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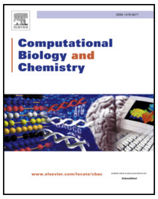

Research Article

Experimental and theoretical spectroscopic studies in relation to molecular structure investigation of para chloro, para fluoro and para nitro maleanilinic acids

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ABSTRACT

In the present work novel para chloro, para fluoro and para nitro maleanilic acid derivatives were prepared and investigated using FT-IR, thermal analyses (TA) measurements (TGA/DTG and DTA) in comparison with mass spectral (MS) fragmentation at 70 eV. The crystallographic structures of studied compounds were investigated by X-ray diffraction (XRD). The vibrational frequencies and the corresponding normal modes were evaluated at the optimized geometry. Vibrational modes were analyzed using GAUSSVIEW software. Experimental FT-IR and Raman spectra of the three newly prepared derivatives, namely, (E) – oxo -4- ((4- nitro phenyl) amino)-4-oxobut-2-enoic acid (p-NMA), (E) -4- ((4- chloro phenyl)amino)-4- oxobut -2- enoic acid (p-ClMA), (E) -4- ((4-fluoro phenyl) amino)- 4- oxobut -2- enoic acid (p-FMA) were compared with the theoretically calculated one. FT-IR and the observed vibrational frequencies were assigned; thermal analysis and mass spectrum measurements of the maleanilic acid derivatives were recorded and discussed. The computational calculations were carried out by DFT - B3LYP method with 6-311 + + G(d,p) basis sets and the corresponding results were tabulated. The alternations of structures of p-FMA, p-ClMA and p-NMA due to the subsequent substitutions were investigated. This correlation between experimental and theoretical calculations provided a good confirmation of the proposed structures of the newly prepared compounds. The derivatives were found to be highly effective against Hepatocellular carcinoma cells > Breast carcinoma cells > colon carcinoma cells. It was recognized, that cancer cells over expression promotes tumorigenic functions; can be suppressed by p-NMA > p-FMA > p-ClMA inhibitors.

1. Introduction

Many publications regarding the synthesis of maleanilic acids using equimolar of aniline or its derivatives with maleic anhydride in the presence of solvents were performed (Cava et al., 1961; Khan et al., 2009; Gowda and Mahendra, 2007; Vukovic et al., 1999; Roth, 1978; Ryttel and Makromol, 1999). Different solvents can be used, ether, acetic acid, acetone, nitro benzene, chloroform or mixed solvents such as equal volume of ethyl alcohol and water. N-substituted maleanilic acid derivatives can be used to prepare the maleimides, which are an important class of substrates for biological as chemical probes of protein structure (Corrie, 1994). It can be used as protective and curative fungicides (Fujinami et al., 1972). In polymer chemistry they can be used as photo initiators for free-radical polymerization (Body et al., 2006; Griffiths et al., 1981; Morder, 1987; Rich et al., 1975; Keller and Rudinger, 1975; Nath et al., 2001; Gielen, 2002; Chandrasekhar et al., 2002). They can also be used as monomers in poly-maleimides or their copolymers synthesis. The computational chemistry (Mulholland and Dundas, 2018) is rapidly growing using home computers and getting faster; which can be used for the interpretation of experimental results (Mura et al., 2002). Therefore this paper is mainly focused on studying molecular structure- reactivity relationship of maleanilic acid- derivatives via investigation of their decomposition mechanisms using thermal (TA) in comparison with the theoretical molecular orbital calculations. Thermogravimetric (TG/DTG) analysis is used to provide quantitative information on weight losses due to decomposition as a function of time and temperature. This work involved structure studies of halogen and nitrogen N-substituted maleanilinic acid by spectroscopic techniqes, also included a correlation between, mass spectral fragmentation and thermal analysis degradation of the selected

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derivatives. The experimental results obtained are also correlated with the theoretical data of MOCS to identify the weakest bonds broken during both mass and thermal studies. Knowing this structural session of bonds can be used to decide the active sites of this derivative responsible for its chemical and biological properties.

2. Experimental

2.1. Materials and procedures

All chemicals were purchased from Arcos, Oxford and Merck Chemical Co. (Germany). Maleic anhydride, p-Fluoroaniline, p-Chloroaniline and p-Nitroaniline were used as received. Three N-maleanilic acids were prepared according to the procedures reported in the literature (Saedi, 2013). Aniline derivative of 12.75 g (0. 1 mol) was mixed and ground at room temperature with maleic anhydride 9.8 g (0. 1 mol) in an agate mortar. During crunching processes greenish-yellow color products appeared. The grinding was continued for 30 min or more. The crude products were crystallized from ethanol and collected greenish-yellow crystals were dried. Melting points of the products were measured.

