**In-vitro Antiproliferative Activity Study of 2, 4, 5-Triphenyl-1H-imidazole Derivatives**

**Abstract**
Various substituted 2, 4, 5-triphenyl-1H-imidazole derivatives were synthesized and evaluated for *in vitro* antiproliferative activity against human non-small cell lung carcinoma-A549 cell lines by MTT assay method. Compound 2-iodo-6-methoxy-4-(4, 5-diphenyl-1H-imidazol-2-yl) phenol (6f) (IC$_{50}$: 15 μM) was found to be most promising which inhibits the growth of cancer cells (90.33% of total population).

**Keywords:** Antiproliferative activity; Imidazoles; Melanoma cells

**Introduction**
Heterocyclic compounds containing nitrogen atom in their structure showed pharmacological interest and belongs to the imidazole family. Substituted 2, 4, 5-triaryl-1H-imidazoles have been gained the remarkable significance due to their wide range of biological activities. Imidazole ring is one of the most important structures found in many natural products, and in pharmacologically active compounds such as anti-ulcerative agent cimetidine, the proton pump inhibitor omeprazole and the benzodiazepine antagonist flumazenil. They reduced the platelet aggregation in numerous animal species and humans.

Imidazoles are playing a vital role in biochemical processes [1]. Imidazole compounds have shown estrogen receptor and cytotoxic inhibitors of the cyclooxygenase [2], antifungal [3], antihepatic [4], analgesic [5], fungicidal [6], anti-inflammatory [7], antithrombotic activities [8]. Substituted imidazole possesses potentially novel therapeutic activities [9], and controlled processes [10]. In addition to these, they are also known for pesticidal and herbicidal activity. Imidazoles are the core structures of various biological systems such as histidine, histamine and biotin which are active components in several drug molecules (e.g., Losartan, Olmesartan and Eprosartan) peptides. Nowadays, scientists are trying to develop new synthetic methodology for the use of chemicals that reduce risk to human and animal species. Some methods are reported for the preparation of trisubstituted imidazoles from pharmacological and synthetic point of view.

In continuation of our previous research work in the development of synthetic methodologies such as, sulfated tin oxide: a reusable and highly efficient heterogeneous catalyst for the synthesis of 2,4,5-triaryl-1H-imidazole derivatives [11], and the bioactivity and synthesis of substituted benzimidazole motifs [12].

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efficient noncatalytic protocol for the synthesis of trisubstituted imidazole in polyethylene glycol using microwaves was studied [13]. An efficient synthesis of 2,4,5-triaryl substituted imidazoles under solvent free conditions at room temperature was reported [14]. Microwave-assisted facile synthesis of 2-substituted 2-imidazolines has been reported [15]. Certain synthetic method has been investigated including, an efficient synthesis of 2,4,5-triaryl-1H-imidazole derivatives catalyzed by boric acid in aqueous media under ultrasound-irradiation [16]. Same type of biological screening is done by our researchers such as; ionic liquid promoted synthesis, antibacterial and in vitro antiproliferative activity of novel α-aminophosphonate derivatives [17] induction of mitochondria mediated apoptosis in human breast cancer cells (t-47d) by Annona Reticulata l. leaves methanolic extracts [18].

Our previous reported research works explain only the synthetic methods of imidazoles. But, in this work, we evaluated antiproliferative activity of the synthesized substituted 2, 4, 5-triaryl-1H-imidazoles. The viability of human non-small cell lung carcinoma-A549 cells have been analyzed by MTT assay method.

Materials and Methods

Sample preparation

Each compound was dissolved in dimethyl sulfoxide (DMSO) to get a final concentration of 10 mg/ml and sterilized by 0.2-micron membrane filtration.

Cell culture conditions

Human non-small cell lung carcinoma A549 cells were maintained in RPMI 1640 (Life technologies Inc. USA) containing 10% heat-inactivated FBS (Life technologies Inc. USA) and 50 μg/mL Gentamicin (Himedia, India). The cells were grown at 37°C temperature in incubator supplied with 5% CO₂. When grow to 65%-80% confluency, the cells were trypsinized with 0.25% TPVG solution (Himedia, India), counted and aliquot at the desired density for growth assays. All the experiments were done using 48 h grown cells.

Determination of cell viability and the MIC

The effect of compounds on viability of A549 cells was determined by MTT cell proliferation assay. The cells were plated at ~ 1 × 10⁴ cells in each well of 96 well plates in 100 μL RPMI 1640 medium. 0 to 20 μM of compound was added to each well. Each concentration of imidazole compounds was repeated in 8 wells. Cell viability was determined after 24 h incubation in CO₂ incubator at 37°C. MTT (5 mg/mL in PBS) was added to each well and incubated for 4 h. The absorbance was recorded at 490 nm for all the 96 well plates Multiscan Ascent (Thermo Inc). The inhibitory effect of imidazole compounds on cell growth was assessed as percent cell viability. Cells without treatment were considered as more than 80% viable. The % viability of carcinoma cell analyzed in presence of substituted 2, 4, 5-triaryl-1H-imidazoles (Graph 1).

