

## CYTOTOXIC STUDIES OF 3-(1-BENZOFURAN-2-YL)-5-(SUBSTITUTED ARYL) ISOXAZOLE

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### ABSTRACT:

The 3-(1-benzofuran-2-yl)-5-(substituted phenyl) isoxazole, I1-I7 was evaluated for *in vitro* cytotoxic activity on HeLa cell lines at the minimum seven concentrations at two fold dilutions. Amongst all the compound I4 has shown good activity. While, Compound I5 has shown moderate activity. The remaining tested compounds were not cytotoxic effect on tested cell lines.

**Keywords:** Benzofuran, Isoxazole, Cytotoxic activity, HeLa cell lines

### INTRODUCTION:

Cancer remains the leading cause of death in the World and as a result there is a pressing need for novel and effective treatments. One of the characteristic of cancer cells, that differs from their normal counterparts in a number of biochemical processes, particularly during the control of cell growth and division. Despite major breakthroughs in many areas of modern medicine over the past 100 years, the successful treatment of cancer remains a significant challenge at the start of the 21st century. Because it is difficult to discover novel agents that selectively kill tumor cells or inhibit their proliferation without the general toxicity, the use of traditional cancer chemotherapy is still very limited. In the field of chemotherapeutic drugs, the search for new, more active, more selective and less toxic compounds is still very intense, and new promising anticancer approaches are being tested<sup>1,2</sup>. Currently, combined anticancer therapies or multi-acting drugs are clinically preferred to traditional cytotoxic treatment, with the aim of overcoming resistance and toxicity drawbacks. These events often prevent successful treatment and are responsible for reduced survival times<sup>3,4</sup>. In the past 50 years, the mass screening of either synthetic derivatives or natural products has led to the

discovery of the currently utilized anticancer drugs.

Benzofuran nucleus may be combined with nitrogen heterocycles in different ways. Several benzofuran compounds are reported to possess antibacterial<sup>5</sup>, antifungal<sup>6</sup>, anti-inflammatory<sup>7</sup>, antidepressant<sup>8</sup>, analgesic<sup>9</sup> and hypoglycemic<sup>10</sup> activities. It has already been pointed out that; benzofuran nucleus is very rarely associated with a nitrogen heterocycle. Several isoxazole derivatives are found to possess antitubercular<sup>11</sup>, antibacterial<sup>11</sup> and antifungal<sup>11</sup> activities.

In our present research work we are synthesizing new compound i.e. benzofuran fused with isoxazole ring. Cytotoxic drugs remain the mainstay of cancer chemotherapy and are being administered with novel ways of therapy such as inhibitor of signals<sup>12</sup>. It is therefore important to discover novel cytotoxic agents with spectra of activity and toxicity that differ from those current agents<sup>12, 13</sup>. On the other hand, benzofuran ring system incorporated with different heterocyclic moieties has wide spectrum of anticancer against different types of carcinoma<sup>14-19</sup>. In the view of the facts mentioned above and as part of our initial efforts, we have reported cytotoxic activity of 3-(1-benzofuran-2-yl)-5-(substituted phenyl) isoxazole, I1-I7 against human cancer cell lines.

**MATERIALS AND METHODS****I] Synthesis of 3-(1-benzofuran-2-yl)-5-(substituted phenyl) isoxazole (I)**

The synthesis of the required derivatives is done by the method followed by Agarwal et al<sup>20</sup> and Manna and Agarwal<sup>21</sup>. Name and Structure of investigated compounds II-I7 is given in Table 1.

**II] *In-vitro* Evaluation of Cytotoxicity activity<sup>22</sup>**

The monolayer cell culture was trypsinized and the cell count was adjusted to  $1.0 \times 10^5$  cells/ml using DMEM medium containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100  $\mu$ l of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO<sub>2</sub> atmosphere and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the sample solutions in the wells were discarded and 50  $\mu$ l of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO<sub>2</sub> atmosphere. The supernatant was removed and 100  $\mu$ l of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC<sub>50</sub>) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth inhibition} = 100 - (\text{OD}_T / \text{OD}_C) \times 100$$

Where, OD<sub>T</sub> = Mean OD of individual test group and OD<sub>C</sub> = Mean OD of control group

