Organochalcogen compounds with electron-deficient heterocyclic ring have shown tremendous potential as biologically active agents, precursors to semi-conducting materials and synthons in organic synthesis. The introduction of chalcogen group into organic moieties is an important aspect of organochalcogen chemistry. A variety of methods on the introduction of elemental chalcogen into the organic moieties have been described in the literature. The chalcogen group in an organic moiety can be incorporated by both nucleophilic and electrophilic reagents. The organochalcogen anions are a powerful nucleophile and are usually prepared *in situ* because of their sensitivity to aerial oxidation. After being introduced in an organic substrate, the carbon-chalcogen bond can be replaced by carbon-hydrogen, carbon-halogen, carbon-lithium or carbon-carbon bond. For example, the organoselenium group can easily be removed by selenoxide elimination and sigmatropic rearrangement reactions leading to the molecules of biological interest.

The biological role of chalcogen is attracting a great deal of interest as several chalcogen-containing proteins have been identified in mammalian tissues. The advent of sulphur drug and later discovery of mesoionic compound, in fact, accelerated the rate of interest in the biological role of chalcogen containing compounds. The biological utility of selenium came of age since the discovery that the bacterial enzymes, formate dehydrogenase and glycine reductase contain selenium. Another factor which contributed in exploring the biochemical role of selenium in mammals is the discovery of selenium containing active site of the antioxidant enzyme glutathione peroxidase (GPx). Glutathione peroxidase is one of the 25 known classes of selenoproteins with antioxidant activity. Antioxidants are enzymes or other organic substances that are capable of counteracting the damaging effect of oxidation (oxidative stress) in animal tissues. Chemical compounds that are capable of generating potentially toxic reactive oxygen species can be referred as pro-oxidants. In normal cell, there is a balance between the pro-oxidants and antioxidants. However, this balance can be shifted towards the pro-oxidants when there is a tremendous increase in the production of oxygen species or when there is a fall in the production of antioxidants. This state in living tissues is called oxidative stress. Oxidative stress is a biochemical condition and is associated with numerous human diseases like cancer, inflammatory and neurodegenerative diseases such as Alzheimer's and Parkinson's disease. The Reactive oxygen species (ROS) are
classically defined as oxygen containing radicals capable of independent existence with one or more unpaired electrons. However, ROS is invariably expanded to include reactive oxygen containing compounds without unpaired electrons such as hydrogen peroxide and singlet oxygen. The consumption and utilization of oxygen in physiological process result in the generation of ROS. Energy production in mitochondria is dependent on oxygen metabolism when molecular oxygen is reduced to water. During this complex electron transfer pathway, incomplete reduction of O₂ can result in generation of highly reactive and damaging ROS, including superoxide radicals (O₂•⁻), singlet oxygen (¹O₂), hydrogen peroxide and hydroxyl radical (OH•⁻).

The present work was an endeavor to synthesize some known and novel organochalcogen compounds containing heterocyclic ring. The heterocyclic ring chosen for the present study includes: 2-methoxypyridine, 3,5-dichloropyridine, pyrazine and substituted and unsubstituted pyridine rings.

All the synthesized compounds were characterized by Elemental analysis, NMR (¹H, ¹³C, ⁷⁷Se and ¹²⁵Te) and Mass spectral techniques. Single X-ray crystallography of some of the representative compounds have also been carried out. Further, a number of substituted pyridylselenium compounds were evaluated for their free radical scavenging activity.

In the present study, the pyridylselenium compounds were evaluated for their free radical scavenging, antimicrobial and anticarcinogenic activity. The slow release of these compounds from the poly(acrylamide) hydrogels (acrylamide = AAm) has also been presented. SEM and TEM characterization was used to investigate the effect of these compounds on the cell morphology of the bacteria. Structural correlations were established with the biological activities of the synthesized compounds by...
comparing their antioxidant, antibacterial and anticarcinogenic activities. The slow release of bis(2-pyridyl) diselenide from poly(acrylamide) hydrogels was also investigated. The effect of the pyridylselenium loading on the antioxidant property of polyacrylamide hydrogels was determined and it was found that the selenium loading increases the magnitude and rate of the radical scavenging activity of poly(acrylamide) hydrogels.

