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SYNTHESIS AND CHARACTERIZATION OF L-AMINO ACID DOPED 2-AMINOPYRIDINE CO-CRYSTALS FOR ANTI CANCER ACTIVITY

A. Sinthiya[1*], M. Koperuncholan[2]

1. PG & Research Department of Physics, Srimad Andavan Arts and Science College (Autonomous), Tiruchirappalli, Tamilnadu, India.

2. Department of Botany, Srimad Andavan Arts and Science College (Autonomous), Tiruchirappalli, Tamilnadu, India.

ABSTRACT: Co-crystals bis-2-aminopyridinium aspartate, 2-aminopyridinium-leucinate, bis-2-aminopyridinuim glutamate and 2-aminopyridinuim-tyrosinate synthesised by the conventional slow evaporation method. The hydrogen bonding interaction and functional groups identified from the FTIR spectrum for the samples prepared in (saturated solution) 1:0.5 molar ratio. The UV spectra for 2-aminopyridinium-leucinate show that the absorbance takes place at lower wavelength 240nm and for the bis-2-aminopyridinium aspartate the absorbance takes place at higher wavelength 290nm compared to other compounds. Bis-2-aminopyridinium aspartate (50µg/mL concentration) act as a good candidate to treating on human osteosarcoma cell compared to other three compounds.

KEYWORDS: 2-aminopyridine, Amino acids, FTIR spectrum, Anticancer activity.

Corresponding Author: Dr. A. Sinthiya* Ph.D.
PG & Research Department of Physics, Srimad Andavan Arts and Science College (Autonomous), Tiruchirappalli 620 005, Tamilnadu, India.

1.INTRODUCTION

Core structural unt in the skeleton of proteins depends on an amide linkage –CO-NH. Amide – based molecules are considered to be the candidate for drug research because of their biological compatability and based on this, the further developments of new amide derivatives has become essental due to their vital application in fungicidal, herbicidal, insecticidal and anticancer [1]. Hydrogen bonding plays a key role in molecular recognition and crystal engineering research [2], [3], [4]. Because of the side chain rings staking with one another in amino acids also play vital role

in the physical properties of peptides and proteins. Amino acids alone or together with insulin were able to regulate expression of several genes involved in the metabolism of carbohydrates and lipids. The properties of α-amino acids area unit advanced, yet simplistic in that every molecule of an amino acid involves two functional groups: carboxyl (-COOH) and amino ($-NH_2$). Side chains having pure organic compound alkyl group or aromatic teams area unit thought of non-polar, and these amino acids are comprised of Phenylalanine, Glycine, Alanine, Isoleucine, Methionine, and Tryptophan. Meanwhile, if the aspect chain contains totally different polar teams like amides, acids, and alcohols, they are classified as polar. Their list includes Serine, Asparagine, Threonine, Glutamine, and Cysteine. Glutamate is involved in Neuron inflammation in autism and is a major excitatory neurotransmitter in the brain [5]. Glutamic acid performs a vital role in brain disorders like Parkinson illness, dementia praecox, and brain disorder and conjointly helps in correcting behavioural disorders of childhood. Aspartic acid, an excitatory neurotransmitter, is a metabolite in the urea cycle and helps in the removal of ammonia [6]. Leucine an essential amino acid helps in the formation of sterols in adipose and muscle tissue [7]. It stimulates the synthesis of muscle protein. Tyrosine is one of the building blocks of protein and is especially important for its role as a precursor to dopamine. It becomes an essential amino acid has provided to the organism [8]. Based on these backgrounds this work report that the through an amide linkage the 2-aminopyridine interact with L-aspartic acid, L-Leucine, L-Glutamic acid, and L-Tyrosine and forms co crystals. Cancer may be the most feared disease of our time [9]. It is second only to heart disease as a leading cause of death in the United States and it is estimated that about one out of every three Americans will develop cancer at some point during his or her life [10]. Currently, although intensive research and some major advances in treatment are attempting to reduce this figure, cancer claims the life of nearly one out of every four Americans [11]. About 1 million cases of cancer are diagnosed every year in this country, and about 500,000 Americans die annually of the disease [12]. Moreover, the number of cancer deaths continues to increase steadily. For example, about 514,000 Americans died of cancer in 1991[13]. The corresponding number [14] has about 510,000 in 1990 and 502,000 in 1989. Over the past 25 years, the United States government, through the National Cancer Institute, has expended a total of approximately $30 billion, and undeniable progress has made [15]. The most important progress that has been made is our understanding of cancer at the cellular and molecular levels. The discoveries of oncogenes and tumor suppressor genes have afforded a conceptual framework to understand the mechanisms that control normal cell growth and differentiation, and the ways in which breakdown of these normal cellular controls leads to the development of cancer. Significant progress has also been made in identifying the causes of several cancers, as well as in detecting some cancers at early, readily treatable statues. If current recommendations for cancer prevention and early detection has put into general practice, they would result in about a two-fold reduction in total cancer mortality [16]. Progress has also been made in

