# SYNTHESIS OF NEW DIARYL DERIVATIVES COMPRISING IMIDAZOTHIADIAZOLE MOIETY AS POTENTIAL ANTICANCER AGENTS

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## ABSTRACT

*Cancer is a class of diseases characterized by out-of-control cell growth. There are so many types of cancer. In case of Women, Breast Cancer ranks second among cancer deaths in women. Approximately 60% of all breast cancer patients have hormone dependent breast cancer, which contains estrogen receptors and requires estrogen for tumor growth. Aromatase, the enzyme responsible for estrogen biosynthesis, is a particularly attractive target in the treatment of hormone-dependent breast cancer.*

*In the present study we have reported the synthesis of some novel Diaryl Derivatives comprising 2-Amino Thiadiazole and Imidazothiadiazole moiety. These moieties are of interest because of structural similarity with the Letrozole, which is the potent Aromatase Inhibitor and their diverse biological activities and clinical applications.*

*We have reported the new series of Letrozole analogues to target Aromatase Enzyme. The reaction was monitored by Thin Layer Chromatography using suitable mobile phase. The Rf values were compared and the Melting Point of the derivatives was determined. It was found that they were different from each others. Further, these derivatives were characterized and confirmed by IR, 1H-NMR, 13C-NMR and Mass Spectral Studies. For Anticancer activity, the selected compounds were submitted to National Cancer Institute (NCI) for in vitro anticancer assay and were evaluated for their anticancer activity. Primary in vitro dose anticancer assay was performed in full NCI 60 Cell panel in accordance with the protocol of the NCI, USA. Compound 1 has a 73.7 % and Compound 4 has a 52.56 % growth Inhibition of Breast Cancer cell lines*

**Keywords:** *Anticancer, Breast Cancer, Aromatase Enzyme, Letrozole, Thiadiazole, Imidazothiadiazole, NCI-USA*

## INTRODUCTION

### Cancer

Cancer is a 2nd leading cause of death in developed countries. Cancer or Neoplasm is the appearance of Tumor, Tumor is a abnormal mass of the cells[1,2]. In Our body activation of the Oncogenes is responsible for the cancer. Generally there is a two type of tumors, Benign Tumor and Malignant Tumor. Tumors can grow and interfere with the digestive, nervous, and circulatory systems, and they can release hormones that alter body function[2,3,4].

In case of Women, There is a high mortality rate in women because of the Breast Cancer. Breast cancer is the Neoplasm of the breast Tissue. A Uncontrolled and abnormal growth in breast tissue[5]. There is two types of Breast Cancer (1) Non-invasive Breast Cancer (2) Invasive Breast Cancer. Pathologicaly activation of BRCA genes is responsible for the development of breast cancer. Breast cancer can have a number of symptoms, but the first noticeable symptom is usually a lump or area of thickened breast tissue. And it can be diagnosis by the biopsy or memography[6,7]

Generally most of the breast tumors are estrogen dependent Tumors[8].

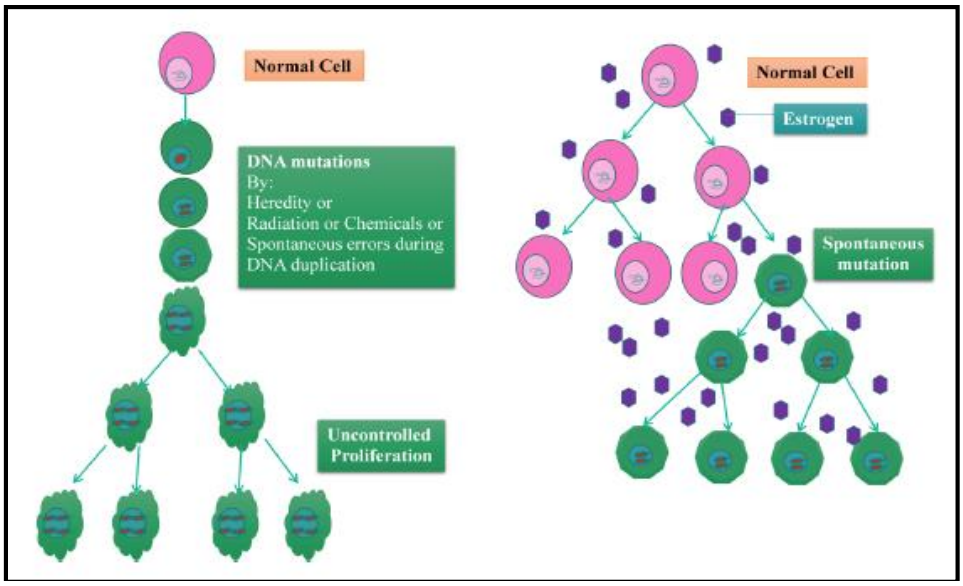



**Figure-1: Estrogen Dependent Breast Cancer**

**RATIONALE FOR DESIGN OF NEW ANTICANCER AGENTS**

Rational approach behind this project is, Proposed molecule have a structural similarity with the Letrozole, which is the potent Aromatase Inhibitor, Due to this reason proposed molecule probably inhibit Aromatase enzyme and will develop as new anti cancer agent.

