Supporting information for Review only

Synthesis and Utilization of Tetrahydronaphthalene-1,3-dicarbonitrile as a Source of Benzo[*f*]quinazoline, Pyridine, Imidazole Derivatives with Antitumor Activity and Molecular Docking and Dynamics Studies

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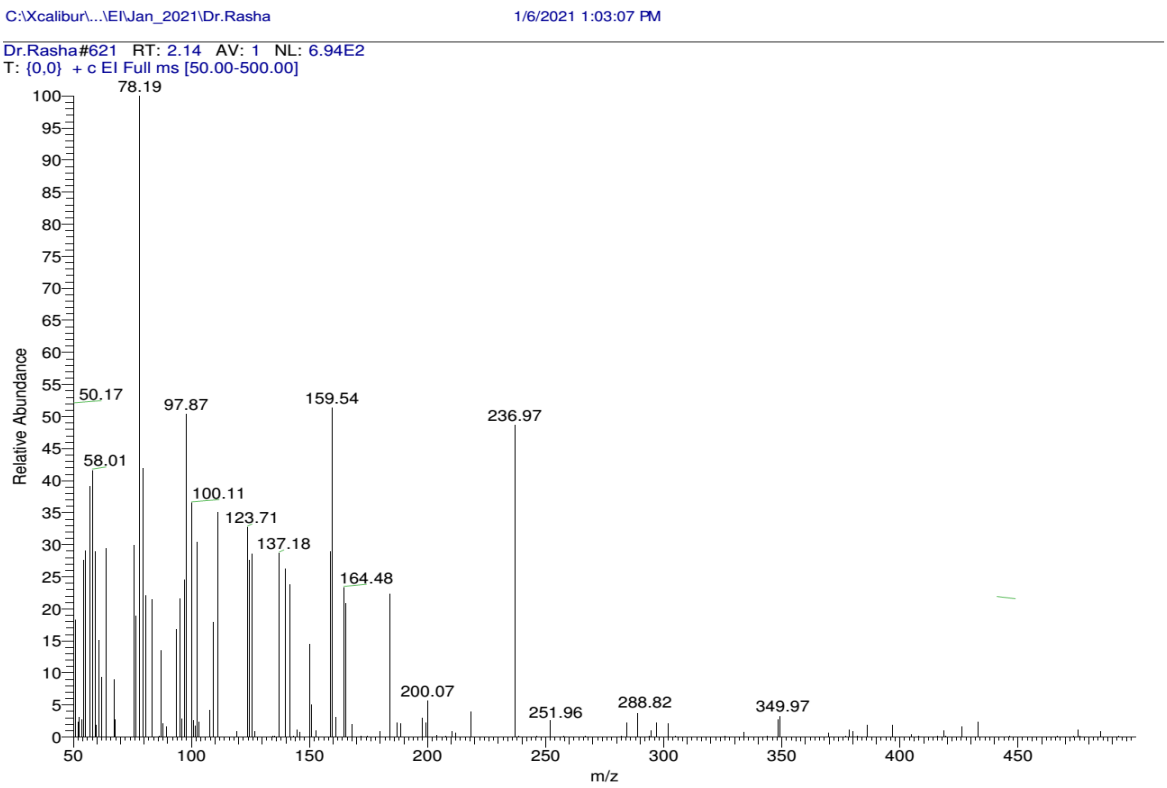
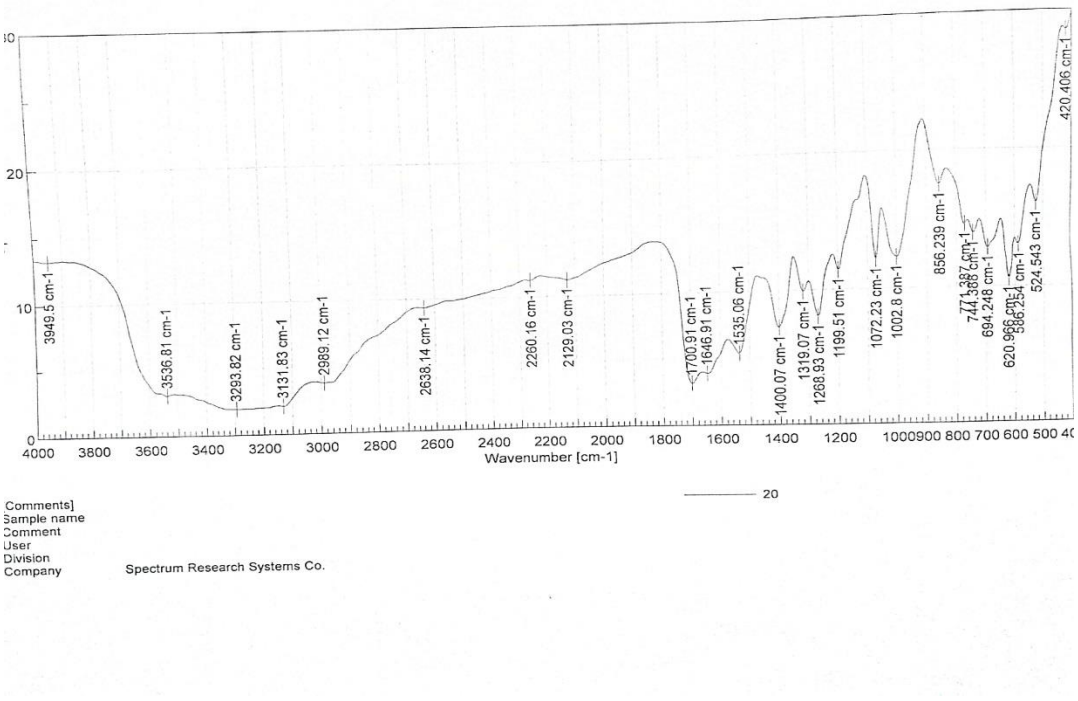
1. Copies of IR, MS, ^{1}H NMR spectra of some final compounds