2.2. Instrumental analyses

2.2.1. FT- IR

The FT-IR spectra were recorded on Thermo Electron Corporation, Madison, WI 53711, USA in the range of 4000-400 cm^{-1}. The spectral resolution is ± 2 cm^{-1} and spectrometer using KBr disc technique. The Rama spectrum was obtained in (Model Sentra, Bruker, Germany) equipment, at laser wave length 532 nm [doubled Nd:YAG laser (neodymium-doped yttrium aluminum garnet)] and power 10 mW. The equipment is fitted with an Olympus metallurgical microscope and the sample was investigated on microscope slide with an x80 lens. The spectrum was obtained at room temperature in the spectral range of 4000-100 cm^{-1}.

2.2.2. Mass spectrometry (MS)

Electron ionization (EI) mass spectrum was obtained using Thermo Finnegan TRACE DSQ quadruple mass spectrometer with electron multiplier detector equipped with GCMS data system. EI mass spectra were obtained at ionizing energy value of 70 eV, ionization current of 60 μA and vacuum is better than 10^{-6} torr.

2.2.3. Thermal analyses (TA)

Thermal analyses (TA) of three N-Maleanilic acid derivatives (I-III) were measured using thermal analyzer of TA Q500. The mass losses of 5 mg sample and the changes of the samples with temperature were measured from room temperature up to 600 °C. The heating rates in argon atmosphere were 5, 10 and 15 °C min^{-1}. These instruments were calibrated using indium metal a thermally stable material. The reproducibility of the instrument reading was tested by repeating each measurement more than twice.

2.2.4. Computational method

The molecular structures of Maleanilic acid derivatives (I-III) in the ground states were optimized (Fig. 1) by a DFT method and using B3LYP functional (Becke, 1993; Lee et al., 1998) combined with 6-311G + +(d,p) basis set (Nassar et al., 2014). In this work, the calculations have been carried out on p-FMA, p-ClMA and p-NMA molecules; neutral molecule (related to TA decomposition) and charged molecular ion (related to MS fragmentation); which have been then used for prediction of the weakest bond broken to follow the fragmentation pathways in both techniques. The calculations were carried out using GAUSSIAN 09 (Frisch et al., 2010) suite of programs. Natural bond orbitals (NBO) analysis and frontier molecular orbitals were performed using NBO 3.1 program (Glendening et al., 1995) implemented in the GAUSSIAN 09 package at the DFT/B3LYP/6-311G + +(d,p) at the same level.

2.2.5. XRD characterization

The crystallite phases of different samples were identified by Powdered X-ray diffraction (XRD) using a step size of 0.04∘ and at a scanning rate of 1.2∘/min with continuous Scan mode on a ARL™ X'TRA Powder diffractometer, Thermo Fisher Scientific Inc. Available at Science and technology of excellence (STCE) with crystallographic data software Winxrd program attached with ICDD laboratory information using metal ceramic tube Copper Target (with Cu-K-alpha wave length = 1.5405981 Å) radiation operating at accelerating voltage and applied current were 44 kV and 45 mA, respectively, Ni Filter and scintillation detector(NaI (TI) scintillation crystal). The diffraction data was recorded for 2Θ values between 10∘ and 70∘.

2.2.6. Cytotoxicity studies and procedures

Cytotoxicity studies and procedures were involved the following (Mosmann, 1983; Gomha et al., 2015):

Mammalian cell lines: MCF-7 cells (human breast cancer cell line), HepG-2 cells (human Hepatocellular carcinoma) and HCT-116 (colon carcinoma) were obtained from VACSERA Tissue Culture Unit.

Chemicals Used: Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dyes were purchase from Sigma St. Louis, Mo., USA. Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with double distilled H2O and filtered through a Whatmann No.1 filter paper.

Cell line Propagation: The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 μg/ml gentamycin. All cells were maintained at 37 °C in a humidified atmosphere with 5% CO2 and were subcultured two times a week.

3. Results and discussion

3.1. Elemental analyses (EA) of N-substituted maleanilic acids

A series of three n-substituted Maleanilinic acid derivatives (I-III) were prepared by reaction between solid maleic anhydride (MA) and various solid N-substituted anilines at room temperature by solvent-less procedure. The prepared Maleanilinic acids are depicted in Scheme 1 . The synthetic method used to prepare the series is simple, clean, economic and nearly quantitative of high yields (Table 1).

The elemental analysis of the prepared compounds and their analytical parameters are given in Table 1.

3.2. Theoretical and experimental FT-IR and Raman bands assignments of derivatives (I-III)

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. Simply, it is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main goal of FT-IR and Raman spectroscopic analyses are to determine the chemical functional groups in the sample. Owing to the different selection rules for Raman and infrared spectroscopy (Ader, 2016); which some molecular functional groups are more sensitive in FT-IR (e.g. high content of polar C=O, C—O, O—H and C—H groups such as in lipids, carbohydrates and proteins). While other groups such as carbon–carbon double and triple bonds in aromatic amino acids are more sensitive in Raman spectra (Socrates, 2001).

Different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids.