SUPPORTING INFORMATION

Design, Synthesis and Biological Evaluation of 1,2-Dihydroisoquinolines as HIV-1 Integrase Inhibitors

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Supplementary Information

General Synthesis of 1,2-dihydroisoquinoline derivatives (4a-s):

To a mixture of 2-(1-alkynyl)benzaldehyde(1.08 mmol), aniline (1.08 mmol) and ketone (5.38 mmol) in EtOH (5 mL) $CoCl_2$ (0.324 mmol, 30 mol %) and *L*-proline (0.108 mmol, 10 mol %) were added.Allow the mixture was stirred at 50-60°C under a nitrogen atmosphere for the completion of reaction as monitored on TLC. Then the compound was purified by column chromatography with EtOAc: Hexane as eluent.

General Synthesis of 1,2-dihydroisoquinoline derivatives (6a-e):

To a mixture of 2-(1-alkynyl)aldimines(1.08 mmol), nitro methane (2equiv.) in DCM (5 mL) $CoCl_2$ (0.324 mmol, 30 mol %) were added. Allow the mixture was stirred at 50-60°C under a nitrogen atmosphere for the completion of reaction as monitored on TLC. Then the compound was purified by column chromatography with EtOAc: Hexane as eluent.

Construction of mutant Integrase gene:

Mutant integrase gene were constructed by site directed mutagenesis protocol using wild type integrase gene in pET15B vector as template The following sense and antisense oligonucleotide were used for introducing single amino acid substitution : G140S FP 5'-CAAGCAGGAATTTAGCATTCCCTACAATC-3'; G140SRP 5'-GATTGTAGGGAATGCTAAATTCCTGCTTG-3'. Sequencing was done to ensure that the appropriate mutation had been transferred to the construct. The construct was transformed into the BL21 strain for expression.

Reference 1: Zhao, XZ,;Steven JS,; Mathieu, M,; Christophe M, Paul LB,; Yves, P,; Stephen, HH,; and Terrence RB, Jr. 4-Amino-1-hydroxy-2-oxo-1,8naphthyridine Containing Compounds Having High Potency against Raltegravir Resistant Integrase Mutants of HIV-1. *J.Med Chem.* 2014, 57, 5190-5202.

HIV-1 integrase strand transfer inhibition assays:

The assay for the detection of HIV-1 integrase strand transfer (ST) inhibitors was adapted from previously described methods. Briefly, a double-stranded biotinylated donor DNA, corresponding to the HIV U5 viral DNA end, was added to the wells of Streptavidin-coated 96-well microtiter plates (R & D systems).a blunt-end donor DNA comprising the U5 top strand oligonucleotide (5'-biotin-GTGTGGAAAATCTCTAGCA-3') annealed to the complementary U5 bottom strand (5'-ACTGCTAGAGATTTTCCACAC-3') was utilised Following 1h incubation at rt and a stringent wash step, purified recombinant integrase (1µM) was assembled onto the pre-processed donor DNA through incubation for 30 minutes at 22ºC. Following a wash step, the test compounds and a positive control inhibitor (Raltegravir, Merck) were titrated into individual wells either at a final concentration of 10µM (for single-dose inhibition assays) or at a minimum of 8 different concentrations ranging from 50-0.39 µM (for dose-response inhibition assays). The microtiter plates were incubated for 30 minutes at 37ºC, washed and the strand transfer reaction was initiated through the addition of FITC-labeled target dsDNA (5'-TGACCAAGGGCTAATTCACT-FITC-3' and 5'-AGTGAATTAGCCCTTGGTCA-FITC-3'). After an incubation period of 1 h at 37ºC, the plates were washed as before and an AP (Alkaline

phosphatase)-conjugated anti-FITC secondary antibody (Sigma) was added. Finally, the plates were washed and chromogenic substrate (BluePhos, KPL) was added to allow for photometric measurement at 620 nm using a microplate reader (xMark, Biorad). Percentage inhibition was calculated utilizing the formula: % inhibition = (1-(A620 compound-A620 No integrase control)/(A620 No inhibitor control-A620 No integrase control))*100. The IC50 values were determined as the concentration required to reduce activity of HIV-1 integrase by 50% and were determined using Origin 6.1 software (Origin Lab Corporation, USA). All inhibition values are the average of triplicate experiments

Reference 2: David, C. A.; Middleton, T.; Montgomery, D.; Lim, H. B.; Kati, W.; Molla, A.; Xuei, X.; Warrior, U.; Kofron, J. L.; Burns, D. J. Microarray compound screening (microARCS) to identify inhibitors of HIV integrase. *J. Biomol. Screen.* **2002**, 7, 259-266.

Cell lines and Transfection:

Jurkat E6.1 cells were maintained in RPMI (Gibco, Invitrogen) media supplemented with glutamine, 100 UmL^{-1} penicillin and 100 μgmL^{-1} streptomycin (Invitrogen), 10% fetal calf serum with 5% CO_2 at 37°C. HEK 293T (Human Embryonic Kidney 293 cells), were maintained in DMEM (Gibco, Invitrogen,) supplemented with glutamine, 100 UmL^{-1} penicillin and 100 μgmL^{-1} streptomycin(Invitrogen), 10% fetal calf serum, with 5% CO_2 at 37 °C. Plasmid were transfected using Lipofectamine 2000 (Invitrogen).Viral stocks of VSV-G-psuedotyped HIV-1 were prepared by co-transfection of pNL4-3 and VSV-G expressing plasmid into HEK 293T cells using lipofectamine 2000 (invitrogen).Medium was replaced 6h post transfection with complete DMEM. The supernatant containing virus particles was collected 48 h post transfection. p24 assay was done by β-galactosidase staining of HIV-1 using TZM-bl reporter cell line. Cells were lysed by RIPA buffer (20 mM Tris-HCl (pH 7.5), 1mM beta-glycerophosphate,1 mM Na_2EDTA,150 mM NaCl, sodium deoxycholate, 1 mM EGTA, 1% NP-40, 1 mM Na_3VO_4, 1%, 2.5 mM sodium pyrophosphate, 1μg/ml leupeptin). Proteins were resolved by polyacrylamide gel electrophoresis and transferred to Immobilon membrane (Millipore, USA). The membranes were blocked with 5% non fat dry milk (Sigma Aldrich) in PBS, washed with PBS containing 0.1% Tween 20 (MERCK, New Jersey) and incubated in the same buffer overnight at 4°C in the presence of primary antibody (1:2000 dilution)(primary antibodies used were against p24 (NIH AIDS Reagent programme) and GAPDH(Santa cruz, USA). The membranes were washed with PBS containing 0.1% Tween 20 and incubated with secondary antibody anti-mouse conjugated with horse radish peroxidase (1:10000 dilution) in 5% non fat dry milk in PBS with 0.1% Tween 20 at room temperature. The desired proteins were detected with EZ western Horse Radish Peroxidase substrate (Biological Industries, Israel). GAPDH was used as a loading control.

HIV-1 pNL4-3 infection and analysis by immune blotting:

Two million Jurkat cells were treated with compounds for 2 h and then infected with VSV-G pseudotyped viruses: pNL4-3 (Wt-HIV) or mock infected (as a control) in the presence of 4 μg/ml Polybrene (Sigma Aldrich) at 37°C. Infection of Jurkat cell line was done by incubating the cells with equal amount of infectious virus (1 MOI) determined by β-galactosidase staining

with the use of HIV-1 indicator TZM-bl cells.[28] The infected cells were harvested after 48 hours and the lysate was used for immunoblotting with indicated antibody.. The compounds were used at 10 μM concentration.

Reference 3: Kari, H,;Bour, S,; Kao, S,; Adachi, A,; Strebel K. The human immunodeficiency virus type 1 accessory protein Vpu induces apoptosis by suppressing the nuclear factor {kappa}-β-dependent expression of anti apoptotic factors. *J. Exp. Med*. **2001**, 194, 1299-1311.

Reference 4: Verma, S,; Ali, A,; Benerjea, A,C,; Inhibition of β-TrcP dependent ubiquitination of p53 by HIV-1 Vpu promotes p53 mediated apoptosis in human T cells. *BLOOD*, **2011**, 117, 6600-6607.

Cytotoxicity assay in TZM-bl cell line:

Serial dilution of each compound (in media, pH 7.4) was prepared in a 96-well plate (Nunc), with concentrations ranging from 0.022862 to 50.0μM. The diluted compounds were incubated with $1x10^4$ TZM-bl cells (in Dulbecco's modified Eagle's medium containing 20 $\mu g ml^{-1}$ DEAE) were then added to each well, and the cells were incubated at 37°C for a further 48 h in a humidified CO_2 incubator. Cell culture toxicity was measured using the Cell Titer-Glo luminescent cell viability assay (Promega) according to the manufacturer's instructions. Cytotoxicity was expressed as a percentage of the reduction in relative luminescence unit values generated in the absence of any compound. Three independent assays using quadruplicate measurements were carried out for each compound.

Cytotoxicity on Jurkat Cell line:

Serial dilution of each compound (in media, pH 7.4) was prepared in a 96-well plate (Nunc), with concentrations ranging from 0.078 to 100μM. The diluted compounds were incubated with $5x10^3$ jurkat cell line (in RPMI 1640 media) and then added to each well, the cells were incubated at 37°C for a further 48 h and 72h in a humidified CO_2 incubator. After incubation, MTT solution (5 mg/mL) was also added to each well 3h prior to the end of the experiment. Water-insoluble dark blue formazan crystals formed in viable cells were solubilized in DMSO, and the absorbance was measured at 570 nm using a microplate reader. Cell survival was determined by comparing the absorbance values obtained for treated and untreated cells. The cytotoxicity was expressed as the concentration of ligand that inhibited 50% of cell growth (CC_{50})

Viral Inhibition Assay:

The inhibitory effect of compounds on virus was assessed by an in vitro phenotypic inhibition assay using an HIV-1 pseudovirus backbone (pSG3renv) complemented with a subtype C CAP210 envelope. Briefly, a 3-fold serial dilution of each compound (in media, pH 7.4) was prepared in a 96-well plate (Nunc), with concentrations ranging from 50.0 to 0.022862 μM. The diluted compounds were incubated with 4000 TCID50 of pseudovirus for 1 h at 37°C. Ten thousand TZM-bl (CXCR4+HeLa cell line) cells (in Dulbecco's modified Eagle's medium containing 20 μg/ml DEAE) were then added to each well, and the cells were incubated at 37°C for further 48 h in a humidified CO_2 incubator. Nonvirus and virus controls were prepared in exactly the same manner, except for the omission of virus or test compounds where ever appropriate. Luciferase activity induced in the TZM-bl cells (measured in relative luminescence

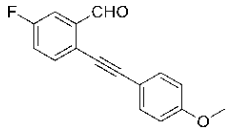
units) was quantified using the Bright-Glo luciferase assay system (Promega) according to the manufacturer's instructions. Inhibition of viral replication was expressed as a percentage of the reduction in relative luminescence unit values generated in the absence of any compound. Two independent assays using duplicate measurements were carried out for each compound.

Reference 5: Li, M.; Gao, F.; Mascola J. R.; Stamatatos, L.; Polonis V. R.; Koutsoukos, M.; Voss, G.; Goefert, P.; Gilbert, P.; Greene, K. M.; Bilska, M.; Kothe, D. L.; Salazar-Gozalez, J. F. ; Wei, X.; Decker, J. M.; Hahn, B. H.; Montefiori, Human immunodeficiency virusttype 1 *env* clones from acute and early subtype b infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J. Virol.* **2005,** *79*, 10108-25

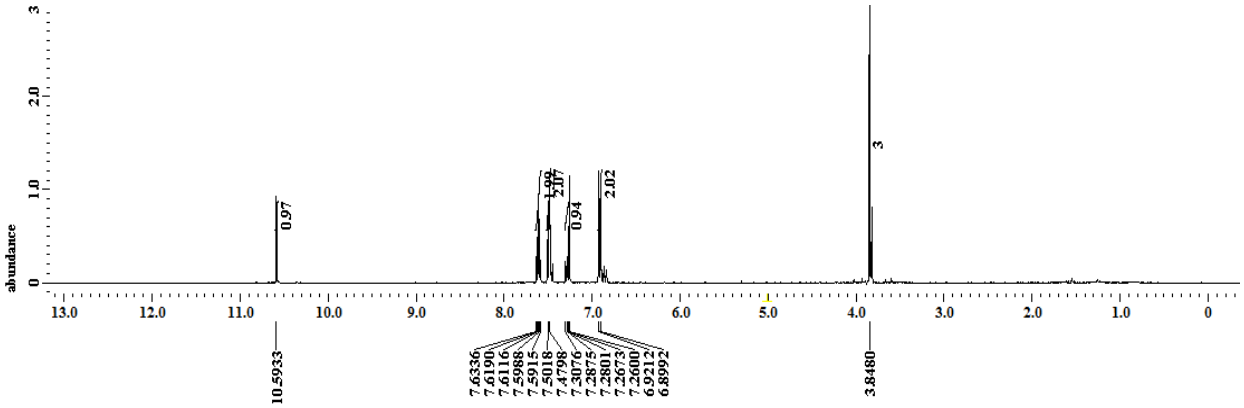
Molecular modeling:

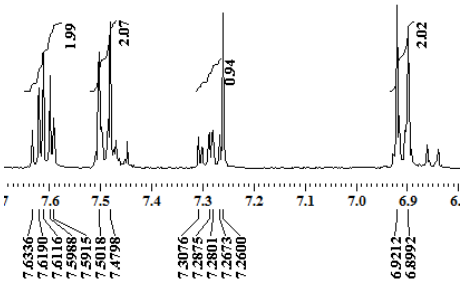
The compounds used in the study were built using sketch module of SYBYL7.1 (Tripos Inc., 1699 S. Hanley Rd., St. Louis, MO 631444 USA, 2005) and subjected to 1000 cycle minimization with standard Tripos force field and 0.005 kcal/mol energy gradient convergence criterion using Powell's method. Three-dimensional structures of these compounds were then prepared using LigPrep (version 2.5, Schrödinger, LLC, New York, NY, 2011) with Epik (version 2.2, Schrödinger, LLC, New York, NY, 2011) to generate metal binding states and tautomeric states for the ligands at pH values of 7.0 ± 2.0. The "metal-binding states" produced by Epik are additional ionization states of ligand-like molecules that are more likely to bind to metals in protein receptors. Adjusted metal state penalty was used in Glide scoring instead of the normal penalty that the ionization state would have in the absence of a metal. The two-metal/IN-CCD/5-CITEP complex was used as a surrogate platform for docking simulations of designed molecules. Protein preparation was done using Maestro (version 9.2, Schrödinger, LLC, New York, NY, 2011). Hydrogen atoms were added during protein preparation wizard. Create zero-order bonds to metals option was activated to replace any covalent bond to metals with zero-order (dashed) bonds, and to adjust the formal charges of the metal and ligating atoms accordingly. Receptor Grid Generation Panel within Glide suite (Glide, version 5.7, Schrödinger, LLC, New York, NY, 2011) was used to set up receptor grid for the prepared HIV-IN model. The grid was defined around metal atom coordinated by catalytic active site residues Asp 64 and Asp116, having outer box and inner box dimension of 20 Å and 10 Å respectively, on each side. The OPLS_2005 force field, which allows a proper treatment of metals, is employed in this molecular docking analysis. Docking was performed using Glide (Grid-based Ligand Docking with Energetics), (Glide, version 5.7, Schrödinger, LLC, New York, NY, 2011) with the standard precision scoring function to estimate protein-ligand binding affinities. The metal binding states of the prepared ligands were docked flexibly into the active site, as specified in receptor grid file of HIV-IN. By default, Schrödinger's proprietary Glide Score multi-ligand scoring function was used to score the poses. A maximum of ten scoring poses were saved for each fragment. The top scoring poses for each fragment were found to possess the expected binding modes with reasonable metal-ligand bond distances based on the docked standard compounds.Selected top scoring molecules binding to the desired core of the Integrase enzyme were selected for the extra precision (XP) mode of docking. Glide uses rigid docking protocol; therefore, to account the conformational dynamics during docking we used AutoDock4.2 inaddition to Glide. We have used standard protocol for receptor grid generation and ligand docking in Auto docks tools.

^{1}H NMR, ^{13}C NMR and HRMS spectra



^{1}HNMR of 5-fluoro-2-((4-methoxyphenyl)ethynyl)benzaldehyde**(1a)**





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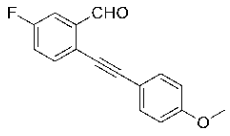

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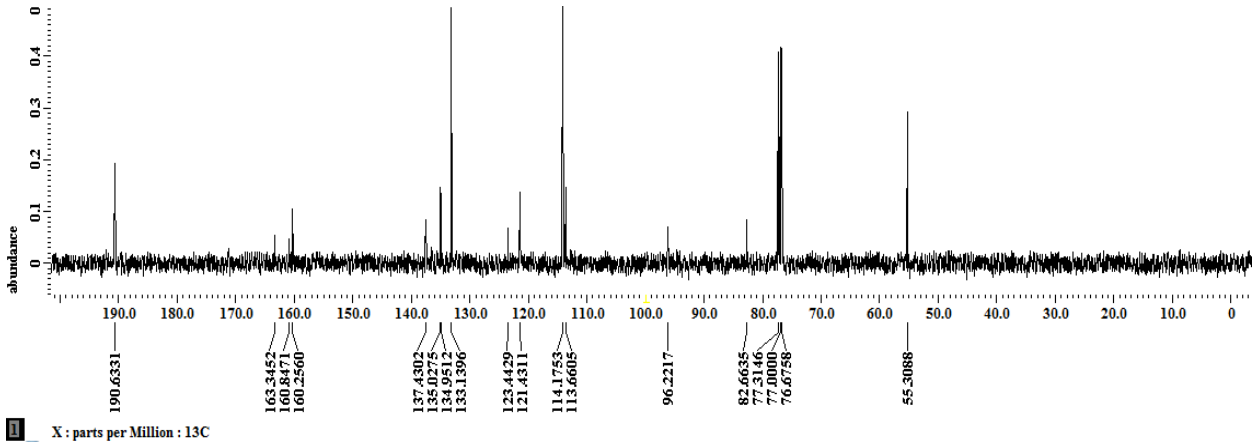
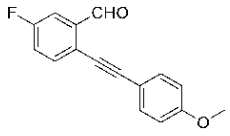
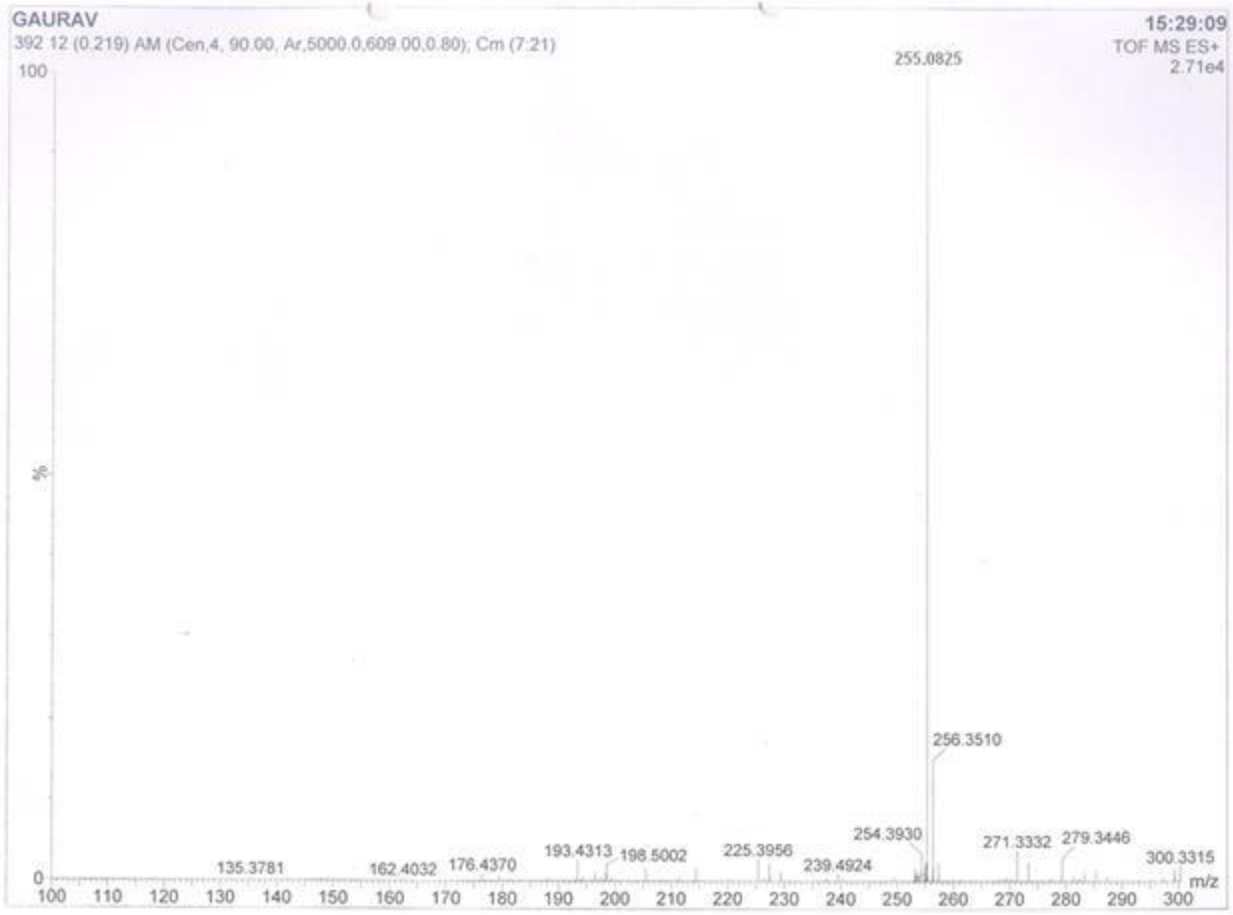
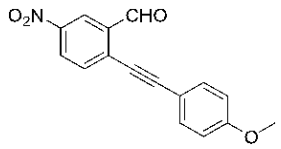


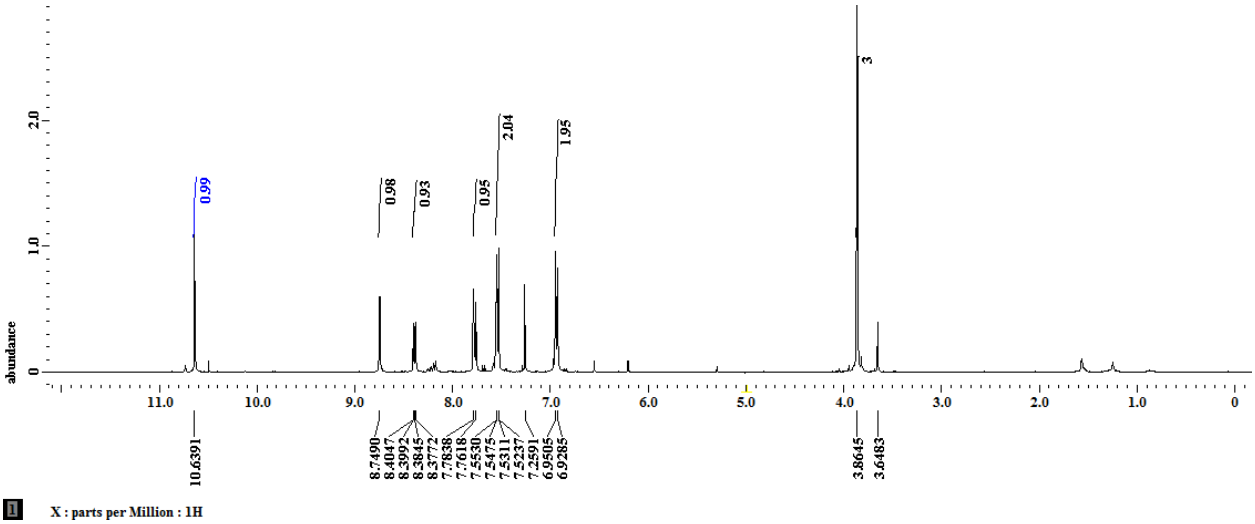


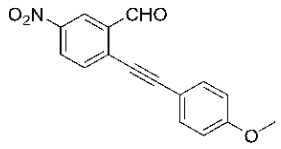
HRMS of 5-fluoro-2-((4-methoxyphenyl)ethynyl)benzaldehyde**(1a)**





[1]HNMR of 2-((4-methoxyphenyl)ethynyl)-5-nitrobenzaldehyde**(1b)**





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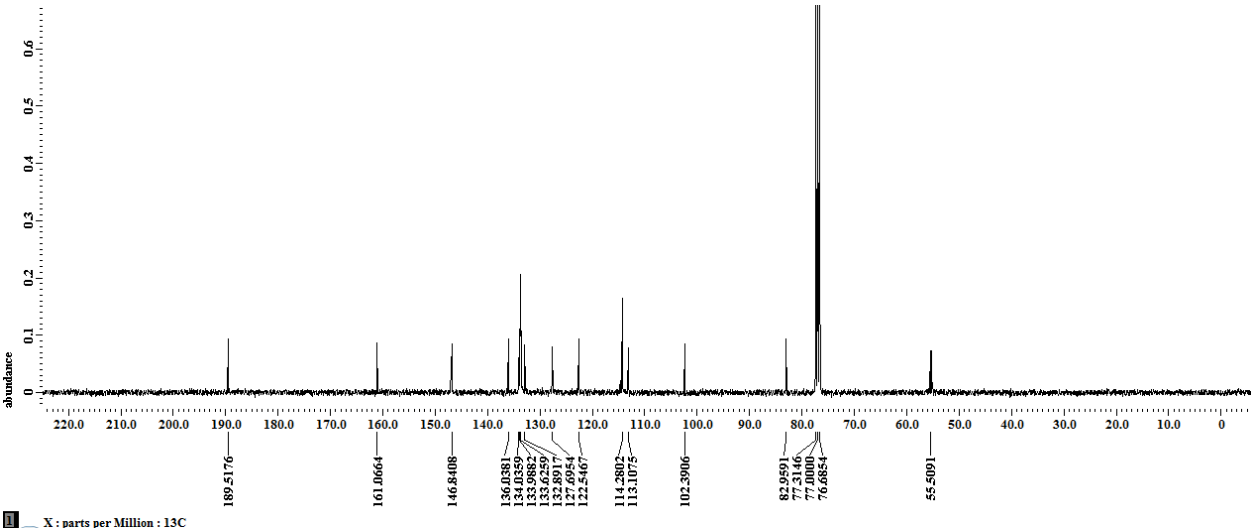

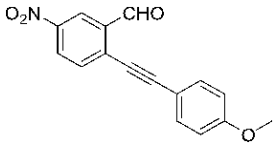

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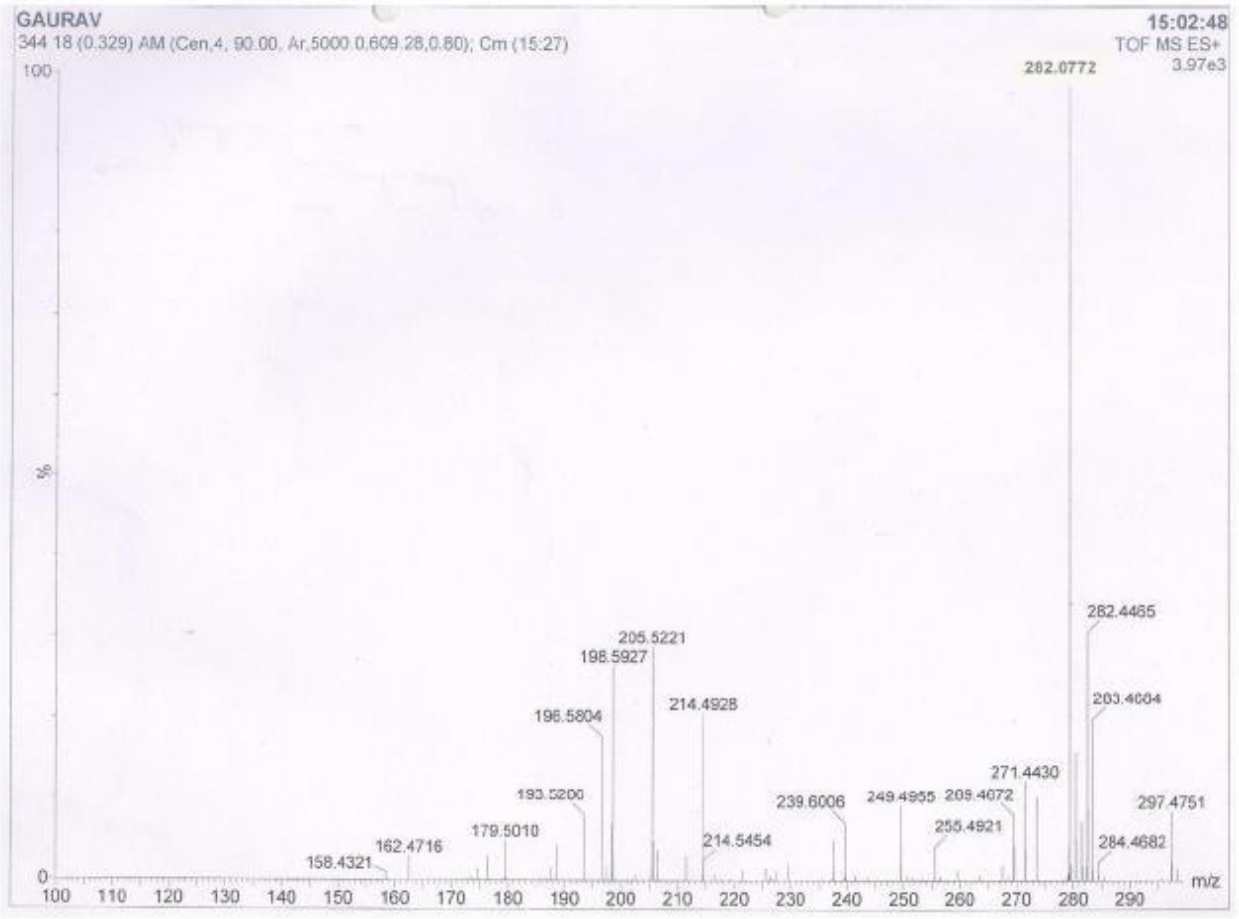
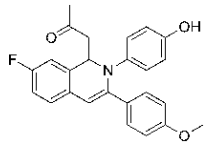
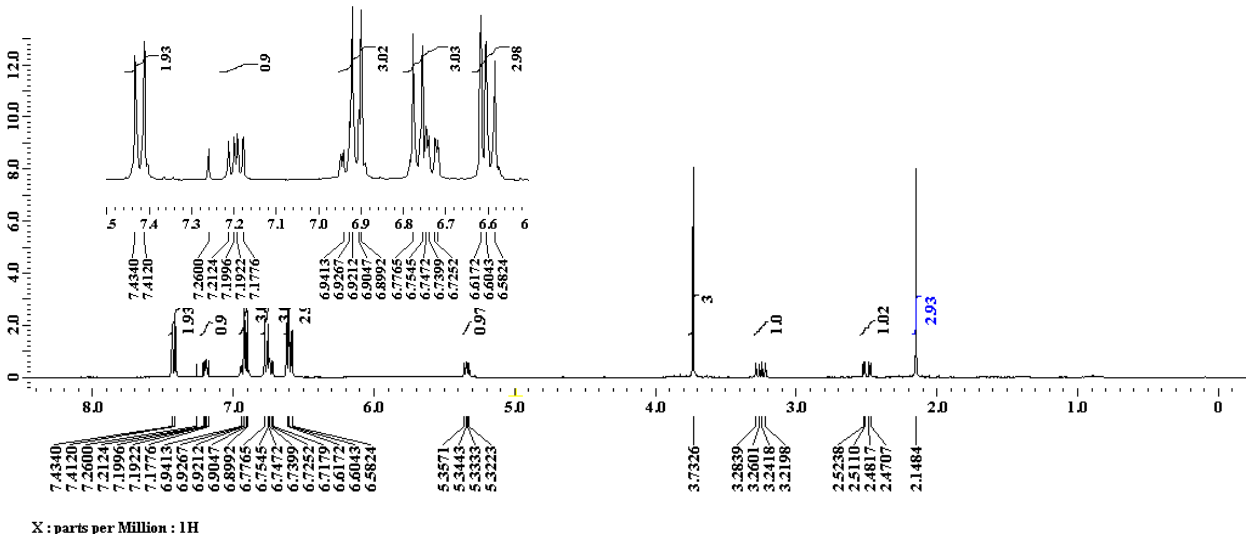
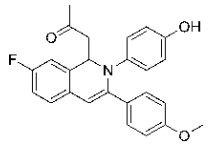