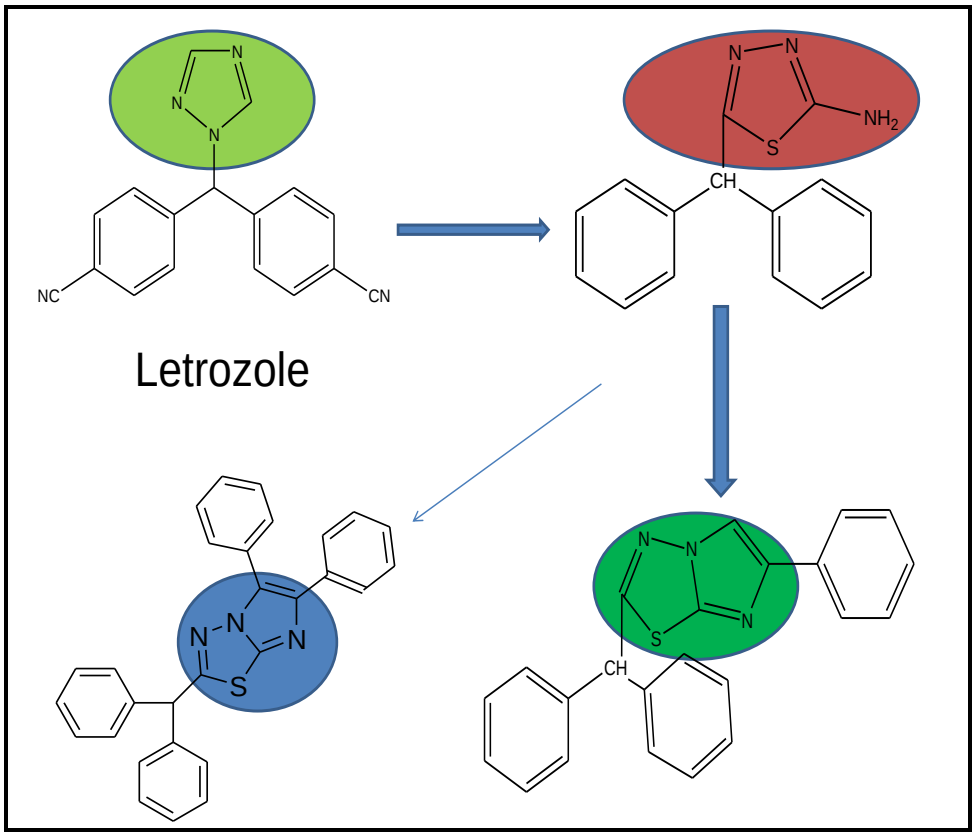



**Figure-2: Design strategy of Proposed compounds**

## TARGET

### Aromatase and its Inhibitors

Aromatase is the cytochrome P450 enzyme that converts androgens including androstenedione and testosterone to the estrogen products, estrone and estradiol respectively[9,10]. This enzyme plays a key role in the regulation of these sex steroids[11].

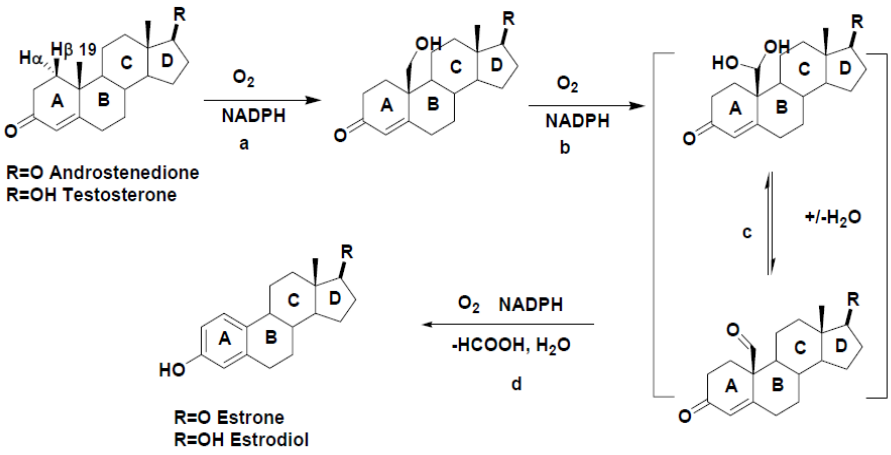



**Figure: 3** Reaction mechanism for estrogen biosynthesis by aromatase

### Aromatase in Breast Cancer

Aromatase activity has been demonstrated in breast tissue in vitro. Furthermore, expression of aromatase is highest in or near breast tumor sites. The regulation of aromatase expression varies due to the different promotors in each tissue. The increased expression of aromatase cytochrome P450arom observed in breast cancer tissues has been associated with a switch in the major promoter region utilized in gene expression[12,13,14].

### Aromatase Inhibitors

These are the agents which inhibit the activity of the Aromatase Enzyme. Estrogens can influence the risk of breast cancer and also the growth of established tumors. Hormone-dependent breast cancer tumors depend of estrogen for growth[15,16]. Two approaches treating these cases of breast cancer are either blocking the mechanism of action of estrogens or inhibiting their synthesis[17]. These therapies are particularly helpful in postmenopausal women in whom hormone responsive is common and estrogen synthesis is primarily peripheral (adipose tissue, muscle and breast tissue) rather than in the ovaries[18].

Letrozole is one of the most effective drug of this class[19]

### Types of Inhibitors:

(1) Type 1 or Steroidal Inhibitors
- Exemestane
- Formestane

(2) Type 2 or Non-steroidal Inhibitors
- Letrozole
- Anastrazole
- Aminoglutethimide

## MATERIALS AND METHODS

The synthetic procedure which we have used in our research work is as below, we had synthesised the 10 compounds and all the synthesise compounds are Structurally analogue of Letrozole, and the all compounds were screen at U.S N.C.I for Anti Cancer Activity And the reaction was monitored by the TLC, here is the composition of the mobile phase