the treatment of some cancers. Certain cancers can effectively controlled by drug combinations, such as acute lymphocytic leukaemia, Hodgkin's disease, some non-Hodgkin's lymphomas, and testicular cancer [17]. Relative to 1971, the consequences of chemotherapy has managed with greater efficacy with antiemetic's and immune-stimulants. In the War on Cancer, and through the history of combating the disease of cancer, natural products have played an important role in the development of contemporary cancer chemotherapy. Between 1960 and 1982 the National Cancer Institute screened around 114,000 extracts from an estimated 35,00 plant samples for anticancer activity. They initiated a new natural products program with a new in vitro human cancer cell line screen in 1987, and as of December, 1991, 28,800 plant samples had been collected from over 20 countries to screen for anticancer activity [18].

2. MATERIALS AND METHODS

Experimental Procedure

The entire chemical purchased from Aldrich Sigma and deionized distilled water used as solvent. Saturated solution of 2-aminopyridine prepared and as a dopant L-aspartic acid L-Leucine, L-glutamic acid and L-tyrosine added in 1:0.5 molar ratio. Co-crystals harvested after one month by conventional slow evaporation method

FTIR spectrum

FTIR spectrum for samples collected from JASCO IR spectrometer, between the ranges 399 cm^{-1} – 4000 cm^{-1} with Scanning Speed - 2 mm/sec.

UV Vis Spectrum

The UV- Vis spectrum collected from ELICO UV Vis spectrometer between ranges 200 nm to 300 nm with the Scan speed 10 nm/min.

Anticancer Activity

For anticancer study, Samples (2-aminopyridine, bis-2-aminopyridinium aspartate, 2-aminopyridinium-leucinate, bis-2-aminopyridinuim glutamate and 2-aminopyridinuim-tyrosinate) has dissolved in DMSO. diluted in culture medium and used to treat the chosen cell line (Hep G2) (obtained from NCCS) over a sample concentration (5 different concentrations – 0.1, 1, 10, 25 and 50 µg/mL) range of 1 - 50 µg/mL for a period of 24 h and 48 h. The DMSO solution has used as the solvent control. A miniaturized viability assay using 3-(4, 5-di-methylthiazol-2-yl)-2, 5-diphenyl-2H-tetra-zolium bromide (MTT) was carried out according to the method described by standard procedure [19]. To each well, 20 µl of 5 mg/mL MTT in phosphate-buffer (PBS) was added and wrapped with aluminium foil, and incubated for 4 h at 37 ^{0}C. The purple Formosan product has dissolved by addition of 100 µl of 100 % DMSO to each well. The absorbance was monitored at 570 nm (measurement) and 630 nm (reference) using a 96 well plate reader (Bio-Rad, Hercules, CA, USA). Data have collected for four replicates. Each and used to calculate the respective means. The percentage of inhibition has calculated, from this data, using the formula:

$$\frac{\text{Mean absorbance of untreated cells (control) – mean absorbance of treated cells (test) x100}}{\text{Mean absorbance of untreated cells (control)}}$$

3. RESULTS AND DISCUSSION

FTIR spectrum

Based on the spectrum analysis show that the 2-aminopyridine was protonated from carbonyl group of L-aspartic acid, L-leucine, L-glutamic acid and L-Tyrosine and resulted as bis-2-aminopyridinium aspartate, 2-aminopyridinium-leucinate, bis-2-aminopyridinuim glutamate and 2-aminopyridinuim-tyrosinate co-crystals respectively. This confirmed from the FTIR spectrum of these co-crystals and shown in figure 2. The absorption peaks at 3448, 3559 cm^{-1}, 3518 cm^{-1}, 3346 cm^{-1} due to the presence of $O_{(carbonyl)}…H_{(pyridine\ N)}$ Hydrogen bond between 2-aminopyridine and carbonyl group of L-aspartic acid, L-leucine, L-glutamic acid and L-Tyrosine respectively.