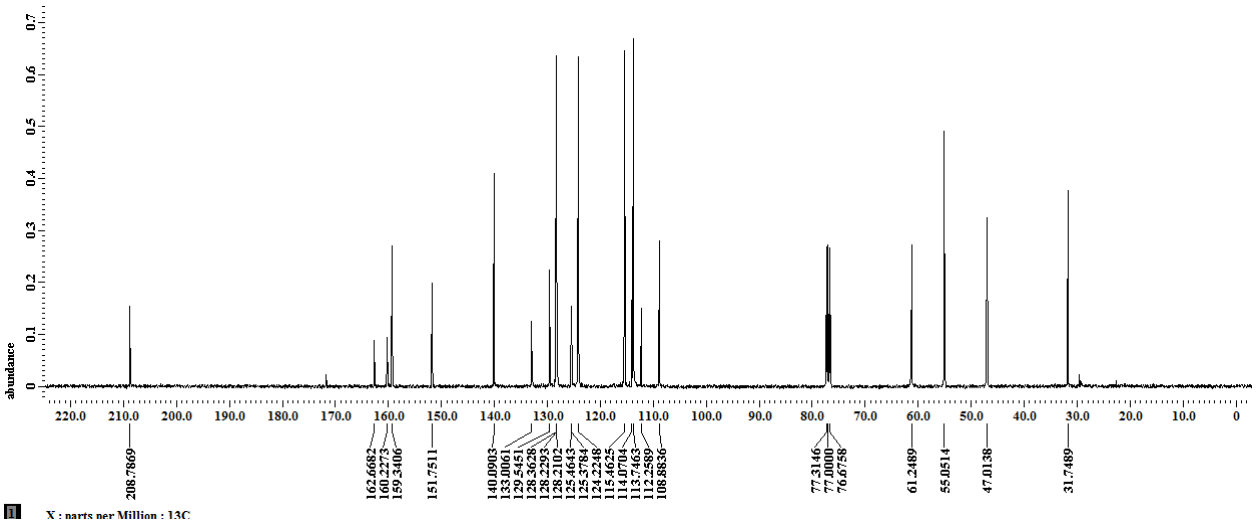


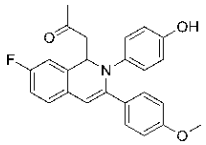
[1]HNMR of 1-(7-fluoro-2-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4a)**



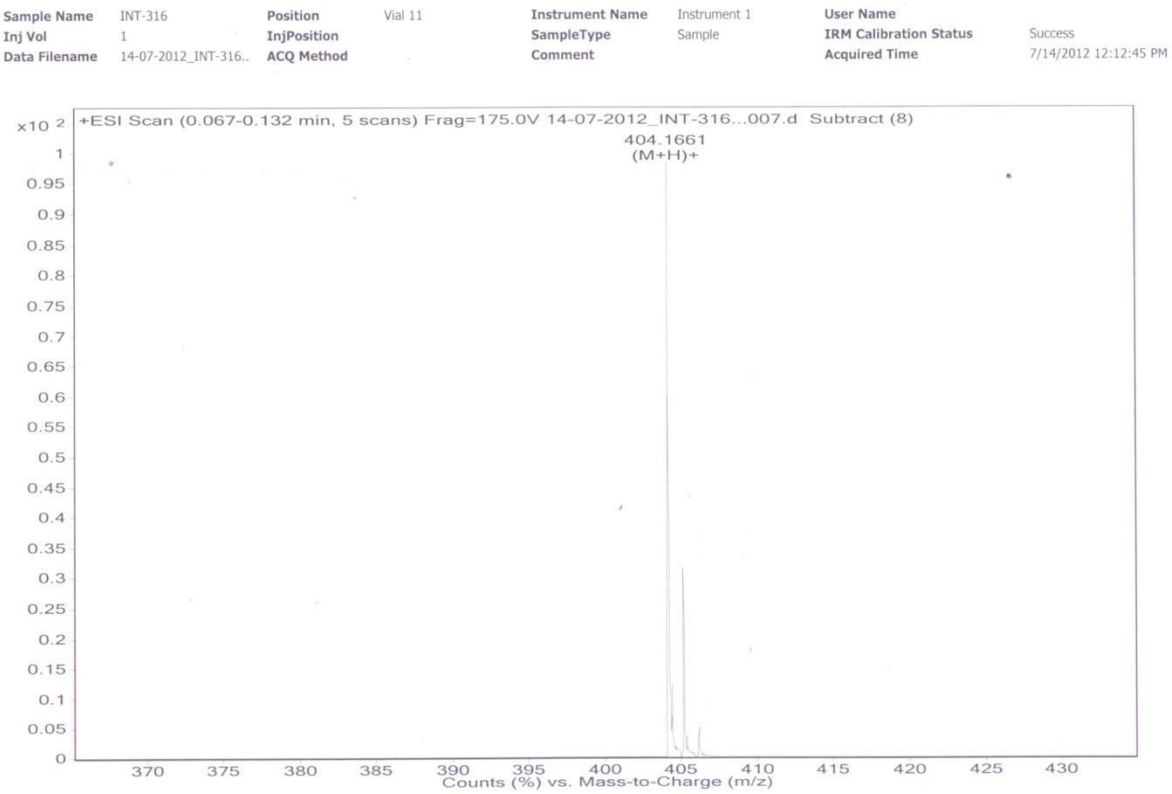


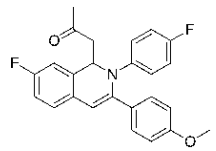
^{13}CNMR of 1-(7-fluoro-2-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4a)**



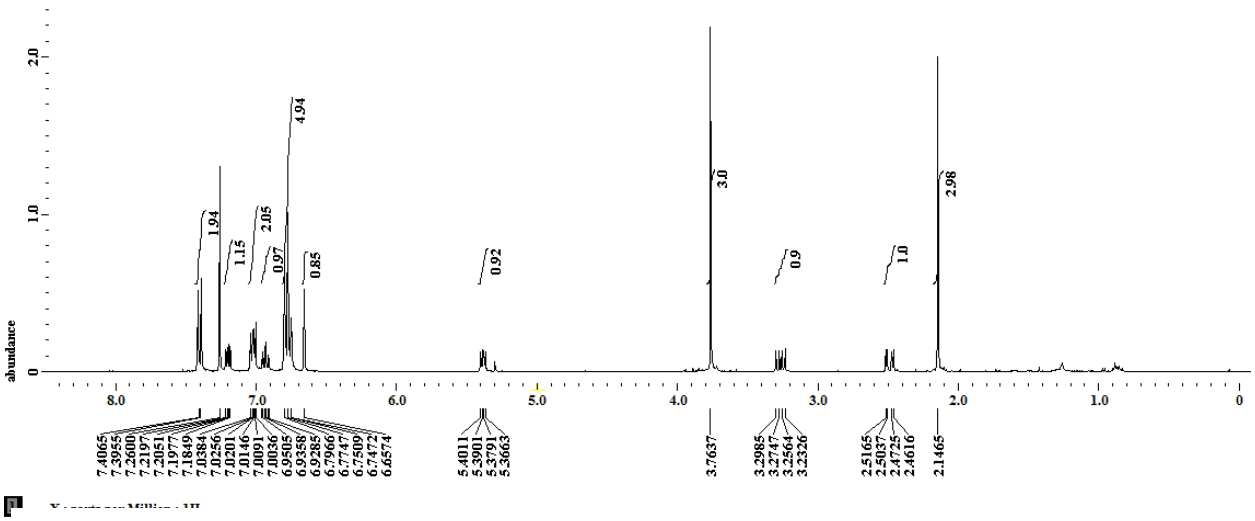


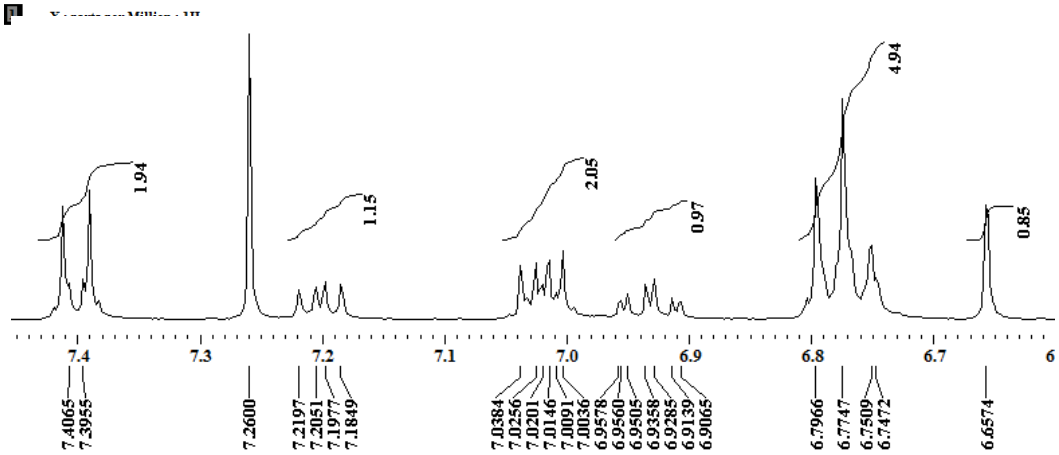
HRMS of 1-(7-fluoro-2-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4a)**

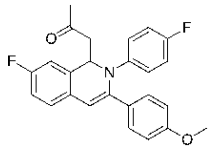




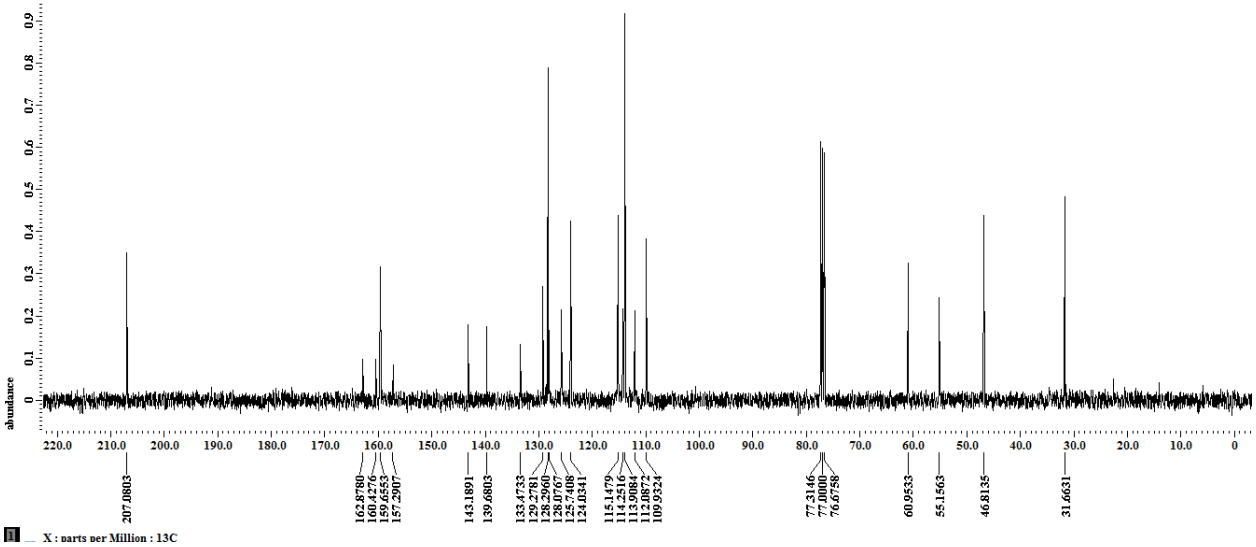
[1]HNMR of 1-(7-fluoro-2-(4-fluorophenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)propan-2-one **(4b)**

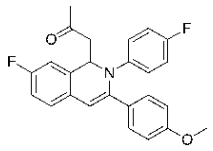




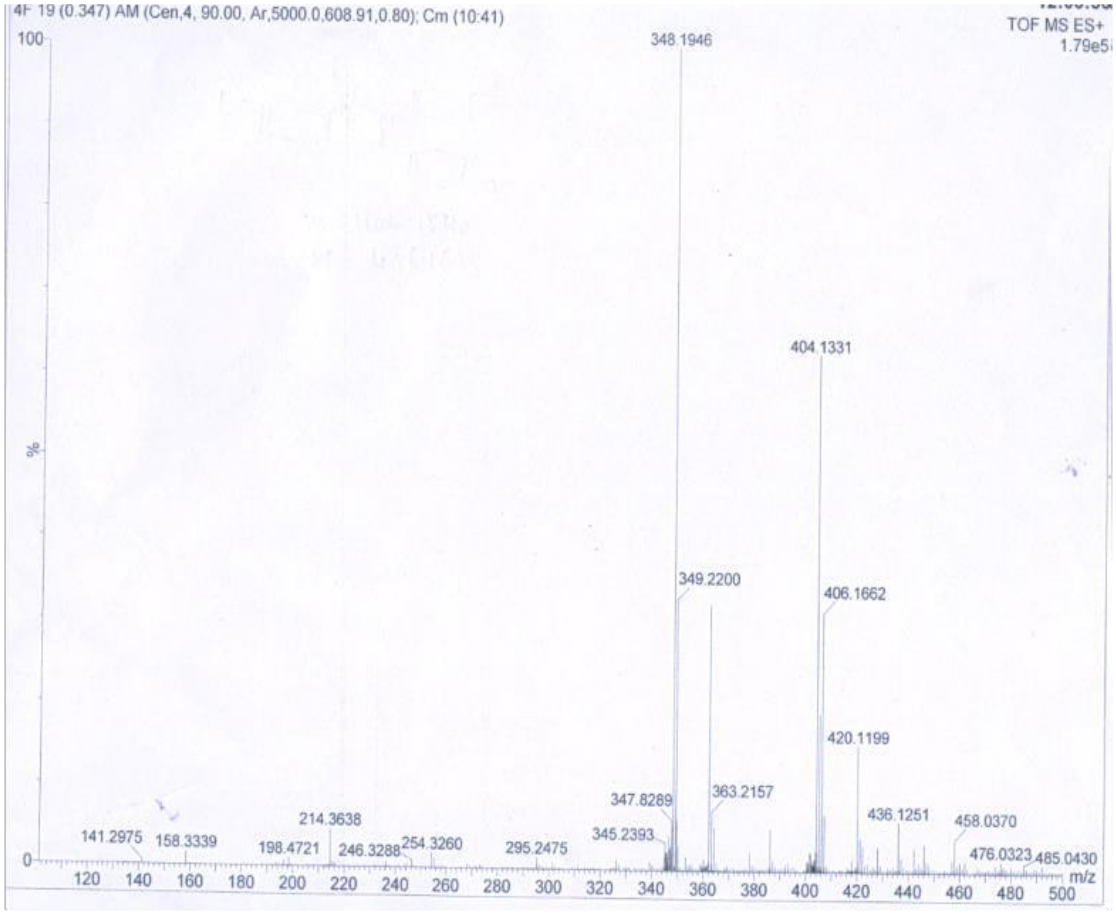


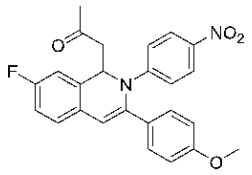
^{13}CNMR of 1-(7-fluoro-2-(4-fluorophenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)propan-2-one **(4b)**



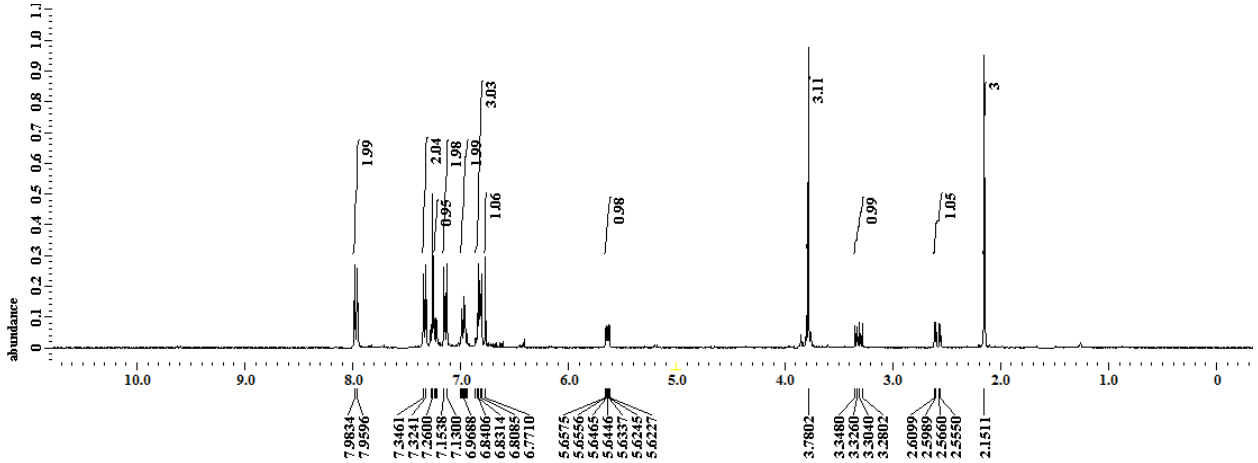


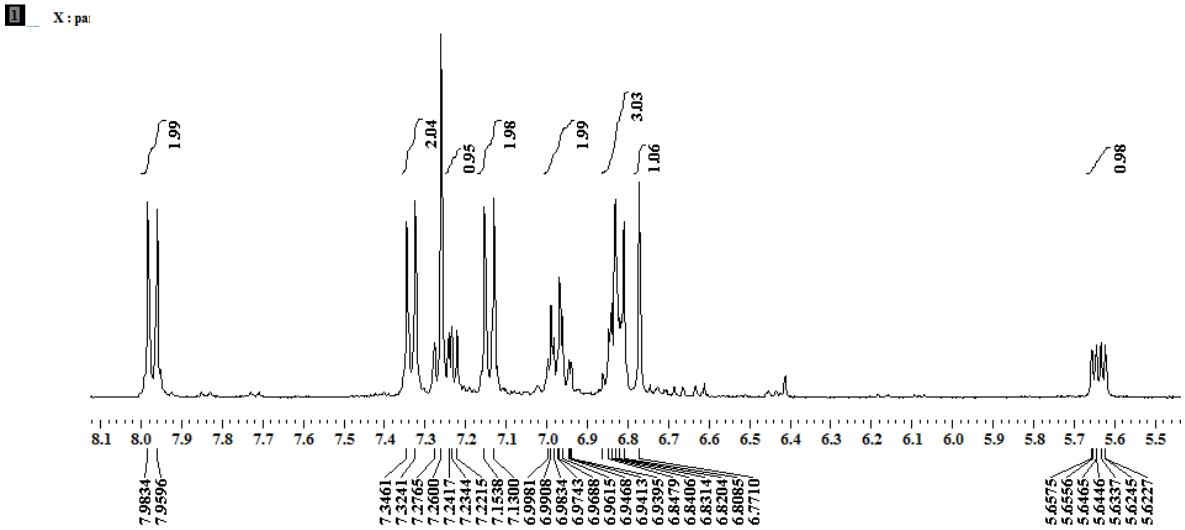
HRMS of 1-(7-fluoro-2-(4-fluorophenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)propan-2-one **(4b)**

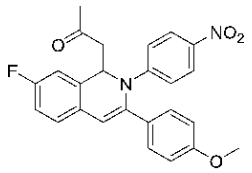




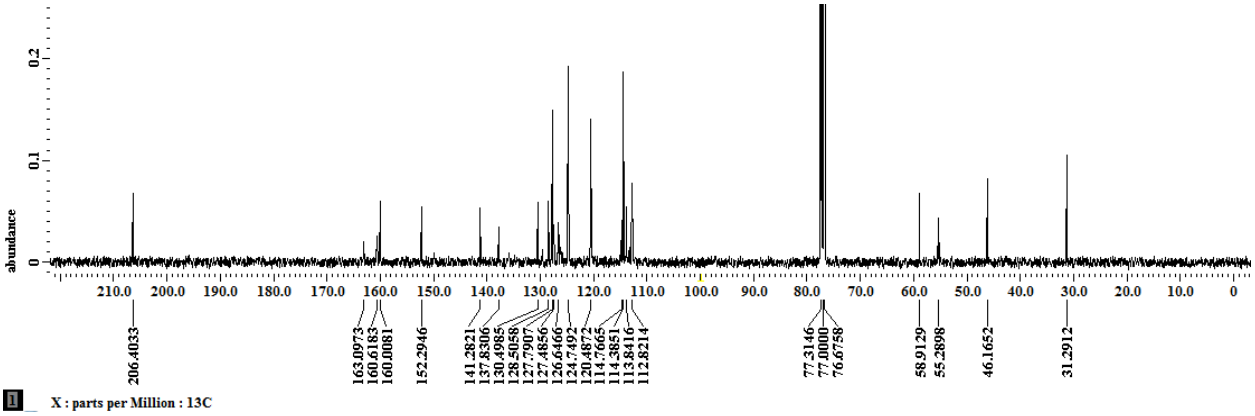
[1]HNMR of 1-(7-fluoro-3-(4-methoxyphenyl)-2-(4-nitrophenyl)-1,2-dihydroisoquinolin-1-yl)propan-2-one **(4c)**

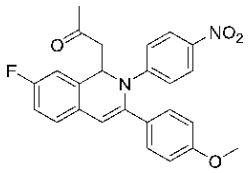




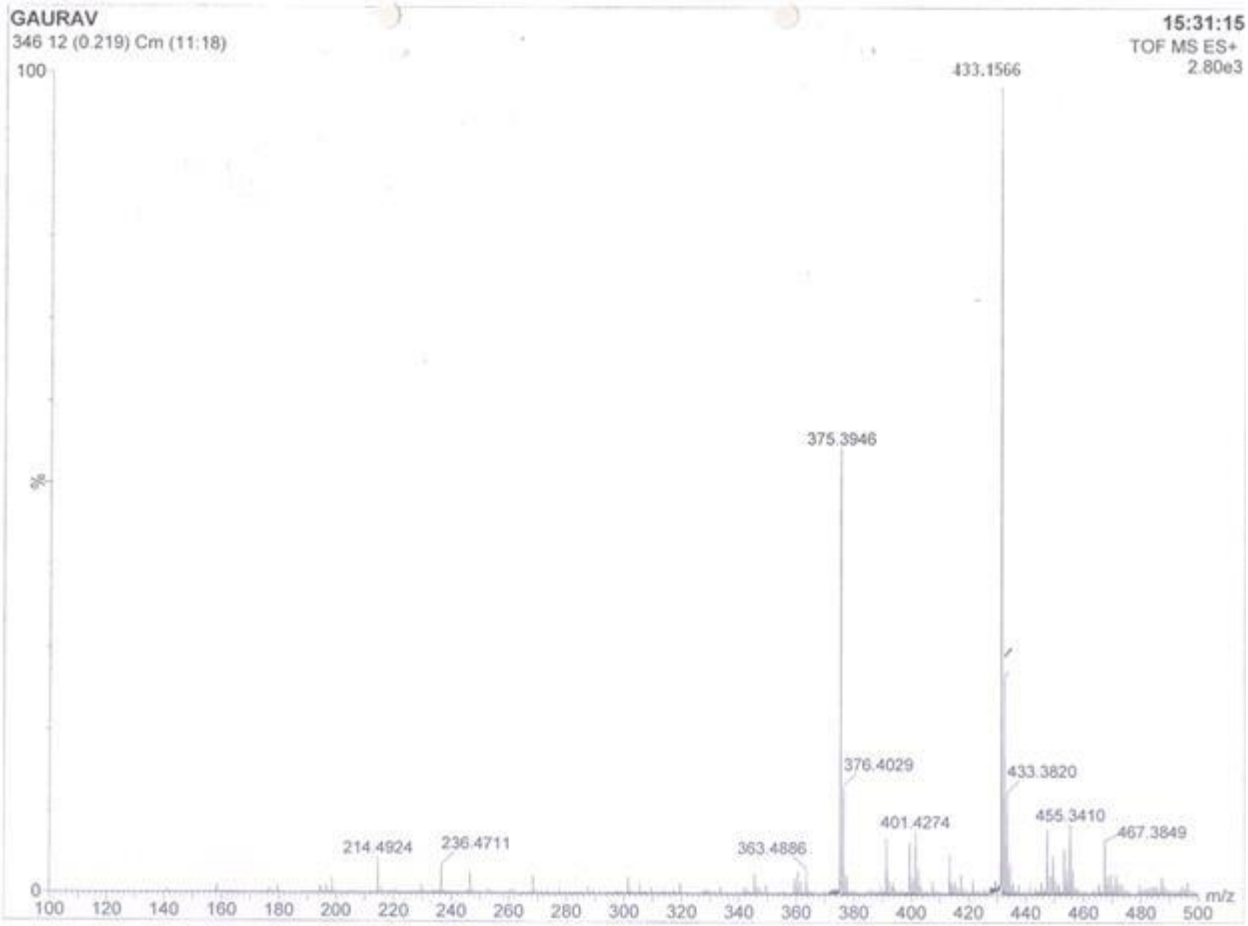


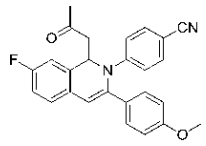
^{13}CNMR of 1-(7-fluoro-3-(4-methoxyphenyl)-2-(4-nitrophenyl)-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4c)**



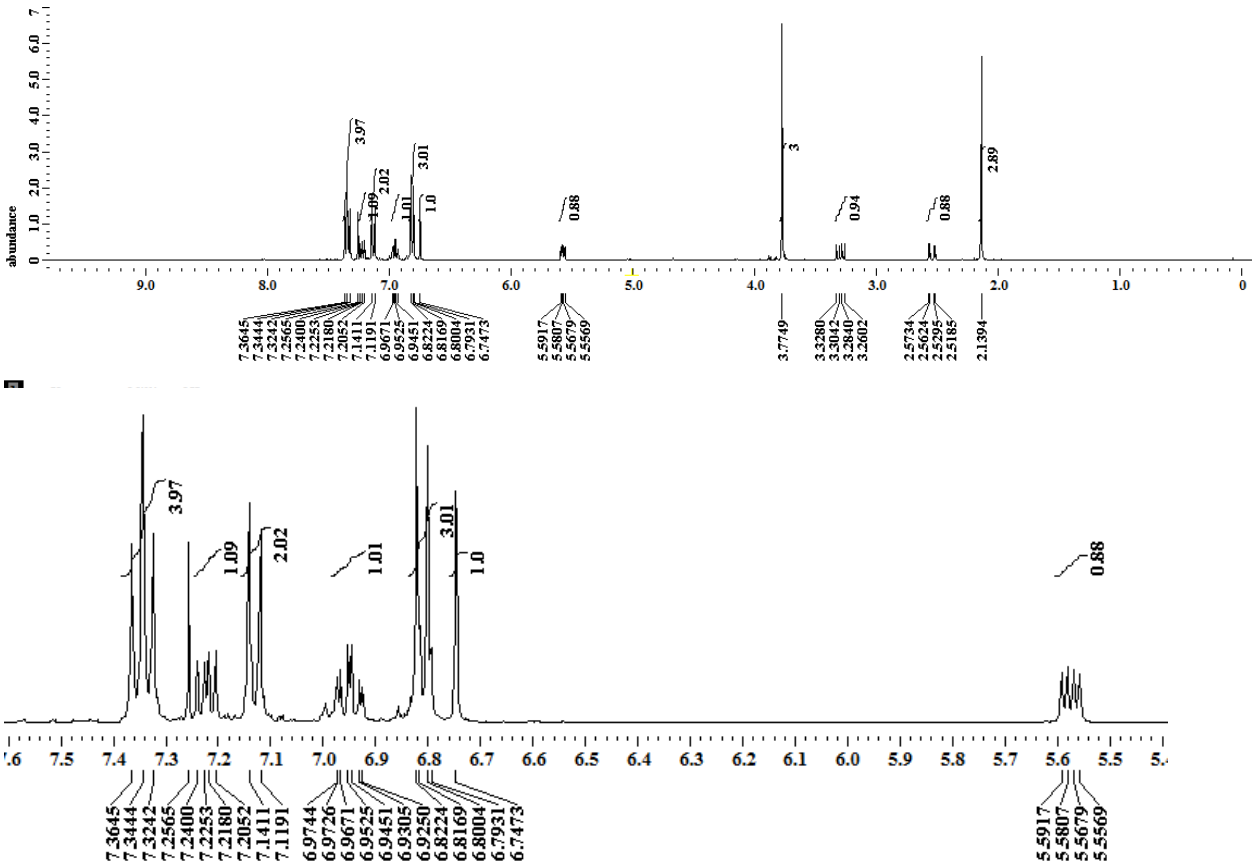


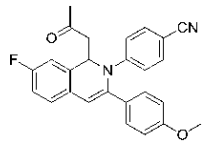
HRMS of 1-(7-fluoro-3-(4-methoxyphenyl)-2-(4-nitrophenyl)-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4c)**



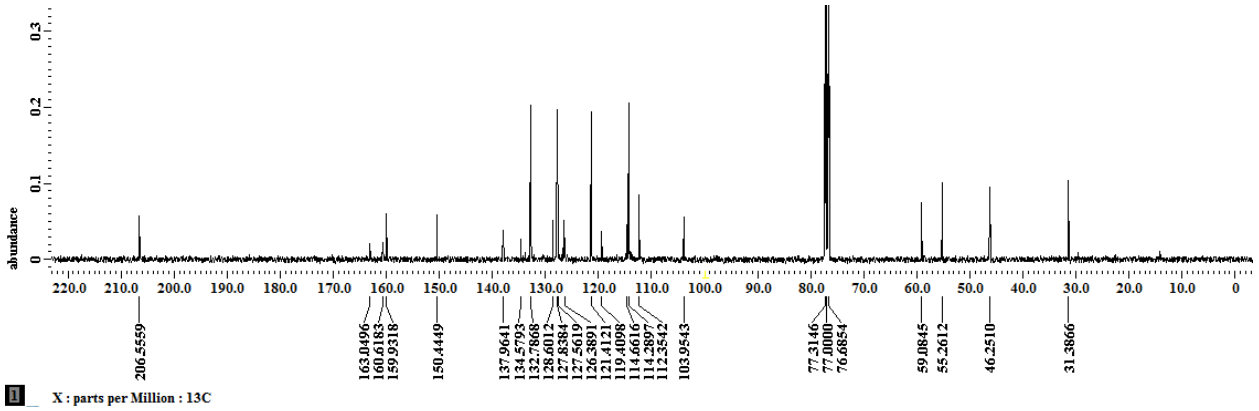


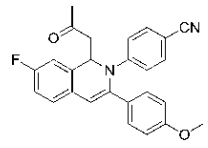
^{1}HNMR of 4-(7-fluoro-3-(4-methoxyphenyl)-1-(2-oxopropyl)isoquinolin-2(1H)-yl)benzonitrile **(4d)**





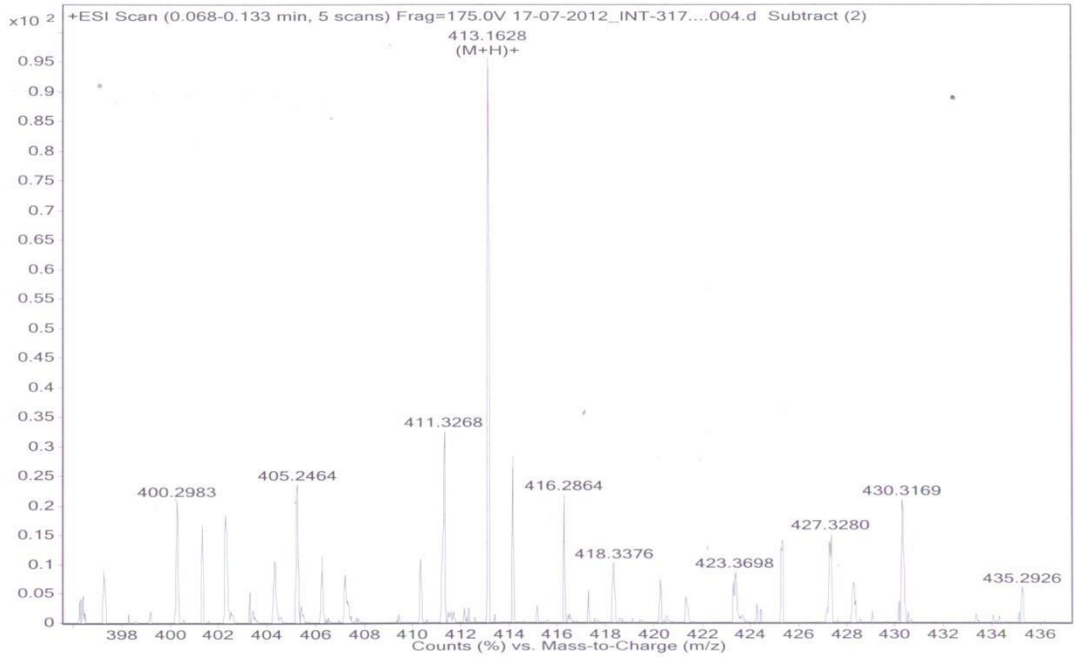
^{13}CNMR of 4-(7-fluoro-3-(4-methoxyphenyl)-1-(2-oxopropyl)isoquinolin-2(1H)-yl)benzonitrile **(4d)**

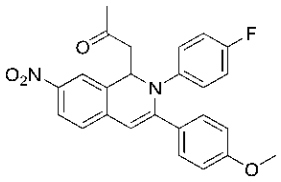




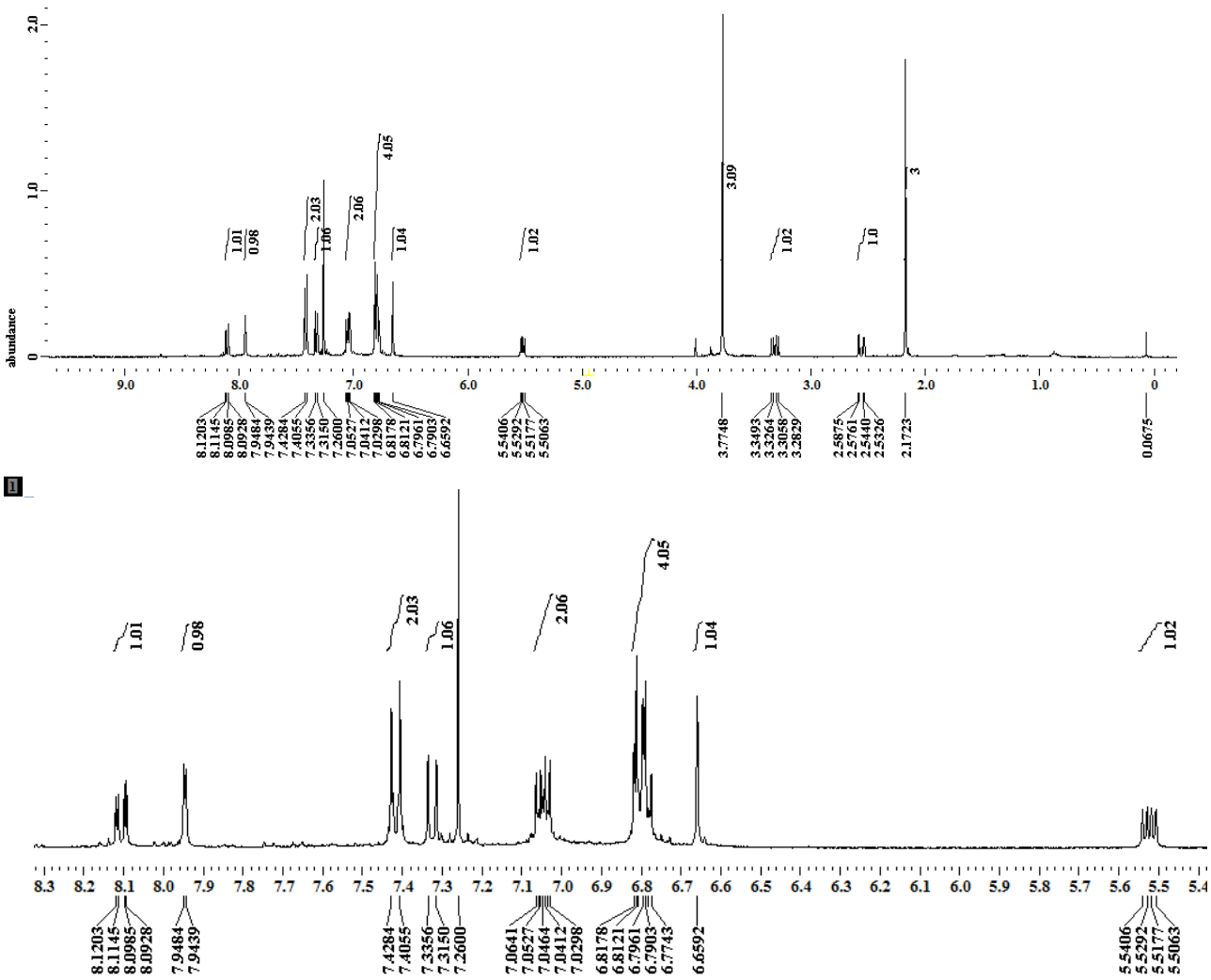
HRMS of 4-(7-fluoro-3-(4-methoxyphenyl)-1-(2-oxopropyl)isoquinolin-2(1H)-yl)benzonitrile **(4d)**

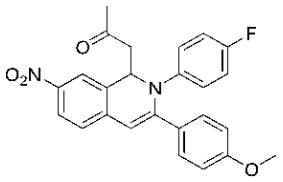
Sample Name	INT-317	**Position**	Vial 4	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	1	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	17-07-2012_INT-317..	**ACQ Method**		**Comment**		**Acquired Time**	7/17/2012 12:28:59 PM



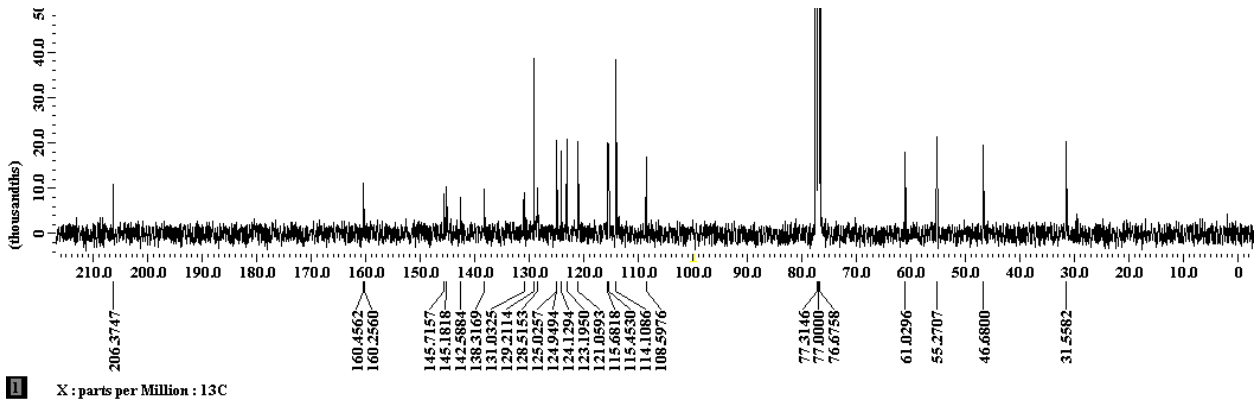


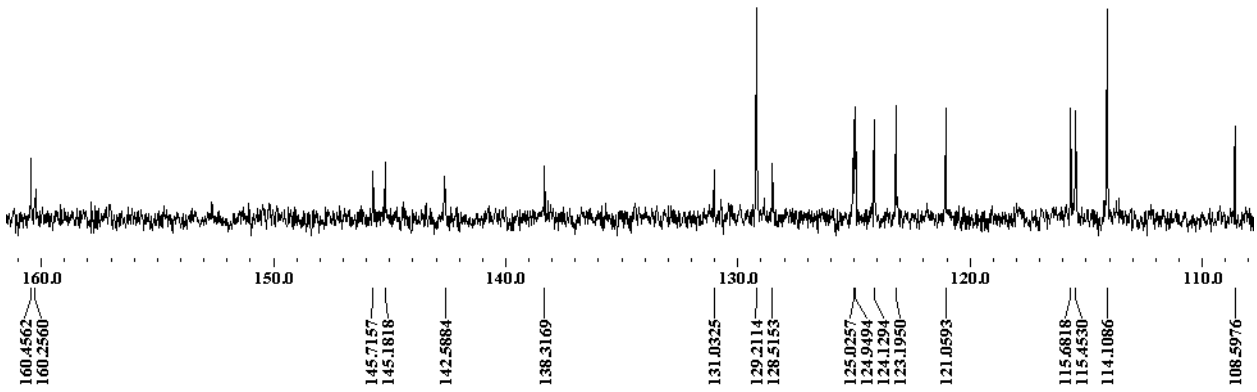
[1]HNMR of 1-(2-(4-fluorophenyl)-3-(4-methoxyphenyl)-7-nitro-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4e)**

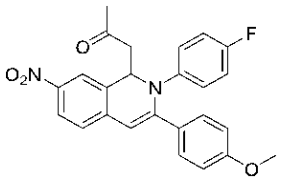




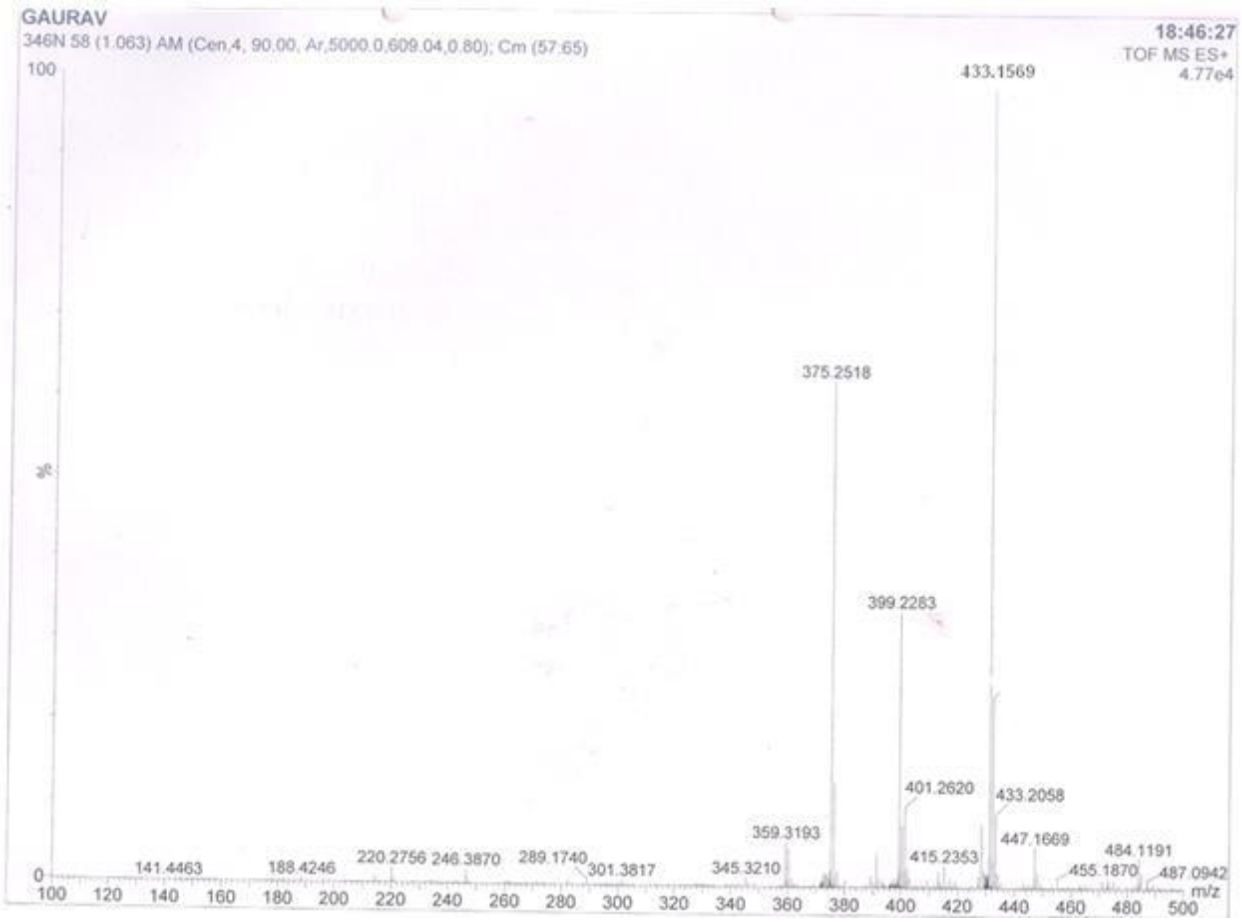
^{13}CNMR of 1-(2-(4-fluorophenyl)-3-(4-methoxyphenyl)-7-nitro-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4e)**

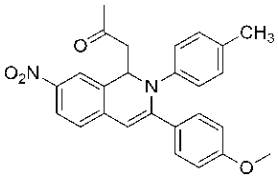




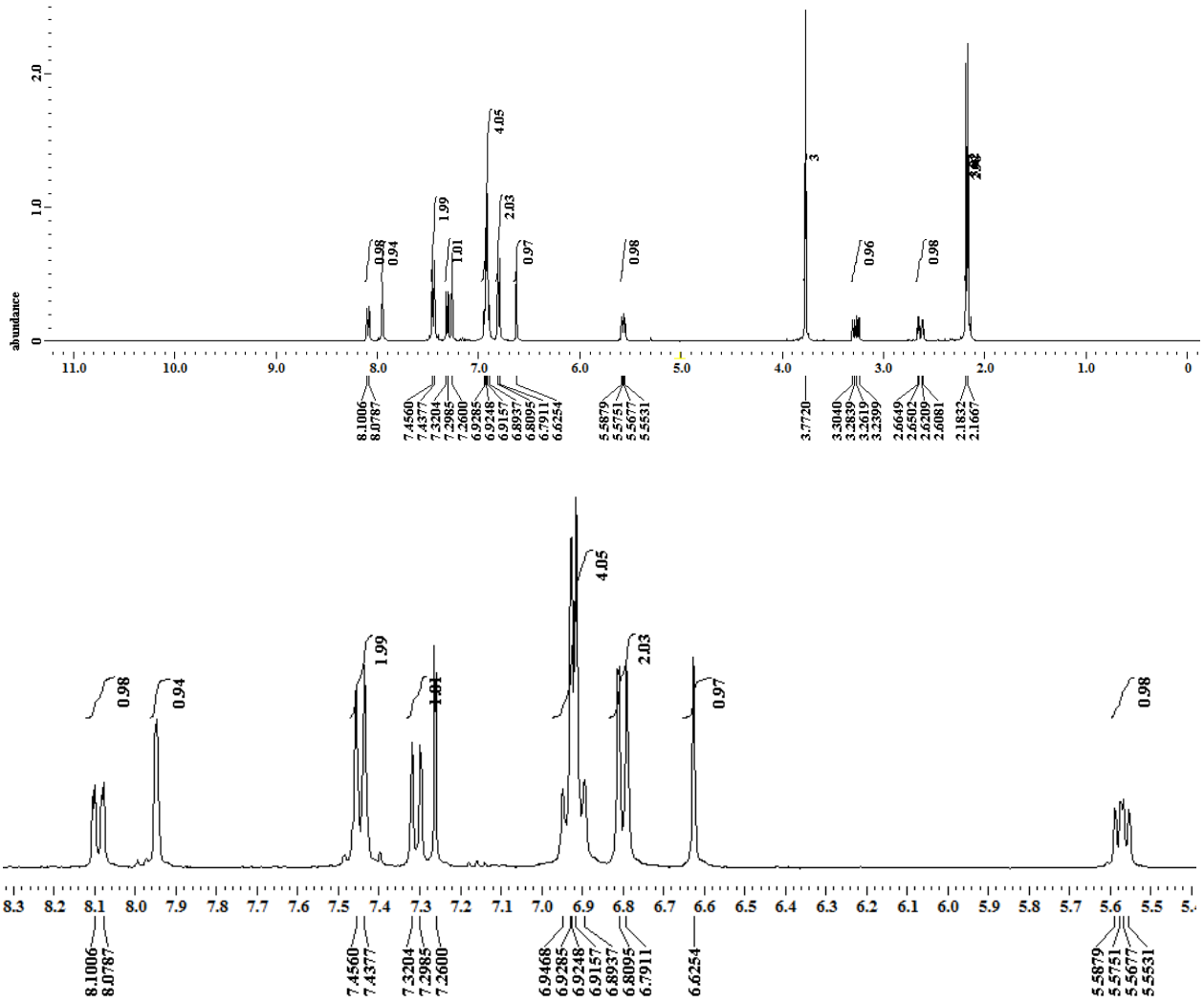


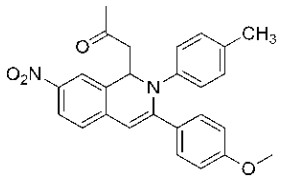
HRMS of 1-(2-(4-fluorophenyl)-3-(4-methoxyphenyl)-7-nitro-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4e)**



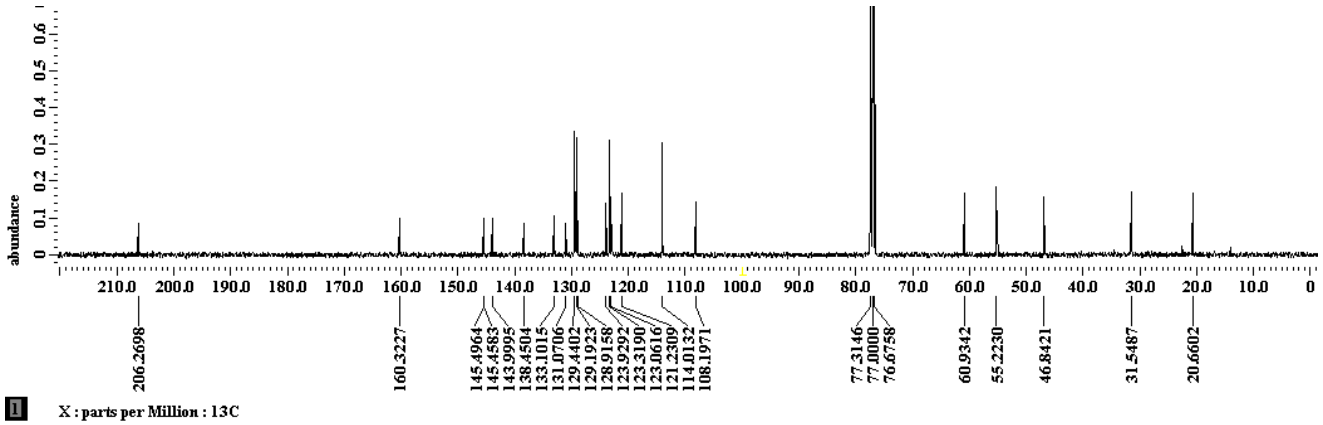


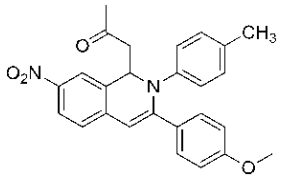
[1]HNMR of 1-(3-(4-methoxyphenyl)-7-nitro-2-p-tolyl-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4f)**



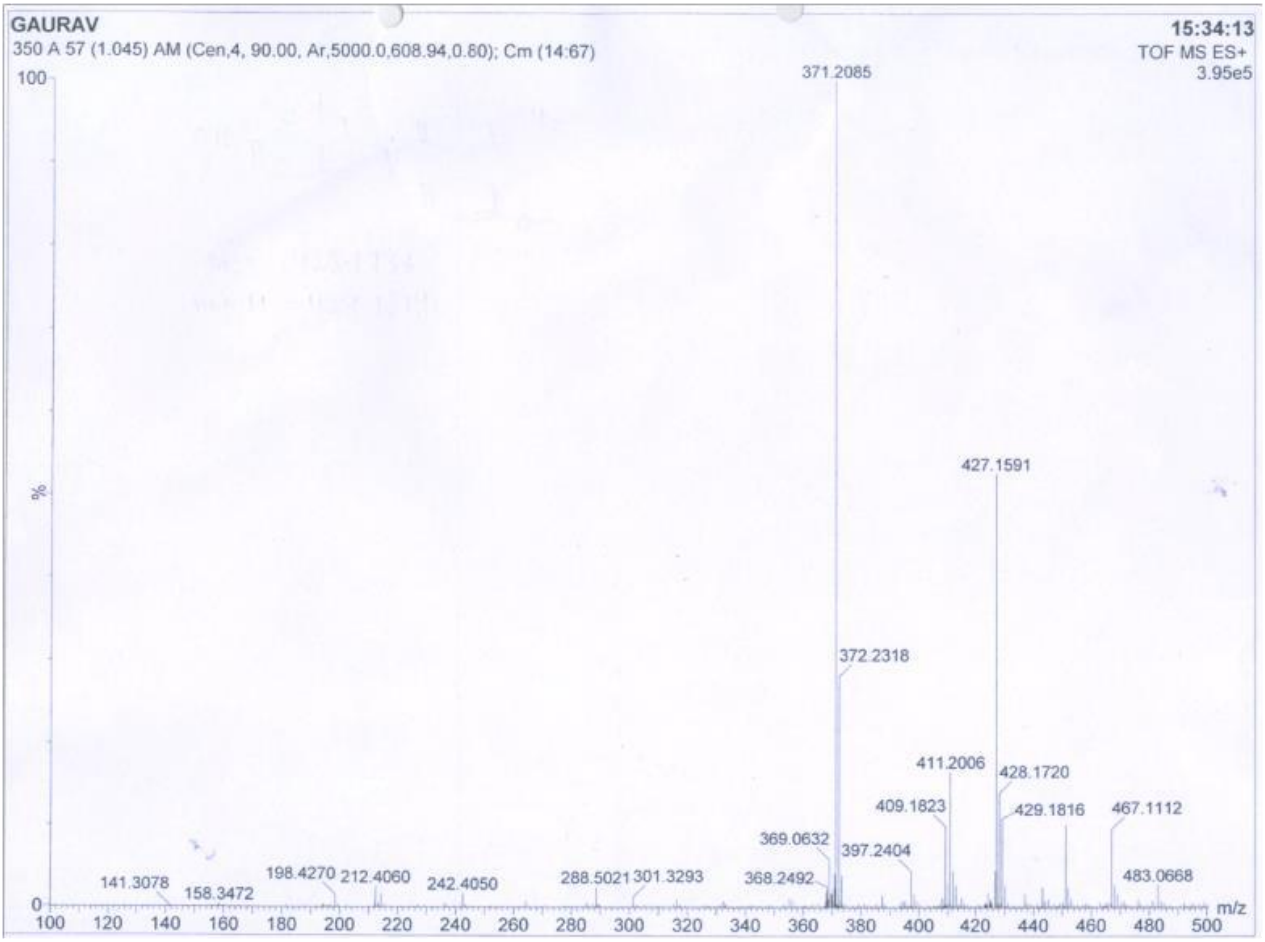


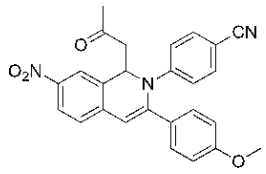
^{13}CNMR of 1-(3-(4-methoxyphenyl)-7-nitro-2-p-tolyl-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4f)**



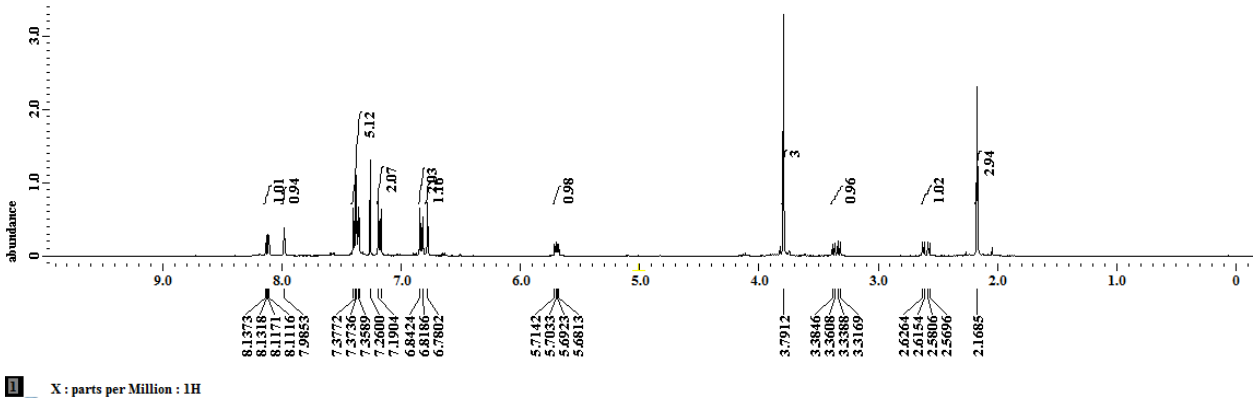


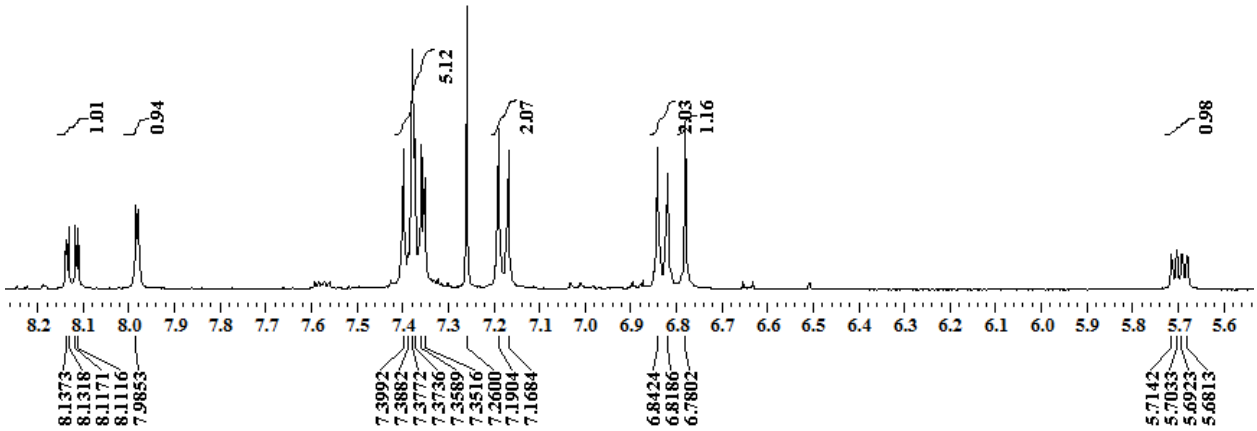
HRMS of 1-(3-(4-methoxyphenyl)-7-nitro-2-p-tolyl-1,2-dihydroisoquinolin-1-yl)propan-2-one**(4f)**

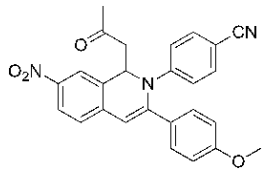




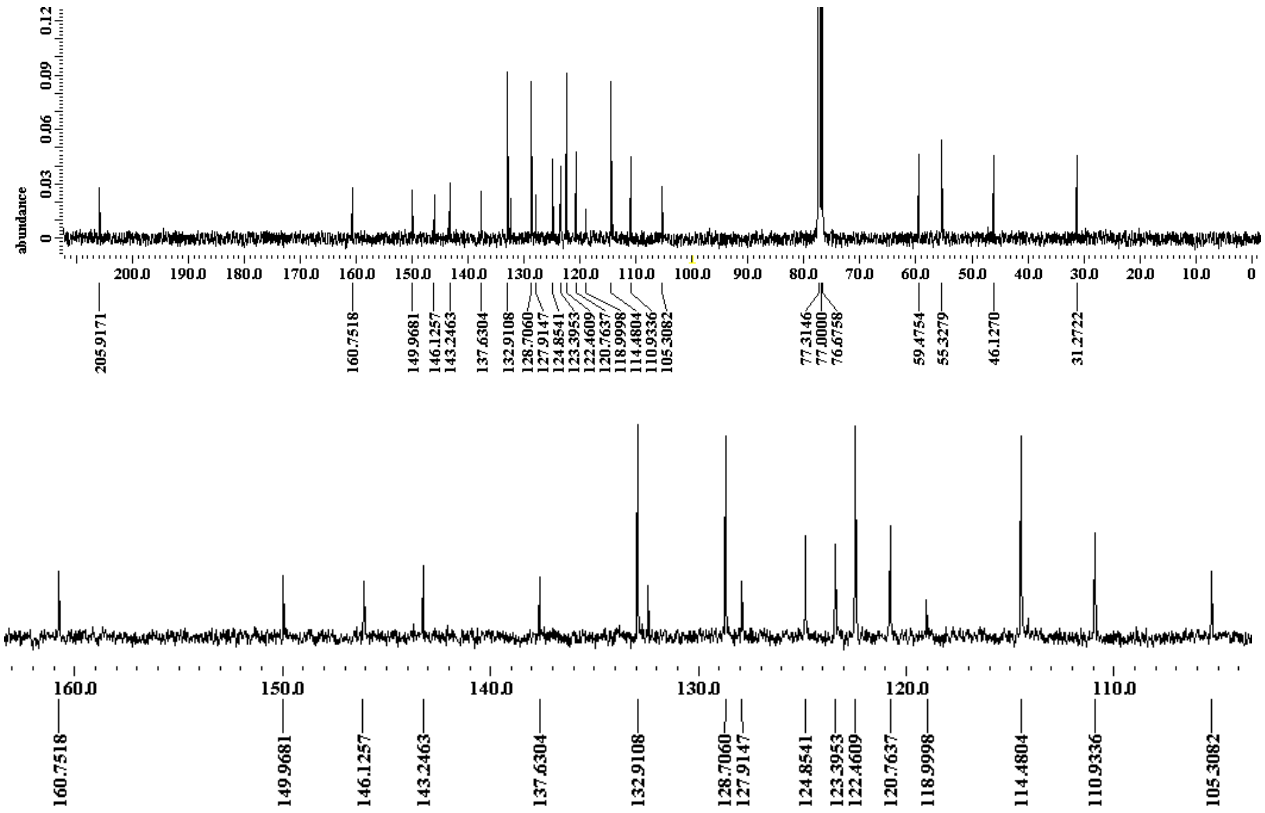
^{1}HNMR of 4-(3-(4-methoxyphenyl)-7-nitro-1-(2-oxopropyl)isoquinolin-2(1H)-yl)benzonitrile**(4g)**

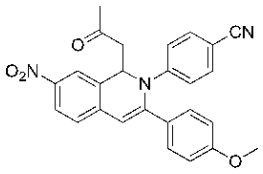




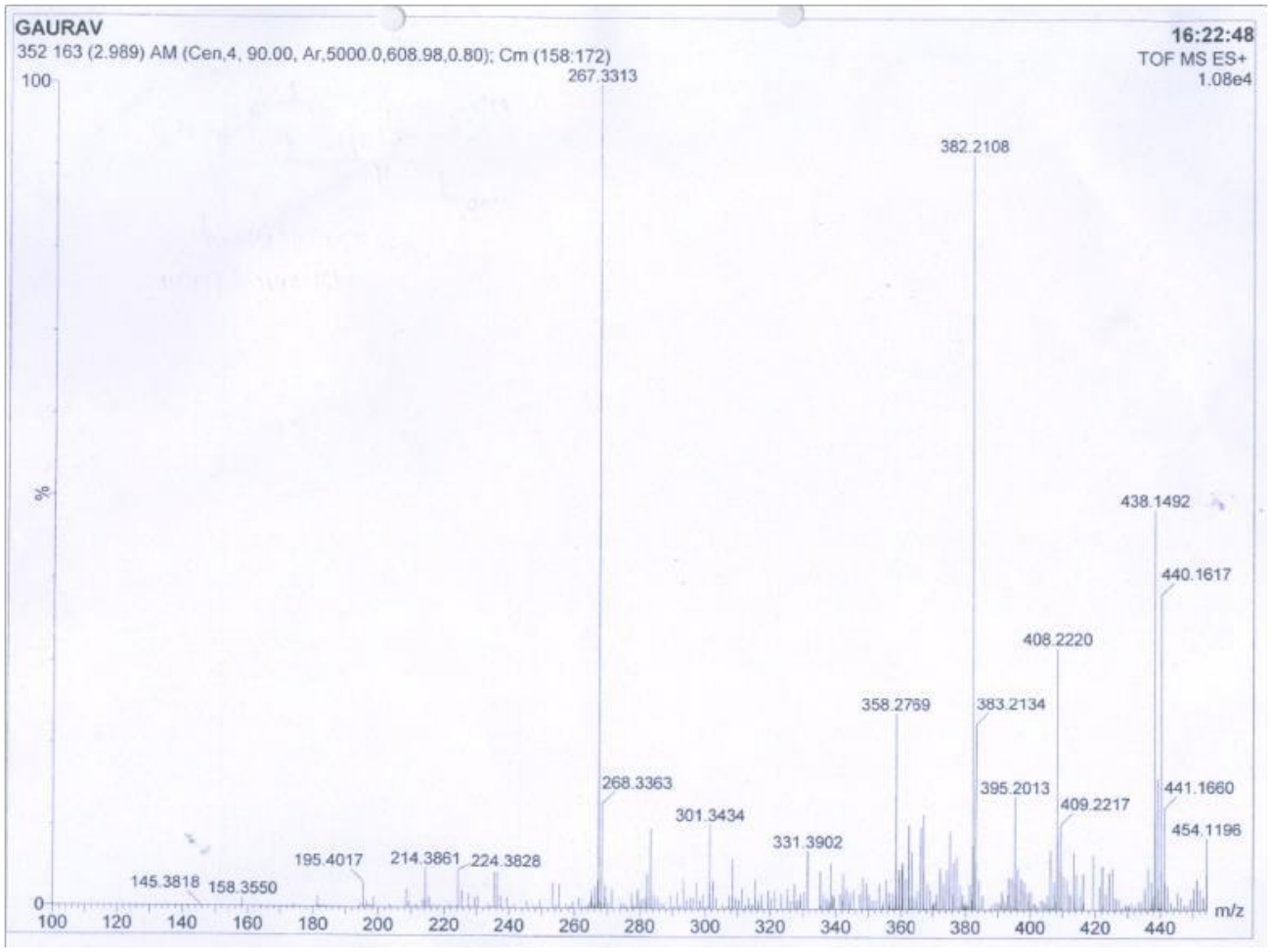


^{13}CNMR of 4-(3-(4-methoxyphenyl)-7-nitro-1-(2-oxopropyl)isoquinolin-2(1H)-yl)benzonitrile**(4g)**

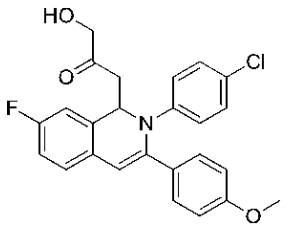




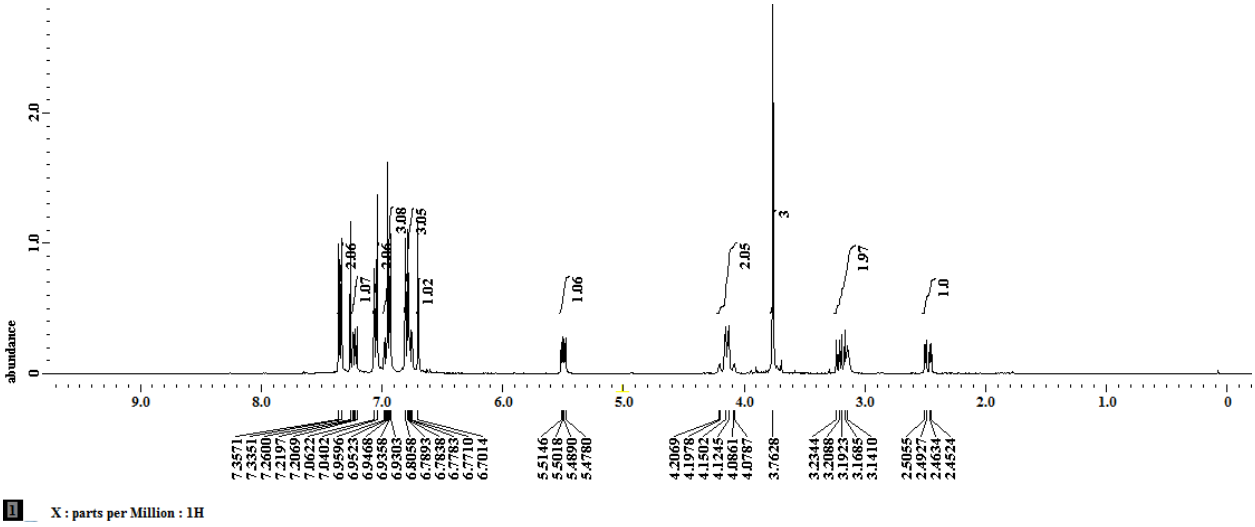
HRMS of 4-(3-(4-methoxyphenyl)-7-nitro-1-(2-oxopropyl)isoquinolin-2(1H)-yl)benzonitrile**(4g)**

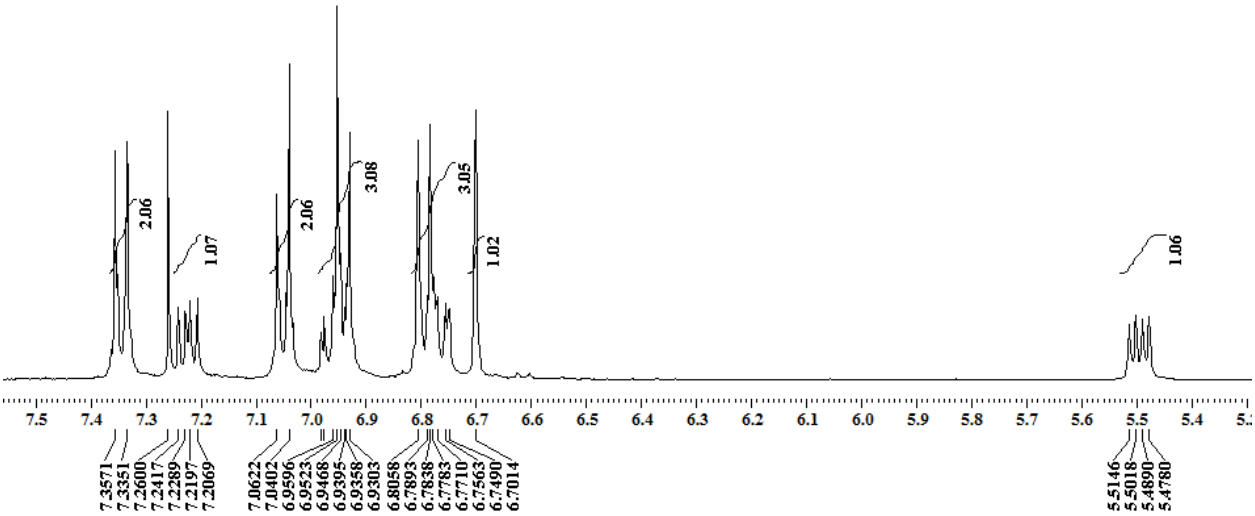


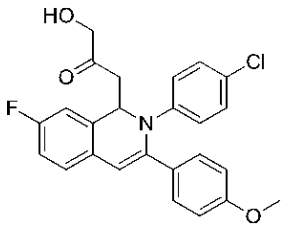
1-(2-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-7-nitro-1,2-dihydroisoquinolin-1-yl)propan-2-one (4i) and 1-(7-Fluoro-3-(4-methoxyphenyl)-2-p-tolyl-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one (4j) was earlier published by Urvashi et. al.[21]



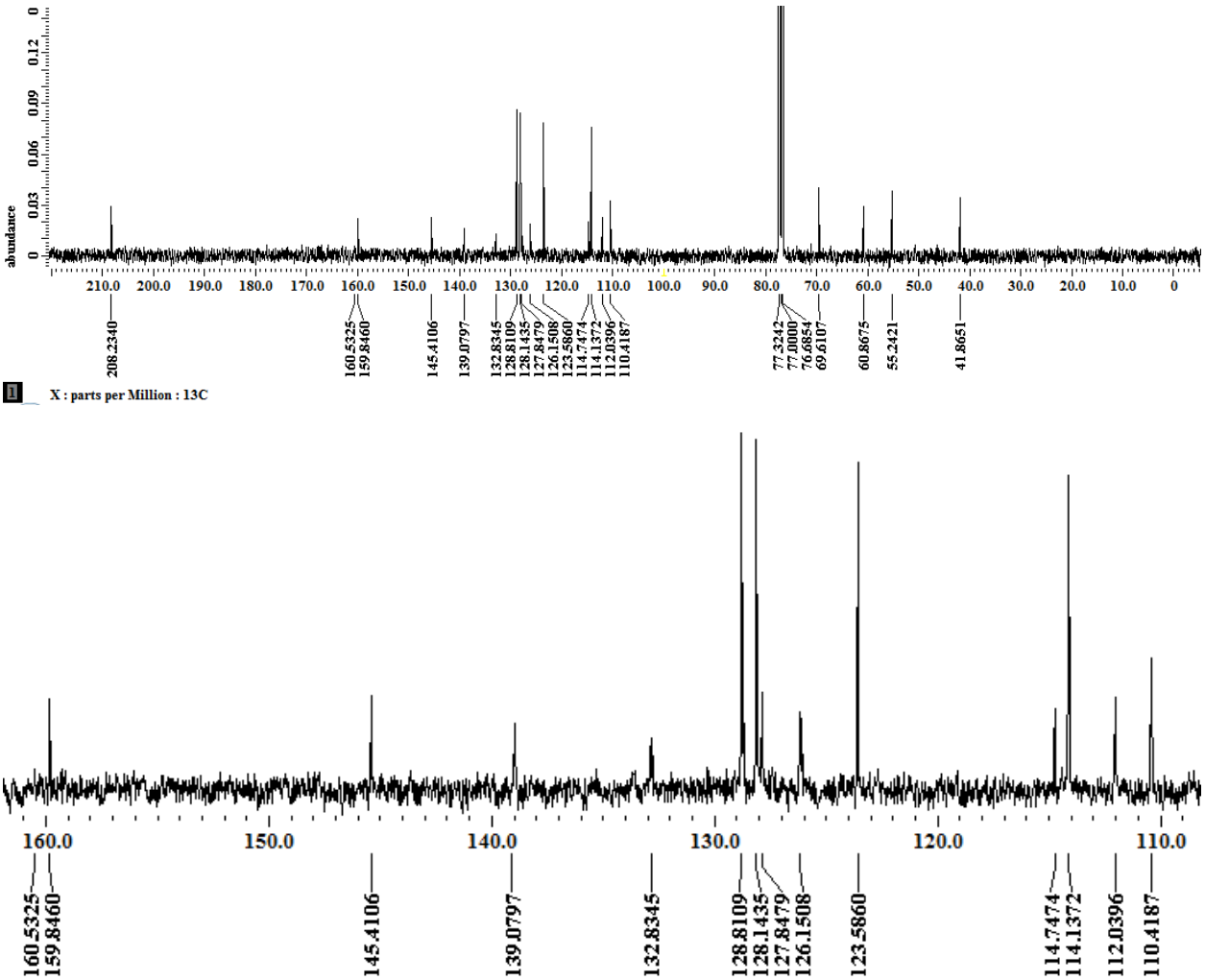
^{1}HNMR 1-(2-(4-chlorophenyl)-7-fluoro-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one**(4j)**

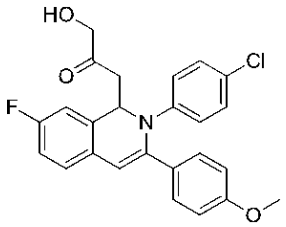




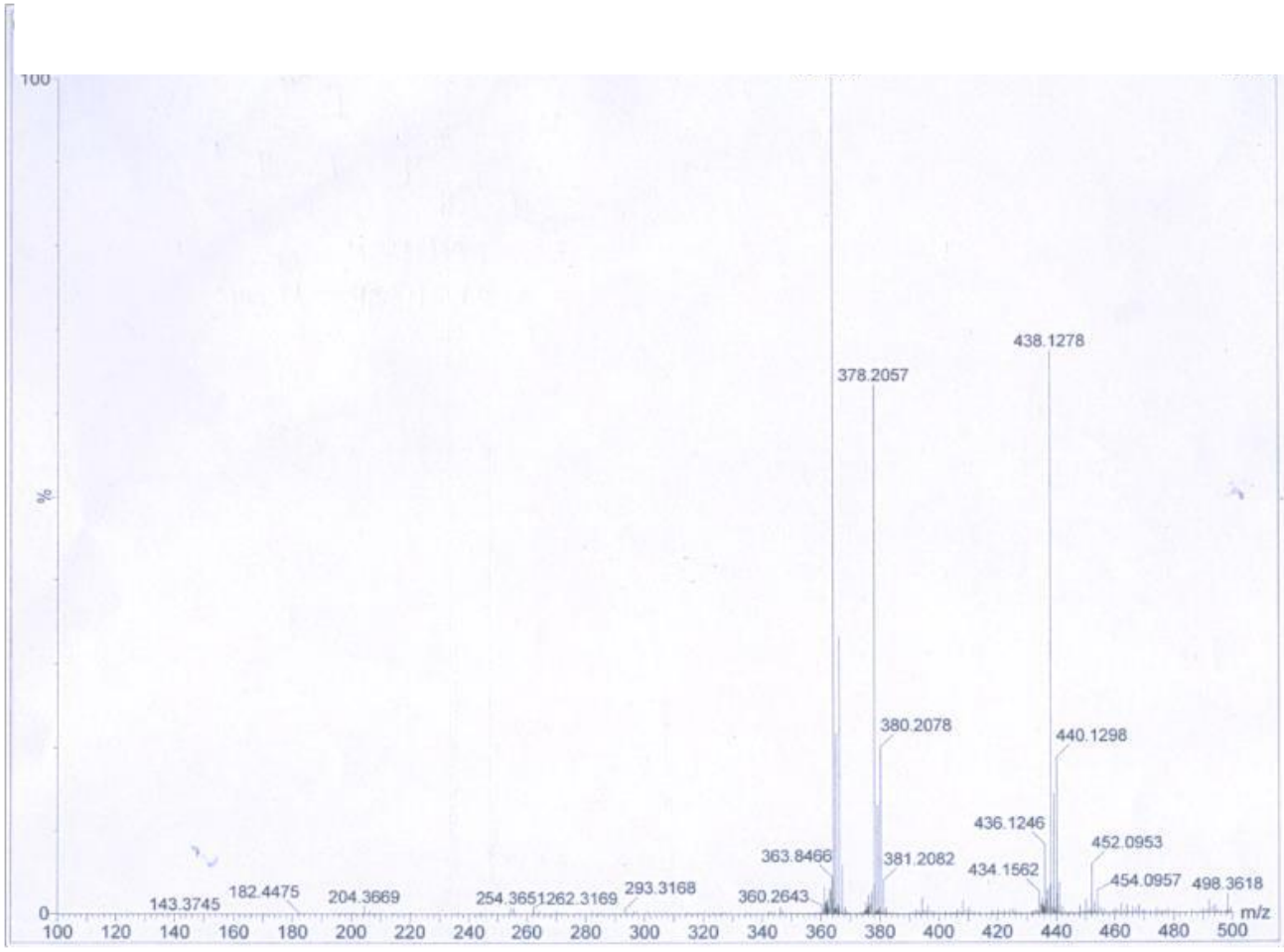


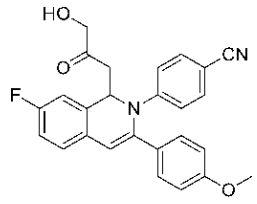
^{13}CNMR of 1-(2-(4-chlorophenyl)-7-fluoro-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one**(4j)**



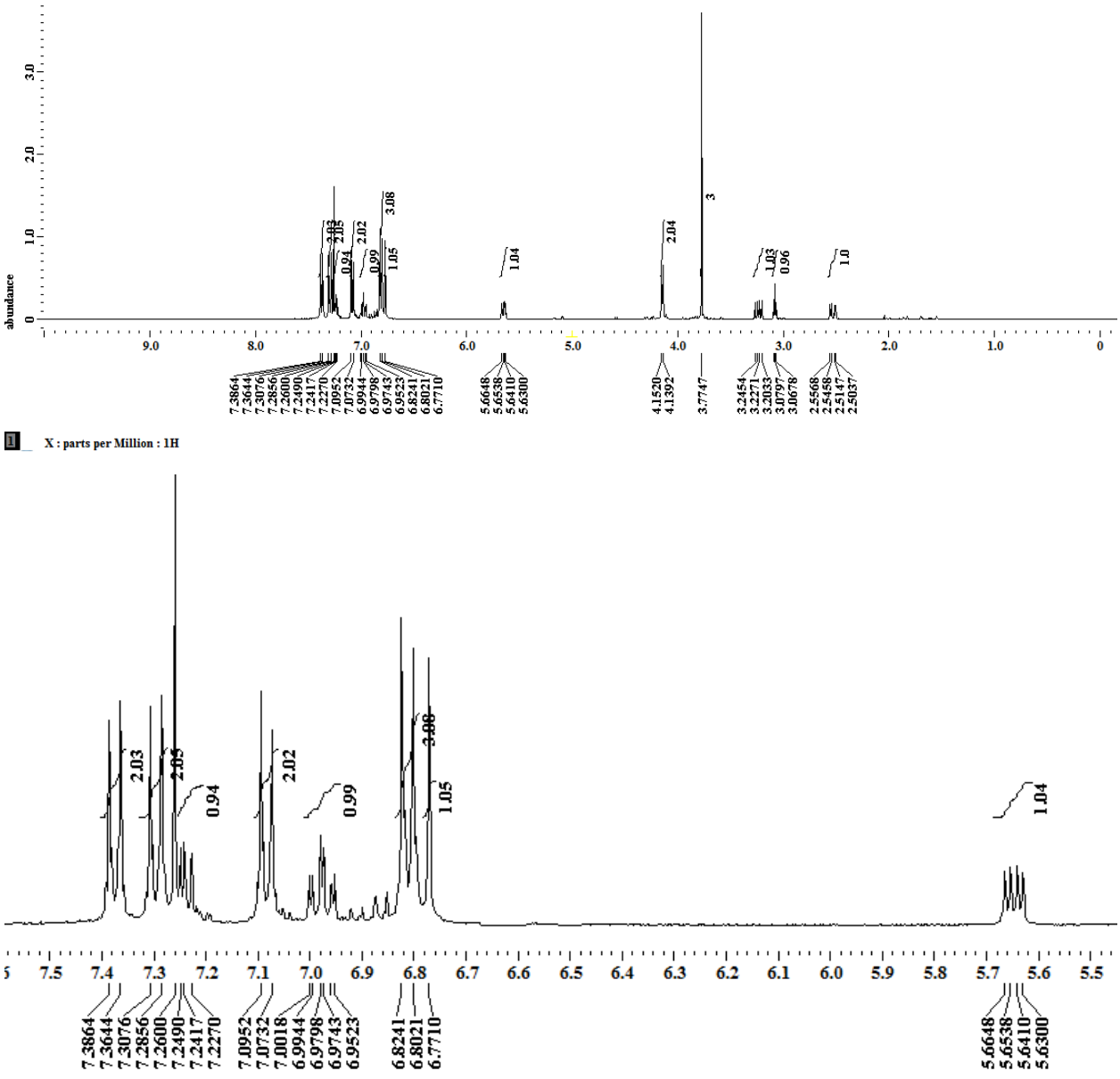


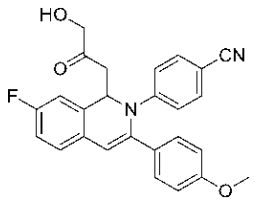
HRMS of 1-(2-(4-chlorophenyl)-7-fluoro-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one**(4j)**



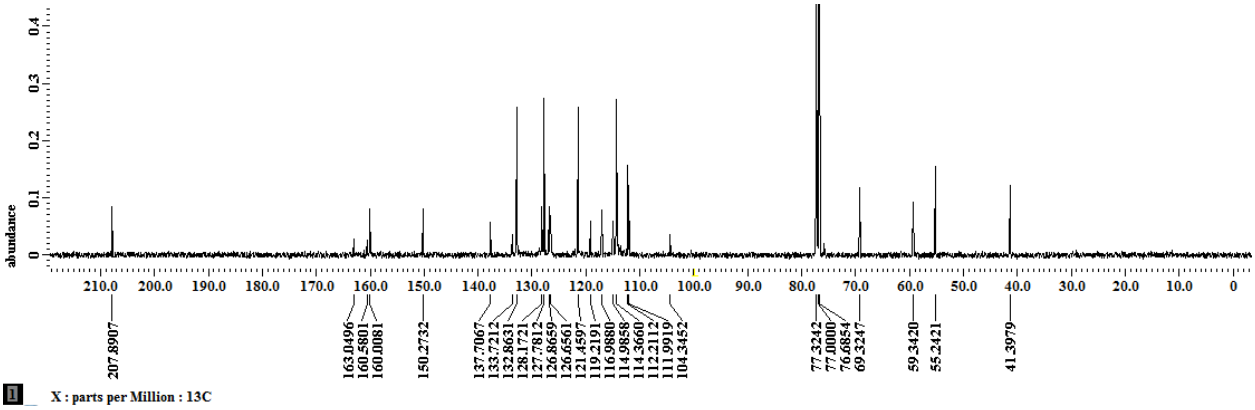


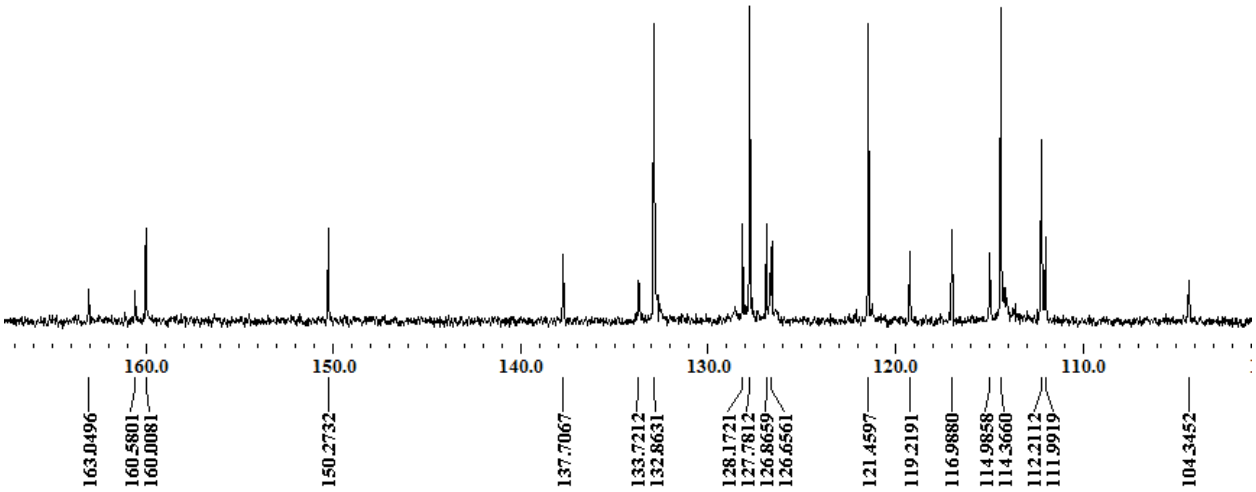
^{1}HNMR of 4-(7-fluoro-1-(3-hydroxy-2-oxopropyl)-3-(4-methoxyphenyl)isoquinolin-2(1H)-yl)benzonitrile**(4k)**