1. Chloroform: Methanol (9.5 : 0.5)
2. Chloroform: Methanol (9 : 1)

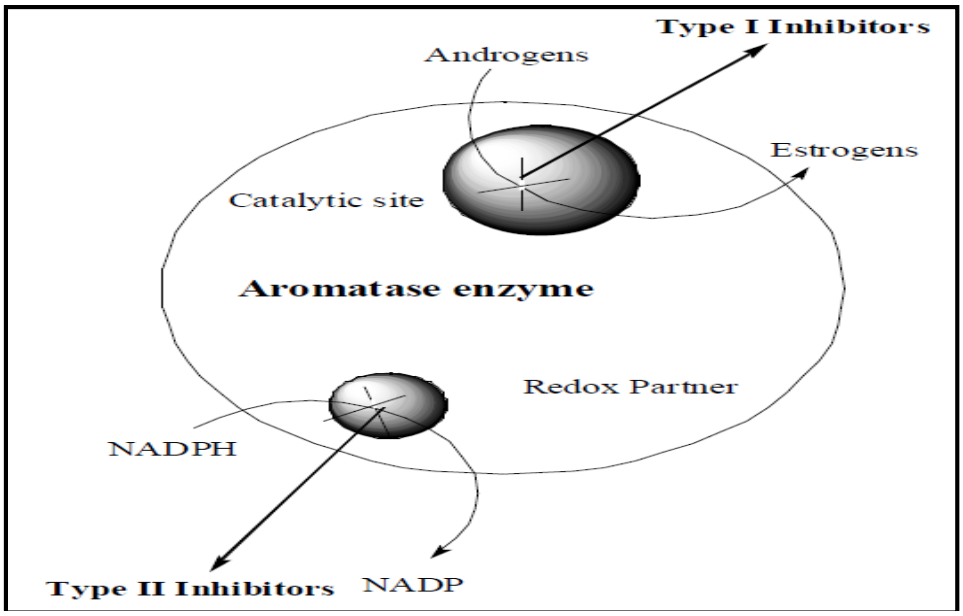
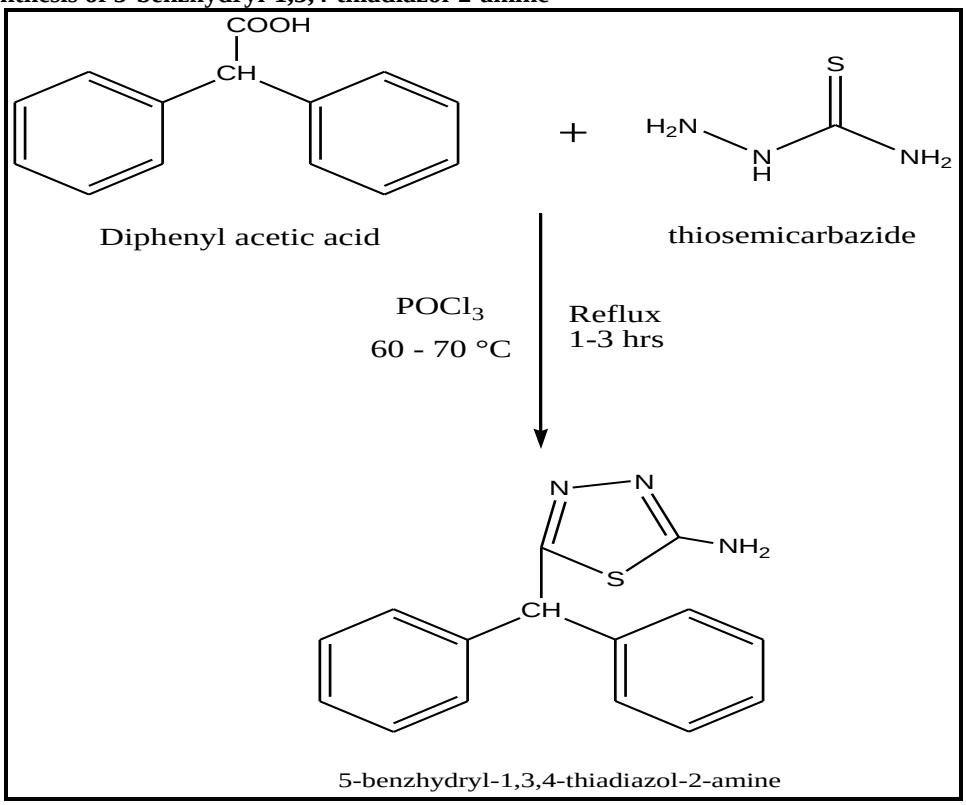
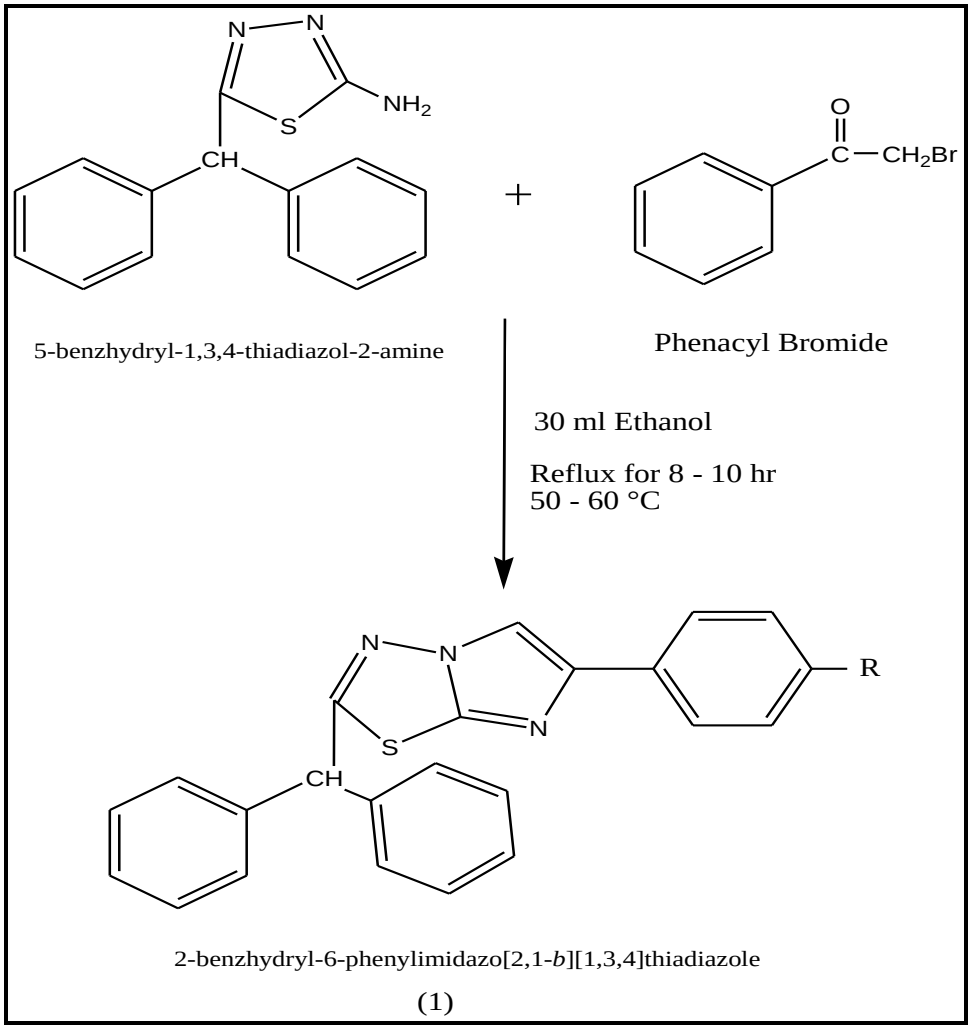



