International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 04 Special Issue: 09 | Sep -2017 www.irjet.net p-ISSN: 2395-0072
One Day International Seminar on Materials Science & Technology (ISMST 2017)
4th August 2017
Organized by
Department of Physics, Mother Teresa Women's University, Kodaikanal, Tamilnadu, India

# Anti-cancer and Cytotoxic studies on DAST crystal

**J. Arul Martin Mani[1], V. Joseph[1], P. Sagayaraj[1*]**

*[1] Department of Physics, Loyola College, Chennai 600034, India*

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***Abstract*** - The title compound 4-N,N-dimethylamino-4'-N'-methyl-stilbazolium tosylate (DAST) has been synthesized by metathesization of 4-N,N-dimethylamino-4'-N'-methyl stilbazolium iodide (DMSI) and sodium p-toluene sulfonate. Single crystal X-ray diffraction analysis confirms the unit cell parameters of the grown crystal. The present study was designed to investigate DAST for its anticancer activity on human breast cancer cells and cytotoxic effect. The results show strong inhibition against the MCF-7 cell lines and weak inhibition against the Vero cell lines which makes it suitable for pharmaceutical applications.

***Key Words***: DAST, Anticancer and cytotoxic activity

## 1. INTRODUCTION

Cancer is the major cause of mortality in the world. Reports say that breast cancer is one of the most common types of cancers in females. Though there are commonly used methods for the treatment of cancer, still there is a significant need to improve current cancer therapies and search for novel compounds [1-2]. Cell viability and cytotoxicity analysis are used for drug screening and to test new compounds [3]. The works on anticancer and cytotoxic activity measurements of various samples by MTT assay method has been reported [4-5]. Heterocyclic derivatives, especially pyridine based crystals were reported as potential materials for biological activities [6]. In this study, anticancer and cytotoxic activities of DAST against MCF-7 and Vero cell lines are investigated by MTT assay method.

## 2. EXPERIMENTAL PROCEDURE

### 2.1 Synthesis

DAST was prepared by metathesization of 4-N,N-dimethylamino-4'-N'-methyl stilbazolium iodide (DMSI) salt with sodium p-toluene sulfonate. DMSI was prepared by the condensation of 1,4-dimethyl pyridinium iodide (2.35 g, 10 mmol), methanol (30 ml) and 4-N, N-dimethylamino benzaldehyde (1.79 g, 10 mmol) in the presence of piperidine (0.2 ml) [7]. DMSI (0.7324 g, 2 mmol) and sodium p-toluene sulfonate (0.3883 g, 2 mmol) were simultaneously dissolved in 70 ml and 30 ml of Millipore water respectively at 70 °C. These two hot solutions were mixed and heated for 30 minutes at 70 °C and then allowed to cool naturally to room temperature. A red precipitate was obtained as a result of exchange reaction between anion and cation. Supersaturated solution of this dried salt was prepared in methanol and crystals were grown by slow evaporation technique. The photograph of the grown crystal is shown in Fig. 1.

**Fig - 1**: Photograph of grown DAST single crystal

## 3. RESULTS AND DISCUSSION

### 3.1 Single crystal XRD

XRD analysis for the grown crystal has been carried out to identify the cell parameters using an ENRAF NONIUS CAD 4 automatic X-ray diffractometer with MoKα (λ=0.7170 Å).

The lattice parameters are a = 10.3648 Å, b = 11.3224 Å, c = 17.8933 Å, α = γ = 90°, β = 92.24° and the grown crystal belongs to monoclinic system with space group Cc, which very well matches with the reported value [8].

### 3.2 Anticancer activity of DAST

The MCF-7 Cells (human breast cancer cells) were plated in 24-well plates (1 × $10^5$/well) and incubated in 37°C with 5% $CO_2$ condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100 µl/well (5 mg/ml) of 0.5 % 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1 ml of DMSO was added in all the wells. The absorbance at 570

nm was measured with a UV- Visible Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50 % inhibition (IC 50) was determined graphically. The effect of the samples on the proliferation of MCF-7 cells was expressed as the % cell viability, using the following formula:

$$\% \text{ Cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100$$

From the table 1, the concentration required for a 50% of inhibition (IC 50) was determined and it was clear that DAST has 49.37% cell inhibition at 31.2 µg/ml which means that nearly 50% of cancer cells were demolished by 31.2 µg/ml of DAST. From the observed values, it is inferred that DAST would be suitable for anticancer activity against MCF-7 cell line. Fig. 2 shows the anticancer effect of different concentration of DAST on MCF-7 cell lines. Chart - 1 shows Plot of % of Cell Viability of MCF-7 cells Vs concentration of DAST.

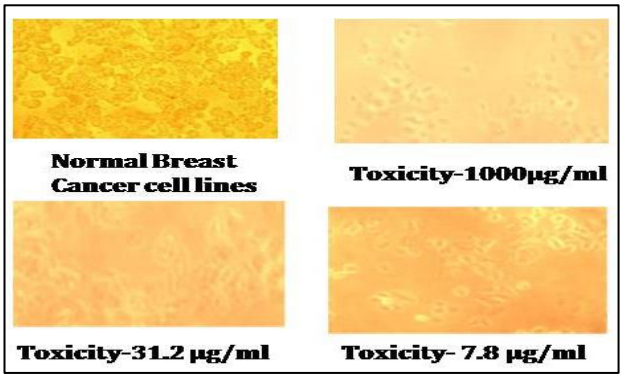


**Fig - 2:** Anticancer effect of Sample on *MCF-7 cell* line

| Concentration (µg/ml) | Dilutions | Absorbance (O.D) | Cell viability (%) |
|---|---|---|---|
| 1000 | Neat | 0.091 | 16.63 |
| 500 | 1:1 | 0.129 | 23.58 |
| 250 | 1:2 | 0.175 | 31.99 |
| 125 | 1:4 | 0.204 | 37.29 |
| 62.5 | 1:8 | 0.248 | 45.33 |
| 31.2 | 1:16 | 0.277 | 50.63 |
| 15.6 | 1:32 | 0.309 | 56.48 |
| 7.8 | 1:64 | 0.328 | 59.96 |
| Cell control | --- | 0.547 | 100 |