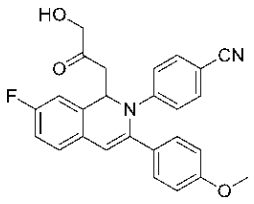




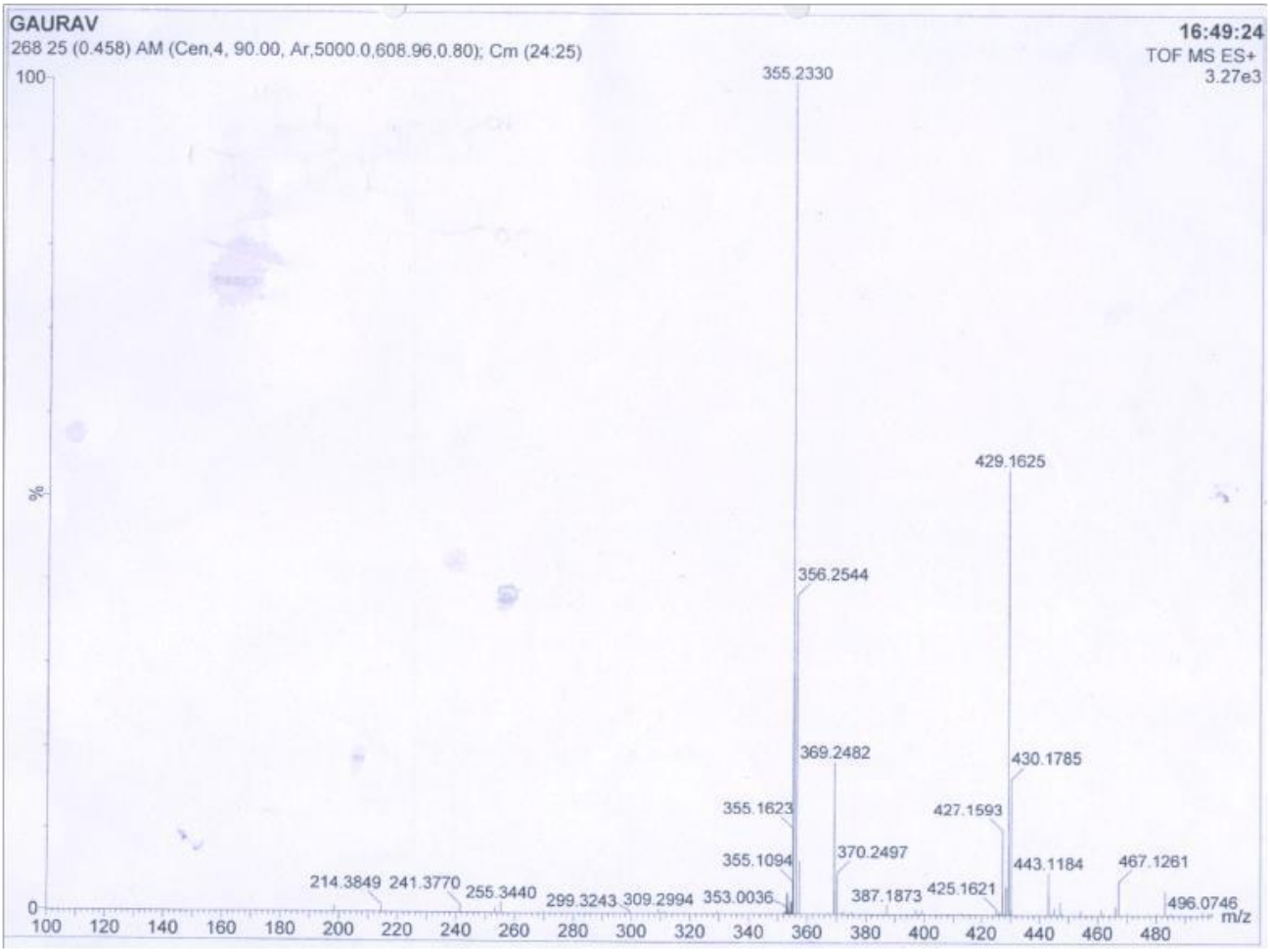
^{13}CNMR of 4-(7-fluoro-1-(3-hydroxy-2-oxopropyl)-3-(4-methoxyphenyl)isoquinolin-2(1H)-yl)benzonitrile**(4k)**

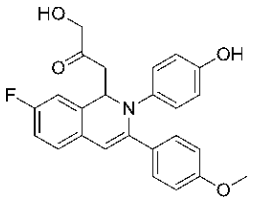




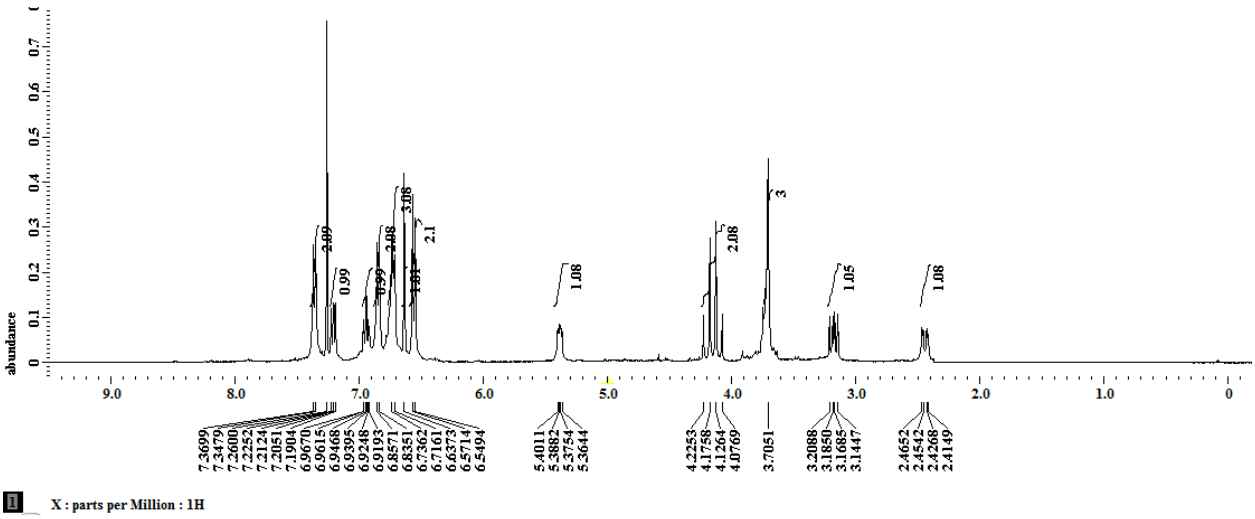


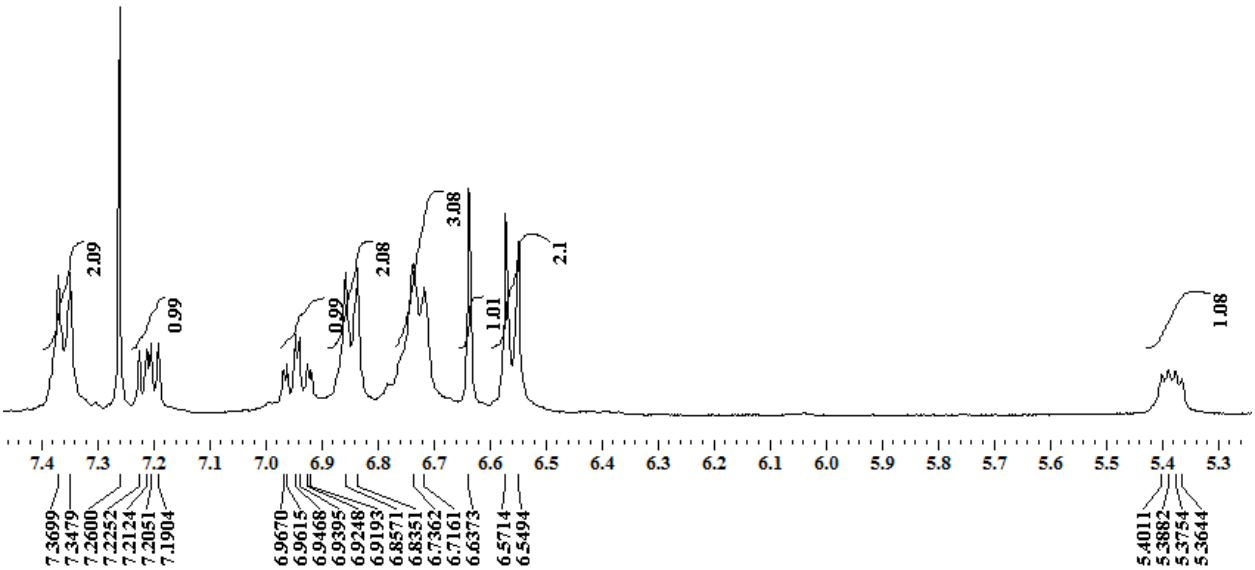
HRMS of 4-(7-fluoro-1-(3-hydroxy-2-oxopropyl)-3-(4-methoxyphenyl)isoquinolin-2(1H)-yl)benzonitrile**(4k)**

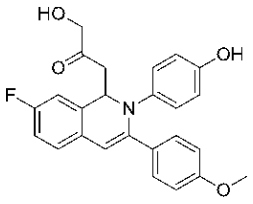




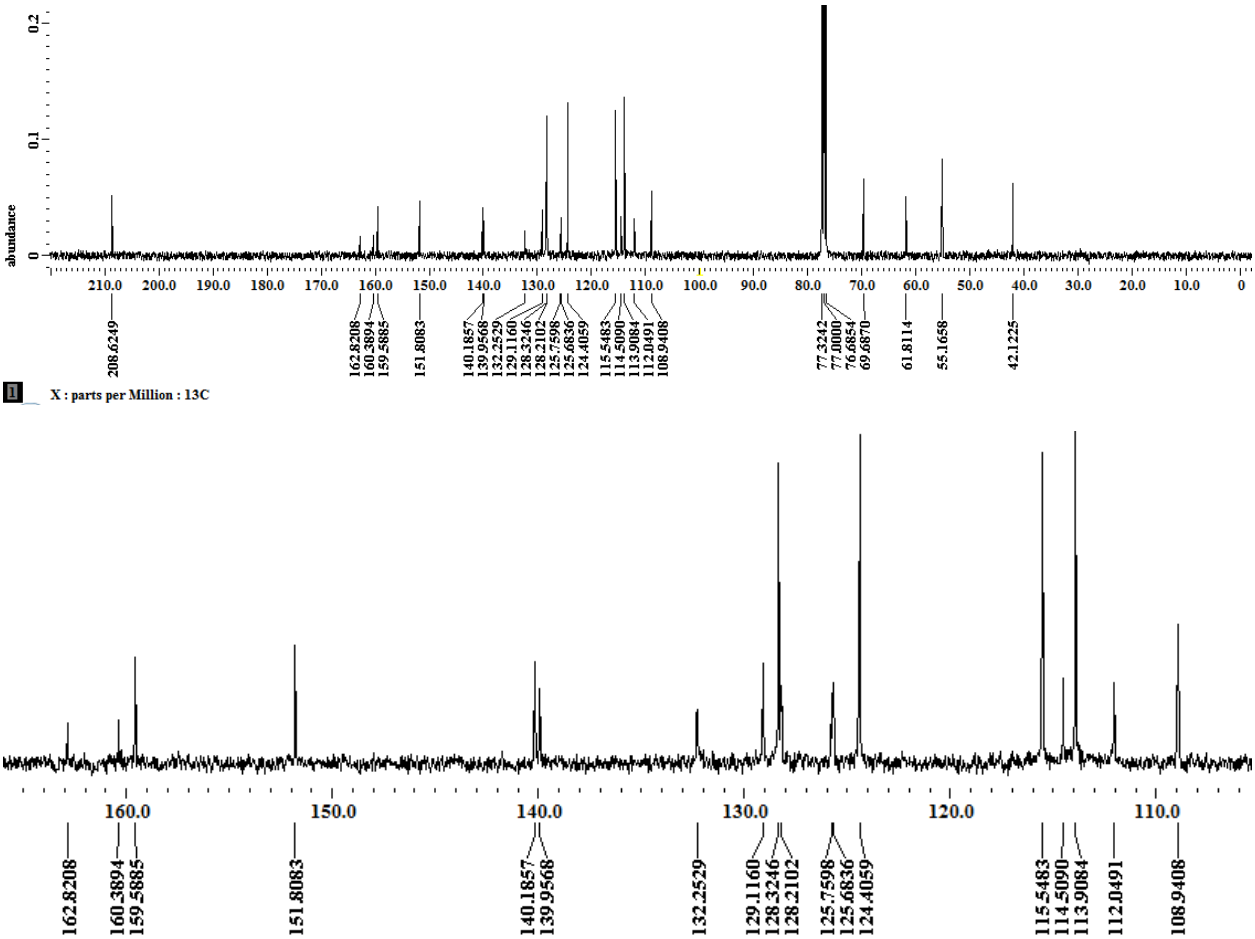
[1]HNMR of 1-(7-fluoro-2-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one**(4l)**

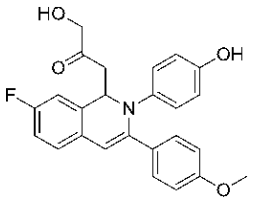




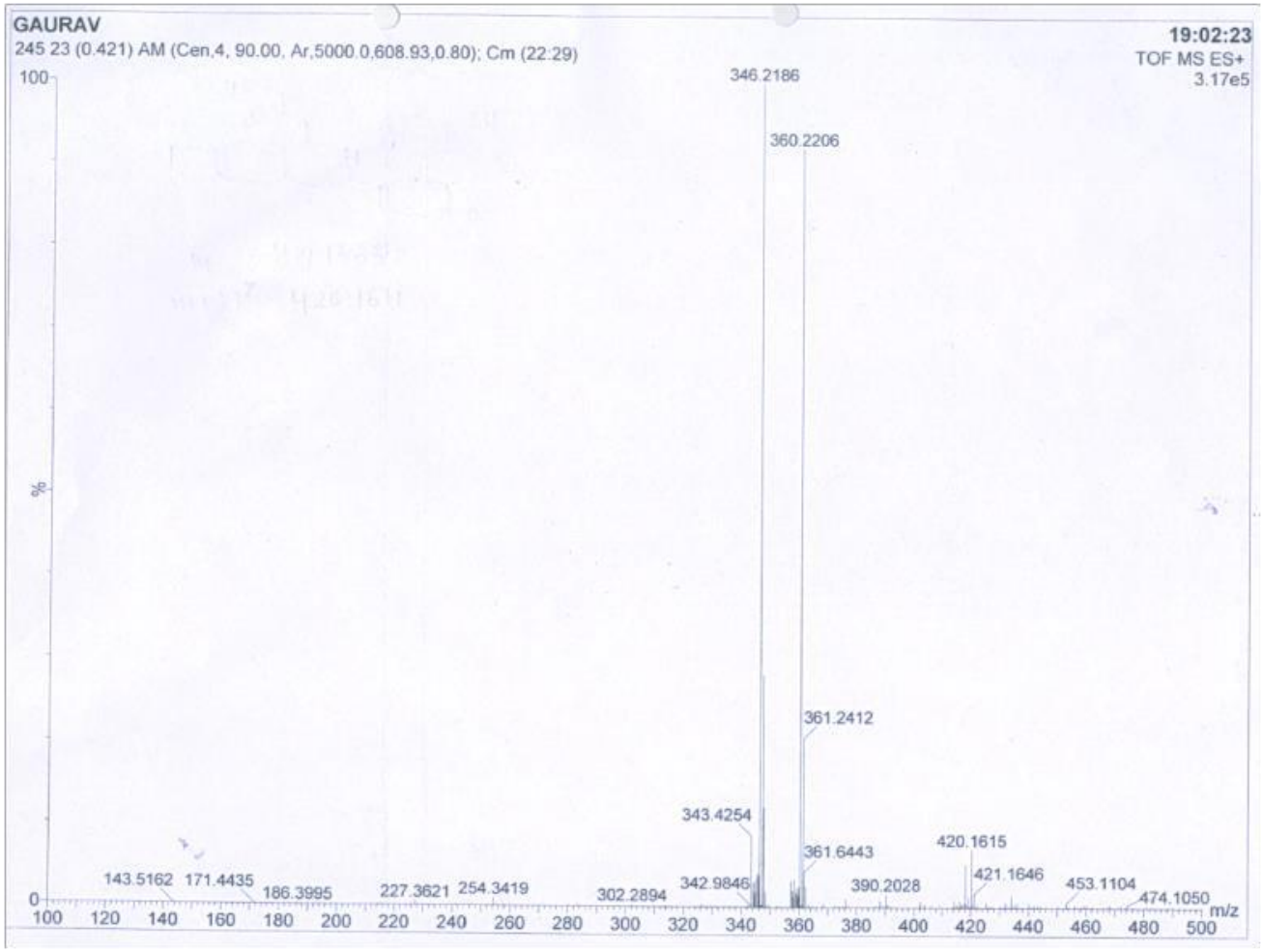


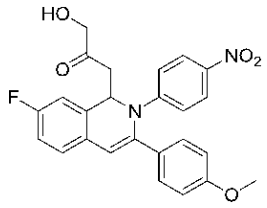
^{13}CNMR of 1-(7-fluoro-2-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one**(4l)**



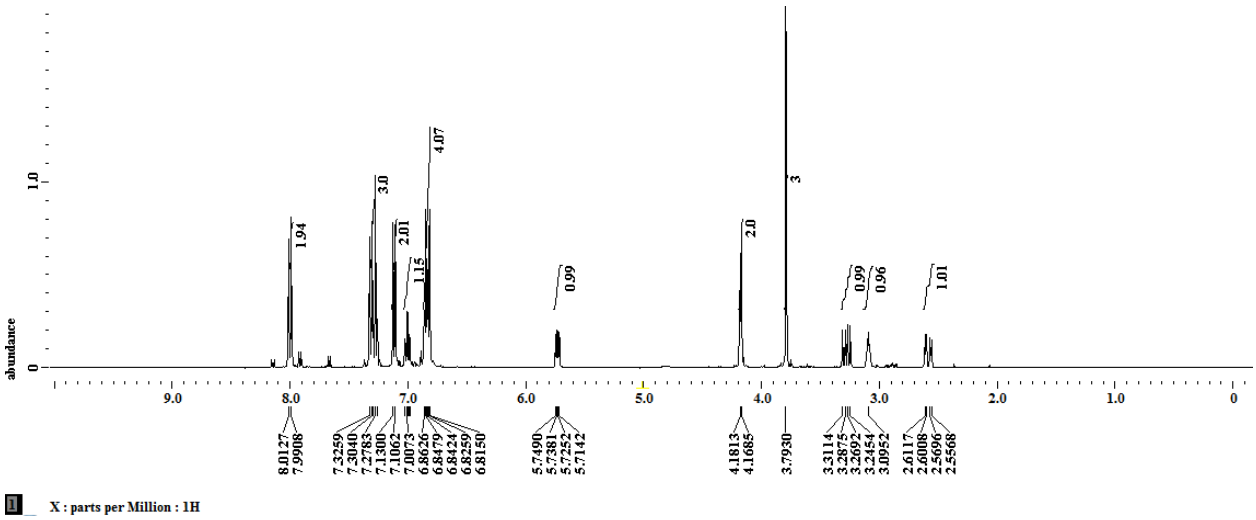


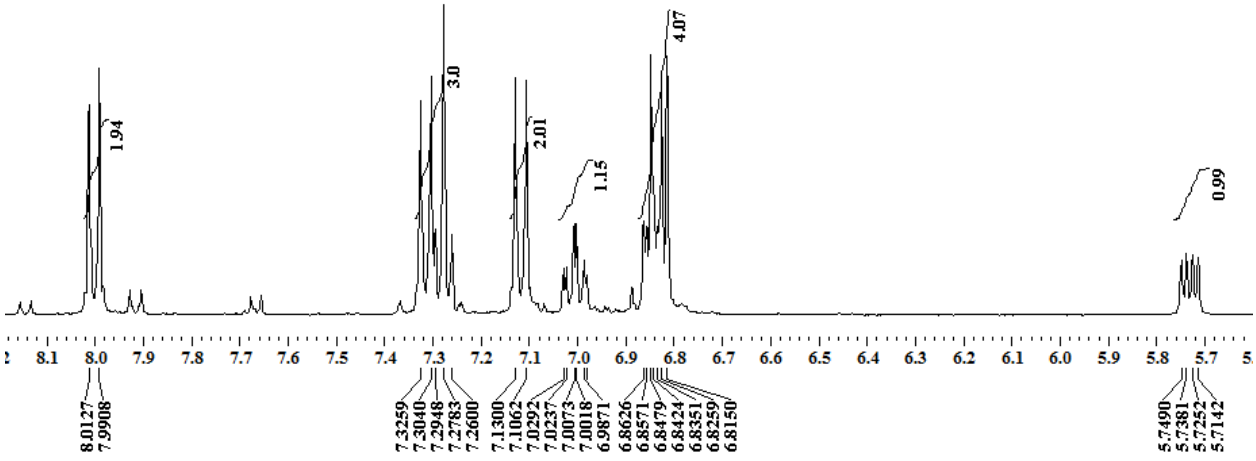
HRMS of 1-(7-fluoro-2-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one**(4l)**

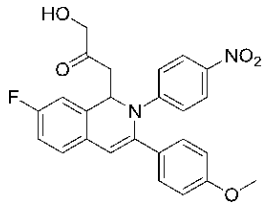




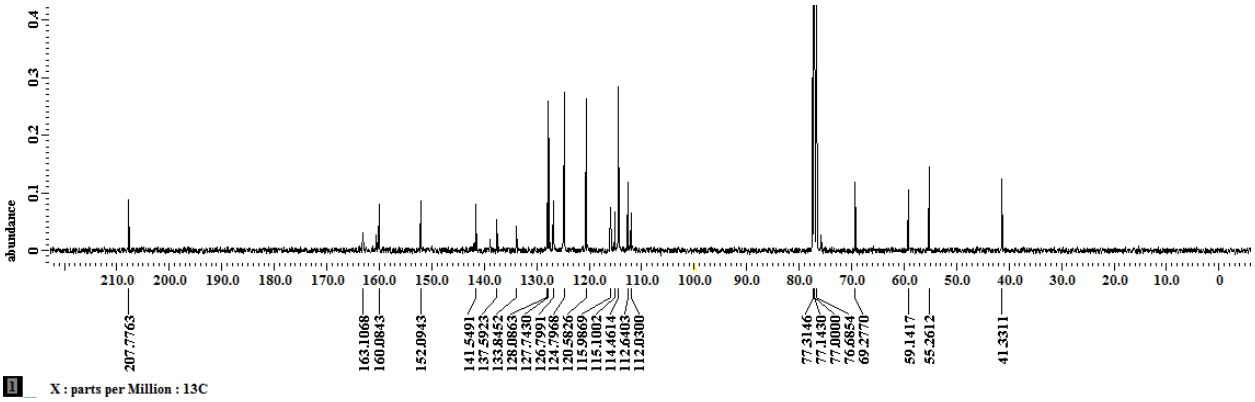
[1]HNMR 1-(7-fluoro-3-(4-methoxyphenyl)-2-(4-nitrophenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one**(4m)**

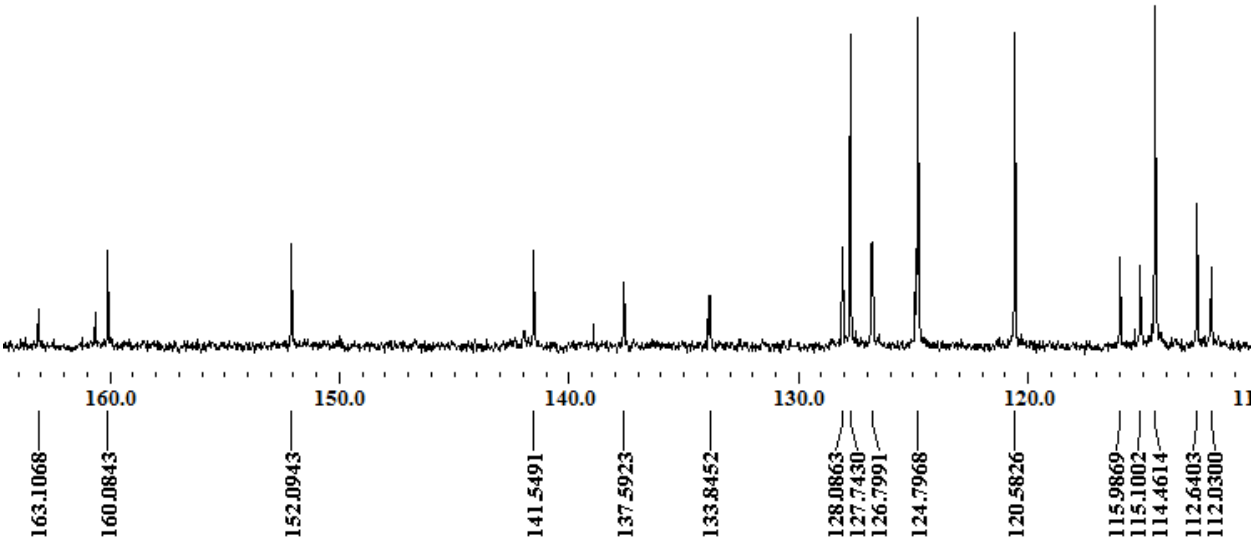


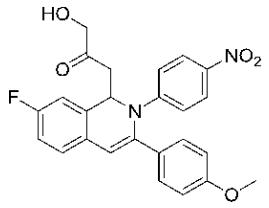




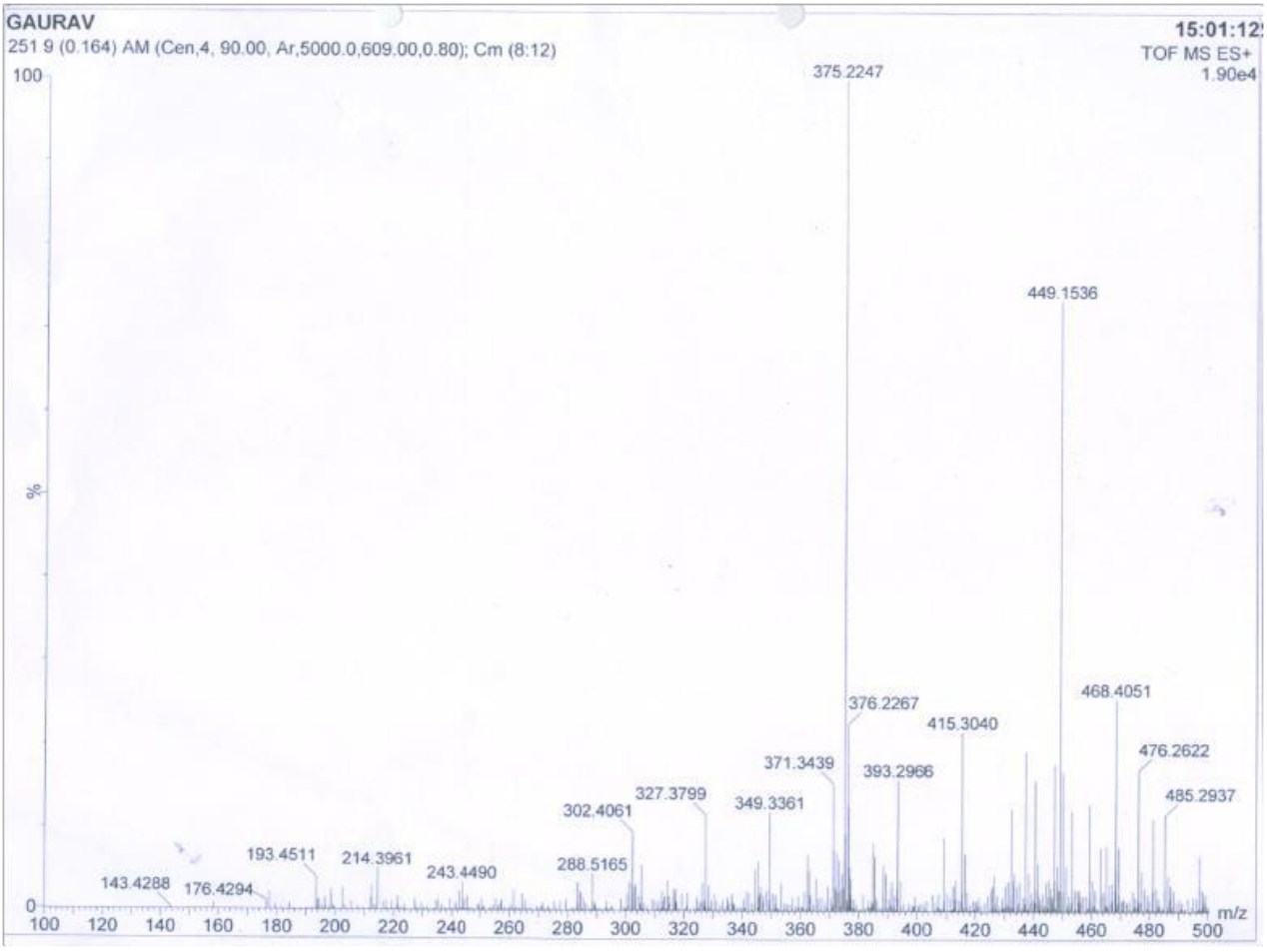
^{13}CNMR of 1-(7-fluoro-3-(4-methoxyphenyl)-2-(4-nitrophenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one**(4m)**