**Figure-4: Types of Aromatase Inhibitors**

**Step – 1 : Synthesis of 5-benzhydryl-1,3,4-thiadiazol-2-amine**



**Step – 2: 2-benzhydryl-5-phenylimidazo[2,1-b][1,3,4]thiadiazole**




(1)

**Derivatives**

| **COMPD.** | **R** |
|---|---|
| 1 | H |
| 2 | $CH_3$ |
| 3 | Br |

- **Types of Phenacyl Bromide Used**
  - ✓ Compound 1 = Plain Phenacyl Bromide
  - ✓ Compound 2 = 4-bromo phenacyl bromide
  - ✓ Compound 3 = 4-methyl phenacyl bromide

**Step-3(a): Synthesis of α-bromo-1-(4-substituted) phenyl-2-(4- substitu ted) phenyl-1-Ethanones or Diaryl acyl bromide**

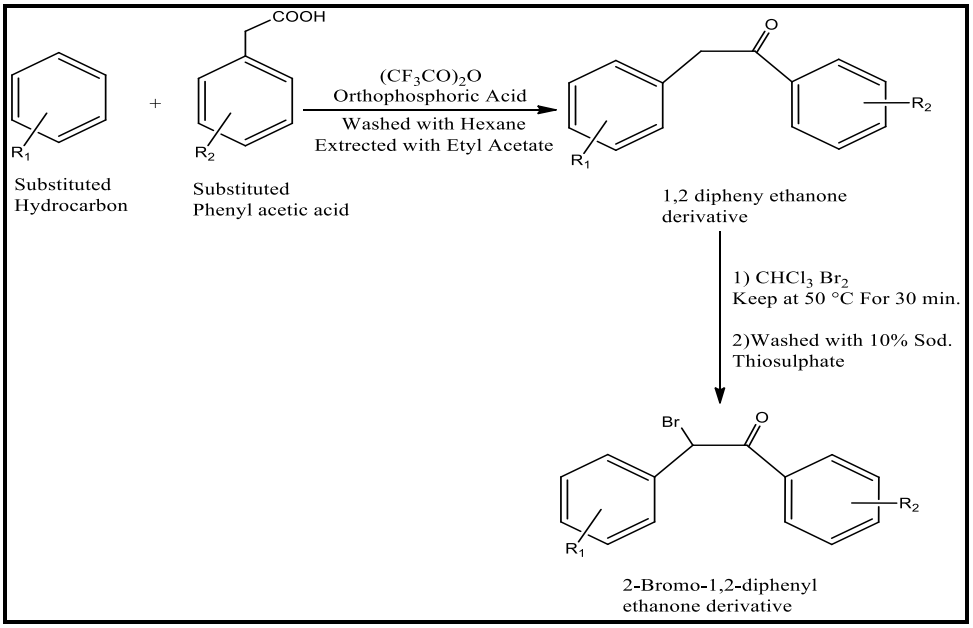
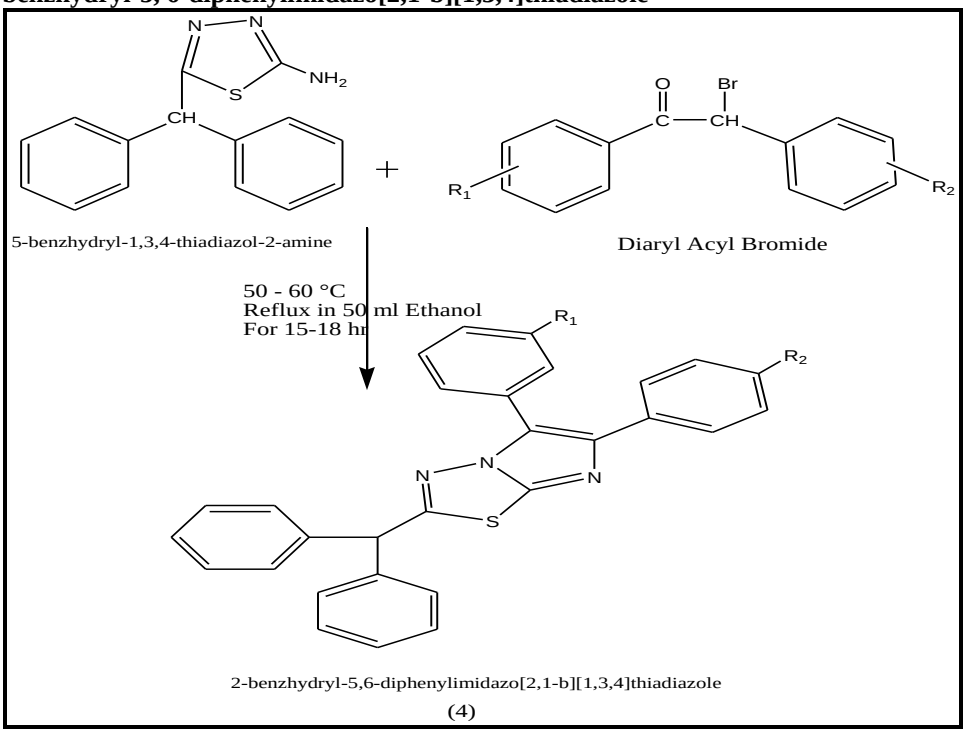