**Table - 1:** Anticancer effect of sample on MCF-7 cell line

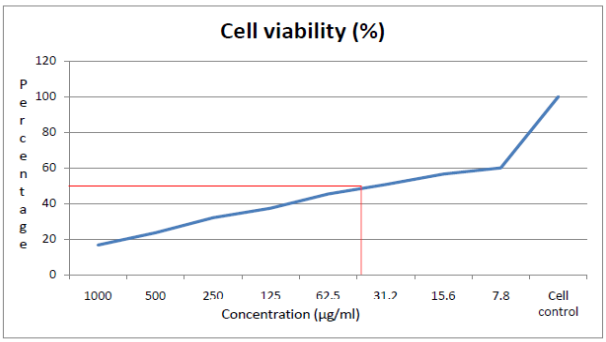


**Chart – 1:** Plot of % of Cell Viability Vs concentration of sample

### 3.3 Cytotoxicity measurement

The cytotoxicity analysis was carried out for DAST on normal Vero cell lines (monkey's kidney cells) to know the level of toxicity of various concentration of the sample and to find the safer value of DAST, which keeps more than 50 % of the normal cells viable when used as a drug ( IC 50 value). *Vero* cell lines were obtained from Veterinary College, Vepery, Chennai. The reagents and procedure was exactly same as in the case of anticancer analysis.

Cell control and sample control is included in each assay to compare the full cell viability assessments. The % cell viability against different concentrations of the sample is given in table 2. From this analysis, it is confirmed that even a higher concentration of DAST (1000 µg/ml) keeps 66.50 **%** of the normal cells viable (IC50 > 1000 µg/ml) while demolishing 83.37 **%** of cancerous cells. Thus, DAST showed a strong inhibition against the MCF-7 cell lines and weak inhibition against the Vero cell lines. In conclusion, DAST can be a better candidate for developing anticancer compounds. Fig. 3 shows the cytotoxic effect of various concentration of DAST on Vero cell lines. Chart-2 shows Plot of % of Cell Viability of Vero cells Vs concentration of DAST

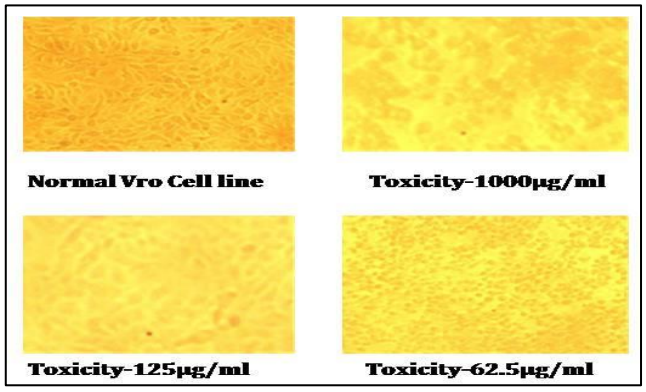


**Fig – 3:** Cytotoxicity effect of Sample on normal Vero cell line

**Table - 2:** Cytotoxicity effect of sample on Vero cell line.

| Concentration (µg/ml) | Dilutions | Absorbance (O.D) | Cell viability (%) |
|---|---|---|---|
| 1000 | Neat | 0.272 | 66.50 |
| 500 | 1:1 | 0.297 | 72.61 |
| 250 | 1:2 | 0.316 | 77.26 |
| 125 | 1:4 | 0.348 | 85.08 |
| 62.5 | 1:8 | 0.365 | 89.24 |
| 31.2 | 1:16 | 0.379 | 92.66 |
| 15.6 | 1:32 | 0.387 | 94.62 |
| 7.8 | 1:64 | 0.392 | 95.84 |
| Cell control | --- | 0.409 | 100 |

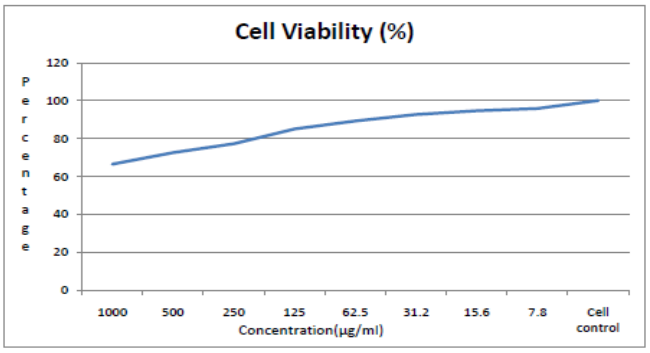


**Chart – 2:** Plot of % of Cell Viability Vs concentration of sample

## 4. CONCLUSION

Good quality single crystals of DAST were grown by slow evaporation technique. The grown crystals were subjected to single crystal XRD to identify its unit cell parameters and space group. The cytotoxic effect of DAST shows that it would be suitable for pharmaceutical use since its % of cell viability is more for normal cells and less for cancer cells (32.1 µg/ml concentration of the DAST kills 49.37 % of cancer cells along with 7.34 % of normal cells). Though DAST is well known for its NLO and THz properties, it is clearly evident from the above studies that this crystal is bioactive and further investigation to find out its potential use in industrial and pharmaceutical applications can be promoted in future.

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