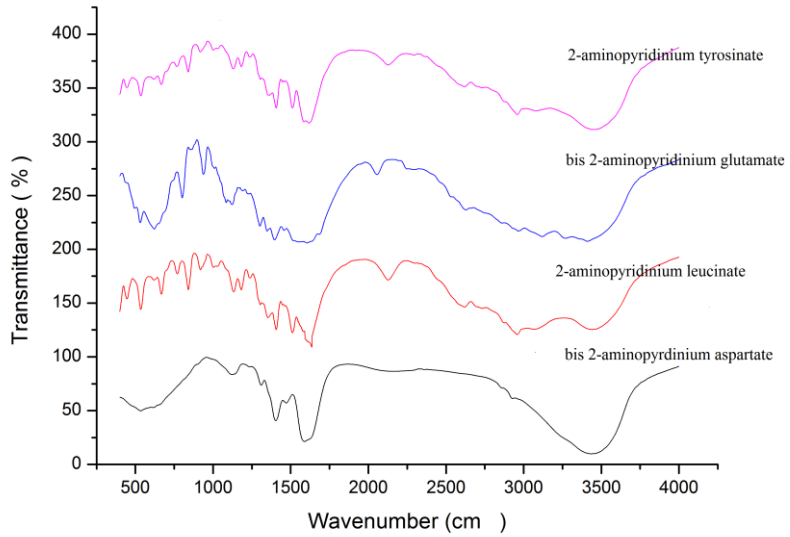

Figure 1: FTIR spectrum of amino acid doped 2-aminopyridine co-crystals

The peak at 3000 cm^{-1} disappears in all co-crystals. And new peak at 1595 cm^{-1}, 1617 cm^{-1}, 1734 cm^{-1}, 1665 cm^{-1} appears in co-crystals of bis-2-aminopyridinium aspartate, 2-aminopyridinium-leucinate, bis-2-aminopyridinuim glutamate and 2-aminopyridinuim-tyrosinate respectively shown in figure 1. This indicates the condensation / interaction of 2-aminopyridine with L-aspartic acid, L-leucine, L-Glutamic acid and L-tyrosine.

UV- Spectrum

The UV spectra show absorbance at 216 nm for standard 2-aminopyridine. The absorbance spectra for individual samples collected for selected wavelength range in order to highlight the absorbance peak accurately. The UV spectra show absorption at 290nm, 243nm, 280nm and 270nm for bis-2-aminopyridinium aspartate, 2-aminopyridinium-leucinate, bis-2-aminopyridinuim glutamate and 2-

aminopyridinuim-tyrosinate respectively shown in figure 2. These absorbance peaks varies for the samples prepared in 2:1 molar ratio (100ml) and 1:0.5(Saturated solution) molar ratio due to the concentration of doped amino acids in the samples. The UV-Vis spectrum shows that the molecules closely packed and undergoes $n \rightarrow \pi^*$ transition in all these co-crystal.