Mass spectrum of compound 2



IR spectrum of compound 2

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Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

Analyzed by	: Dr. Mai Younis
Analyzed	: 25/07/2018 10:02:49 ص
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Sample ID	:
Customer Name	: Dr. Mansora Ali - NRC
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Report File	:
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Modified	: 25/07/2018 10:10:24 ص

Method

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End Time	:10.00min
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Event Time	:0.50sec
Scan Speed	:1250
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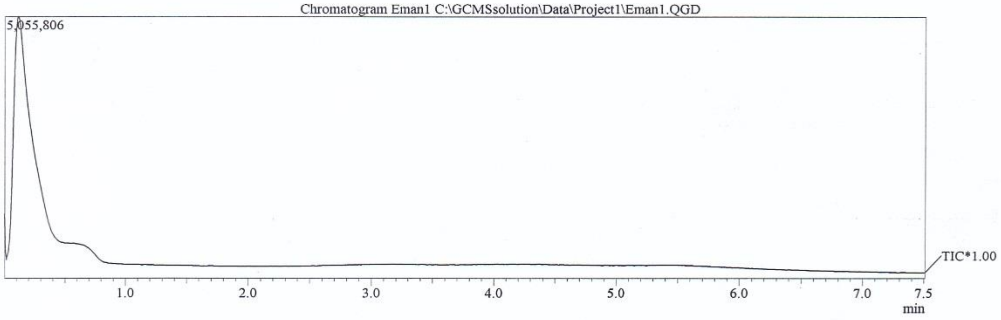
End m/z	:600.00
Electron Voltage	: 70 eV
Ionization Mode	: EI

C:\GCMSsolution\Data\Project1\Eman1.QGD

Chromatogram Eman1 C:\GCMSsolution\Data\Project1\Eman1.QGD



Spectrum

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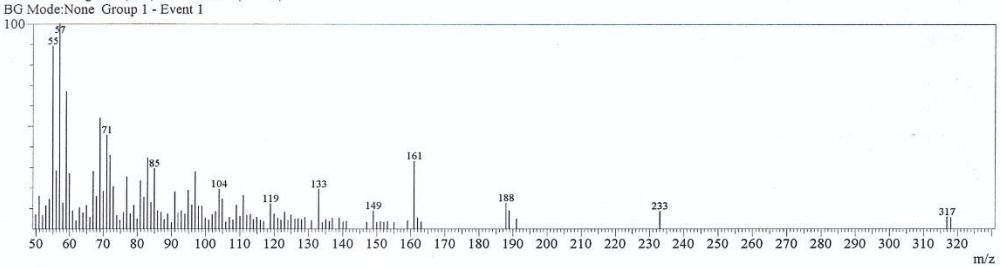