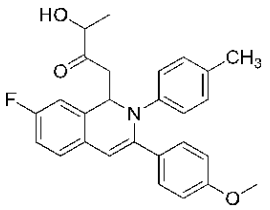




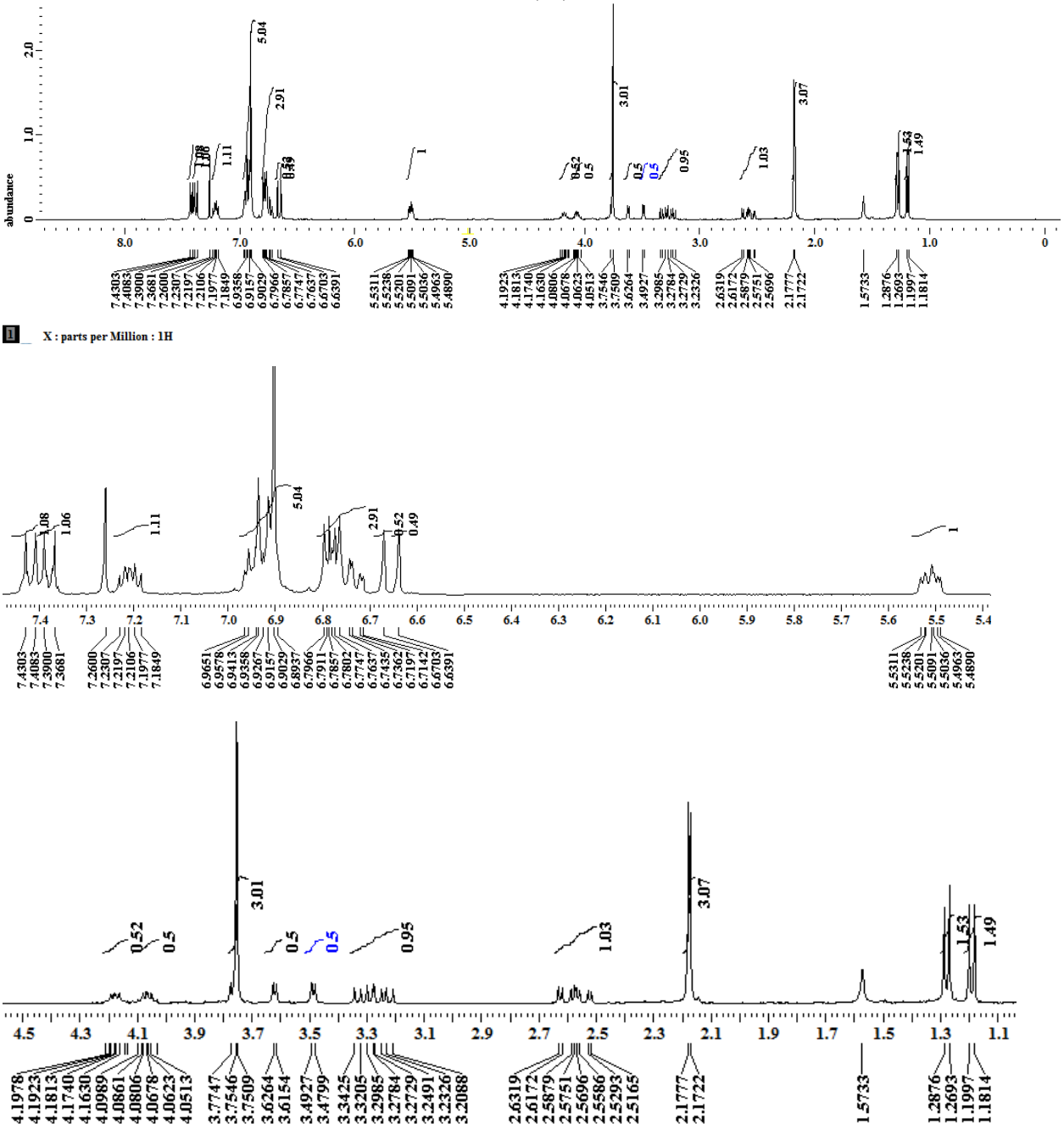


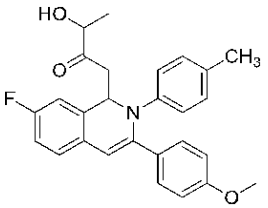
HRMS of 1-(7-fluoro-3-(4-methoxyphenyl)-2-(4-nitrophenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxypropan-2-one**(4m)**



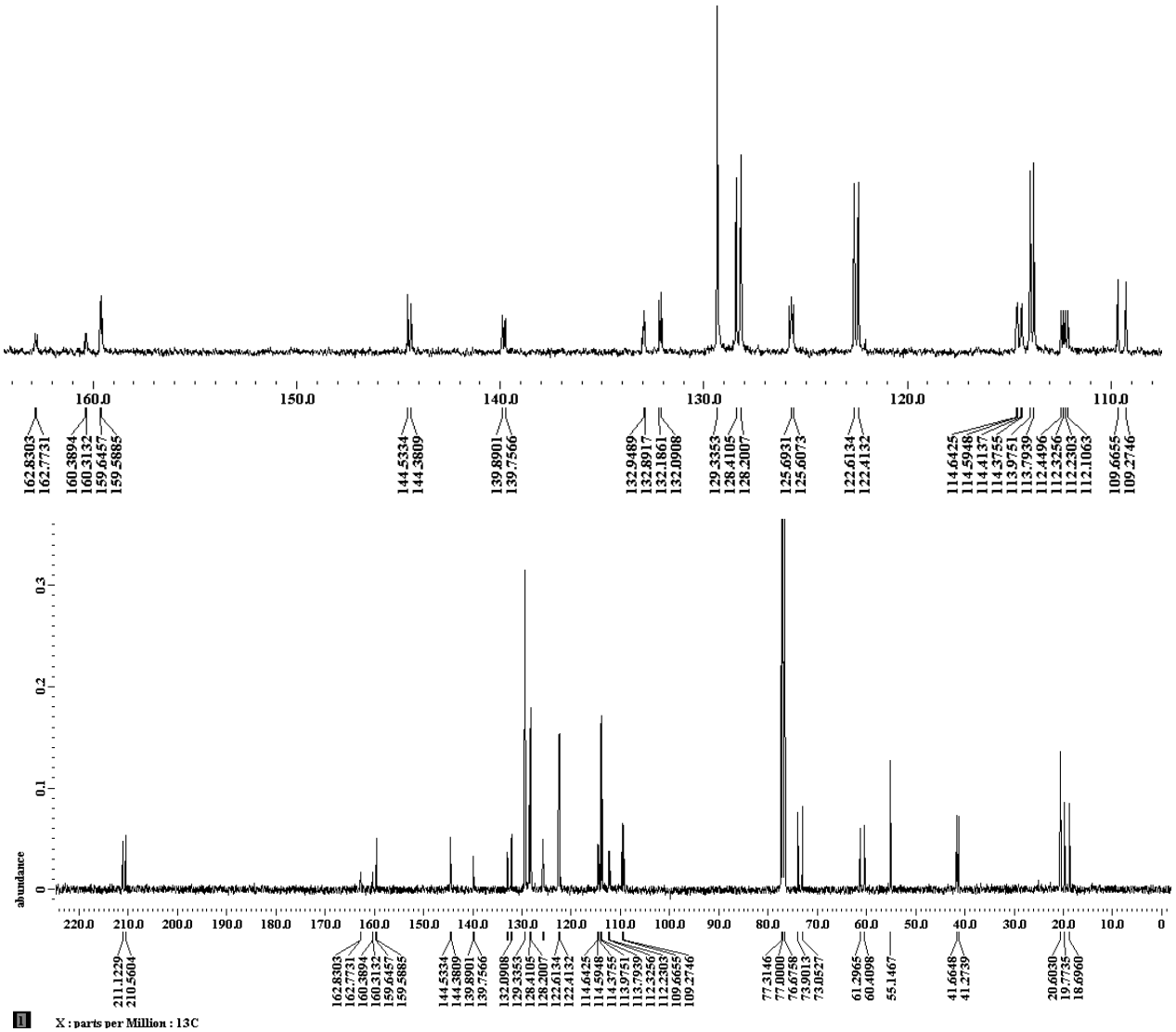


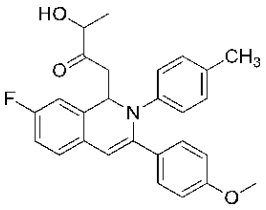
^{1}HNMR of 1-(7-fluoro-2-(4-fluorophenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4n)**



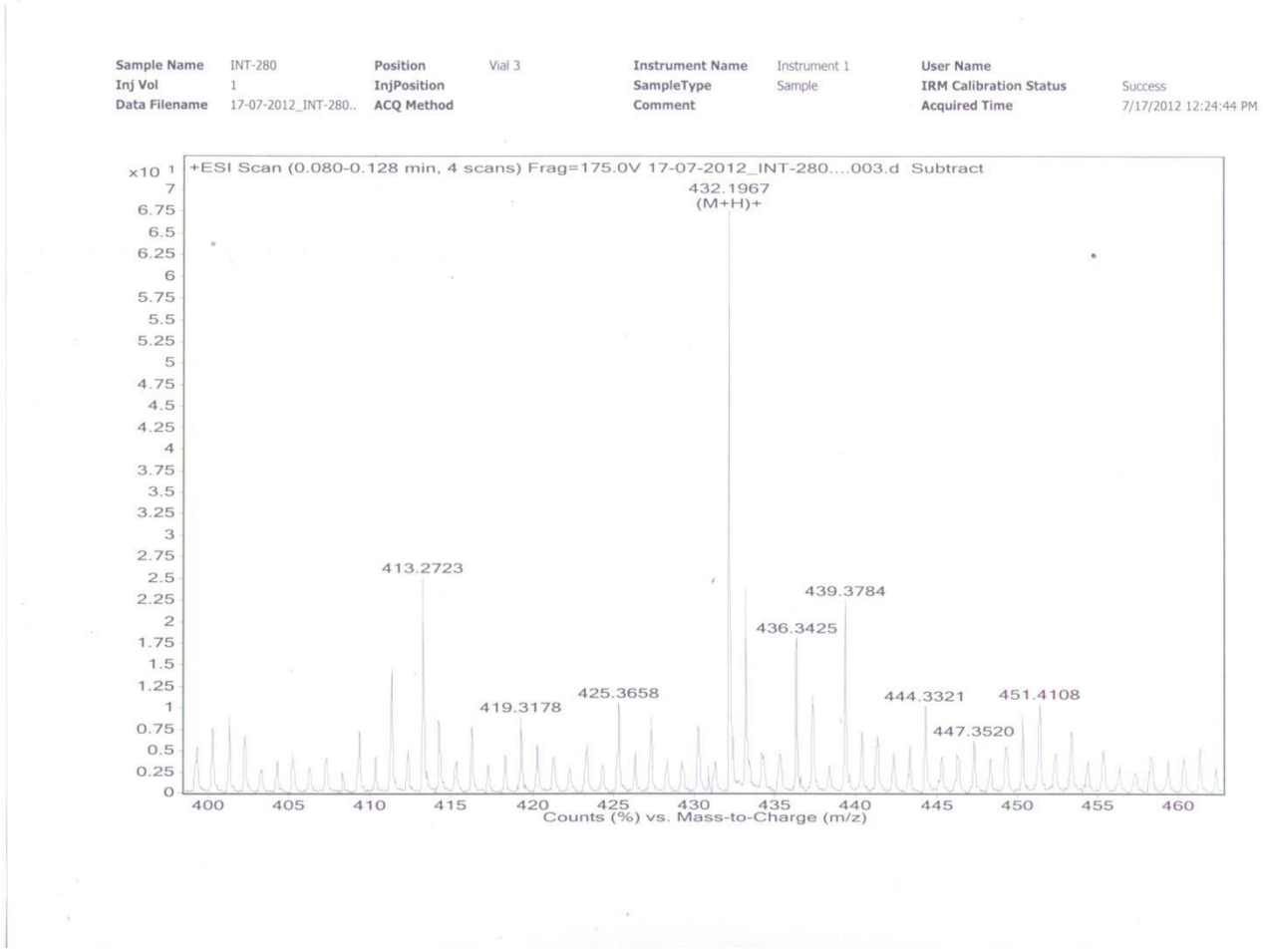


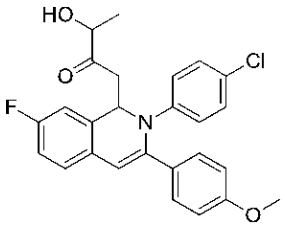
^{13}CNMR of 1-(7-fluoro-2-(4-fluorophenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4n)**



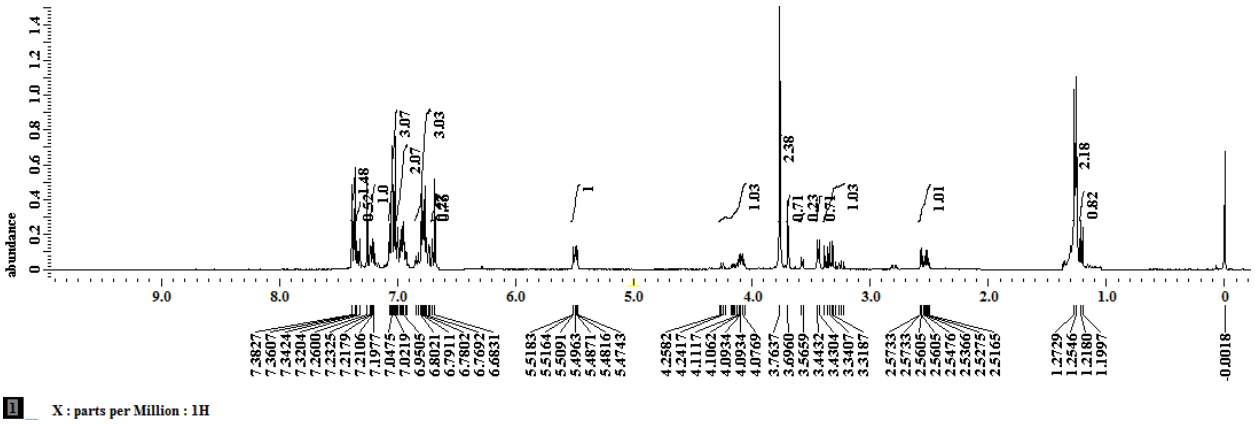


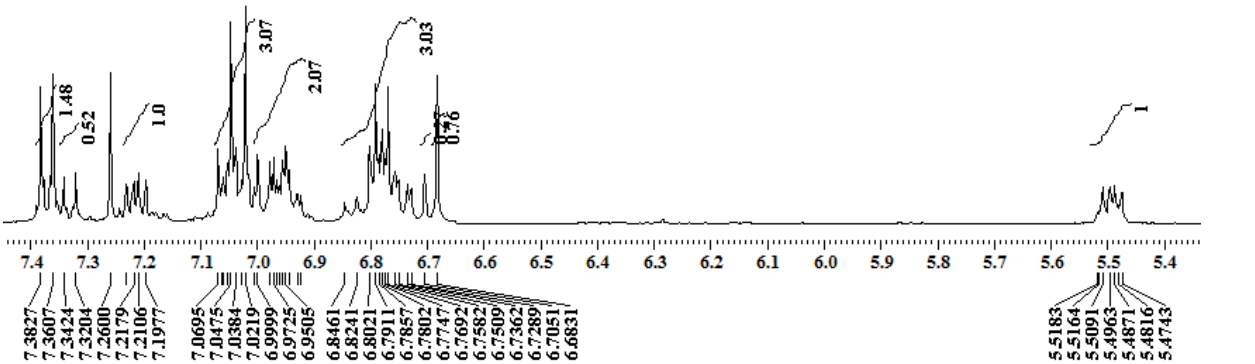
HRMS of 1-(7-fluoro-2-(4-fluorophenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4n)**

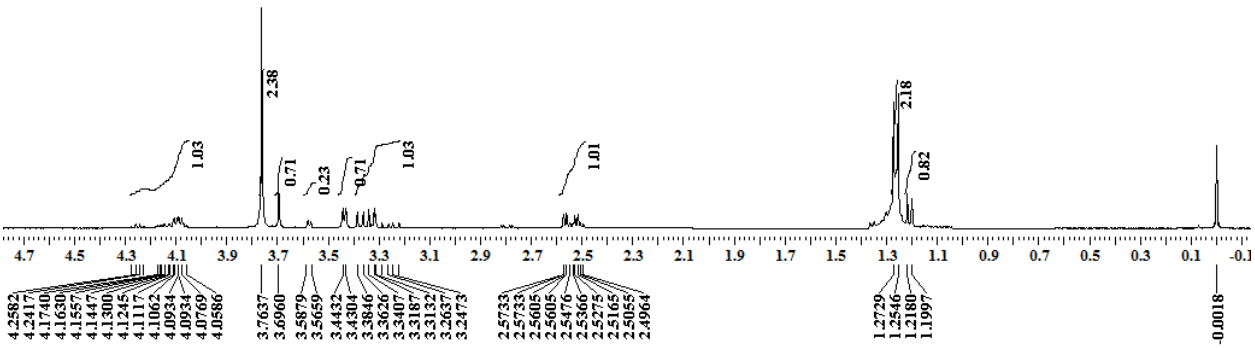


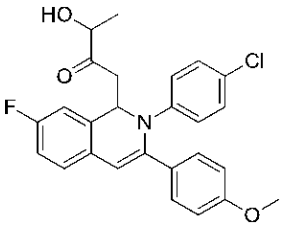


[1]HNMR of 1-(2-(4-chlorophenyl)-7-fluoro-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4o)**

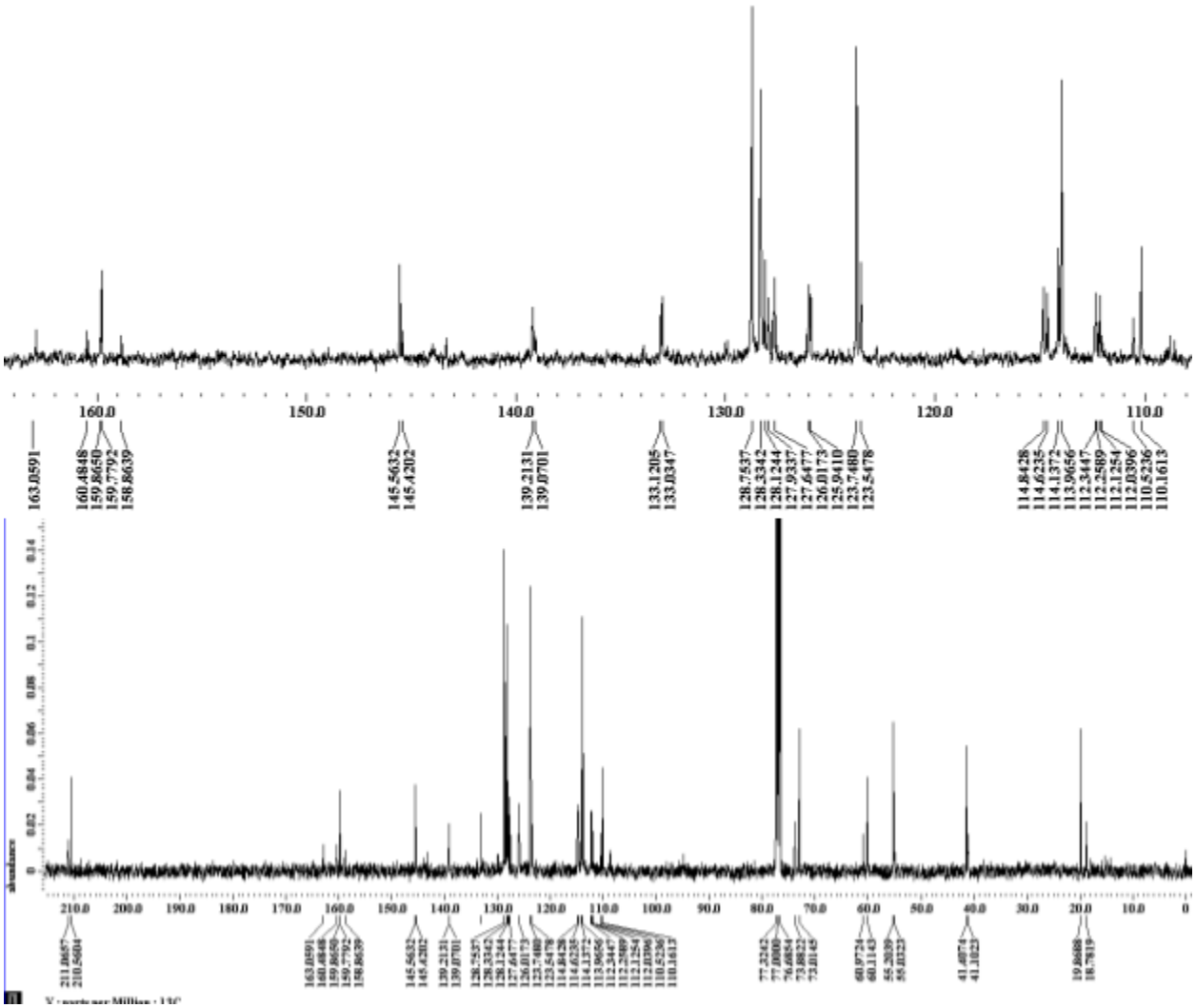


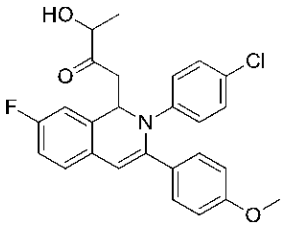




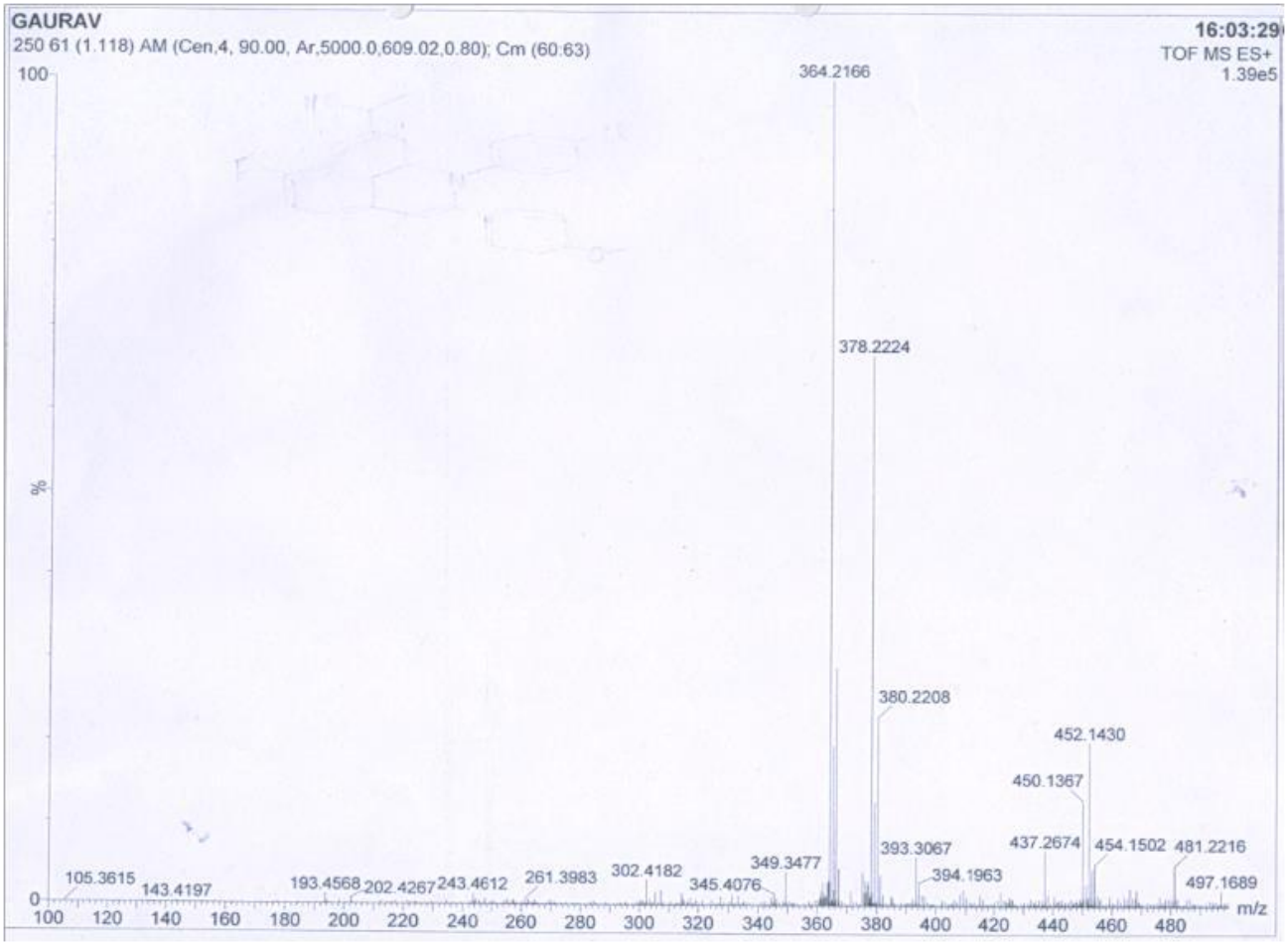


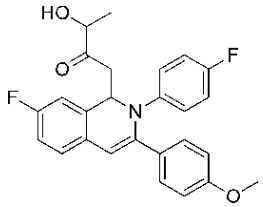
^{13}CNMR of 1-(2-(4-chlorophenyl)-7-fluoro-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4o)**



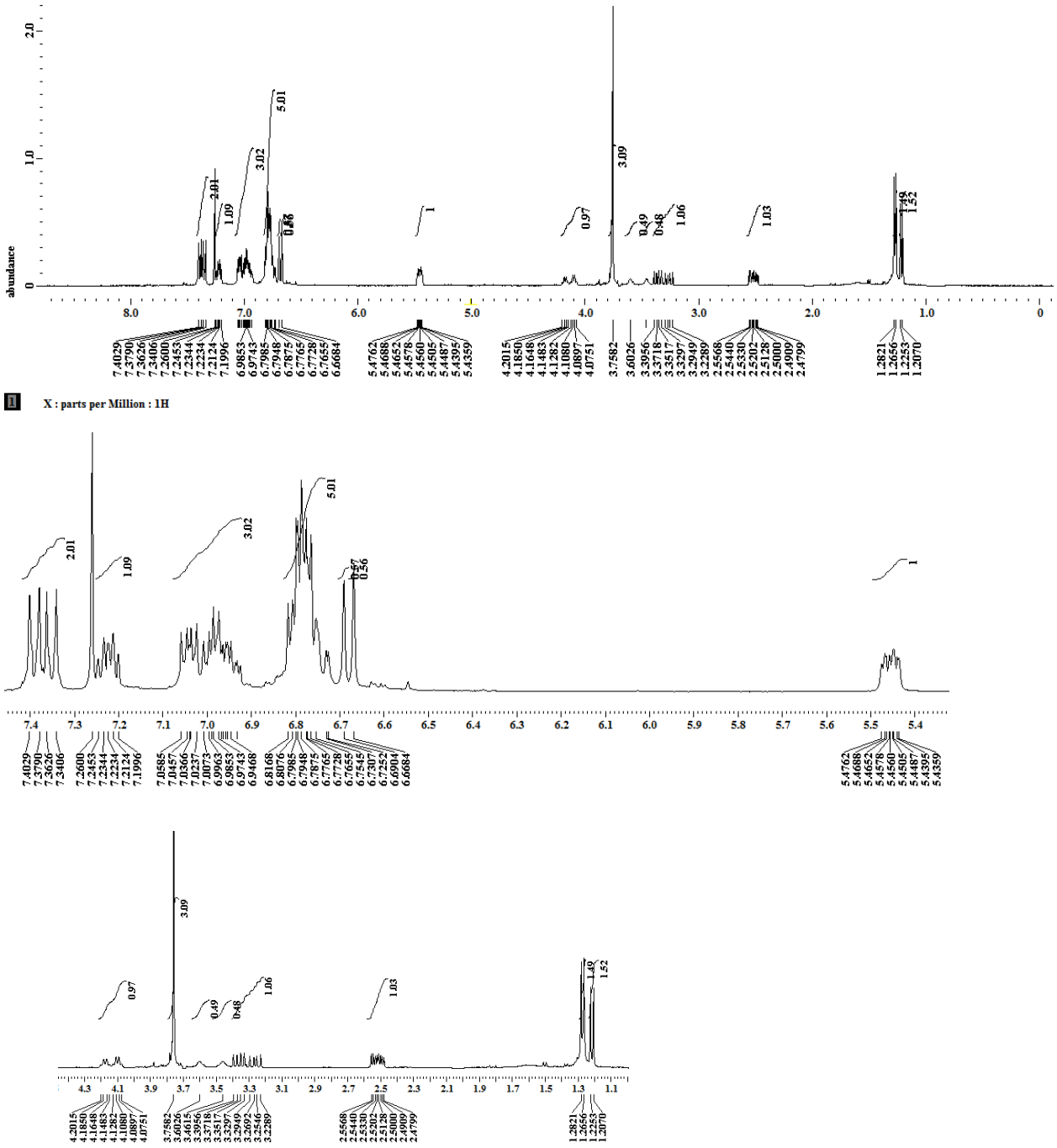


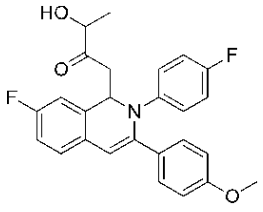
HRMS of 1-(2-(4-chlorophenyl)-7-fluoro-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4o)**



