyl) ethylidene] thiosemicarbazone which showed considerable activity with the standard. The presence of electron withdrawing groups namely nitro, bromo and chloro may be responsible for exhibiting significant cytotoxic activity.

8.6 Anti-tubercular activity

The Anti-tubercular activity of compounds were tested against *Mycobacteria tuberculosis* (Vaccine strain, H37 RV strain). few molecules, (**10c)-** 2-{[2-{[(4-chlorophenyl) methylidene] hydrazono} propyl] sulfanyl}- 6-nitro-1,3-benzoxazole, **(10d)-** 2 -{[2-{[(4- bromophenyl) methylidene] hydrazono} propyl] sulfanyl} -6-nitro-1,3-benzoxazole, **(11)-** 4,5,6,7-tetrabromo-N'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] ethylidene} -1,3-dioxo- 1,3-dihydro-2H-isoindole-2-carbothiohydrazide, **(16)-** 7- chloro-N'- {1-methyl- 2-[(6-nitro -1,3-benzoxazol-2-yl)sulfanyl] ethylidene}-2,4-dioxo-4,4a-dihydroquinazoline-3(2H)-carbothiohydrazide, **(17a)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-one N-[1-(4-chlorophenyl) ethylidene] thiosemicarbazone and **(17f)-** 1-[(6-nitro- 1,3-benzoxazol-2-yl) sulfanyl] propan-2-one N-[1-(4-nitrophenyl) ethylidene] thiosemicarbazone exhibited potent Anti- tubercular activity when compared with standard drug Pyrazinamide, Ciprofloxacin and Streptomycine. The presence of groups namely nitro, methoxy and bromo may be the reason for the significant antitubercules activities in the synthesized compounds.

8.7 DNA cleavage Studies

The DNA cleavage Studies of synthesized various benzoxazole derivatives were evaluated against Calf-thymus DNA. Few of the molecules (**10c)-** 2-{[2-{[(4-chlorophenyl) methylidene] hydrazono} propyl] sulfanyl}-6-nitro-1,3-benzoxazole, **(10d)-** 2-{[2- {[(4-bromophenyl) methylidene] hydrazono} propyl] sulfanyl}-6-nitro-1,3-benzoxazole, **(11)-** 4,5,6,7-tetrabromo- N'-{1-methyl-2-[(6-nitro-1,3- benzoxazol-2-yl) sulfanyl] ethylidene} -1,3-dioxo-1,3-dihydro-2H-isoindole-2- carbothiohydrazide, **(14)-** N-{1-methyl -2-[(6-nitro -1,3-benzoxazo l-2-yl)sulfanyl] ethylidene}-2,4-dioxo-4,4a-dihydroquinazoline-3(2H)-carbothiohydrazide, (**17a)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan- 2-one N-[1-(4-chlorophenyl) ethylidene] thiosemicarbazone, (**17b)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-one N-[1-(4-bromophenyl)

ethylidene] thiosemicarbazone were showed good DNA cleavage activity. The good DNA Cleavage exhibited by the synthesized compound may be due to existence of the substituents groups like bromo, chloro.

8.8 Molecular docking studies

The molecular interactions of the synthesized compounds with amino acid receptor were studied by molecular docking, in which the molecular binding energy have been calculated. Few of the compounds (**5a)-** 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-phenylmethylidene]thiosemicarbazone **(5g)-** 1-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]propan-2-one N-[(4-hydroxyphenyl) methylidene] thiosemicarbazone, **(8c)-** 2- ({[1-(chloroacetyl)-1*H*-benzimidazol-2-yl]methyl}sulfanyl)-6-nitro- 1,3-benzoxazole, **(8b)-** 2-{[(1-ethyl-1H-benzimidazol-2-yl)methyl]sulfanyl}-6-nitro-1,3-benzoxazole, **(10g)-** 2-{[2-{(2E)-[(4-chlorophenyl) methylidene] hydrazono} propyl] sulfanyl} -6-nitro-1,3-benzoxazole, **(10d)-** 2-{[2- {[(4-bromophenyl) methylidene] hydrazono} propyl] sulfanyl}-6-nitro-1,3-benzoxazole, **(13)-** N'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl]ethylidene}-1,3-dioxo-1,3-dihydro-2H-benzo[f]isoindole-2-carbothiohydrazide, **(16)-** N'-{1-methyl-2-[(6-nitro-1,3-benzoxazol-2-yl)sulfanyl] ethylidene}-2,4-dioxo-1,4-dihydroquinazoline-3(2H)-carbothiohydrazide, (**17a)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-one N-[1-(4-chlorophenyl) ethylidene] thiosemicarbazone and **(17d)-** 1-[(6-nitro-1,3-benzoxazol-2-yl) sulfanyl] propan-2-one N-[1-(4-bromophenyl) ethylidene] thiosemicarbazone showed higher binding energy towards the receptors. The synthesized few compound showed considerable molecular docking values may be due to the presence of nitro, chloro, bromo and methoxy groups.