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Research Article

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A study on the cytotoxic effect of certain organic crystals 4-methylanilinium-4-hydroxybenzenesulfonate, 2-amino-5-nitropyridiniumtrifluoroacetate and 2-amino-4-methylpyridiniumtartratemonohydrate

J. V. Jovita[1]*, D. Reuben Jonathan[2], S. Chidambaravinayagam[2], A. Ramanand[1] and P. Sagayaraj[1]

[1]PG & Research Department of Physics, Loyola College, Chennai, India
[2]PG & Research Department of Chemistry, Presidency College, Chennai, India

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ABSTRACT

A series of three organic crystals were generated by slow evaporation solution technique from the organic compounds 4-Methylanilinium-4-hydroxybenzenesulfonate, 2-Amino-5-nitropyridinium trifluoroacetate and 2-Amino-4-methylpyridinium tartrate monohydrate. Their cytotoxic character was investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method. These crystals in solution displayed potential cytotoxic activity.

Key words: Organic crystals, Cytotoxic activity, MTT assay method, % Cell viability

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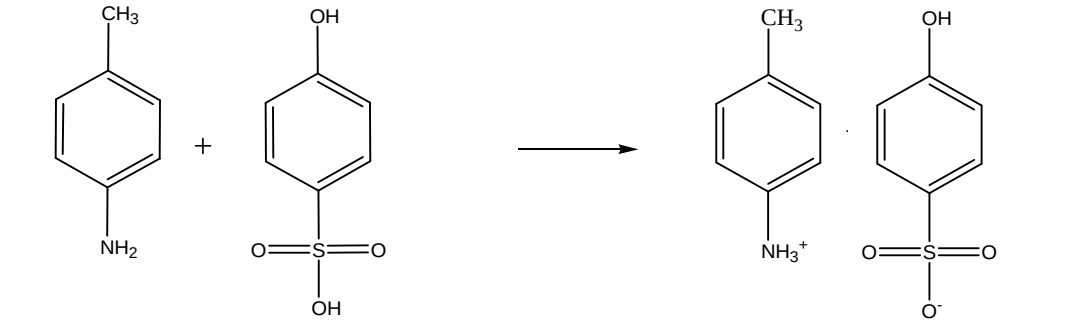
INTRODUCTION

Organic crystals [1-3] have attracted the attention of scientific community for more than three decades for their wide range of technological applications. Literature survey indicates that organic crystals are employed as meaningful materials in the development of device fabrication such as light emitting diodes [4], semiconductor optics [5], microwave photonics [6], telecommunication [7], optical information storage [8] optical computing [9] and in the field of nonlinear optics[10]. However, there is no much information available to indicate the interaction of organic crystalline materials with that of abnormal cell growth. Therefore, we present herein a study dealing with the investigation of three organic crystals derived from the organic compounds 4-Methylanilinium-4-hydroxybenzenesulfonate (MAPS), 2-Amino-5-nitropyridiniumtrifluoroacetate (ANPTFA) and 2-amino-4-methylpyridinium tartratemonohydrate (AMPTM) by slow evaporation solution method [11,12] for their cytotoxic activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method [13, 14].

EXPERIMENTAL SECTION

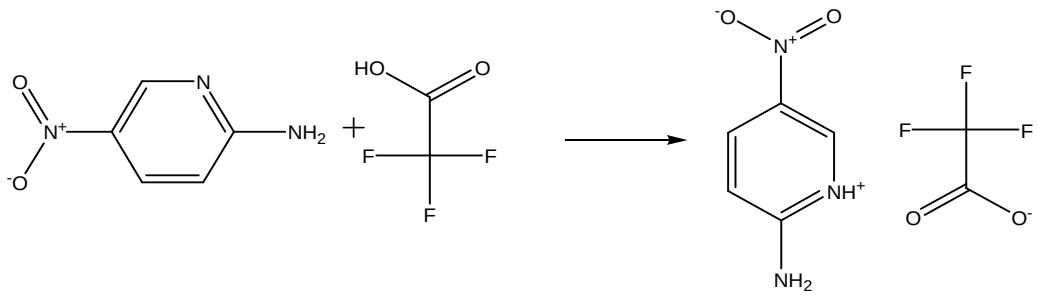
The organic crystals namely MAPS, ANPTFA and AMPTM were developed by slow evaporation solution method and reported earlier by Jovita and coworkers [15-17]. The reaction schemes of the compounds MAPS, ANPTFA and AMPTM are shown in figures 1, 2 and 3 respectively.