The current research work has been presented in five chapters.

**Chapter 1: Introduction and Review of Literature**

In this chapter, a brief introduction and review of literature on organochalcogen compounds have been provided. The discussion includes synthesis and biological evaluation of these compounds.

**Chapter 2: Experimental**

The second chapter deals with the material and methods used for the present study. Crystal structure refinement data is also provided in the present chapter.

There are three chapters that are devoted to the results and discussion of the experimental findings, i.e., Chapter 3, 4 and 5

**Chapter 3**

The results and discussion described in Chapter 3 has been divided into two sections.

**Section A: Synthesis and characterization of 2-methoxypyridylchalcogens**

In this section, synthesis of different organochalcogen derivatives of 2-methoxypyridine have been reported. Two methods were investigated for synthesizing these compounds. The methodologies involve lithiation of 2-methoxypyridine with and without prior complexation with boron trifluoride (BF₃).

Organochalcogen compounds with different functionality at different sites are synthesized regioselectively by different research groups and evaluated for their various application in different models. BF₃ directed lithiation of 2-methoxypyridine was investigated and developed for the synthesis of corresponding selenium compounds. Treatment of BF₃-complexed 1a with LDA exclusively afforded C-6 lithiation. Quenching of the resulting anion with selenium and an electrophile resulted
in the formation of the respective selenium derivative of 2-methoxypyridine (Scheme S1).

**Scheme S1**: Synthesis of selenium derivatives of 2-methoxypyridine at C-6 position

Next, the lithiation of 2-methoxypyridine was attempted without prior complexation with boron trifluoride (BF₃). Lithiation at ortho-position, facilitated by
ortho-directed ability of the methoxy group, has been used to synthesize various chalcogen derivatives of 2-methoxypyridine at C-3 position at 0 °C. This lithiated species further quenching with selenium and an electrophile. Using this protocol a number of chalcogen derivatives of 2-methoxypyridine have been synthesized (Scheme S2). Contrary to the lithiation of complexed 1a, the lithiation of BF3-uncomplexed 1a could not be achieved at -78 °C. The lithiation of 1a was achieved at the C-3 position at 0 °C. Clearly, there is a total change in the selectivity of the reaction when carried out with and without BF3 complexation.

Quantum mechanical analysis was carried out to understand the cause of observed regioselectivity in these reactions. The deprotonation energy and pKa value calculations, along with relative stability of the lithiated species suggest that the C-3 position in the uncomplexed 1a is the most likely position to get lithiated. Under the BF3 complexed condition, the relative stability of the lithiated species achieved through Li···F interaction guides the lithiation and subsequent chalcogenation at the C-6 position. Clearly, the coordination between the lithium (on C-6) and the fluoride of BF3 overshadows the directed metalation ability of the methoxy unit in affecting the lithiation in the BF3 complexed 2-methoxypyridine.

Section B: Synthesis and characterization of 3,5-dichloropyridylselenium compounds

In continuation of the study concerning lithiation of substituted pyridyl derivatives, the first ever study on the BF3 directed lithiation of 3,5-dichloropyridine with LDA to get the resulted organoselenium compounds with mono- and dilithiation have been reported in this section. BF3 controlled lithiation gave lithiated species at C-2/C-6 position, quenching with selenium and an electrophile resulted in the formation of the respective selenium derivative of 3,5-dichloropyridine (Scheme S3). No product corresponding to C-2/C-6 lithiation formed when 1.2 equiv. of LDA has been used, only coupled product of 3,5-dichloropyridine formed. In order to get the resulted product 2.2 equiv. of LDA has been used.