**RESULTS AND DISCUSSION:**

The entire compounds were tested for cytotoxic assay in seven different concentrations and repeated twice. The compound which having average CTC<sub>50</sub> ( $\mu$ g/ml) value greater than 1000 is considered as

nontoxic to cells and below this value the compound confirmed cytotoxic property. The cytotoxic and/or growth inhibitory effect of the compound, II-7, were tested against HeLa cell lines of two fold dilution of seven concentration ranging from 1000-15.5  $\mu$ g/ml. The percentage growth was evaluated spectrophotometrically against control not treated with test agents. A 24 h continuous drug exposure protocol was followed and cell viability by MTT assay was used to estimate cell viability or growth. For the compound, the 50% cytotoxic concentration (CTC<sub>50</sub>) and total growth inhibition percentages (TGI) were obtained for each cell lines. The concentration of test drug needed to inhibit cell growth by 50% (CTC<sub>50</sub>) values is generated from the dose-response curves for each cell line. The percentage growth concentration which is greater than CTC<sub>50</sub> value is considered as cytotoxic active concentration. The percentage growth concentration which is minimum than the CTC<sub>50</sub> value was considered as nontoxic concentration.

Among the compounds II-I7 tested for cytotoxic activity in HeLa, the compound I4 shows potent cytotoxic effect, while compound I5 shows moderate cytotoxic effect on HeLa cells with CTC<sub>50</sub> value of 376.25 and 762.25  $\mu$ g/ml respectively. All the other compounds proved to be nontoxic with CTC<sub>50</sub> values above 1000  $\mu$ g/ml (Figure 1, Table 2 and 3).

**CONCLUSION:**

Benzofuran with fused isoxazole compounds were reported to possess, antibacterial, antifungal, anti-inflammatory and antitubercular activities. Here these moieties were screened for cytotoxic activity. Among these derivatives 4-Hydroxy benzaldehyde, 3-ethoxy,4-Hydroxy benzaldehyde substituted derivative (I4 and I5) showed good cytotoxic activity. Above results establish the fact that benzofuran with fused isoxazole can be a rich source for exploitation.

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Figure 1: Cytotoxicity effect of various compounds

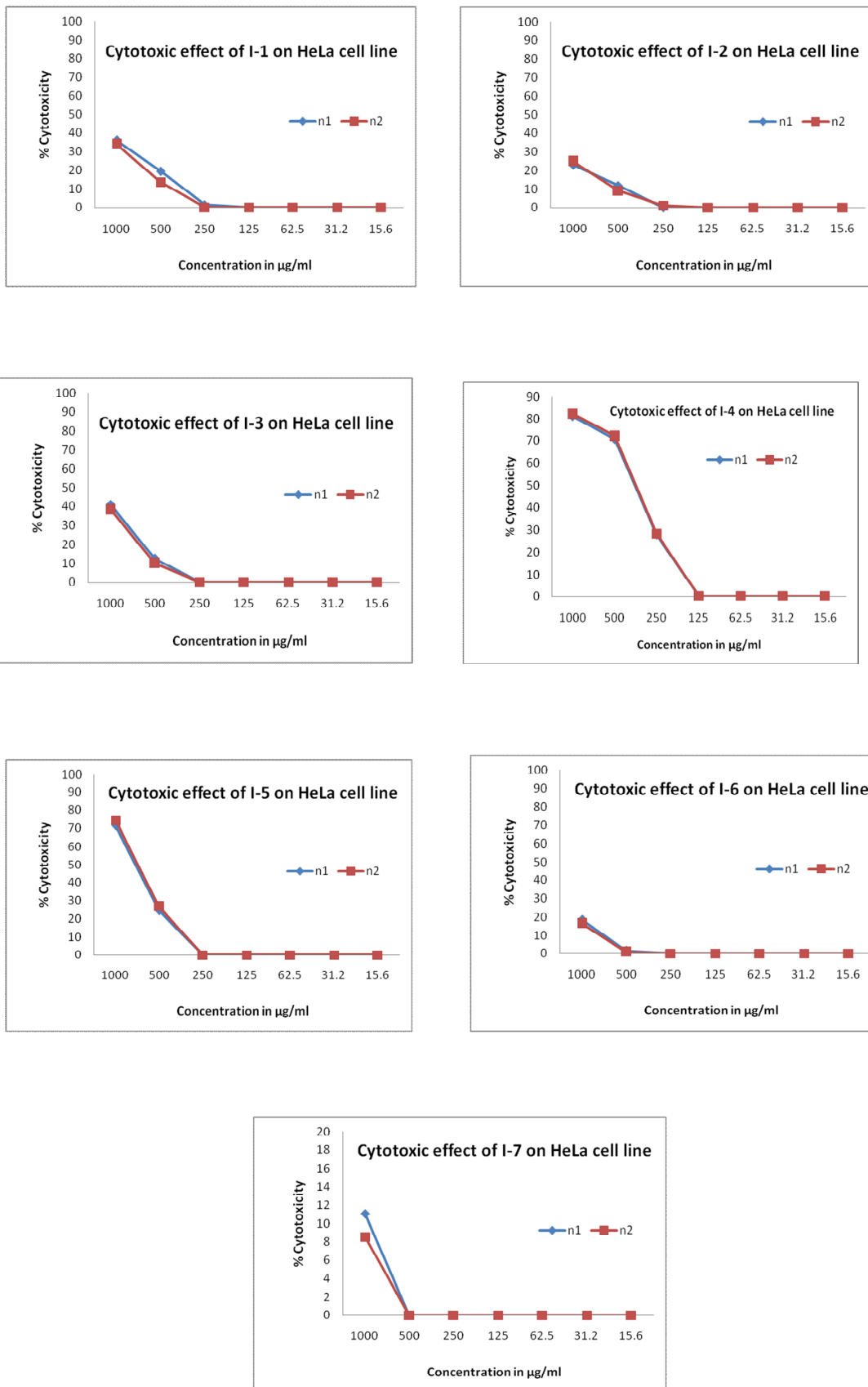
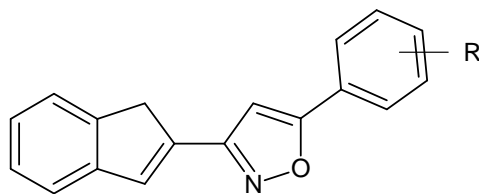