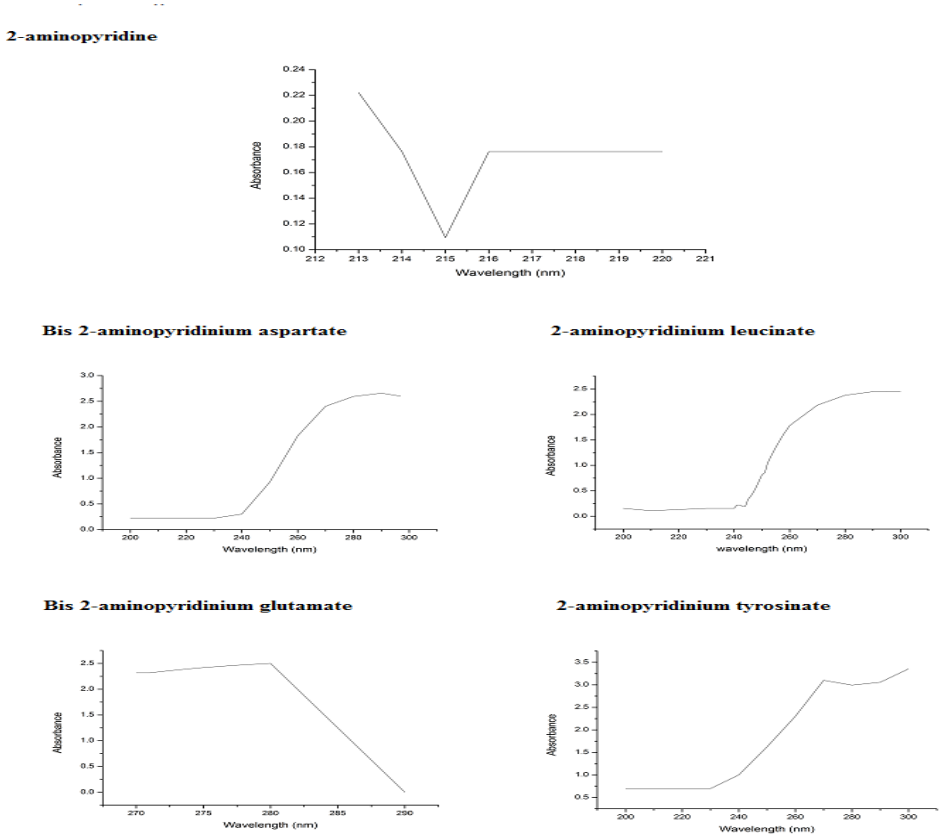

Figure 2: UV -Vis spectrum of amino acid doped 2-aminopyridine co-crystals

Anti-cancer activity

To assess for preliminary anticancer activity in terms of cell viability, the MTT and MTS in vitro cytotoxicity assays are considered two of the most economic, reliable and convenient methods. In late stages of programmed cell death, cells split to form apoptotic bodies. Each apoptotic body contains only function of the original cell's DNA content. When stained with PI, this population has known as the sub-G1 population and has qualified by receiving a DNA content of less than 2n chromosomes. In addition, apoptotic cells demonstrate specific morphological changes such as chromatin condensation and plasma membrane blebbing [20], [21], [22]. In the MTT assay, 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenylte-trazolium-bromide is bio reduced by dehydrogenase inside living cells to make a colored Formosan dye, while in the MTS assay, a similar bioconversion takes places utilizing 3-(4,5-dimethylthiazol-2-yl) -5-(3-carboxymethoxyphenyl) -2-(4-sulfophenyl) -2H-tetrazolium, inner salt and an electron coupling reagent [23], [24], [25]. The cytotoxic effect of the, 2-aminopyridine, bis-2-aminopyridinium aspartate, 2-aminopyridinium-leucinate, bis-2-aminopyridinuim glutamate and 2-aminopyridinuim-tyrosinate examined on human osteosarcoma

cell lines (Hep G2) for 24 h and 48 h (Sample conc. = 0.1 – 50 µg/mL). The cytotoxicity effect very high in bis-2-aminopyridinium aspartate than all other compounds. In all concentrations against Hep G2 cell lines and stamped down the development of the cancer cells significantly, in a back breaker and a duration dependent manner. Due to its Amphiphilic nature and hence would penetrate the cell membrane easily, thin out the energy status in tumor and alter hypoxia status in the cancer cell. It should point out that one of the primary problems of conventional anticancer therapy is multidrug resistance (MDR), whereby cells acquire resistance to structurally and functionally unrelated drugs following chemotherapeutic treatment [26], [27], [28]. One of the primary causes of MDR is overexpression of the P-glycoprotein transporter [29], [30], [31], [32]. The cytotoxic activity, according to the dose values of the exposure of the complex required to reduce survival to 50% (IC50), compared to untreated cells shown in image 3.