The compound 4-Methylanilinium-4-hydroxybenzenesulfonate was prepared from 4-methylaniline and 4-hydroxybenzenesulfonate. The compound 2-Amino-5-nitropyridinium trifluoroacetate was synthesized from 2-Amino-5-nitropyridine and trifluoroacetic acid. The compound 2-Amino-4-methylpyridiniumtartrate was prepared from 2-amino-4-methylpyridine and tartaric acid.



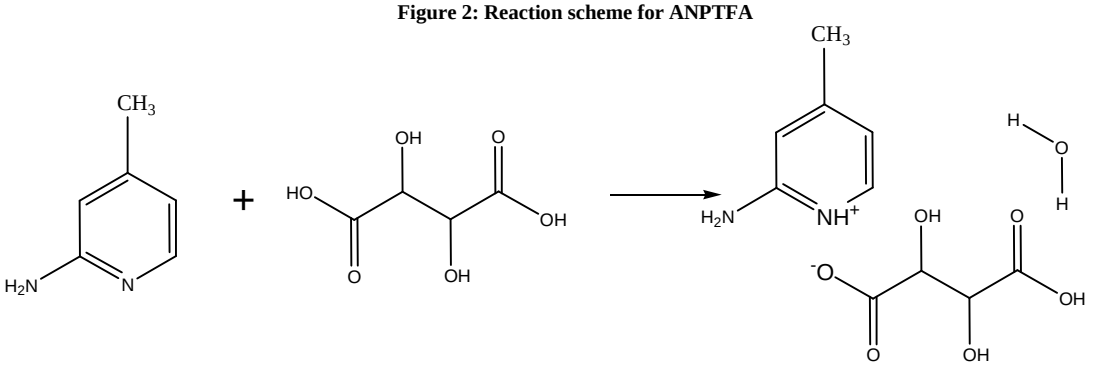
p-methylaniline p-phenol sulfonic acid 4-methylanilinium p-phenolsulfonate

Figure 1: Reaction scheme for MAPS



2-amino5-nitropyridine trifluoroacetic acid 2-amino-5- nitropyridinium trifluoroacetate

Figure 2: Reaction scheme for ANPTFA



2-Amino 4-methyl pyridine L-tartaric acid 2-Amino-4-methyl pyridinium tartrate monohydrate

Figure 3: Reaction scheme for AMPTM

Cytotoxicity Studies: (MTT Assay Method)

The cytotoxicity of sample crystals namely MAPS, ANPTFA and AMPTM was determined by the MTT assay method reported by Mosmann [18].

Procedure:

Cells (1×10^5/well) were plated in 5ml of medium/well in 6-well plates (Costar Corning, Rochester, NY). After 48 hours of incubation, the cell reaches the confluence. Then, cells were incubated in the presence of samples for 24-48 hours at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 1 ml/well (5 mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide cells (MTT) phosphate-buffered

saline solution was added. After 4 hours of incubation, 0.04M HCl/isopropanol was added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570nm was measured with a UV-Visible spectrophotometer using wells without sample containing cells as blank. The effect of the samples on the proliferation of Chang liver cell lines/Vero cell lines was expressed as the % cell viability, using the following formula:

% cell viability = (A570 of treated cells/A570 of control cells) × 100%

RESULTS AND DISCUSSION

The organic crystals MAPS, ANPTFA and AMPTM developed by slow evaporation solution method and reported earlier [15-17] were investigated for their cytotoxic activity by the MTT assay method.

MTT Assay method report on the crystal MAPS:

The organic crystal MAPS was investigated for its cytotoxic activity on Chang liver cell lines [19, 20] by the MTT assay method. The figure 4(a) shows the image of the normal Chang liver cell lines and 4(b) represents the image of cytotoxic effect of MAPS on and 62.5µg/ml concentrations.

The measurements were obtained and the % cell viability at different concentrations is represented in table 1. The plot of % cell viability of MAPS versus concentration in µg/ml is shown in figure 4(c).