Lithiation of 3,5-dichloropyridine in the absence of BF3 gave lithiated species at C-4 position, quenching with selenium and an electrophile gave resulted product (Scheme S4). It is interesting that as 2.2 equiv. of LDA has been used, two products were formed containing mono- and disubstituted at C-2/C-4 in case of quenching with
selenium and iodomethane. In case of other electrophile like iodoethane and (chloromethyl)benzene, only one product are formed at C-4 position.

Scheme S3: Synthesis of selenium derivatives of 3,5-dichloropyridine with BF$_3$-complexation.

Scheme S4: Synthesis of selenium derivatives of 3,5-dichloropyridine without BF$_3$-complexation.
Quantum chemical analysis was carried out to understand the cause of the observed regioselectivity in these reactions. The deprotonation energy and pKa value calculations, along with the relative stability of the lithiated species suggest that the C-4 position in uncomplexed 2a is the most likely position to get lithiated. Under the BF3 complexed condition, the relative stability of the lithiated species achieved through Li···F interaction guides the lithiation and subsequent chalcogenation at the C-6/C-2 position.

Chapter 4: Synthesis and characterization of pyrazineselenium compounds

A survey of the literature revealed that there is no study on the BF3-directed lithiation of pyrazine (3a) with LDA or LTMP as the lithiating reagent. The present study is an attempt to explore this aspect of the pyrazine chemistry. The lithiation of pyrazine in the presence and absence of BF3.Et2O with LDA and LTMP was investigated. The developed procedure was subsequently employed for the synthesis of pyrazinylselenium compounds.

In the present study, lithiation of pyrazine (3a) in the presence and absence of BF3.Et2O with lithium diisopropylamide (LDA) has been discussed. The developed procedure was subsequently employed for the synthesis of pyrazinylselenium compounds. The BF3-directed metallation of substituted pyrazines (2-chloro-, 2-bromopyrazine, etc.) has been reported with highly expensive bimetallic tetramethylpiperdine (TMP) bases, TMPZnCl2LiCl and (TMP)2Mg2LiCl. However, there is no study on the BF3-directed lithiation of 3a with LDA or LTMP. Pyrazine in the presence of BF3.Et2O did not give the desired products. A number of variations, like the use of 1.1 or 2.2 equiv. of BF3.Et2O, LDA or LTMP (1.1/2.2 equiv.) as the lithiating reagent, THF or diethyl ether as the solvents and different reaction temperatures had no effect on the outcome of the reaction. Different electrophile (selenium, dimethyl sulfide, hexachloroethane, benzaldehyde and acetone) were used but none gave the products corresponding to the lithiation of 3a. It appears that the BF3 complexation prevents the lithiation of 3a with LDA or LTMP.

The effort to lithiate 3a through prior complexation with BF3.Et2O completely failed to facilitate the lithiation of the pyrazine ring. Then, direct lithiation of 3a with LDA were attempted. Treatment of a solution of 3a with LDA (1.1 equiv.) at –78 °C
Summary

afforded the carbanion 3b (Scheme S5). The reaction of 3b with selenium and electrophile to afford the formation of the respective selenium derivative of pyrazine in good yield suggest exclusive lithiation at C-2. The dilithiation of 3a was also achieved with 2.2 equiv. of LDA, which was used to synthesize 2,5-bis(methylselenenyl)pyrazine (3k). The position of the second –SeCH₃ unit in 3k was determined by single crystal X-ray crystallography as the NMR spectral data was inconclusive.

Scheme S5. Synthesis of pyrazinylselenium compounds.

Chapter 5

Chapter 5 has been divided into two sections.