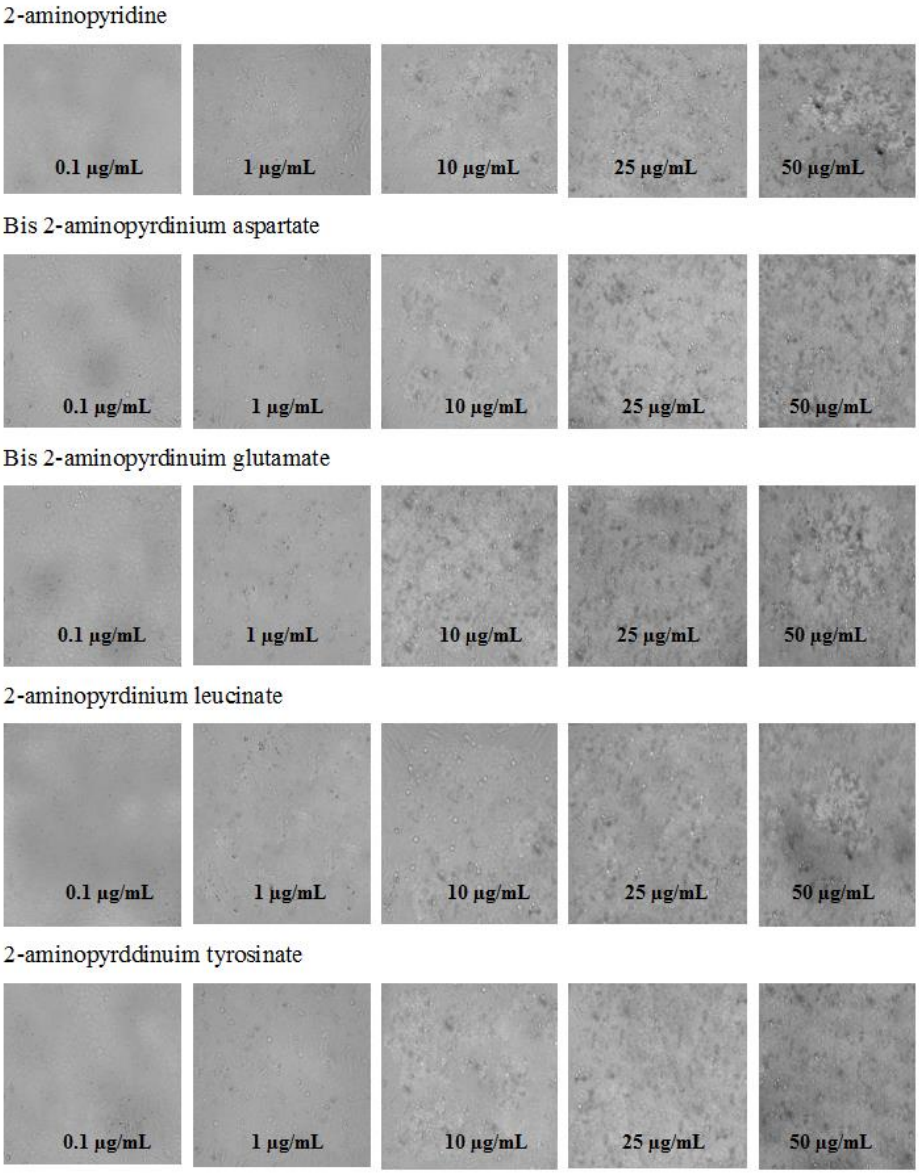

Figure 3: Anticancer activity of amino acid doped 2-aminopyridine co-crystals

4. CONCLUSION

The bis-2-aminopyridinium aspartate, 2-aminopyridinium-leucinate, bis-2-aminopyridinuim glutamate and 2-aminopyridinuim-tyrosinate co-crystals formed due to the protonation of 2-aminopyridine and deprotonating of carbonyl group present in amino acids. These co-crystals characterized by FTIR spectrum and UV spectrum. Among these samples bis-2-aminopyridinium aspartate kill the cancer cells compared to other synthesised compounds.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

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