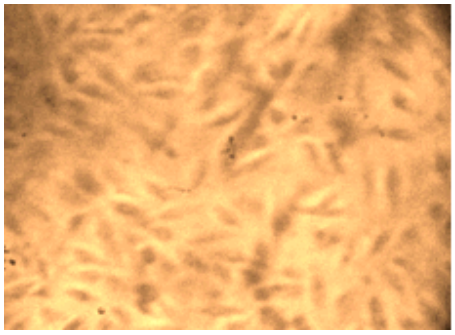


Figure 4(a): Normal Chang liver cell lines

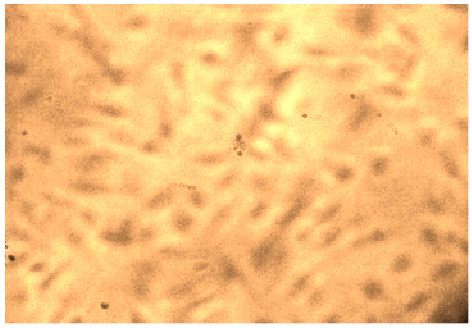


Figure 4(b): Cytotoxic effect of MAPS at 62.5µg/ml concentration

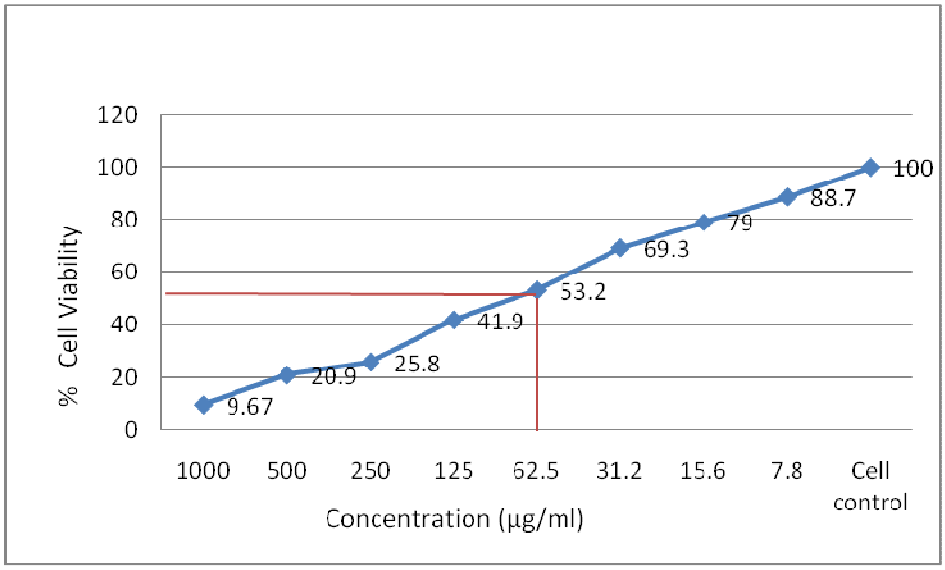


Figure 4(c): Plot of % Cell Viability versus Concentration

Table 1: Cytotoxic effect of MAPS on normal Chang liver cell lines

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.06	9.67
2	500	1:1	0.13	20.9
3	250	1:2	0.16	25.8
4	125	1:4	0.26	41.9
5	**62.5**	1:8	0.33	**53.2**
6	31.2	1:16	0.43	69.3
7	15.6	1:32	0.49	79.0
8	7.8	1:64	0.55	88.7
9	Cell control	-	0.62	100

The concentration required for a 50% of viability (IC50) was determined graphically by making use of MTT Cell Proliferation Assay Instruction Guide. It was clear that MAPS has 53.2% cell viability at 62.5µg/ml. From the observed values, it is inferred that MAPS may be a suitable material for pharmaceutical application with the concentration less than 62.5µg/ml. The crystal has a substantial cytotoxic effect, which may be a potential material for anticancer treatment. Similar observations were made by Wan Norlzzah Wan Mohd Zain *et al.* [21] in a chemical compound named Clausine-B.

MTT Assay method report on the crystal ANPTFA:

The organic crystal ANPTFA was investigated for its cytotoxic activity on Vero cell lines [22, 23] by the MTT assay method. The figure 5(a) represents the image of the normal Vero cell lines and 5(b) shows the image of the cytotoxic effect of ANPTFA on normal Vero cell lines at a concentration of 125µg/ml.