Table 1: Name and Structure of investigated compounds



I1-7

| Compound Code | Chemical Name   | R   |
|---------------|---|---|
| I1            | 3-(1-benzofuran-2-yl)-5-(phenyl)isoxazole                 | -H  |
| I2            | 3-(1-benzofuran-2-yl)-5-(4-nitrophenyl)isoxazole          | -NO <sub>2</sub> (p)                          |
| I3            | 3-(1-benzofuran-2-yl)-5-(3-nitrophenyl)isoxazole          | -NO <sub>2</sub> (m)                          |
| I4            | 4-[3-(1-benzofuran-2-yl)isoxazol-5-yl]phenol              | -OH (p)                                       |
| I5            | 4-[3-(1-benzofuran-2-yl)isoxazol-5-yl]-2-ethoxyphenol     | -C <sub>2</sub> H <sub>5</sub> O (m), -OH (p) |
| I6            | 3-(1-benzofuran-2-yl)-5-(3-methoxyphenyl)isoxazole        | CH <sub>3</sub> O (m)                         |
| I7            | 3-(1-benzofuran-2-yl)-5-(3,4,5-trimethoxyphenyl)isoxazole | -CH <sub>3</sub> O (o, m, p)                  |

Table2: Cytotoxicity properties of test compounds of I1-I7 on HeLa cell line

| Compound Code | Test Concentration | % Cytotoxicity |       | CTC <sub>50</sub> (µg/ml) |       | Average CTC <sub>50</sub> (µg/ml) |
|---------------|--------------------|----------------|-------|---------------------------|-------|-----------------------------------|
|               | (µg/ml)            | n1             | n2    | n1                        | n2    |                                   |
| <b>I1</b>     | 1000               | 36             | 33.85 | <1000                     | <1000 | <1000                             |
|               | 500                | 19.56          | 13.65 |                           |       |                                   |
|               | 250                | 1.42           | 0     |                           |       |                                   |
|               | 125                | 0              | 0     |                           |       |                                   |
|               | 62.5               | 0              | 0     |                           |       |                                   |
|               | 31.2               | 0              | 0     |                           |       |                                   |
|               | 15.6               | 0              | 0     |                           |       |                                   |
| <b>I2</b>     | 1000               | 23             | 24.75 | <1000                     | <1000 | <1000                             |
|               | 500                | 11.85          | 9.06  |                           |       |                                   |
|               | 250                | 0              | 1.05  |                           |       |                                   |
|               | 125                | 0              | 0     |                           |       |                                   |
|               | 62.5               | 0              | 0     |                           |       |                                   |
|               | 31.2               | 0              | 0     |                           |       |                                   |
|               | 15.6               | 0              | 0     |                           |       |                                   |
| <b>I3</b>     | 1000               | 41.09          | 38.65 | <1000                     | <1000 | <1000                             |
|               | 500                | 12.35          | 9.87  |                           |       |                                   |
|               | 250                | 0              | 0     |                           |       |                                   |
|               | 125                | 0              | 0     |                           |       |                                   |
|               | 62.5               | 0              | 0     |                           |       |                                   |
|               | 31.2               | 0              | 0     |                           |       |                                   |
|               | 15.6               | 0              | 0     |                           |       |                                   |
| <b>I-4</b>    | 1000               | 81.18          | 82.28 | 372.5                     | 380.0 | <b>376.25</b>                     |
|               | 500                | 70.88          | 72.08 |                           |       |                                   |
|               | 250                | 27.78          | 28.32 |                           |       |                                   |
|               | 125                | 0              | 0     |                           |       |                                   |