Mass Table
Line#:1 R.Time:1.9(Scan#:227)
MassPeaks:105
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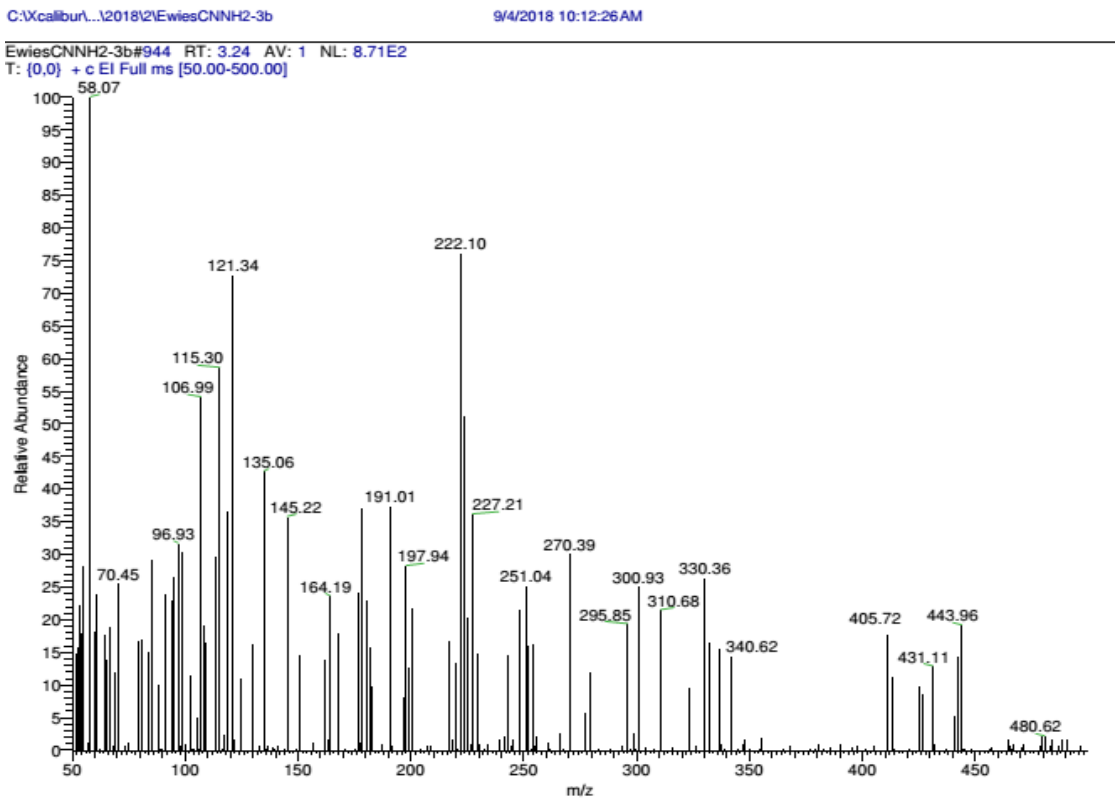
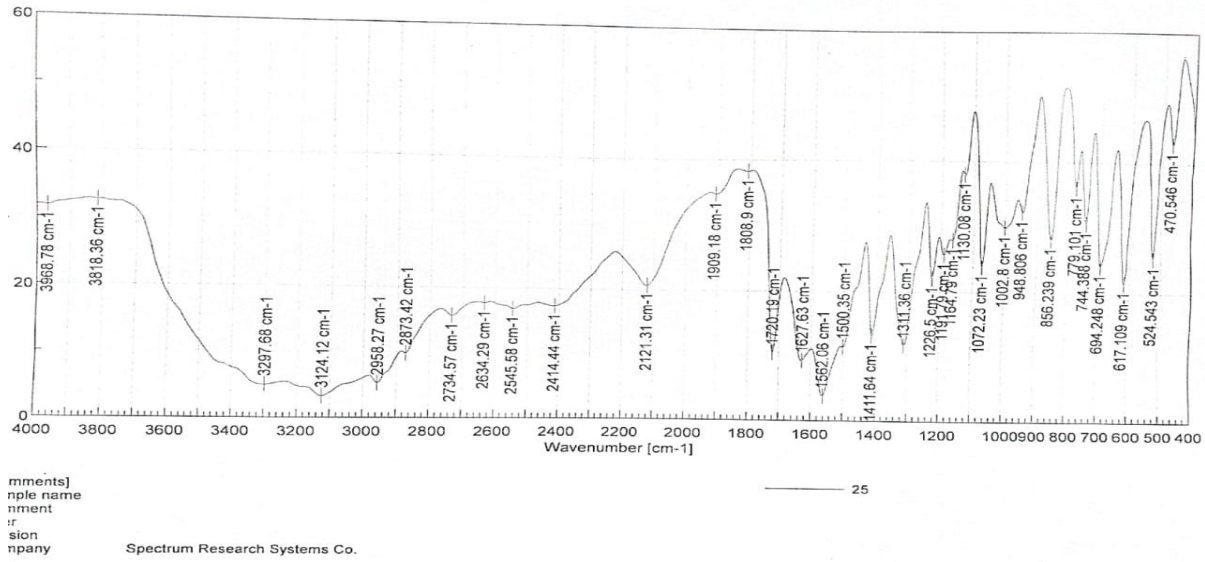
BG Mode:None Group 1 - Event 1

#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.
1	49.90	1196	6.89	4	52.95	1952	11.24	7	56.00	4913	28.30
2	50.90	2756	15.87	5	54.00	2478	14.27	8	56.95	17362	100.00
3	51.95	1141	6.57	6	54.95	15440	88.93	9	57.95	2175	12.53