Section A: Synthesis, characterization and X-ray structure of pyridylselenium compounds

This section concerns with the preparation of different series of substituted and unsubstituted pyridylselenium compounds. To explore the chemistry of pyridylselenium compounds, an efficient synthesis of bis(2-pyridyl)selenides and -diselenide by utilizing LiAlH₄ as a reducing agent has been discussed.
LiAlH₄ (1.0 equiv.) was added to a suspension of elemental selenium in dry DMF. A grayish suspension indicating the formation of LiAlSeH₂ was obtained. Addition of 2-bromopyridine (1.0 equiv.) to this suspension and 3 h refluxing gave bis(2-pyridyl)selenide (4a) and bis(2-pyridyl)diselenide (5a) in 72% and 23% yields, respectively (Scheme S6). Following the same procedure, other bis(2-pyridyl)selenides (4b-4e) and bis(2-pyridyl)diselenides (5b-5e) were also prepared. Interestingly, in all these reactions the monoselenide was the major product formed even when the proportion of 2-bromopyridine was increased from 1.0 equiv. to 2.0 equiv. This is contrary to all the reported methodologies where the diselenide is the major product formed.

Scheme S6: Synthesis of mono- and diselenides from elemental selenium and LiAlH₄

Also, bis(2-pyridyl)selenides were directly prepared from the corresponding diselenides by reacting with LiAlH₄ (Scheme S7). The later methodology afforded analytically pure symmetrical monoselenide without any contamination of the respective diselenide. The reaction involves the formation of selenenylaluminato complex that undergoes pyridine shift reaction leading to the generation of LiAlSeH₂ and symmetrical pySepy. The proposed mechanism has been supported by experimental observation and ¹H NMR analysis.

Scheme S7: Synthesis of bis(2-pyridyl)selenides by using LiAlH₄.
In order to find out correlation of biological activity with number of methyl substitution on the ring of pyridylselenium compounds, the compounds of 3,5-, and 3,4-lutidine were synthesized with previously reported method.

The prepared diselenides are soluble in conventional organic solvents like THF, ethanol etc. but are insoluble in water. Since we wanted to test the biological activities of some of these compounds in water, we prepared their hydrochloric salt by adding a solution of HCl to a cooled immiscible mixture of the respective diselenide in water (Scheme S8). The hydrochloric salts thus obtained are dark yellow in colour and readily soluble in water.

![Scheme S8: Synthesis of hydrochloric salt of the diselenidespyridyl and selenylbromides.](image)

Further in this chapter, methyl substituted and unsubstituted 2-pyridylselenylbromides were prepared and characterized. The reaction of the respective diselenides with bromine in ethyl acetate gave resulted product in a near quantitative yield by (Scheme S9). All the 2-pyridylselenylbromides are yellow in color and decompose before melting. These compounds are insoluble in diethyl ether, ethyl acetate, THF but soluble in DMSO and DMF.

![Scheme S9: Synthesis of pyridylselenylbromides.](image)
X-ray structures of some of the synthesized compounds were also analyzed. The molecule bis(5-bromo-2-pyridyl)diselenide (5i) exhibit C-H···N hydrogen bonds and Br···Br intermolecular secondary interactions and bis(3-bromo-2-pyridyl) diselenide (5j) display π···π stacking and π···Br secondary interactions. These intermolecular interactions lead to supramolecular self-assembly and suprapolymeric architecture in 5i and 5j. A search on Cambridge Structural Database was undertaken to identify π···π stacking interactions in bis(2-pyridyl)diselenides reported in the literature. Through this study it was established that the planar bis(2-pyridyl) diselenide with a cis-cis conformation, average angle Se–Se–C of 92.3°, and sterically less demanding ortho-substituent exhibit π···π stacking interaction leading to a supramolecular self-assembly.

Section B: Biological evaluation of pyridylselenium compounds and its slow release from polyacrylamide hydrogels

Antioxidant activity studies

In this section antioxidant property of the pyridylselenium compounds were evaluated the by two methods, DPPH free radicals and NO scavenging methods. The concept that selenium containing molecules may be better antioxidants than the classical antioxidants has led to the design of new organoselenium compounds. There are relatively few reports on the study of antioxidant property of the organoselenium compounds by these two methods. Most of the studies are based on the NADPH-glutathione reduction methodology.