**Table-1: Combination of Phenyl Acetic Acid and Hydrocarbon**

| Sr.No | Types of Phenyl acetic acid | Types of Hydrocarbon |
|---|---|---|
| 1 | Plain Phenyl acetic acid | Benzene |
| 2 | Plain Phenyl acetic acid | Toluene |
| 3 | Plain Phenyl acetic acid | Chlorobenzene |
| 4 | P-methoxy phenyl acetic acid | Benzene |
| 5 | P-methoxy phenyl acetic acid | Toluene |
| 6 | P-methoxy phenyl acetic acid | Chlorobenzene |

**Step-3(b): 2-benzhydryl-5, 6-diphenylimidazo[2,1-b][1,3,4]thiadiazole**

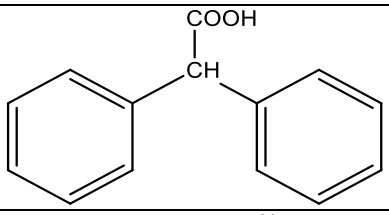
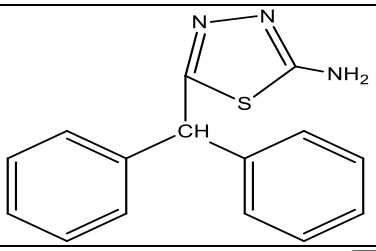
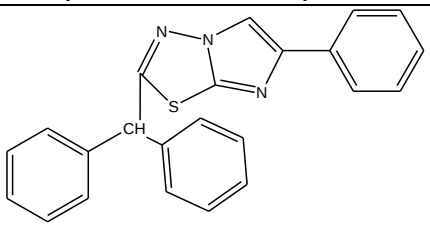
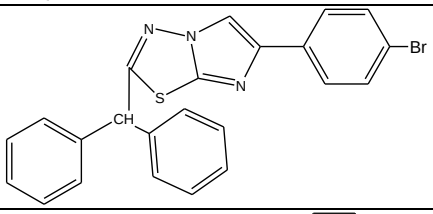
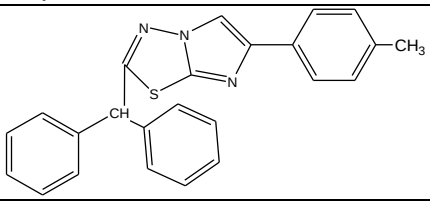


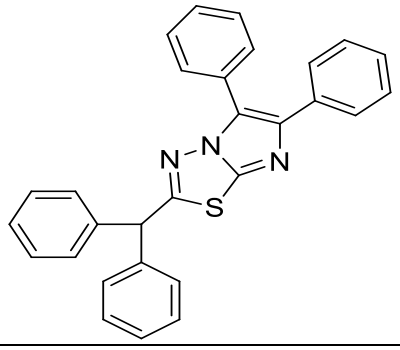
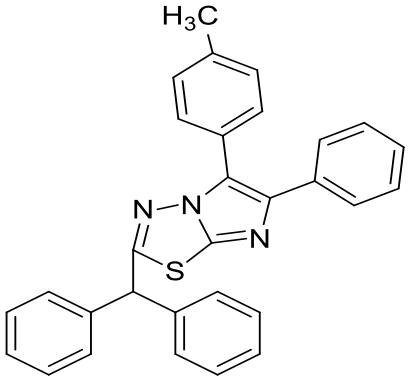
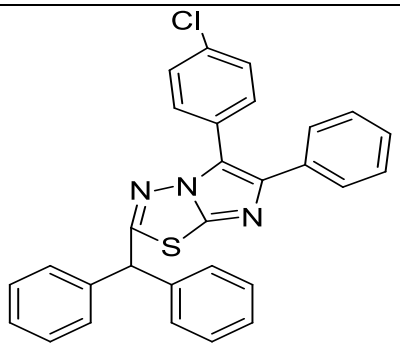
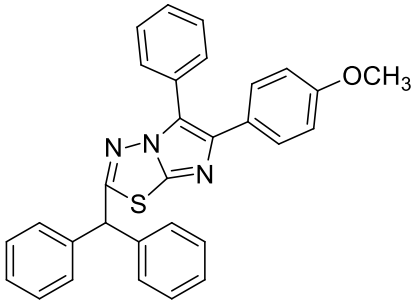
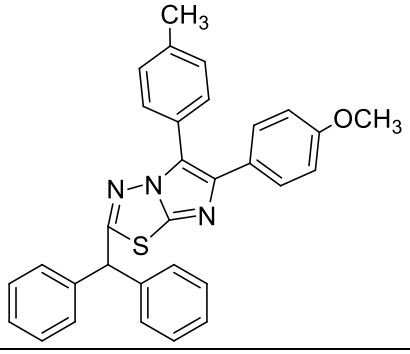
**Derivatives**