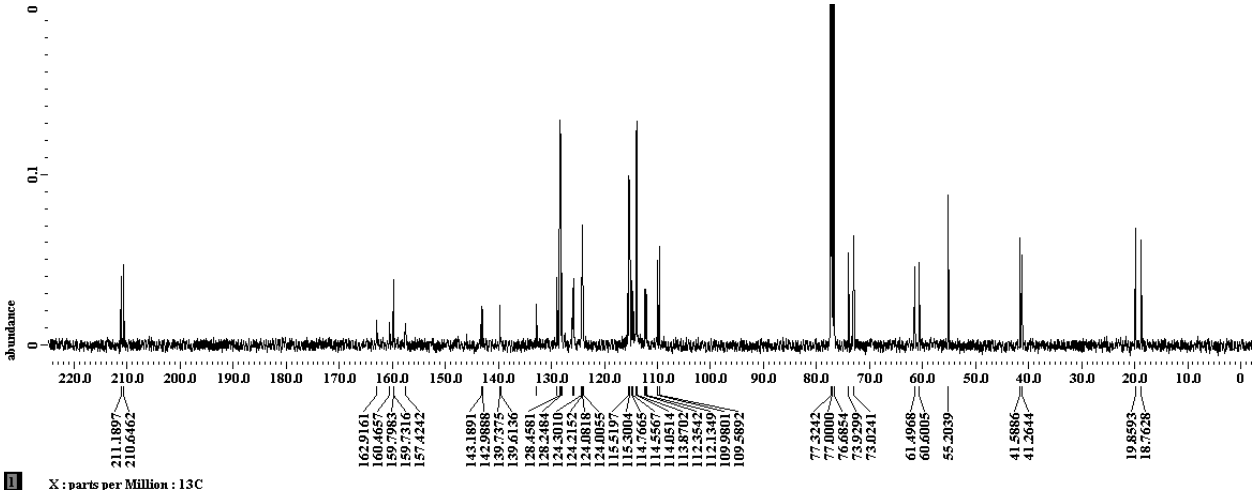


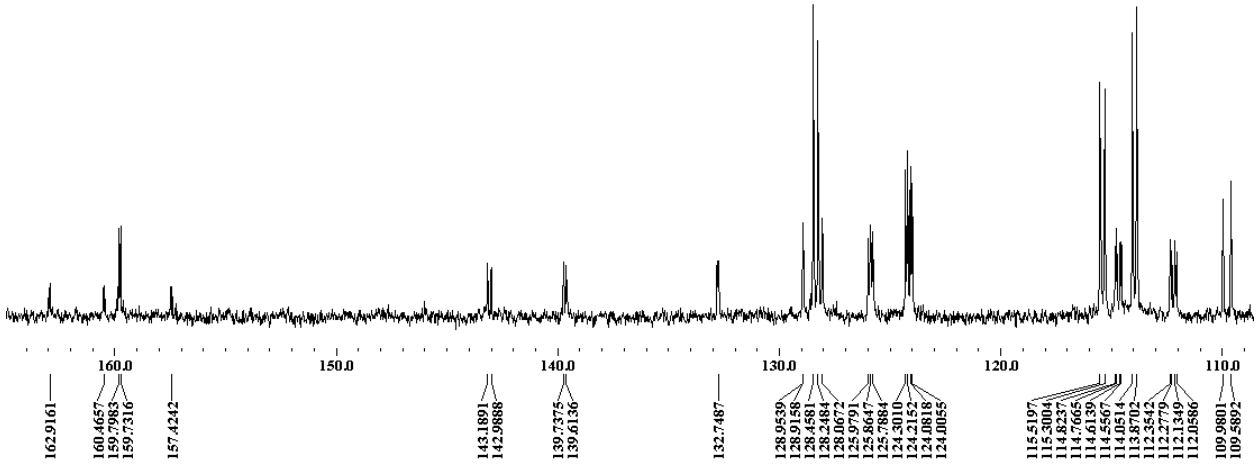
^{1}HNMR of 1-(7-fluoro-2-(4-fluorophenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4p)**

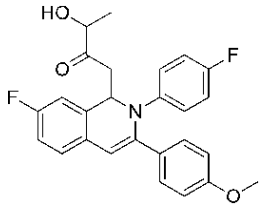




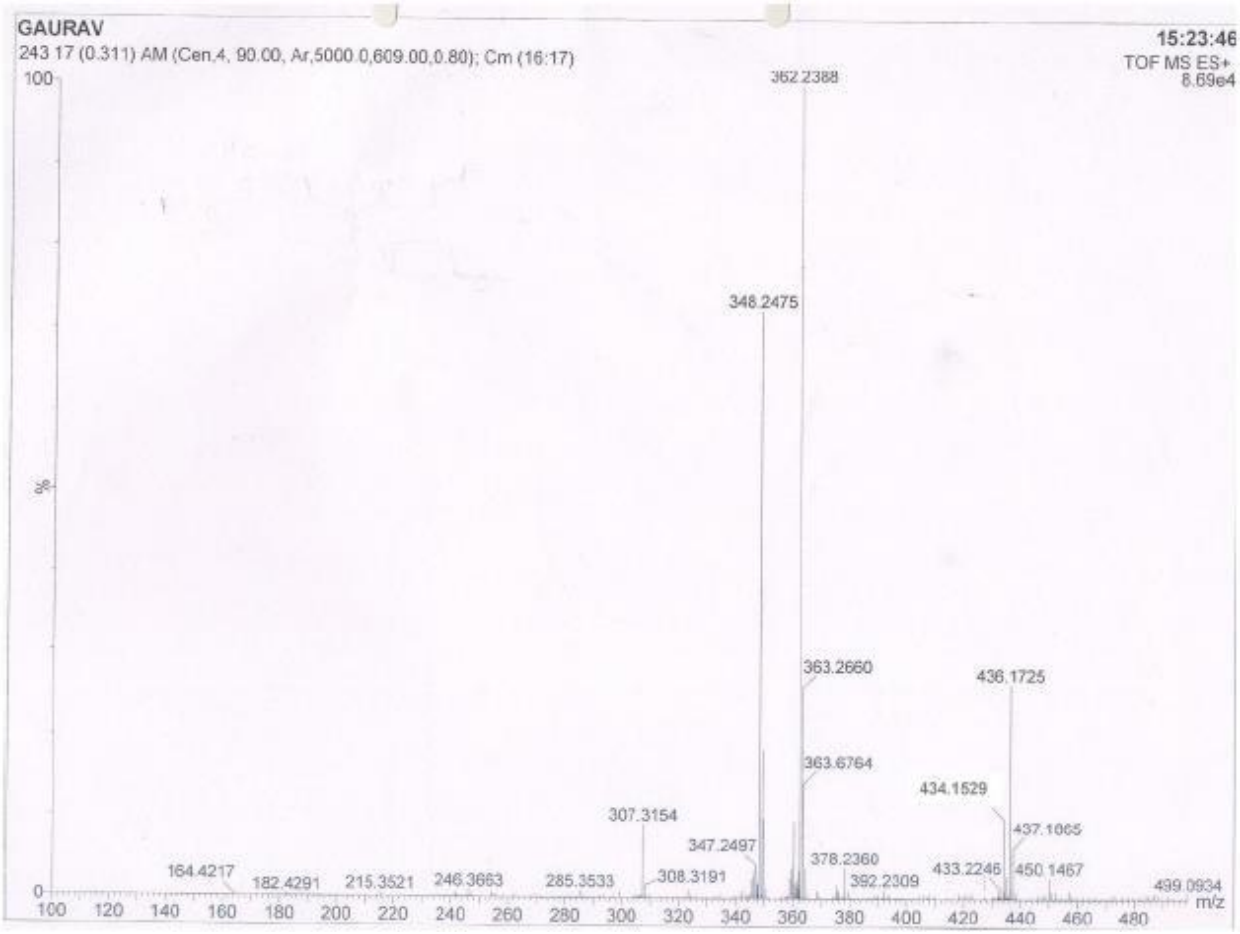
^{13}CNMR of 1-(7-fluoro-2-(4-fluorophenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4p)**

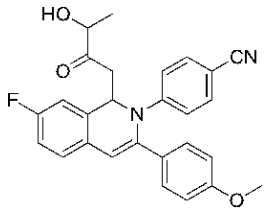




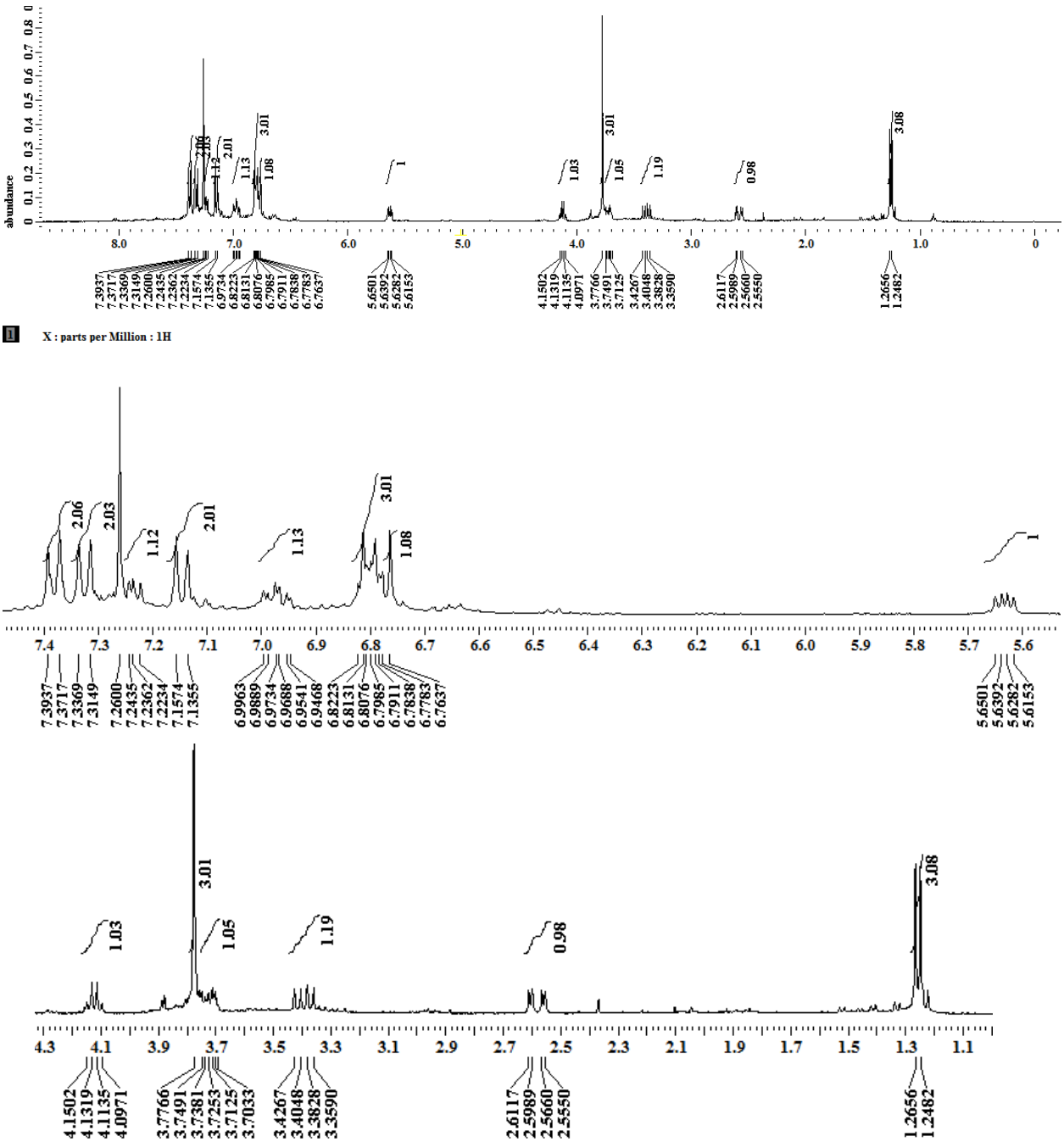


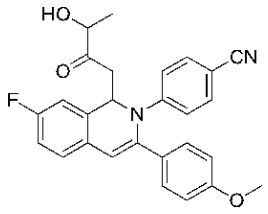
HRMS of 1-(7-fluoro-2-(4-fluorophenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4p)**



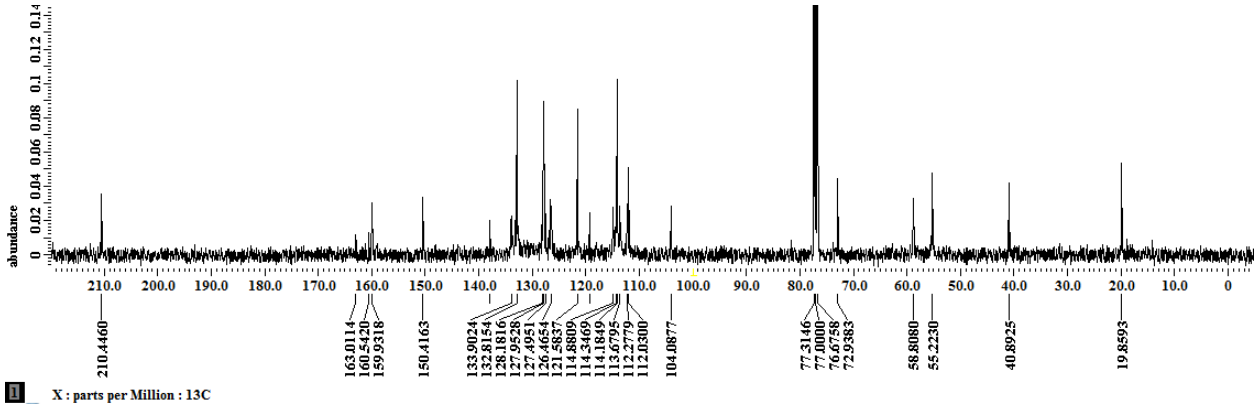


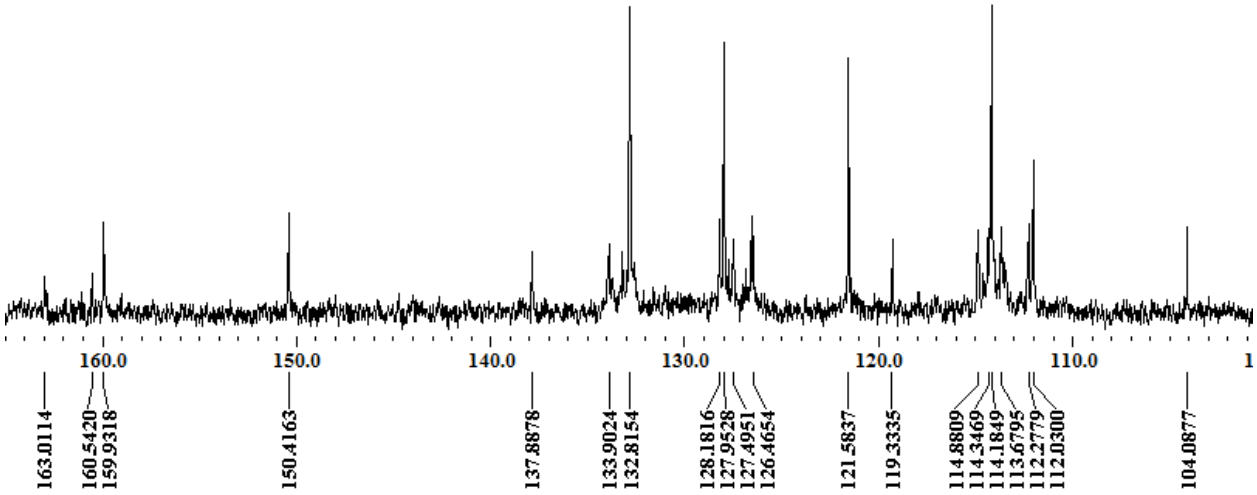
^{1}HNMR of 4-(7-fluoro-1-(3-hydroxy-2-oxobutyl)-3-(4-methoxyphenyl)isoquinolin-2(1H)-yl)benzonitrile**(4q)**

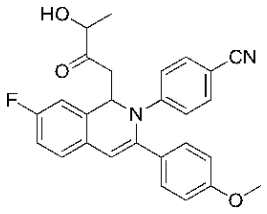




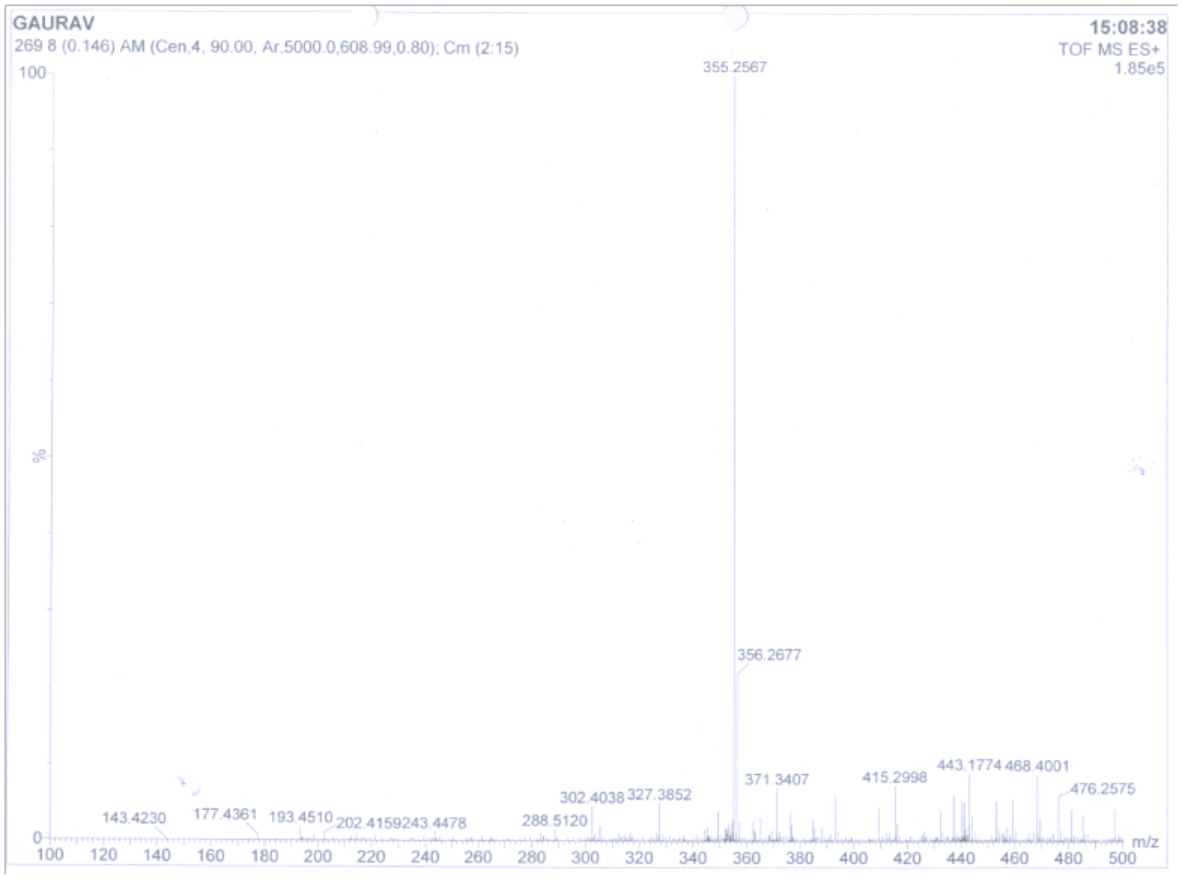
^{13}CNMR of 4-(7-fluoro-1-(3-hydroxy-2-oxobutyl)-3-(4-methoxyphenyl)isoquinolin-2(1H)-yl)benzonitrile**(4q)**

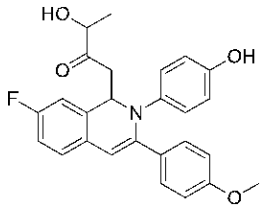




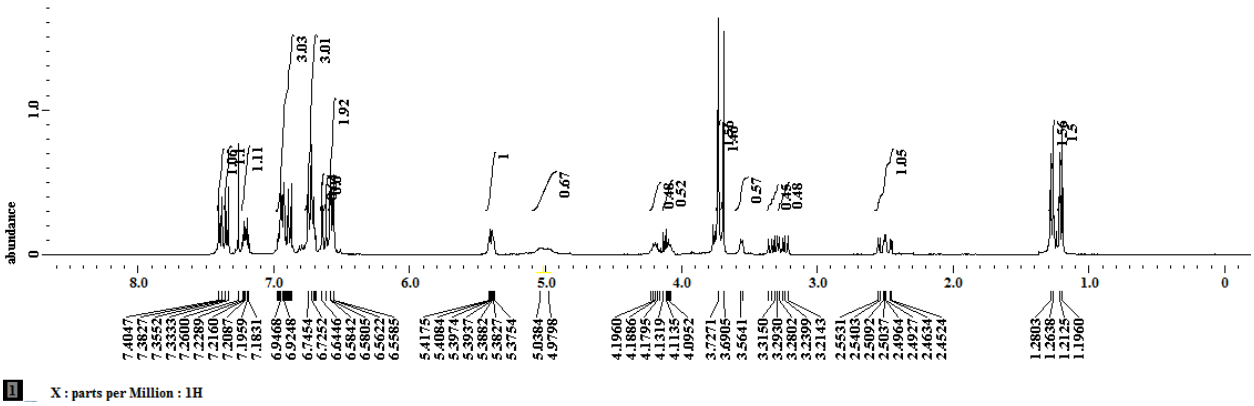


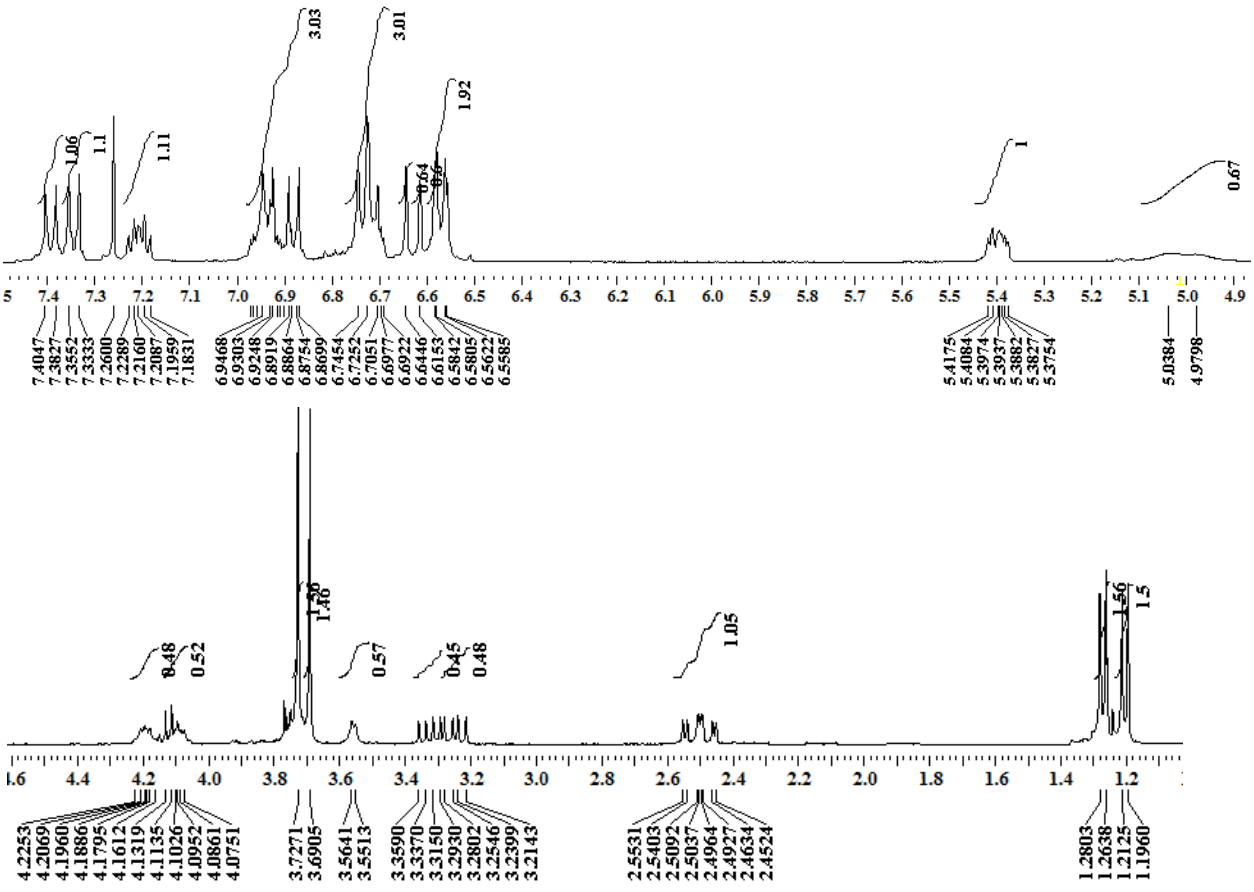
HRMS of 4-(7-fluoro-1-(3-hydroxy-2-oxobutyl)-3-(4-methoxyphenyl)isoquinolin-2(1H)-yl)benzonitrile**(4q)**

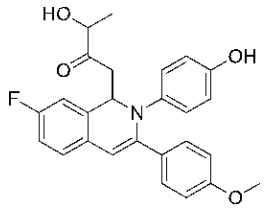




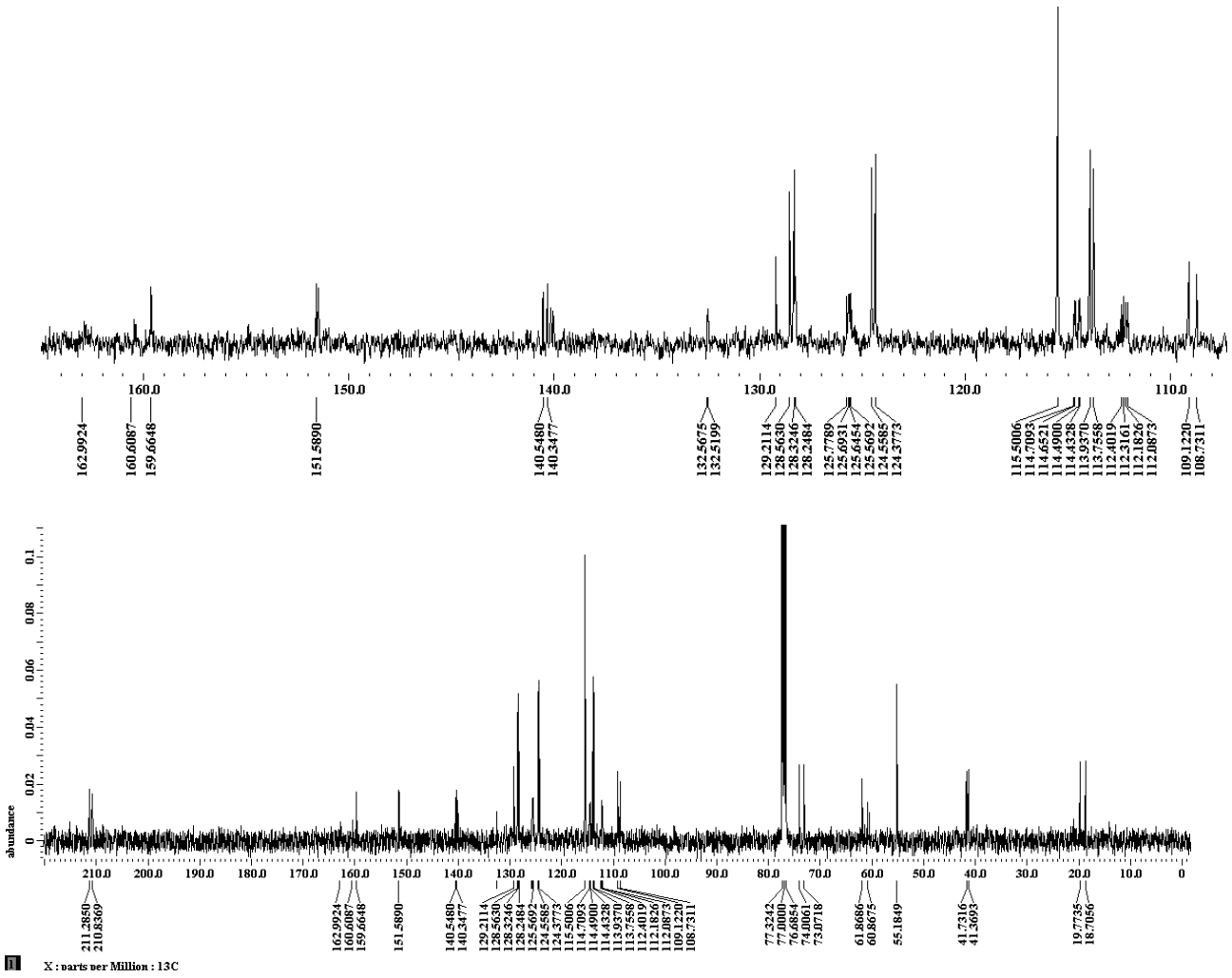
[1]HNMR of 1-(7-fluoro-2-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4r)**