DPPH radical scavenging method

![Fig. S1. Comparison of DPPH % scavenging activity: a) diselenides at 5.0 × 10⁴ μM and b) 2-pyridylselenenyl bromides at 1.0 × 10² μM. Data are reported as mean±SD of three readings per conc.](image-url)
A careful analysis of the tested compounds containing one methyl group attached to the pyridine ring (picolyseleniums, 5b-5e and 8b-8e) reveals a clear trend in the free radical scavenging activity which is mentioned below and displayed in Fig. S2.

(5b/8b) ortho-methyl (C-3) > (5e/8e) meta-methyl (C-6) > (5c/5c) meta-methyl (C-4) > (5d/8d) para-methyl (C-5)

Fig. S2. Relation between Se–CH₃ distance (Å) and DPPH reduction activity of diselenides (5b-5e). The bond distances have been calculated from the crystal structure of the respective diselenides. Data are reported as mean±SD of three readings per conc.

Two-way ANOVA of DPPH activity yielded a significant Se–CH₃ distance (Å) × concentration of pyridylselenium compounds interaction (p<0.05 by Turkey test). This result indicates a significant role of the electron-releasing inductive effect of the methyl group in enhancing the antioxidant activity of the titled compounds. Interestingly, 2,6-bis(3,5-dimethylselenenyl)pyridine (6f), which contains two selenium atoms at two different position and no Se–Se bond, did not exhibit the DPPH radical scavenging activity at any concentration. From these observations it could be inferred that the presence of Se–X (X = Sepy, Br) bond is essential for the antioxidant activity. It also appears that the antioxidant quenching cycle involves the generation of free radicals (pySe´ and X´) due to the cleavage of Se–Br and Se–Se bond induced by the DPPH radical in the solution. It is postulated that the electron-releasing methyl group stabilizes the pySe´ radical which drives the cleavage of Se–X bond. The lower bond dissociation energy for the Se–Br (297 KJ/mole) bond than for
the Se–Se (332.6 KJ/mole) bond partly explains the greater antioxidant activity of the selenenylbromides than the corresponding diselenides.

**NO scavenging method**

Contrary to the results obtained with DPPH reduction method, the diselenides shows greater NO scavenging activity than the corresponding pyridylselenenylbromides (one-way ANOVA/Turkey, p<0.05). In fact the diselenides are approximately $10^2$ times more active than the pyridylselenenylbromides. This indicate different mode of action of organoselenium compounds in the quenching of NO and DPPH radicals. In the case of NO scavenging method also, the antioxidant activity is found to increase with the increase in the number of methyl groups attached to the pyridine ring (Fig. S3). The scavenging efficacy of the picolyselenium compounds (5b-5e and 8b-8e) has been found to decrease with the increase in distance of the methyl group from the selenium moiety. Importantly, all the diselenides shows greater (1.5-2 times) NO antioxidant activity than ebselen.

![Comparison of NO scavenging activity](image)

**Fig. S3.** Comparison of NO scavenging activity: a) diselenides at $4.0 \times 10^2$ μM and b) 2-pyridylselenenyl bromides at $2.0 \times 10^4$ μM. Data are reported as mean±SD of three readings per conc.

In addition to the certain beneficial properties, the organoselenium compounds have been reported to be toxic in nature especially, at relatively higher concentrations which can lead to a number of health related complications. At higher levels of concentration, organoseleneniums are considered to be cytotoxic and genotoxic in nature as they have been shown to generate free radicals, act as prooxidant leading to decrease in free glutathione, and break DNA strand at elevated concentrations. Only a narrow therapeutic window exists for the organoselenium compounds and the conventional drug delivery systems would not provide ideal pharmacokinetic profiles for these compounds. The ideal pharmacokinetic profile can be attained by reaching
the therapeutic levels without exceeding the maximum endurable dose while maintaining the required concentration. The later objective could be achieved by use of a polymer matrix as a drug-releasing device which releases organoselenium compounds in a controlled and sustained manner.