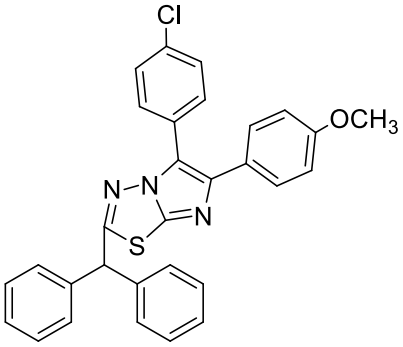
| COMPD. | $R_1$ | R |
|---|---|---|
| 4 | H | H |
| 5 | H | $CH_3$ |
| 6 | H | Cl |
| 7 | $OCH_3$ | H |
| 8 | $OCH_3$ | $CH_3$ |
| 9 | $OCH_3$ | Cl |

Following is the summary of the synthesize compounds:

**Table- 2: Compound code and details of Synthesize compounds**

| COMPOUND | STRUCTURE | CHEMICAL NAME |
|---|---|---|
| Starting Material |  | Diphenyl acetic acid |
| Intermediate |  | 5-benzhydryl-1,3,4-thiadiazol-2-amine |
| Compound 1 |  | 2-benzhydryl-6-phenylimidazo[2,1-b][1,3,4]thiadiazole |
| Compound 2 |  | 2-benzhydryl-6-(4-bromophenyl)imidazo[2,1-b][1,3,4]thiadiazole |
| Compound 3 |  | 2-benzhydryl-6-p-tolylimidazo[2,1-b][1,3,4]thiadiazole |

| | | |
|---|---|---|
| Compound 4 |  | 2-benzhydryl-5,6-diphenylimidazo[2,1-b][1,3,4]thiadiazole |
| Compound 5 |  | 2-benzhydryl-6-phenyl-5-p-tolylimidazo[2,1-b][1,3,4]thiadiazole |
| Compound 6 |  | 2-benzhydryl-5-(4-chlorophenyl)-6-phenylimidazo[2,1-b][1,3,4]thiadiazole |
| Compound 7 |  | 2-benzhydryl-6-(4-methoxyphenyl)-5-phenylimidazo[2,1-b][1,3,4]thiadiazole |
| Compound 8 |  | 2-benzhydryl-6-(4-methoxyphenyl)-5-p-tolylimidazo[2,1-b][1,3,4]thiadiazole |

| | | |
|---|---|---|
| Compound 9 |  | 2-benzhydryl-5-(4-chlorophenyl)-6-(4-methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazole |

## ANTICANCER ACTIVITY

All the synthesised compounds were screen for the detection of Anti Cancer Activity at the U.S.A National Cancer Institute by the 60 cell line assay[22].

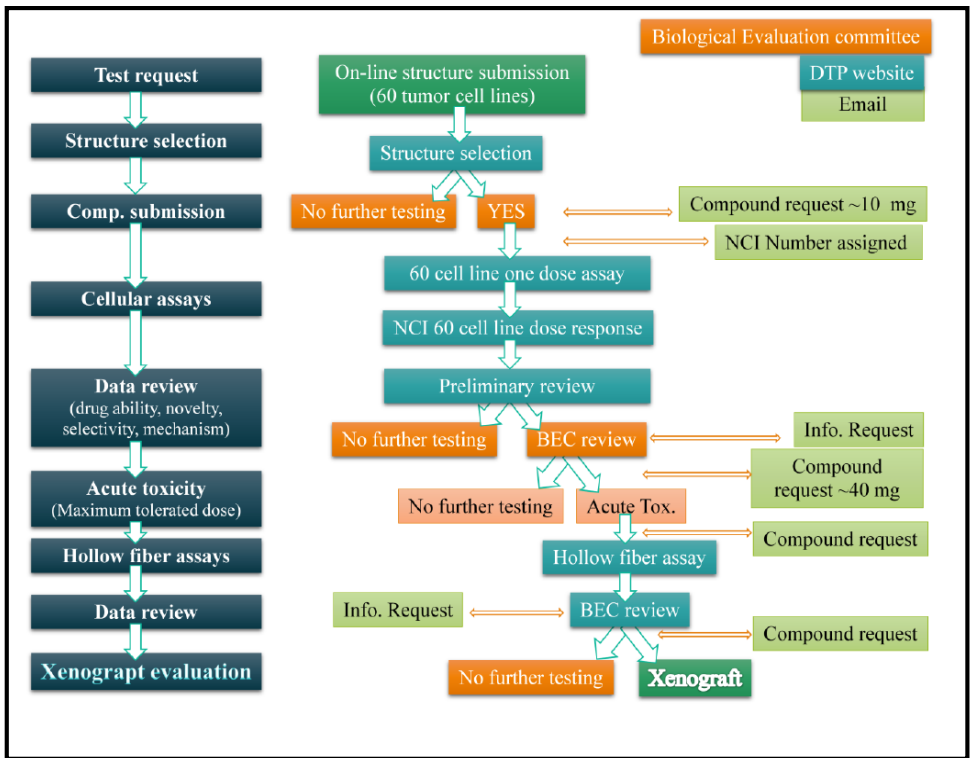



**Figure-3: NCI-60 Cell Line Assay Procedure**

## RESULT

In this, we have synthesized some novel Diaryl Derivatives comprising 2-Amino Thiadiazole and Imidazothiadiazole moiety, which are structurally analogues of Letrozole, Which is potent Inhibitor of Aromatase Enzyme, used in treatment of Breast Cancer.

Thiadiazole derivative was synthesized using the reaction between the Diphenyl Acetic Acid and Thiosemicarbazide in presence of $PoCl_3$, The in-situ cyclazation gives the 5-benzhydryl 1,3,4 thiadiazole 2-amine, since it is reported compound so it was confirmed by TLC, Melting point, and IR spectra.