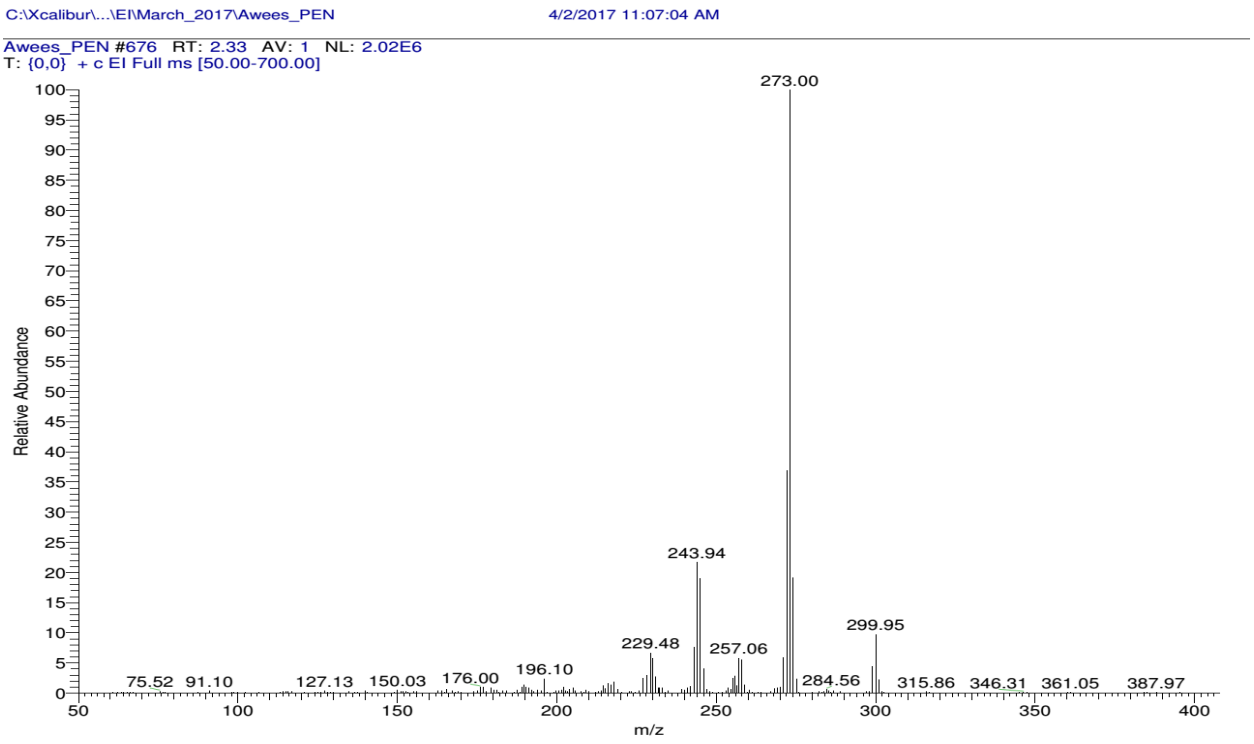
Mass spectrum of compound 3a



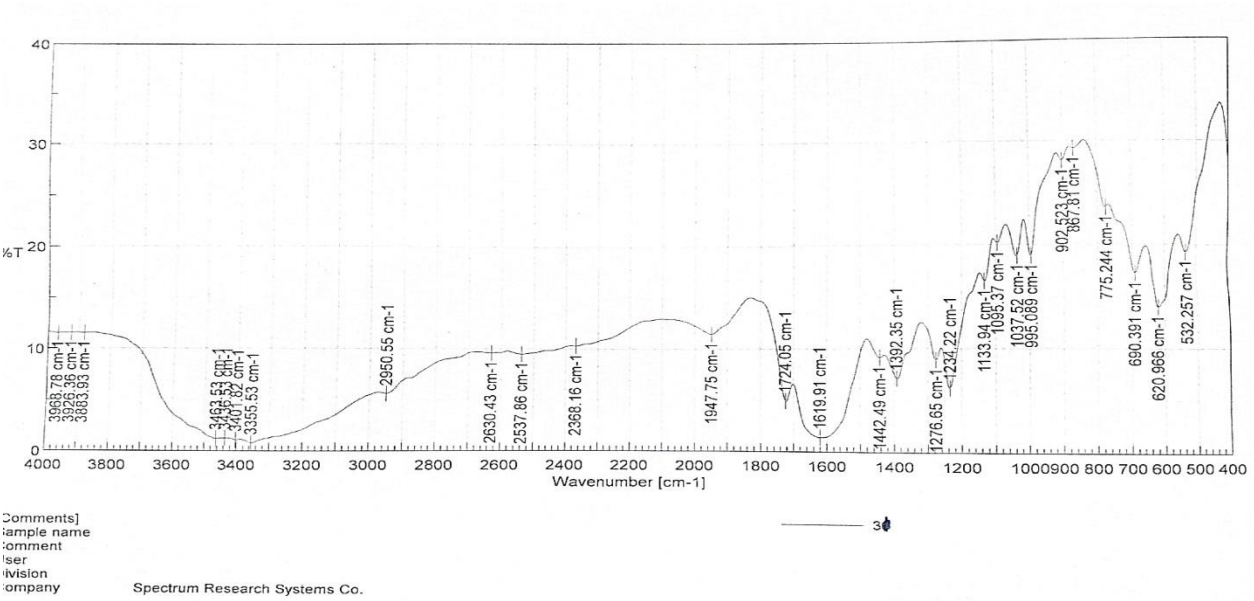
Mass spectrum of compound 3b



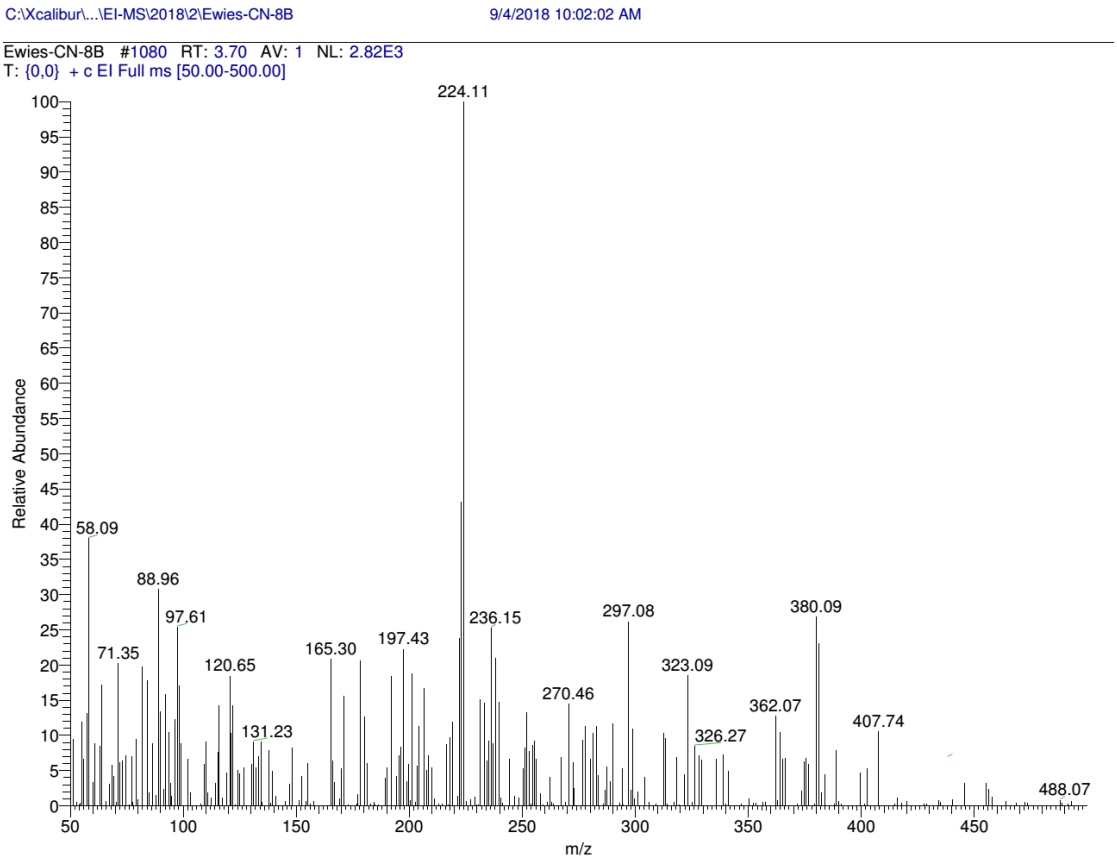
IR spectrum of compound 3b



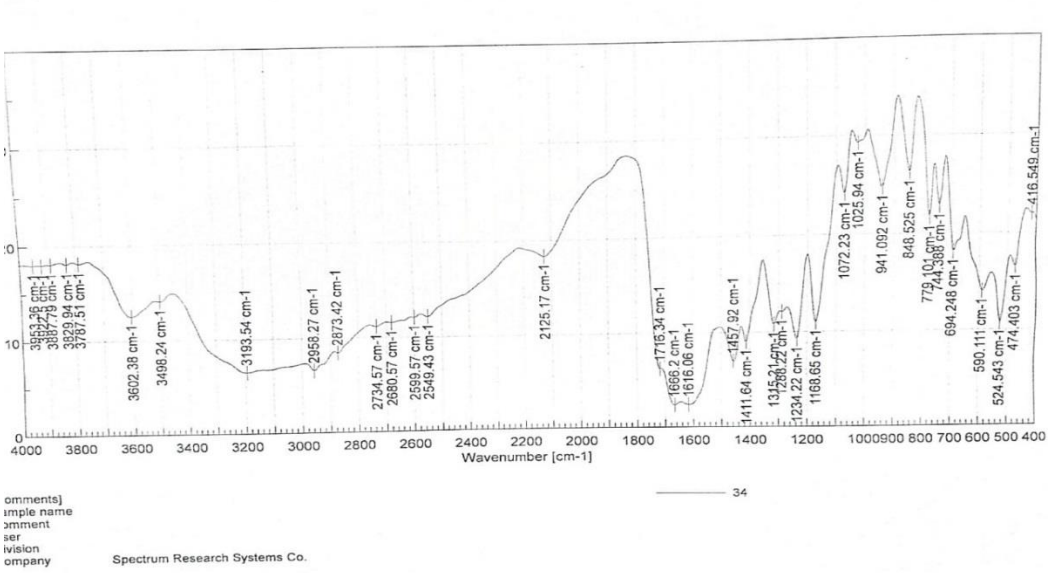
Mass spectrum of compound 5



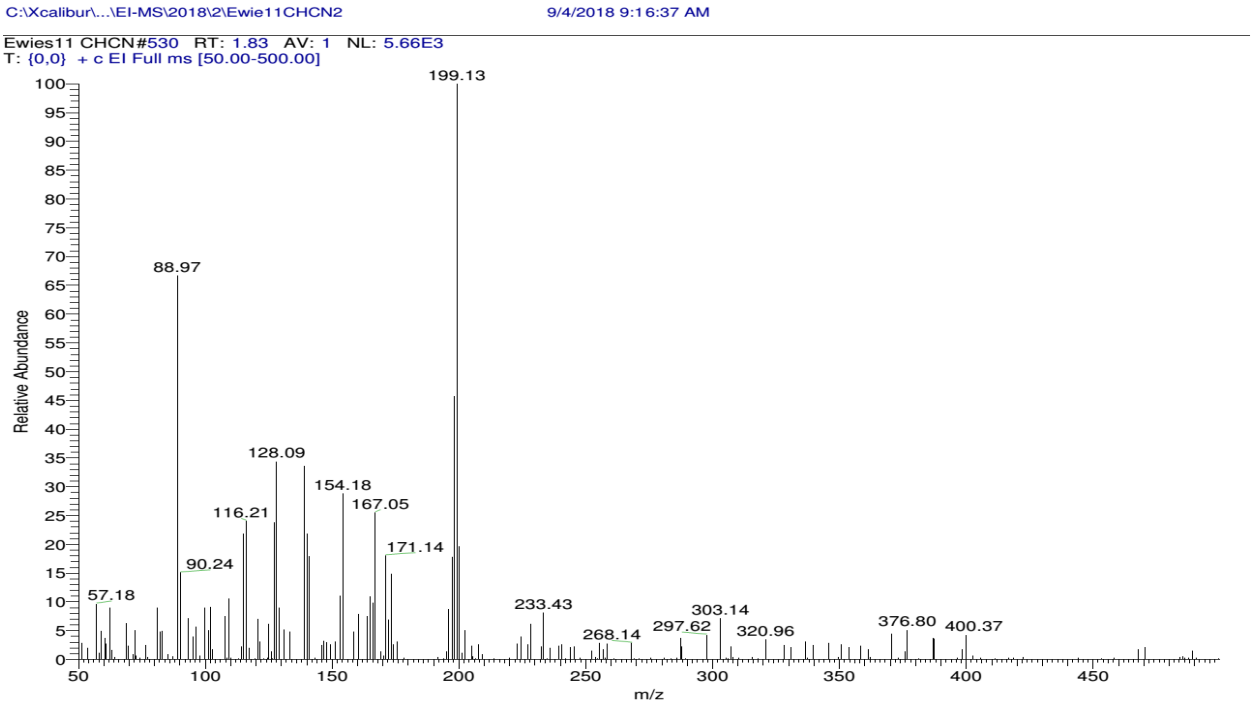
IR spectrum of compound 5



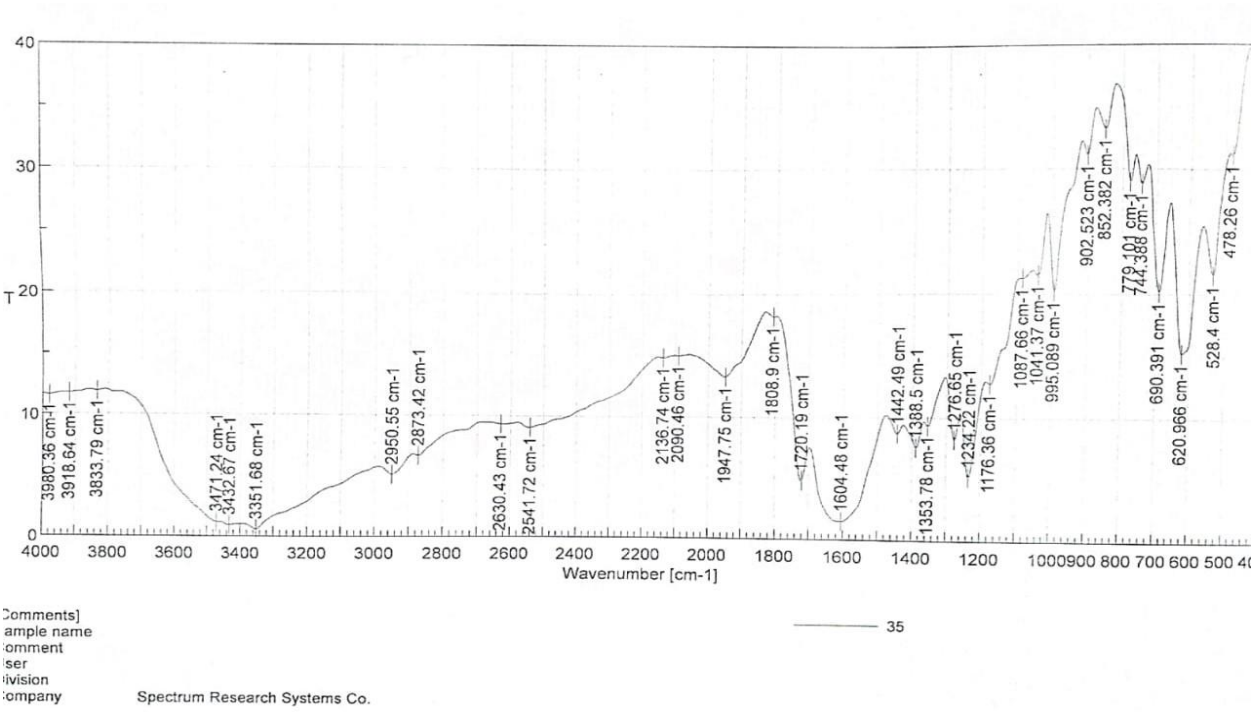
Mass spectrum of compound 8b



IR spectrum of compound 8b