**Antioxidant activity of unloaded and selenium-loaded poly(AAm) hydrogel**

In this part, the slow release of pyridylselenium compounds from poly(AAm) hydrogels has been discussed. Before the control release study, the antioxidant activity of poly(AAm) hydrogels with and without the loading of the pyridylselenium compounds. The Poly(AAm) hydrogel was loaded with the compound 3-methyl-2-pyridylselenenylbromide \( 8b \) and the DPPH scavenging activity was determined after fixed interval of time. It can be inferred from the experimental results (Fig. S4a) that the loading of selenium compounds not only increases the magnitude of the antioxidant activity of poly(AAm) hydrogel but also enhances its rate of radical scavenging. This is due to slow release of the selenium compound from the polymer matrix which augments the antioxidant activity of the hydrogel.

**Release dynamics of 5a from the loaded poly(AAm) hydrogels in different medium**

The release profile of 5a from the loaded polymer matrix in different release medium at 37 °C was also investigated. The amount of 5a released in pH 2.2 buffer was higher than the release in pH 7.4 buffer solution. After 24 h, the total amount of 5a released in pH 2.2 buffer and pH 7.4 buffer was found to be 2.86 ±0.11 and 2.06 ±0.18 mg/10mL/g of hydrogel respectively. The greater release of 5a in pH 2.2 buffer may be due to its higher solubility in the acidic conditions. From the Fig. S4b it is clear that release of 5a from the drug loaded polymer occurred in a controlled manner.

![Fig. S4.](image)

**Fig. S4.** a) DPPH radical % scavenging activity of 8b-loaded and non-loaded poly(AAm) hydrogel; b) release profile of 5a from loaded poly(AAm) hydrogels in different release medium at 37 °C.
Antibacterial activities

Three gram positive bacteria *Bacillus pumilus* (MTCC 1607), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 737) and two gram negative bacteria *Escherichia coli* (MTCC 1687), *Pseudomonas oleovorans* (MTCC 617) were used for the present antibacterial investigations. All these bacterial strains are responsible for health hazards in humans. The preliminary screening of antibacterial activities of the synthesized compounds was done with disk diffusion method. All these compounds were further characterized by their MIC values with dilution method.

Pyridylselenium compounds were dissolved in DMSO which does not show any zone of inhibition. All the diselenides show antibacterial activity against the five tested bacterial strains. In case of bis(2-pyridyl)diselenide (5a), 29.23 mm and 26.30 mm zone of inhibition was observed with 100 μg and 30 μg concentration, respectively against *B. pumilus*. Bis(5-chloro-2-pyridyl)diselenide (5h) shows 15.22 mm and 13.18 mm zone of inhibition at 100 μg/disk and 30 μg/disk against *B. pumilus*. In the case of pyridylselenylbromides, the compound 2-pyridylselenenylbromide (8a) showed 21.66 mm zone of inhibition with 100 μg/disk and 19.16 mm zone of inhibition with 30 μg/disk concentrations against *B. pumilus*. 16.16 mm zone of inhibition was recorded with 100 μg/disk against gram-negative bacteria *E. coli*.

In relation to the MIC values also, 5a is found to be the most potent against all the bacterial strains and bis(4,5-dimethyl-2-pyridyl)diselenide (5g) is the least potent of all pyridylselenium compounds (Fig. S5). The significant MIC value of 6 ppm was obtained for 5a on *B. pumilus*. The hydrochloride salt of bis(2-pyridyl)diselenide (7a) have shown remarkable antibacterial property. Compounds, 7a and 7b show minimum inhibitory concentration of 2 ppm against *Bacillus pumilus* and *Bacillus subtilis*, and 40 and 50 ppm against *E. coli*. The antibacterial activity of all the diselenides is more significant on the gram positive bacteria than the gram negative bacteria. The same trend was observed in case of a series of pyridylselenenylbromides (Fig S6).
**Fig. S5.** Comparison of MIC values of bis(2-pyridyl)diselenides (5a-5g, 7a-7c).