Time dependence and sensitivity assay

The cells were seeded at 10000 cells/well in 12-well plates. A fixed concentration of 10 μM was added to each well and incubated for 48 h. After an interval of 6 h, the % survival of each well was calculated. Time and concentration dependent analysis was done at 20 μM concentration of novel derivative of imidazoles (Graph 2).

Results and Discussion

In this study, a series of novel substituted 2,4,5-triphenyl-1H-imidazole derivatives (1a, 2b, 3c, 4d, 8h, 6f, 7g, 9i, 11j, 12k, 13l, 18m, 19n, 21o) have been evaluated for in vitro antiproliferative activity against human A549 cancer cell lines. We have examined the effect of various 2,4,5-triphenyl-1H-imidazole compounds on the viability of cultured carcinoma cells which showed promising antiproliferative activity against A549 cancer cell lines by MTT method. Melanoma cells were incubated with 20 μM 2, 4, 5-triphenyl-1H-imidazole derivatives for 24 hours and their viability was assessed. The cell viability was assessed by MTT assay after 24 h of growth at 37°C. The % viability of carcinoma cell analyzed in presence of substituted 2, 4, 5-triphenyl-1H-imidazoles. Time and concentration dependent analysis was done at 20 μM concentration of the novel derivative of imidazoles.

The compound 2-iodo-6-methoxy-4-(4,5-diphenyl-1H-imidazol-2-yl) phenol (6f) showed predominant growth inhibition of the melanoma cells 90.33% due to the presence of heteroatom nitrogen, phenolic -OH, -I and -OCH₃ groups.

We have not only explored promising antiproliferative activity of 6f compounds but also, other imidazole compounds exhibited potent in vitro antiproliferative activity. Newly substituted 2, 4, 5-triphenyl-1H-imidazole derivatives are (7g), (9i), (12k), (13l), (18m), (19n) and (21o) as compared to the standard (control C4p) have been showed promising lung carcinoma-A549 cell growth inhibition activity (Table 1).

![Graph 1](http://organic-inorganic.imedpub.com/archive.php)

**Graph 1**

Effect of imidazole derivatives on cancer cell line A549. The MTT assay was performed on A549 cell.

![Graph 2](http://organic-inorganic.imedpub.com/archive.php)

**Graph 2**

Time and dose dependent assay of imidazole derivatives on human lung carcinoma cell.
Table 1 Antiproliferative activity of 2,4,5-triphenyl-1H-imidazole derivatives against melanoma cell A549.

| Compounds                                                                 | Antiproliferative Activity |
|---------------------------------------------------------------------------|----------------------------|
| ![Compound 6f](image) 2-iodo-6-methoxy-4-(4,5-diphenyl-1H-imidazol-2-yl)phenol | ![Image](image)            |
| ![Compound 7g](image) 2-(3-chlorophenyl)-4,5-diphenyl-1H-imidazole         | ![Image](image)            |
| ![Compound 9i](image) 2-(3,4-dimethoxyphenyl)-4,5-diphenyl-1H-imidazole    | ![Image](image)            |
| ![Compound 12k](image) 2-bromo-6-methoxy-4-(4,5-diphenyl-1H-imidazol-2-yl)phenol | ![Image](image)            |
2-(4,5-diphenyl-1H-imidazol-2-yl)phenol

4-(4,5-diphenyl-1H-imidazol-2-yl)benzonitrile

2-(2-methyl-1H-imidazol-4-yl)-4,5-diphenyl-1H-imidazole

2-(3,4-difluorophenyl)-4,5-diphenyl-1H-imidazole

Control C4p
Among them, compounds 2-methoxy-4-(4,5-diphenyl-1H-imidazol-2-yl) phenol (4d) and 2-(3-nitrophenyl)-4,5-diphenyl-1H-imidazole (11j) showed moderate activity 48.18% and 45.16% respectively which is less than 50% of inhibition of cancer cell line as compared to the standard (control). Compound 2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazole (8h) showed very less antiproliferation of cells i.e., 29%, while compounds 2,4,5-triphenyl-1H-imidazole (1a), 4-(4,5-diphenyl-1H-imidazol-2-yl) phenol (2b) and 2-(4-methoxy phenyl)-4,5-diphenyl-1H-imidazole (3c) does not showed any inhibition activity against cancer cell line. Compounds 6f, 7g, 9i, 12k, 13l, 18m, 19n and 21o were found to be promising antiproliferative against melanoma cancer cell lines.

Conclusion
In the present study, some new imidazoles were evaluated for in vitro antiproliferative activity against human non-small cell lung carcinoma-A549 cell lines by MTT assay method. Tested compounds 6f, 7g, 9i, 12k, 13l, 18m, 19n and 21o showed significant growth inhibitory effects. Compound 2-iodo-6-methoxy-4-(4,5-diphenyl-1H-imidazol-2-yl) phenol (6f) (IC$_{50}$ 15 μM) was found to be most promising, inhibit growth of cancer cells (90.33% of total population).

This study provides innovative idea about in vitro antiproliferative activity of 2, 4, 5-triphenyl-1H-imidazole derivatives and provide a valuable information for further development of more potent anticancer agents.

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Conflicts of Interest
The authors confirm that this article content has no conflict of interest.

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