Mass spectrum of compound 11



Comments]
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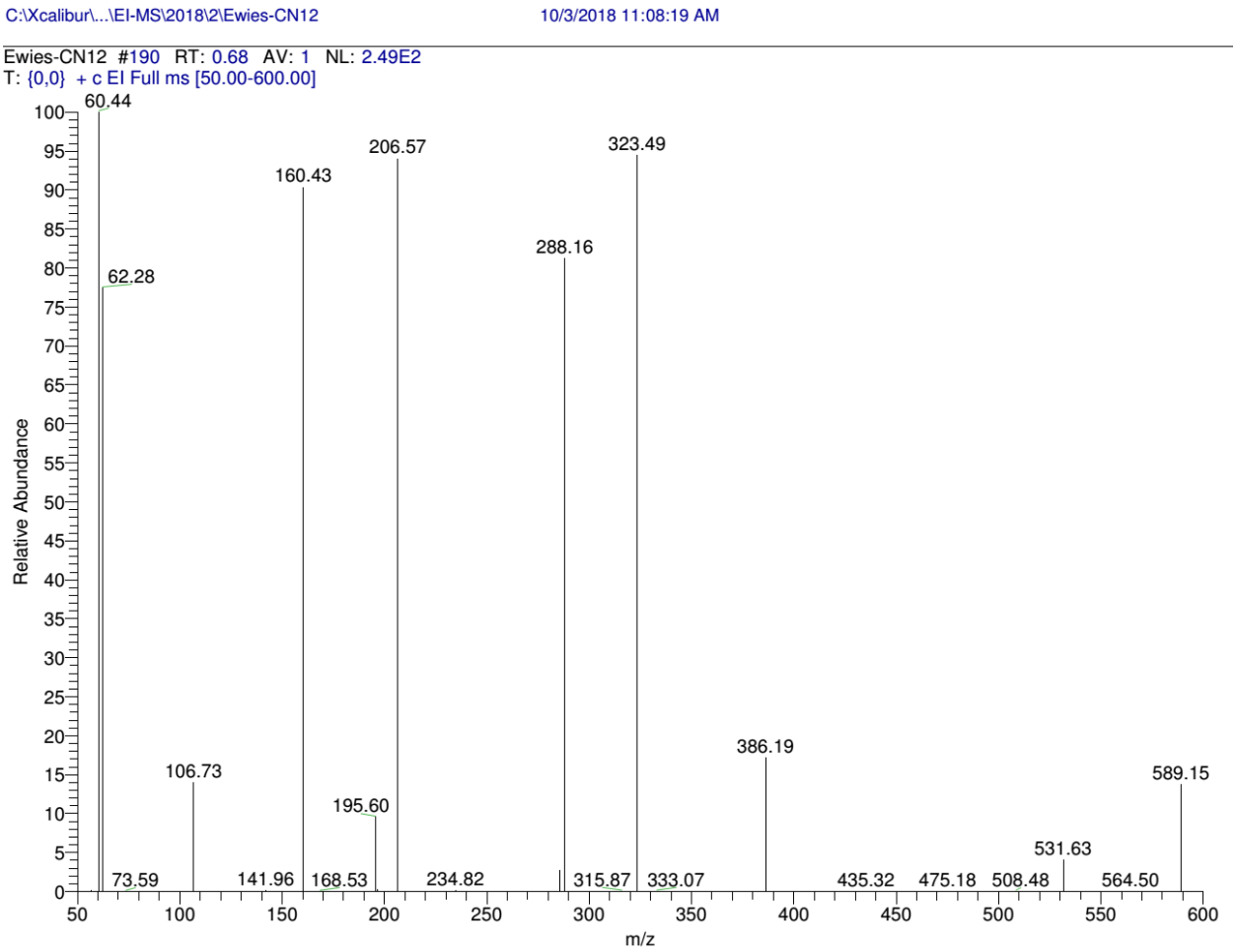
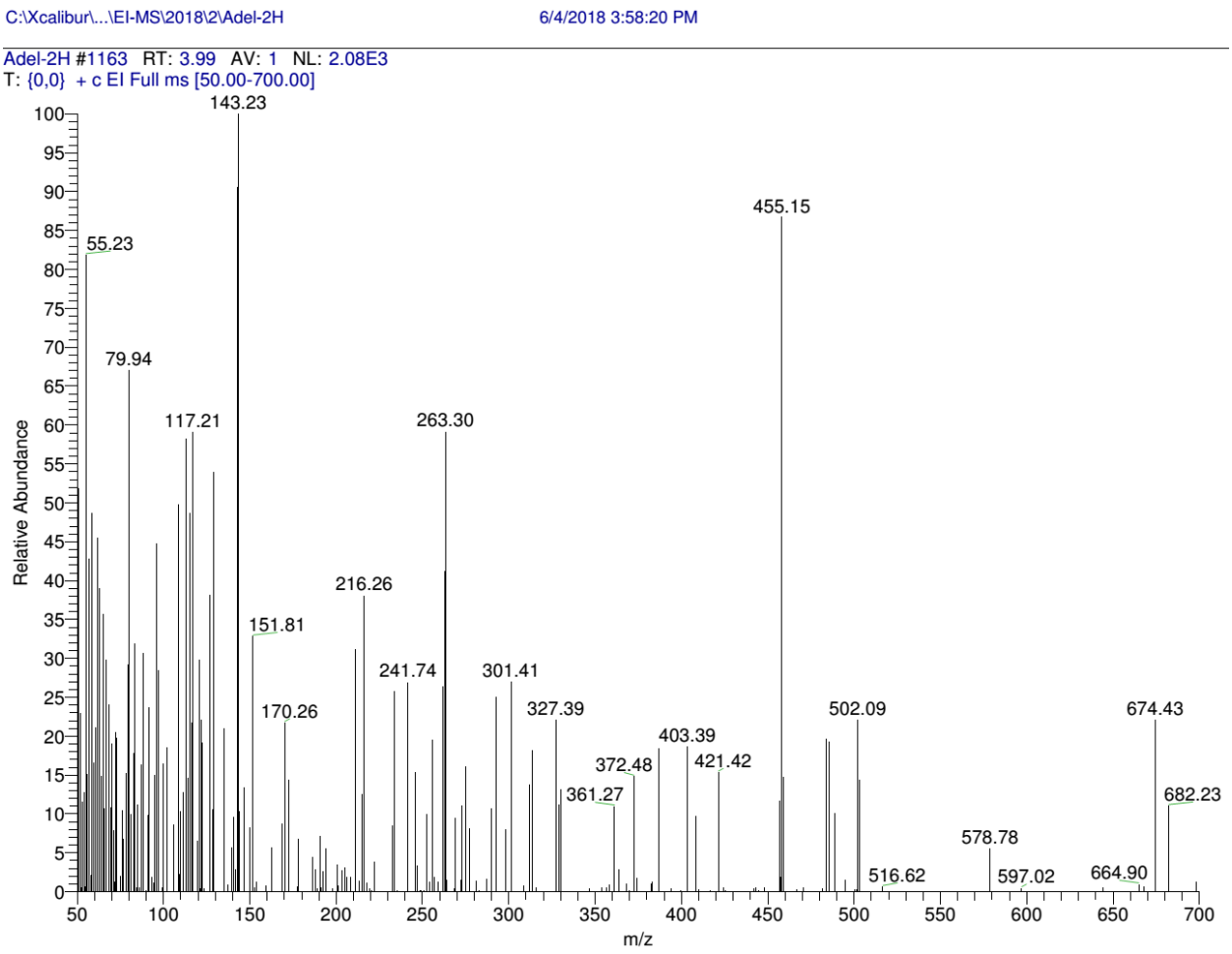
IR spectrum of compound 11



Mass spectrum of compound 12



Mass spectrum of compound 13d

Experimental

Melting point of compounds were measured and uncorrected by digital apparatus of Electrothermal IA 9000 series (Electro-thermal, Essex, UK). Infrared of compounds were recorded on Spectrophotometer of Perkin-Elmer Infracord using KBr pellets. Nuclear magnetic resonance of ^{1}H and ^{13}C NMR in a solvent of DMSO or $CDCl_3$ on Bruker Avance TM 400 MHz Spectrometer. Its chemical shift in δ values that related to TMS. MS spectra were measured on Spectrometer of Shimada GCS-OP 1000 Ex at 70eV. Elemental analysis of new compounds were determined via

Instrument of Elmenter-Varu EL Germany. The pure isolated materials is the net yields. Thin-layer chromatography (TLC) was used to monitor the reactions and assess the compounds purity using the silica gel-Al sheet (Type 60, F 254). The spots were identified by exposing the sheets to a UV lamp at 254/366 nm. The chemical names given for the prepared compounds are according to the IUPAC system. The reported yields are based upon pure materials isolated. Solvents were dried/purified according to conventional procedures.