**Fig. S6.** Comparison of MIC values of pyridylselenylbromides (8a-8f).
In order to investigate the active site in these compounds, the antibacterial properties of the parent compounds, namely, pyridine, 3-methyl, 4-methyl, 5-methyl and 6-methylpyridine were also evaluated. It was found that none of the aforementioned compounds were active against any of the five tested strains of bacteria. It suggests that the presence of selenium moiety in the pyridine ring is essential for the antibacterial activity of the pyridylselenium compounds. To confirm further, the compound 6f, 3,5-dimethyl-2,6-bis(methylselenenyl)pyridine, which does not have the Se–Se bond, was evaluated for antibacterial activity. This compound did not show any antibacterial property even at 10000 ppm of its concentration indicating the Se–Se bridge as the active site in the bis(2-pyridyl)diselenides.

Electron microscopic studies

Electron microscopy is an essential technique for the visualization of the cytoplasmic organization and cell wall structure of the bacteria. The use of Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) techniques to characterize different microorganism and different antimicrobial agents has often been exploited by many research groups. In the present study, SEM and TEM techniques were used to investigate the mode of interaction of the pyridylselenium compounds with the bacteria. SEM and TEM images indicate the damage of cytoplasmic membrane over the entire surface resulting in the leakage of cell constituents from many places resulting in the death of the cell.

β-Galactosidase Inhibition in B. pumilus

Based on MIC values and their ability to get dissolves in water, hydrochloride salt of bis(2-pyridyl)diselenide, 7a (5a-HCl) and hydrochloride salt of bis(6-methyl-2-pyridyl)diselenide, 7b (5e-HCl) were selected for enzyme profiling study with respect to β-galactosidase induction in B. pumilus. The ability of the pyridylseleniums to suppress the protein synthesis was determined by exposing the biomass to an inducing substrate and observing the extent of inhibition of induced enzyme formation. In line with the results obtained with the disk-diffusion method, the introduction of methyl group on the pyridine ring significantly reduces the inhibition
activity of the diselenides. The intracellular inhibition potency of $7a$ is found to be 1.3-fold stronger than that of $7b$.

At a concentration of 3 ppm
\[
\frac{\text{% inhibition in intracellular enzyme induction due to } 7a \ (5a-\text{HCl})}{\text{% inhibition in intracellular enzyme induction due to } 7b \ (5e-\text{HCl})} = \frac{73.33}{56.42} = 1.3
\]

At a concentration of 100 µg/disc with *B. Pumilus*
\[
\frac{\text{Zone of Inhibition of } 7a}{\text{Zone of Inhibition of } 7b} = \frac{29.23}{22.56} = 1.3
\]

Anticarcinogenic activity
Anticarcinogenic activity of the synthesized compounds was screened at different concentrations against cancer cell line, Raji (acute lymphoid leukemia) cells using the MTT colorimetric assay. Curcumin a known anticancer compound has been considered as positive control in the whole study. (2-pyridyl)diselenide ($5a$) showed the maximum activity and possessed IC$_{50}$ at 25 µM. The anticarcinogenic activity of the pyridylseleniums decreases with the increase in the number of methyl group. Among the tested compounds containing one methyl group, i.e., 3-methyl, 4-methyl, 5-methyl and 6-methyl substituted diselenides, bis(6-methyl-2-pyridyl)diselenide shows the minimum IC$_{50}$ value of 40 µM against the cancer cell line, Raji. Bis(3,5-dimethyl-2-pyridyl)diselenide ($5f$) was the least active compound out of bis(2-pyridyl)diselenides, it showed 50% inhibition concentration at 205 µM. The corresponding bis(2-pyridyl)selenides are 2.5-5 times less active and induced 50% cell death only at >100 µM concentration. The standard drug Curcumin shows IC$_{50}$ at about 17 µM against the cancer cell line, Raji (acute lymphoid leukemia).