We have reacted the 5-benzhydryl 1,3,4 thiadiazole 2-amine with different Phenacyl Bromide and Diary Acyl Bromide, which gives Diaryl Derivatives containing di-substituted and tri-substituted Imidazothiadiazole moiety respectively, The derivatives were characterized by spectral studies using IR, $^1$H NMR, $^{13}$C NMR

The structures of final derivatives **(1,2,4,5,6)** were confirmed through the following spectral data. disappearance of primary amine peak above 3200 $cm^{-1}$,Ar C-H peak around 3000 $cm^{-1}$, Ar C=C peak between 1450 – 1600 $cm^{-1}$ and C=N of thiadiazole and imidazole are identified between 800 – 600 $cm^{-1}$

$^1$H NMR spectra revealed all the corresponding peaks at δ=6-8 for aromatic protons

while -CH protons shows peak at δ= 5-6. $^{13}C$ NMR gave valuable information to confirm cyclisation about Imidazothiadiazole ring system.

The synthesized compounds were evaluated for their *in-vitro* anticancer activity at NCI, USA. **Compound 1** and **Compound 4** showed encouraging anticancer activity.

Compound 1 has a 73.7 % growth Inhibition of Breast Cancer cells

Compound 4 has a 52.56 % growth Inhibition of Breast Cancer cells

**Following is the Result/Mean graph of the anti Cancer Screening by U.S NCI :**

**Figure-4: Mean Graph of One Dose Screen of the Compound 1**

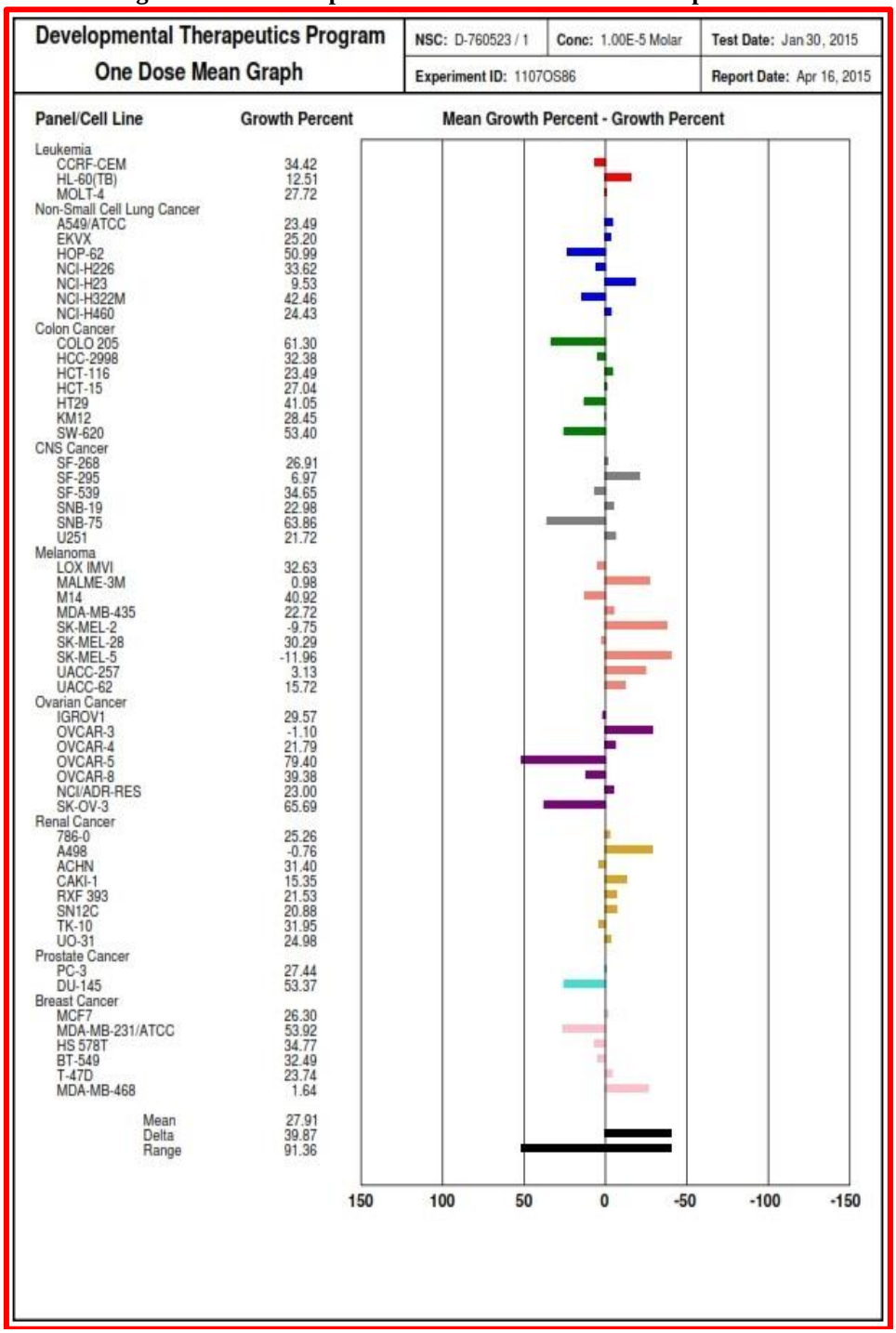
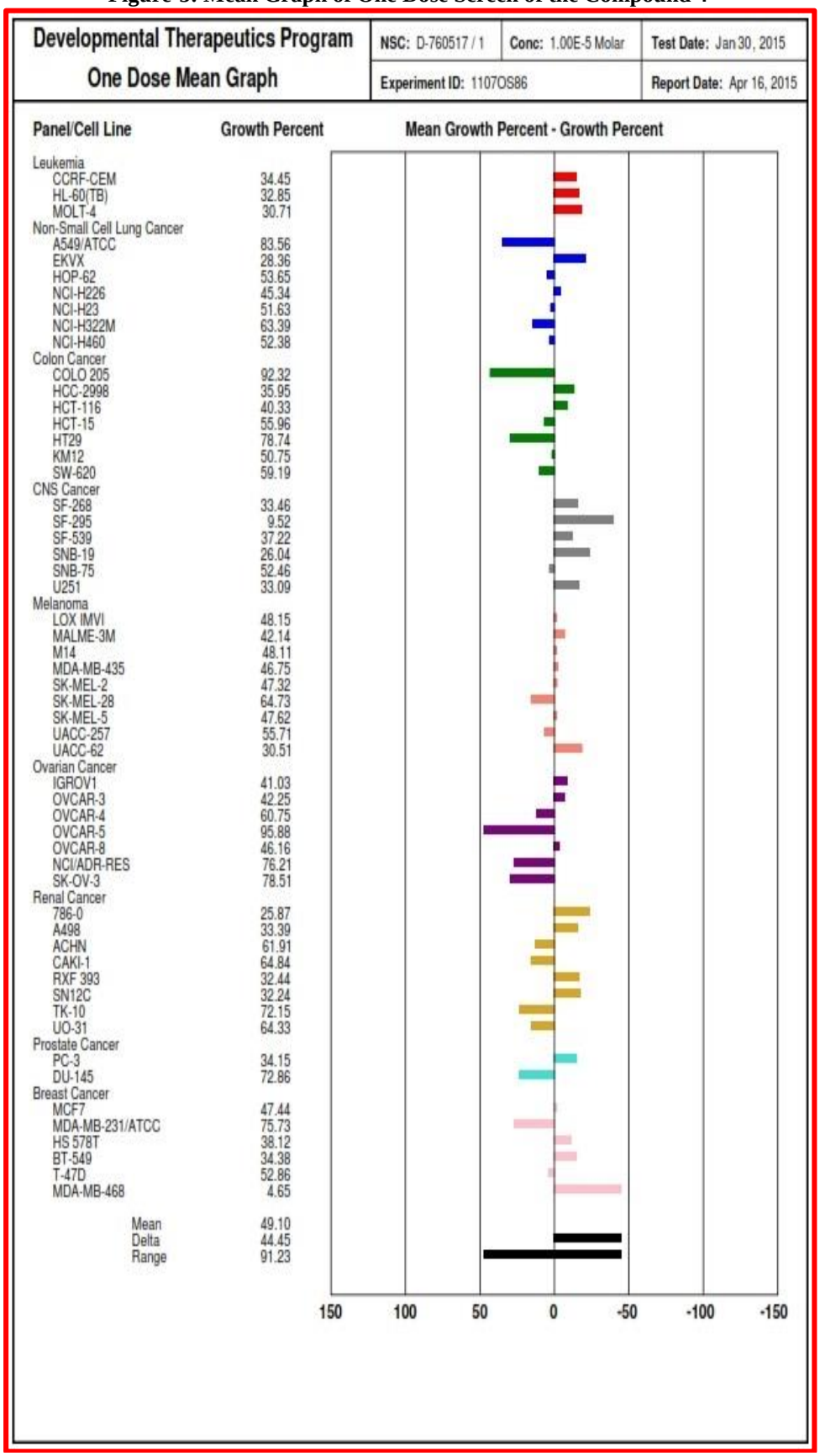