|               | 62.5               | 0              | 0     |                           |       |                                   |
|               | 31.2               | 0              | 0     |                           |       |                                   |
|               | 15.6               | 0              | 0     |                           |       |                                   |

| Compound Code | Test Concentration | % Cytotoxicity |       | CTC <sub>50</sub> (µg/ml) |       | Average CTC <sub>50</sub> (µg/ml) |
|---------------|--------------------|----------------|-------|---------------------------|-------|-----------------------------------|
|               | (µg/ml)            | n1             | n2    | n1                        | n2    |                                   |
| <b>I5</b>     | 1000               | 71.35          | 74.35 | 775.5                     | 750   | 762.25                            |
|               | 500                | 24.56          | 26.58 |                           |       |                                   |
|               | 250                | 0              | 0     |                           |       |                                   |
|               | 125                | 0              | 0     |                           |       |                                   |
|               | 62.5               | 0              | 0     |                           |       |                                   |
|               | 31.2               | 0              | 0     |                           |       |                                   |
|               | 15.6               | 0              | 0     |                           |       |                                   |
| <b>I6</b>     | 1000               | 18.56          | 16.5  | <1000                     | <1000 | <1000                             |
|               | 500                | 1.54           | 0.8   |                           |       |                                   |
|               | 250                | 0              | 0     |                           |       |                                   |
|               | 125                | 0              | 0     |                           |       |                                   |
|               | 62.5               | 0              | 0     |                           |       |                                   |
|               | 31.2               | 0              | 0     |                           |       |                                   |
|               | 15.6               | 0              | 0     |                           |       |                                   |
| <b>I7</b>     | 1000               | 11.05          | 8.56  | <1000                     | <1000 | <1000                             |
|               | 500                | 0              | 0     |                           |       |                                   |
|               | 250                | 0              | 0     |                           |       |                                   |
|               | 125                | 0              | 0     |                           |       |                                   |
|               | 62.5               | 0              | 0     |                           |       |                                   |
|               | 31.2               | 0              | 0     |                           |       |                                   |
|               | 15.6               | 0              | 0     |                           |       |                                   |

Table 3: Cytotoxicity property (CTC<sub>50</sub>) of test compounds of I1-I7 on HeLa cell line

| Concentration (µg/ml) | CTC <sub>50</sub> (µg/ml) |       |       |               |               |       |       |
|-----------------------|---------------------------|-------|-------|---------------|---------------|-------|-------|
|                       | I1                        | I2    | I3    | I4            | I5            | I6    | I7    |
| <b>1000</b>           | >1000                     | >1000 | >1000 | <b>376.25</b> | <b>762.25</b> | >1000 | >1000 |
| <b>500</b>            |                           |       |       |               |               |       |       |
| <b>250</b>            |                           |       |       |               |               |       |       |
| <b>125</b>            |                           |       |       |               |               |       |       |
| <b>62.5</b>           |                           |       |       |               |               |       |       |
| <b>31.2</b>           |                           |       |       |               |               |       |       |
| <b>15.6</b>           |                           |       |       |               |               |       |       |