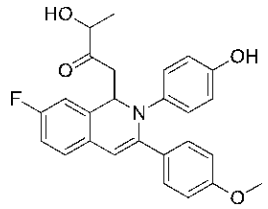




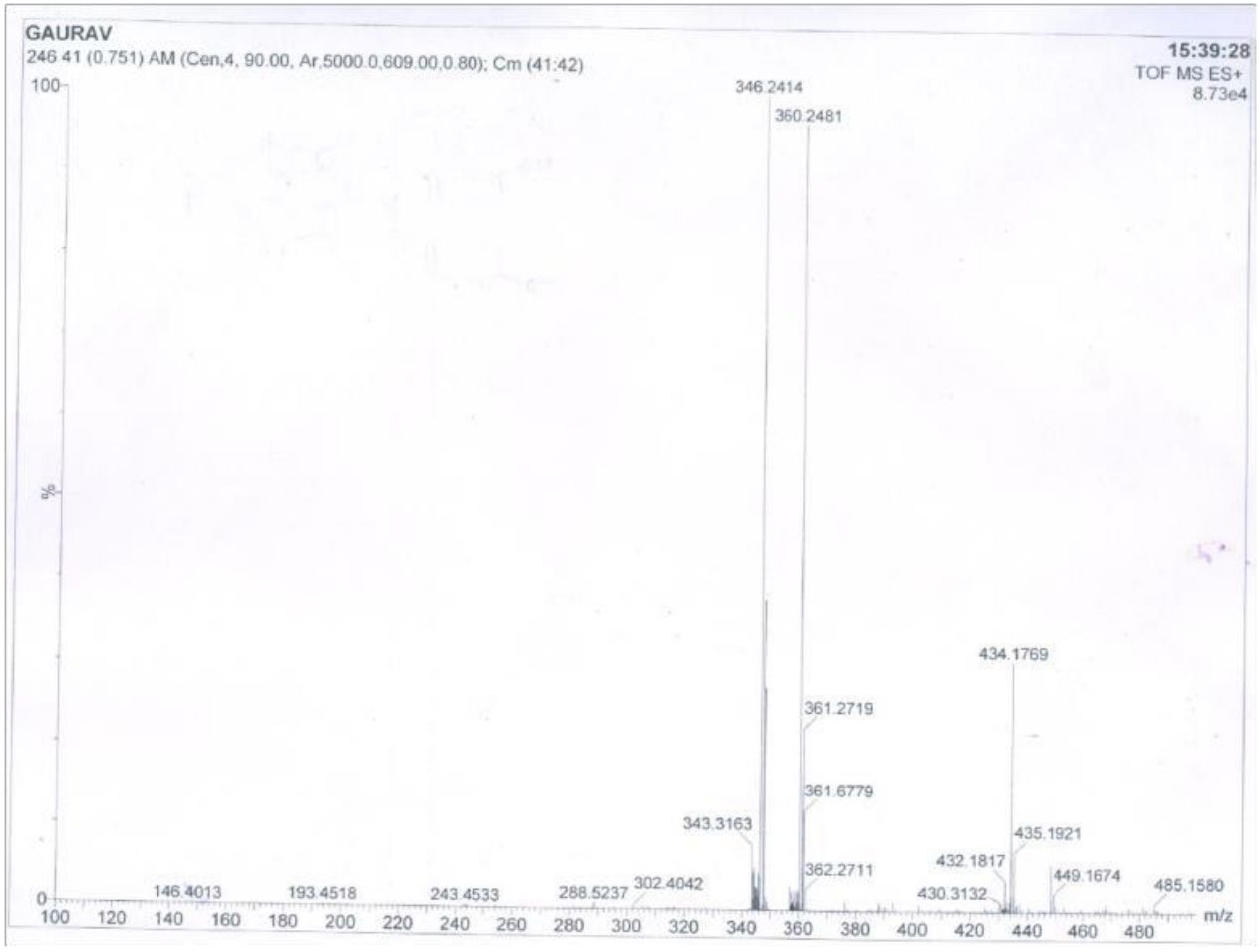


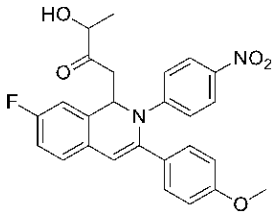
^{13}CNMR of 1-(7-fluoro-2-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4r)**



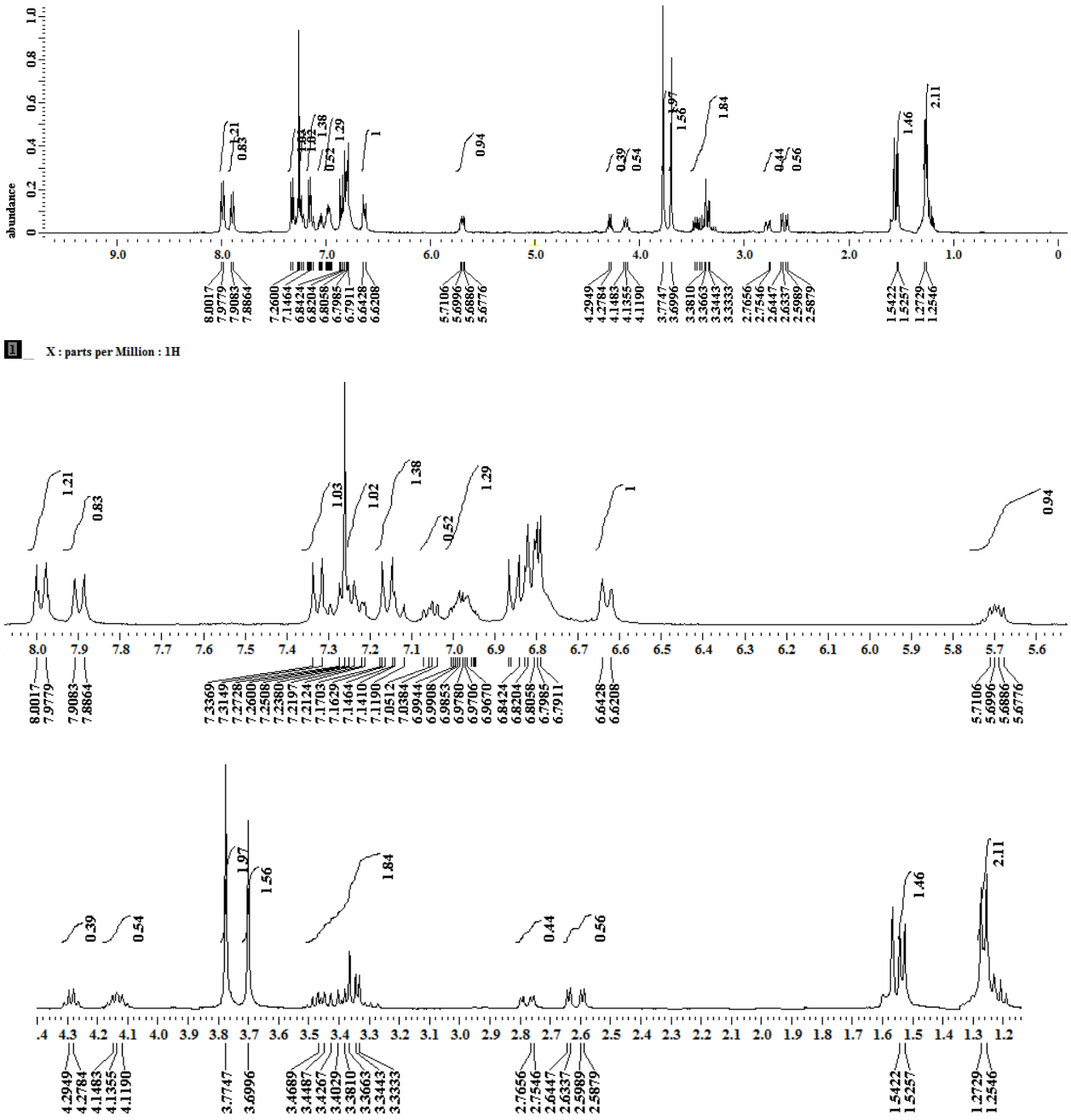


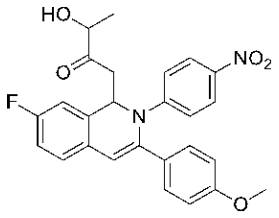
HRMS of 1-(7-fluoro-2-(4-hydroxyphenyl)-3-(4-methoxyphenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4r)**



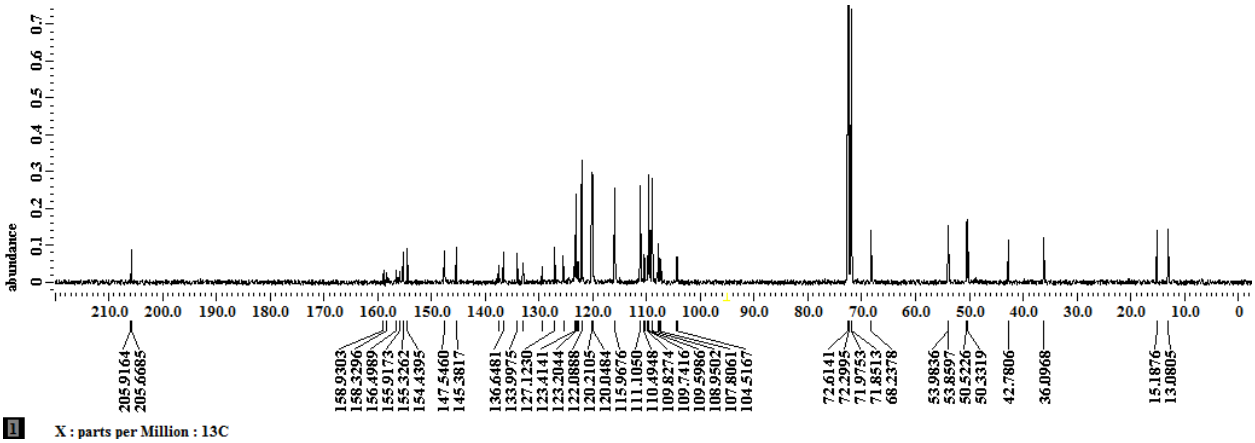


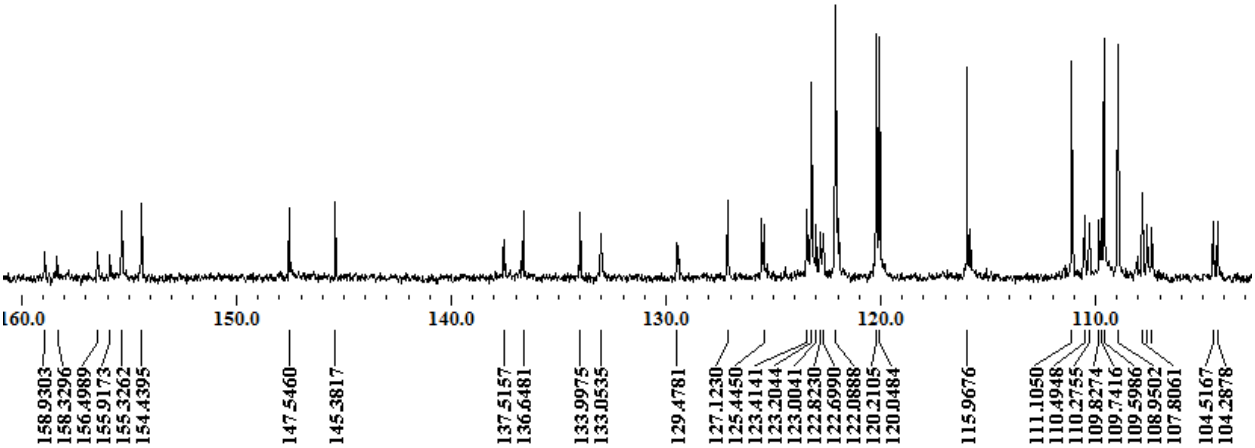
[1]HNMR 1-(7-fluoro-3-(4-methoxyphenyl)-2-(4-nitrophenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4s)**

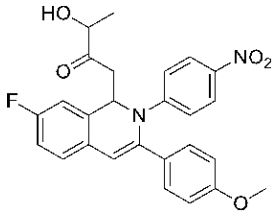




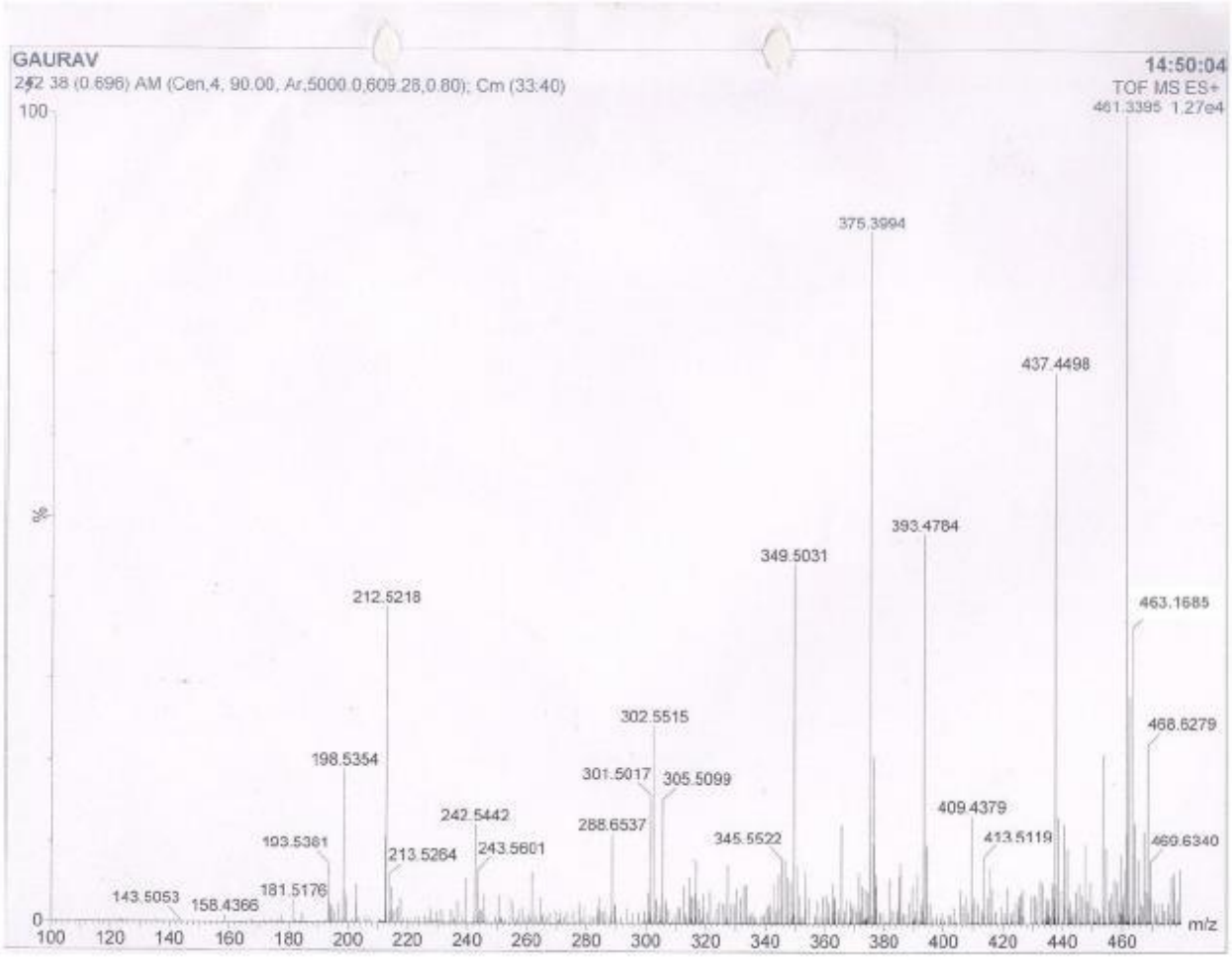
^{13}CNMR of 1-(7-fluoro-3-(4-methoxyphenyl)-2-(4-nitrophenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4s)**







HRMS of 1-(7-fluoro-3-(4-methoxyphenyl)-2-(4-nitrophenyl)-1,2-dihydroisoquinolin-1-yl)-3-hydroxybutan-2-one**(4s)**



1,2-dihydro-1-(nitromethyl)-2,3-diphenylisoquinoline (7a), 2-(4-fluorophenyl)-1,2-dihydro-1-(nitromethyl)-3-p-tolylisoquinoline (7b), 2-(4-chlorophenyl)-7-fluoro-1,2-dihydro-3-(4-methoxyphenyl)-1-(nitromethyl)isoquinolines (7c), 2-(4-chlorophenyl)-1,2-dihydro-7-nitro-1-(nitromethyl)-3-phenylisoquinoline (7d), 1,2-dihydro-7-methoxy-3-(4-methoxyphenyl)-1-(nitromethyl)-2-phenylisoquinoline (7e) were earlier published by Urvashi et. al.[21]

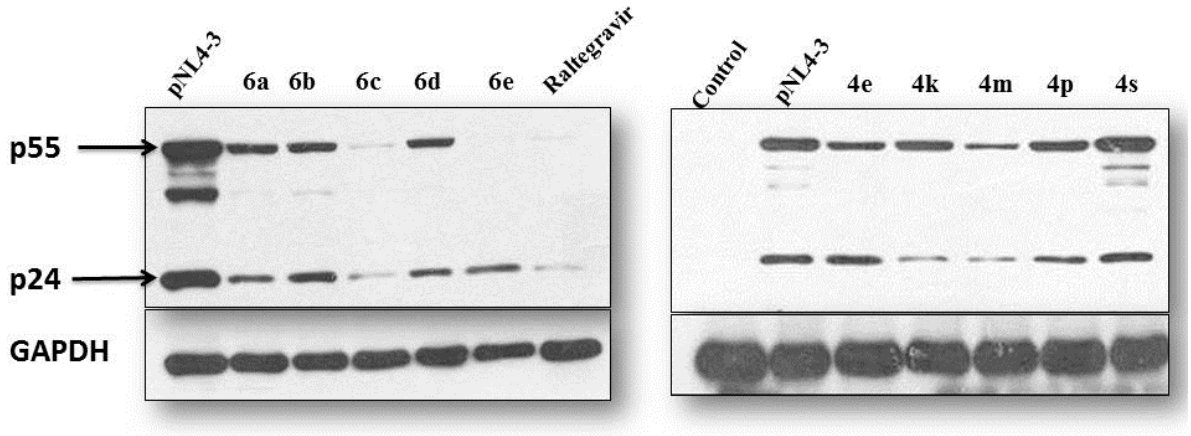


Figure S1. Antiviral Assay of Compounds

Table S1. Glide Scores, Electrostatic interactions and van der Waals interaction of Raltegravir and designed compounds **4a, 4e-4s, and 6a-e**

Compound	Glide Score	Electrostatic interactions	van der Waals interactions	AutoDock Score	Electrostatic interactions	van der Waals interactions
Raltegravir	-7.47	K159, D64,C65, N117, E152	T66, H67, D116, K156	-8.16	D64, E152, K156, K159, E92, D116, C65	G118, T66, N155, H67, F121
4a(R)	-6.08	E152, D116, D64, E92	K156, N155, T66, K159, C65, H67, G118	-6.58	N117, D116, E92, D64, C65	G118, F121, H67, T66
4a(S)	-6.03	K156, E152, D64, C65, D116, E92	N155, K159, T66, H67	-6.45	K159, D64, D116, E152, Q148	H67, N155, T66, K156
4e(R)	-6.23	S119, D116, E152, D64, T66, K159	G118, E92, C65, H67, K156, N155	-6.89	N117, D116, E152, D64, C65, E92	H67, G118, N120
4e(S)	-5.76	E152, K159, K156, D116, D64, E92	N155, T66, C65, H67, G118	-6.15	K159, E152, D64, C65, D116, E92, K156	N155, T66, H67, G118, F121
4f(R)	-6.19	T66, K159, S119, D64, E152	C65, H67, E92, N155, K156, D116, G118	-6.35	K159, H67, E152, D64, D116, G118	T66, C65, E92, F121, N120

4f(S)	-5.85	E152, E92	K156, N155, K159, T66, C65, H67	-5.96	E152, D64, E92, D116, C65	H67, K159, G118, F121, N120
4g(R)	-5.93	T66, K159, D64, D116, S119	E92, G118, C65, H67, E152, N155, K156	-6.46	N117, D116, E152, D64, E92, C65	H67, G118, N120
4g(S)	-5.59	N120, S119, D116, D64, E152, K159	G118, E92, C65, H67, T66	-6.24	N117, D116, D64, E92, H67, E152	G118, C65
4h(R)	-5.98	K159, T66, D64, D116, S119	N155, K156, E152, C65, H67, E92, G118	-6.56	E152, D64, D116, C65, E92, K159	H67, K156, N155, T66, S119, N120, G118, F121
4h(S)	-5.56	K159, K156, E152, E92	L68, T66, H67, N155, C65	-5.84	K156, E152, D64, D116, K159	T66, H67, L68
4i(R)	-5.17	H67, K159, D116, D64	F121, N120, G118, E92, C65, T66	-6.31	N117, D116, E152, D64, C65, E92	H67, G118, N120, F121
4i(S)	-5.13	H67, D64, C65, D116, E152, E92	K159, T66, N155, K156	-6.15	K159, E152, E92, D64, C65, D116	K156, N155, T66, H67, G118, N120, F121
4j(R)	-5.74	H67, C65, D64, E92, D116, K159, N117	F121, G118, T66, E152	-6.89	N155, T66, K159, H67, E152, C65, D64, D116	K156, L68
4j(S)	-5.53	N117, D116, E152, D64, C65, H67	G118, E92	-6.73	D116, C65, D64, K159, T66, N155, E152, H67	K156
4k(R)	-5.49	K159, K156, E152, D64, D116	N155, H67, T66, C65, Q148	-6.34	D64, D116, E152, T66, K159	K156, N155, H67, E92
4k(S)	-5.95	N117, S119, K159, T66, H67, C65, D64	N120, G118, E92, D116	-6.18	T66, H67, D64, C65, K159, D116, E92, N120	G70, F121, G118
4l(R)	-5.42	K156, E152, D64, D116, C65, E92, K159	H67, F121, T66	-6.23	H67, K159, K156, T66, E152, D64, D116	N155, Q148
4l(S)	-5.68	D116, N117, E92, E152, D64	G118, S119, H67	-5.88	D64, D116, N117, H67, E92, E152	C65, G118
4m(R)	-6.51	E152, D64, D116, G118, H67, K159	T66, C65, F121, N120, E92	-6.94	N120, S119, C65, D64, E152, N117, E92	F121, G118, D116, H67
4m(S)	-6.43	D116, E152, D64, N117, E92, H67	G118, C65	-6.86	K159, T66, H67, C65, N155, D64, D116, E92	Q148, E152
4n(RR)	-5.68	E92, D64, E152, C65, D116	K159, K156, N155, T66, H67, G118, F121, N120, S119	-6.11	D64, E152, K156, K159, E92, D116, C65	G118, T66, N155, H67, F121
4n(RS)	-5.70	D64, E152, E92, C65, D116	T66, K156, N155, K159, H67, G118,	-5.97	K156, E152, D64, D116, C65, E92, K159	H67, F121, T66

			F121, N120			
4n(SR)	-5.71	Y143, D116, E92, D64, C65, S119	G140, N117, G118, F121, N120, H67	-6.24	D116, N117, E92, E152, D64	G118, S119, H67
4n(SS)	-5.74	D64, E152, K156, K159, E92, D116, C65	G118, T66, N155, H67, F121	-5.91	E152, D64, D116, G118, H67, K159	T66, C65, F121, N120, E92
4o(RR)	-5.56	N117, D116, E92, D64, C65	G118, F121, H67, T66	-6.17	D116, E152, D64, N117, E92, H67	G118, C65
4o(RS)	-5.62	K159, D64, D116, E152, Q148	H67, N155, T66, K156	-5.98	E92, D64, E152, C65, D116	K159, K156, N155, T66, H67, G118, F121, N120, S119
4o(SR)	-5.61	N117, D116, E152, D64, C65, E92	H67, G118, N120	-5.85	D64, E152, E92, C65, D116	T66, K156, N155, K159, H67, G118, F121, N120
4o(SS)	-5.73	K159, E152, D64, C65, D116, E92, K156	N155, T66, H67, G118, F121	-6.15	Y143, D116, E92, D64, C65, S119	G140, N117, G118, F121, N120, H67
4p(RR)	-5.35	K159, H67, E152, D64, D116, G118	T66, C65, E92, F121, N120	-5.78	D64, E152, K156, K159, E92, D116, C65	G118, T66, N155, H67, F121
4p(RS)	-5.61	E152, D64, E92, D116, C65	K156, N155, T66, H67, K159, G118, F121, N120	-5.89	N117, D116, E92, D64, C65	G118, F121, H67, T66
4p(SR)	-5.65	N117, D116, E152, D64, E92, C65	H67, G118, N120	-5.99	K159, D64, D116, E152, Q148	H67, N155, T66, K156
4p(SS)	-5.82	N117, D116, D64, E92, H67, E152	G118, C65	-6.13	N117, D116, E152, D64, C65, E92	H67, G118, N120
4q(RR)	-5.50	E152, D64, D116, C65, E92, K159	H67, K156, N155, T66, S119, N120, G118, F121	-5.91	K159, E152, D64, C65, D116, E92, K156	N155, T66, H67, G118, F121
4q(RS)	-4.93	K156, E152, D64, D116, K159	T66, H67, L68	-5.57	K159, H67, E152, D64, D116, G118	T66, C65, E92, F121, N120
4q(SR)	-5.74	N117, D116, E152, D64, C65, E92	H67, G118, N120, F121	-5.99	E152, D64, E92, D116, C65	K156, N155, T66, H67, K159, G118, F121, N120
4q(SS)	-5.72	K159, E152, E92, D64, C65, D116	K156, N155, T66, H67, G118, N120, F121	-5.84	N117, D116, E152, D64, E92, C65	H67, G118, N120
4r(RR)	-5.44	N155, T66, K159, H67, E152, C65, D64, D116	K156, L68	-6.54	N117, D116, D64, E92, H67, E152	G118, C65
4r(RS)	-5.13	D116, C65, D64, K159, T66, N155,	K156	-6.23	E152, D64, D116, C65, E92, K159	H67, K156, N155, T66, S119, N120,

		E152, H67				G118, F121
4r(SR)	-5.46	N155, T66, D116, E152, H67, D64, C65	K159, K156, E92	-5.98	K156, E152, D64, D116, K159	T66, H67, L68
4r(SS)	-5.10	E92,N120,C65,D64, D116,N117	G118,F121,H67	-5.94	E152, D116, D64, E92	K156, N155, T66, K159, C65, H67, G118
4s(RR)	-5.33	D64, D116, E152, T66, K159	K156, N155, H67, E92	-6.54	K156, E152, D64, C65, D116, E92	N155, K159, T66, H67
4s(RS)	-5.06	T66, H67, D64, C65, K159, D116, E92, N120	G70, F121, G118	-5.97	S119, D116, E152, D64, T66, K159	G118, E92, C65, H67, K156, N155
4s(SR)	-5.53	H67, K159, K156, T66, E152, D64, D116	N155, Q148	-5.74	E152, K159, K156, D116, D64, E92	N155, T66, C65, H67, G118
4s(SS)	-5.40	D64, D116, N117, H67, E92, E152	C65, G118	-5.36	K159, E152, D64, C65, D116, E92, K156	N155, T66, H67, G118, F121
6a(R)	-6.24	E152, K159, K156, D116, E92	F121, G118, D116, H67	-7.60	E152, K159, T66, D64, C65, D116, E92, N120, S119	N155, K156, H67, G118, F121
6a(S)	-6.39	Y143, D116, E92, D64, C65, S119	G140, N117, G118, F121, N120, H67	-7.34	E92,N120,C65,D64,D116,N117	G118,F121,H67
6b(R)	-6.03	D64, E152, K156, K159, E92, D116, C65	G118, T66, N155, H67, F121	-6.96	S119, N120, C65, K159, H67, T66, E152	G118, E92, D64
6b(S)	-6.36	N117, D116, E92, D64, C65	G118, F121, H67, T66	-6.75	G118, N120, E92, D64	F121, G118, D116, H67
6c(R)	-6.89	K159, D64, D116, E152, Q148	H67, N155, T66, K156	-7.93	N117, D116, E152, D64, C65, E92	K156, N155, T66, H67
6c(S)	-6.65	N117, D116, E152, D64, C65, E92	H67, G118, N120	-7.87	D116, E152, D64, C65, E92	K156, G118, N120, F121, N155,
6d(R)	-5.94	E152, D116, D64, E92	K156, N155, T66, K159, C65, H67, G118	-7.76	H67, D64, C65, K159, D116, E92, E152,	K156, H67, E92, G118, D116, H67
6d(S)	-5.83	K156, E152, D64, C65, D116, E92	N155, K159, T66, H67	-7.46	H67, D64, C65, K159, D116, E92	H67, E92, G118, D116, H67
6e(R)	-6.32	S119, D116, E152, D64, T66, K159	G118, E92, C65, H67, K156, N155	-6.96	K156, E152, D64	T66, H67, L68
6e(S)	-6.18	E152, K159, K156, D116, D64, E92	N155, T66, C65, H67, G118	-7.51	K156, E152, D64, D116, K159	T66, H67, L68, D116