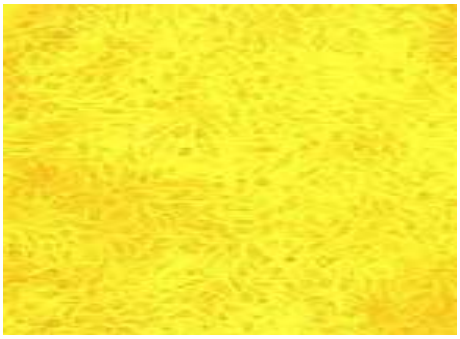


Figure 5(a): Normal Vero cell lines

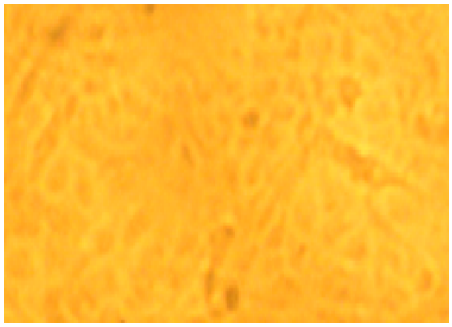


Figure 5(b): Cytotoxic effect of ANPTFA at 125 µg/ml concentration

The table 2 indicates the % cell viability at different concentrations of ANPTFA. The plot of % cell viability versus concentration in µg/ml is shown in figure 6(c).

Table 2: Cytotoxic effect of ANPTFA on Vero cell lines

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.11	18.96
2	500	1:1	0.19	32.75
3	250	1:2	0.24	41.37
4	**125**	1:4	0.29	**50**
5	62.5	1:8	0.36	62.06
6	31.2	1:16	0.42	72.41
7	15.6	1:32	0.48	82.75
8	7.8	1:64	0.54	93.1
9	Cell control	-	0.58	100

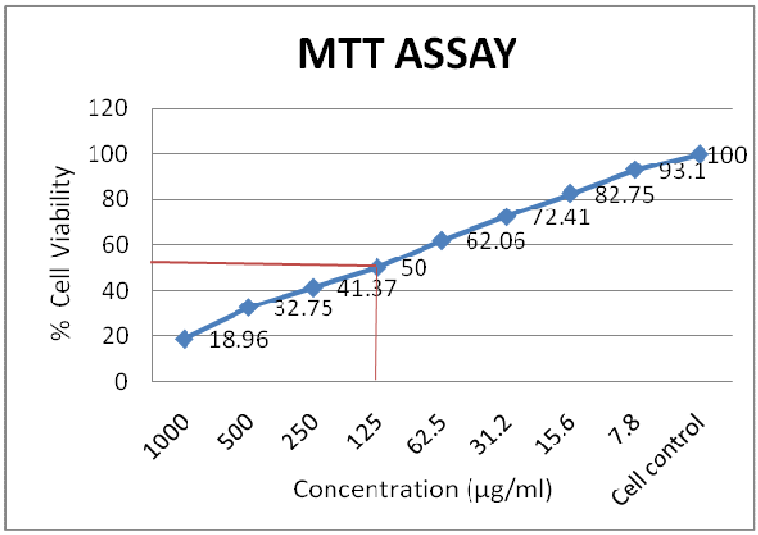


Figure 5(c): Plot of % Cell Viability versus Concentration

The concentration required for a 50% of viability (IC50) was determined graphically by the usual method. It is clear that ANPTFA has 50% cell viability at a concentration of 125µg/ml. From the observed values, it is understood that the ANPTFA has a considerable cytotoxic effect; therefore it may serve as an appropriate material in the anti-cancer treatment at a concentration less than 125µg/ml.

MTT Assay method on the crystal AMPTM:

The organic crystal AMPTM was investigated for its cytotoxic activity on Vero cell lines by the MTT assay method. Figure 6(a) represents the image of the normal Vero cell lines and 6(b) shows the image of the cytotoxic effect of AMPTM on normal Vero cell lines at a concentration of 125µg/ml.