**Figure-5: Mean Graph of One Dose Screen of the Compound 4**



**DISCUSSION**

The present work, which has undertaken is bonafied, for the "**SYNTHESIS OF NEW DIARYL DERIVATIVES AS POTENTIAL ANTI-CANCER AGENTS",** A novel series of Diaryl Derivatives were synthesized comprising Imidazothiadiazole.

The Imidazothiadiazole derivatives were prepared by refluxing 5-benzhydryl 1,3,4 thiadiazole 2-amine with different Phenacyl Bromide and Diary Acyl Bromide respectively in Dry Ethanol.

The yield of the synthesized compounds was found to be in range from 40-85%. Tri-substituted Imidazothiadiazole Derivatives were obtained in good yield as compared to the di-substituted Imidazothiadiazole Derivatives. All the newly synthesized compounds were characterized on the basis of their Physical, Spectral and Analytical data. All synthesised compounds are structurally analogues to the Letrozole, Which is potent Inhibitor of Aromatase Enzyme, used in treatment of Breast Cancer

- ✓ The IR spectra, $^{1}H$ NMR spectra, $^{13}C$ NMR spectra and Mass spectra of the representative compounds were analyzed, studied.

**ACKNOWLEDGMENTS**

**Dear god, I wonna take a minute,**
**Not to ask for anything from you,**
**But simply to say thank you for all I have.**
**"To mom and the dad, for their love, their humour, their ethics, their inspiration but also for their genes"**

I would like to thank my parents Mr. Jagdishchandra N. Gohil and Mrs. Vanitaben J. Gohil for their love and support. The spirit of kindness and forgiveness that you deposited in me will be kept in my heart for all of my life.

I am eternally grateful for the support given to me from my sweet younger sister Jigna Gohil.

My heart felt thanks to my guide Dr. M.N.Noolvi, for his support over the course of my study, Many thanks for your guidance, patience, trust, and provision of unconditional freedom throughout my academic progress.

I am lucky to have an opportunity to carry out my research project at University of Kwazulu-Natal, Durban-South Africa during my study and it is solely because of Dr. M.N.Noolvi sir and his sincere effort.
I sincerely thank to all my colleagues and friends.

**Mr.Chirag J. Gohil**

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