Materials and Method of antitumor screening

In vitro cytotoxic activity

Cell culture of HepG2 (human hepatocellular carcinoma) and MCF-7 (human breast adenocarcinoma) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

MTT cytotoxicity assay

The cytotoxicity activity against HepG2 and MCF-7 human cancer cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells [1-3]. Cells were dispensed in a 96 well sterile microplate (5 x 10^4 cells/well), and incubated at 37^{o}C with series of different concentrations, in DMSO, of each tested compound or Doxorubicin (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 µL of MTT (2.5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 µL of DMSO. The absorbance was measured at 570 nm using a Spectra Max Paradigm Multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. All experiments were conducted in triplicate and repeated on three different days. All the values were represented as mean ± SD. IC_{50}s were determined by probit analysis by SPSS Inc probit analysis (IBM Corp., Armonk, NY, USA).

1. **Methods:**

1.2.System preparation

The X-ray crystal structures of DNA dodecamerd (CGCAAATTTGCG) with a bifurcated hydrogen-bonded conformation of the AT base pairs and its complex Distamycin were retrieved from the protein data bank with codes 2DND [4]. These structures were then prepared for molecular dynamics (MD) studies using UCSF Chimera [5]. Using PROPKA, pH was fixed and optimized to 7.5 [6]. 3b and 6 compounds were drawn using ChemBioDraw Ultra 12.1 [7]. Altogether, all two prepared systems were subjected to 50 ns MD simulations as described in the simulation section.

1.3.Molecular dynamic (MD) simulations

The integration of Molecular dynamic (MD) simulations in biological systems' study enable exploring the physical motion of atoms and molecules that cannot be easily accessed by any other means [8]. The insight extracted from performing this simulation provides an intricate perspective into the biological systems' dynamical evolution, such as conformational changes and molecule association [8]. The MD simulations of all systems were performed using the GPU version of the PMEMD engine present in the AMBER 18 package [9].

The partial atomic charge of each compound was calculated with ANTECHAMBER's General Amber Force Field (GAFF) technique [10]. The Leap module of the AMBER 18 package implicitly solvated each system within an orthorhombic box of TIP3P water molecules within 10 Å of any box edge. The Leap module was used to neutralize each system by incorporating Na+ and Cl- counter ions. A 2000-step initial minimization of each system was carried out in the presence of a 500 kcal/mol applied restraint potential, followed by a 1000-step full minimization using the conjugate gradient algorithm without restraints.

During the MD simulation, each system was gradually heated from 0K to 300K over 500ps, ensuring that all systems had the same amount of atoms and volume. The system's solutes were subjected to a 10kcal/mol potential harmonic constraint and a 1ps collision frequency . Following that, each system was heated and equilibrated for 500ps at a constant temperature of 300K.To simulate an isobaric-isothermal (NPT) ensemble, the number of atoms and pressure within each system for each production simulation were kept constant, with the system's pressure maintained at 1 bar using the Berendsen barostat [11].

For 50 ns, each system was MD simulated. The SHAKE method was used to constrain the hydrogen bond atoms in each simulation. Each simulation used a 2fs step size and integrated an SPFP precision model. An isobaric-isothermal ensemble (NPT) with randomised seeding, constant pressure of 1 bar, a pressure-coupling constant of 2ps, a temperature of 300K, and a Langevin thermostat with a collision frequency of 1ps was used in the simulations.

1.4.Post-MD Analysis

After saving the trajectories obtained by MD simulations every 1 ps, the trajectories were analyzed using the AMBER18 suite's CPPTRAJ [12] module. The Origin [13] data analysis program and Chimera [5] were used to create all graphs and visualizations.

1.5.Thermodynamic calculation

The Poisson-Boltzmann or generalized Born and surface area continuum solvation (MM/PBSA and MM/GBSA) approach has been found to be useful in the investigation of a wide range of nucleic acid systems.[14-17]. The DNA-Ligand complex molecular simulations used by MM/GBSA and MM/PBSA compute rigorous statistical-mechanical binding free energy within a defined force field [18, 19].

Binding free energy averaged over 500 snapshots extracted from the entire 50 ns trajectory. The estimation of the change in binding free energy (ΔG) for each molecular species (complex, ligand, and receptor) can be represented as follows [20]:

$$\Delta G_{bind} = G_{complex} - G_{receptor} - G_{ligand} \quad (1)$$

$$\Delta G_{bind} = E_{gas} + G_{sol} - TS \quad (2)$$

$$E_{gas} = E_{int} + E_{vdw} + E_{ele} \quad (3)$$

$$G_{sol} = G_{GB} + G_{SA} \quad (4)$$

$$G_{SA} = \gamma SASA \quad (5)$$

The terms Egas, Eint, Eele, and Evdw symbolize the gas-phase energy, internal energy, Coulomb energy, and van der Waals energy. The E_{gas} was directly assessed from the FF14SB force field terms. Solvation-free energy (G_{sol}) was evaluated from the energy involvement from the polar states (G_{GB}) and non-polar states (G). The non-polar solvation free energy (G_{SA}) was determined from the Solvent Accessible Surface Area (SASA) [21] using a water probe radius of 1.4 Å. In contrast, solving the GB equation assessed the polar solvation (GGB) contribution. Items S and T symbolize the total entropy of the solute and temperature, respectively.

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