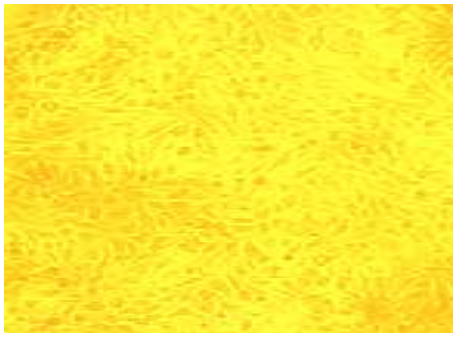


Figure 6(a): Cytotoxic effect of AMPTM on Vero cell lines

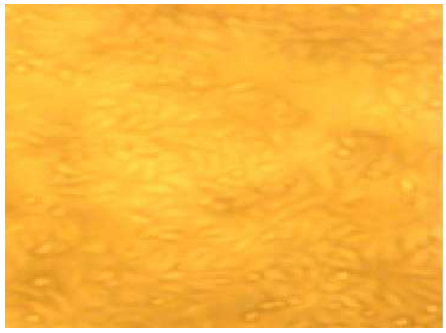


Figure 6(b): Cytotoxic effect of AMPTM on Vero cell lines 125µg/ml concentration

Table 3: Cytotoxic effect of AMPTM on Vero cell lines

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.10	17.24
2	500	1:1	0.17	29.31
3	250	1:2	0.23	39.65
4	**125**	1:4	0.28	**48.27**
5	62.5	1:8	0.34	58.62
6	31.2	1:16	0.41	70.68
7	15.6	1:32	0.46	79.31
8	7.8	1:64	0.52	89.65
9	Cell control	-	0.58	100

The table 3 indicates the % cell viability at different concentrations of AMPTM. Plot of % cell viability versus concentration is given in figure 6(c).

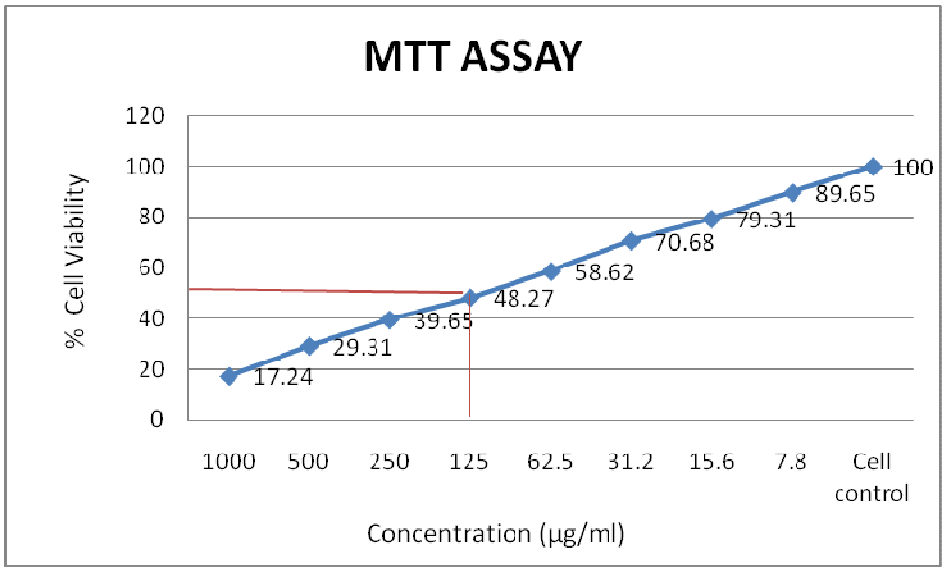


Figure 6(c): Plot of % Cell Viability versus Concentration

The concentration required for a 50% of viability (IC50) was determined graphically by the usual method. The observed cell viability of 48% for the crystal AMPTM is less when compared to the 50% for the crystal ANPTFA and 53.2% for that of the crystal MAPS which were investigated in this present work. Thus, AMPTM has the combined advantages of nonlinearity and bioactive properties.

CONCLUSION

The organic crystals MAPS, ANPTFA and AMPTM developed by slow evaporation solution method and reported earlier by Jovita and coworkers were investigated for their cytotoxic activity by the MTT assay method. The organic crystal MAPS was investigated for its cytotoxic activity on Chang liver cell lines and had 50% inhabitation of viability at a concentration less than 62.5µg/ml. The organic crystals ANPTFA and AMPTM were investigated for their cytotoxic activity on Vero cell lines and had 50% and 48% inhabitation of viability respectively at a concentration of 125µg/ml. So these novel organic crystals may emerge as suitable resources for anti-cancer treatment.

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