Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supplementary Information

Dual-Action Inhibitors of HIF Prolyl Hydroxylases That Induce Binding of a Second Iron Ion

Kar Kheng Yeoh,[a‡†] Mun Chiang Chan,[a†] Armin Thalhammer,[a] Marina Demetriades,[a] Rasheduzzaman Chowdhury,[a] Ya-Min Tian,[b] Ineke Stolze,[b] Luke A. McNeill,[a] Myung Kyu Lee,[c] Esther C. Y. Woon,[a¶] Mukram M. Mackeen,[a∥] Akane Kawamura,[a] Peter J. Ratcliffe,[b] Jasmin Mecinović[a§]* and Christopher J. Schofield[a]*

[a] Chemistry Research Laboratory, Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford OX1 3TA, United Kingdom. Phone: +44 (0) 1865 275674. Fax: +44 (0) 1865 275625. E-mail: christopher.schofield@chem.ox.ac.uk

[b] Henry Wellcome Building for Molecular Physiology, University of Oxford, Oxford OX3 7BN, United Kingdom.

[c] BioNanotechnology Research Centre, Korea Institute of Bioscience & Biotechnology, Yuseong 305-333, Daejeon, Korea.

[†] These authors contributed equally to this study.

Current addresses:

[‡] School of Chemical Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia.

[§] Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands. Email: j.mecinovic@science.ru.nl

[¶] Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore.

[‖] School of Chemical Sciences, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

TITLE RUNNING HEAD Prolyl hydroxylase inhibitors that bind two Fe-ions.

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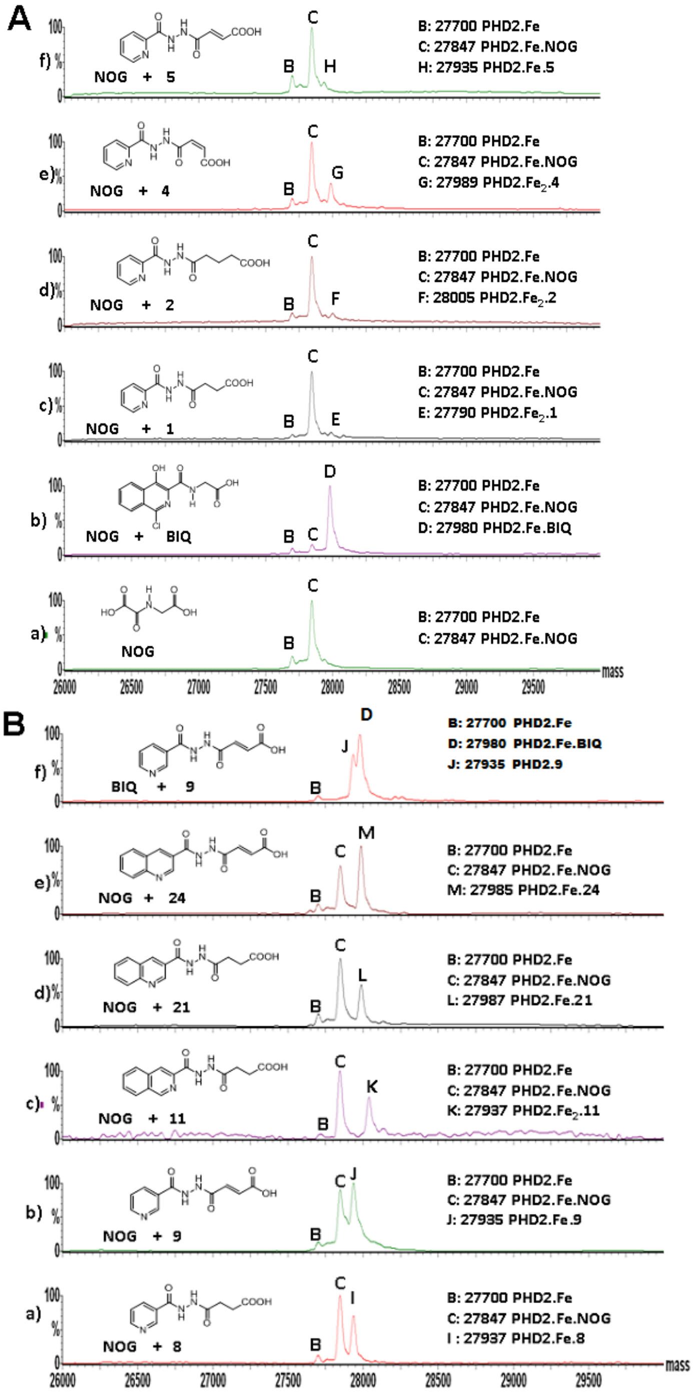
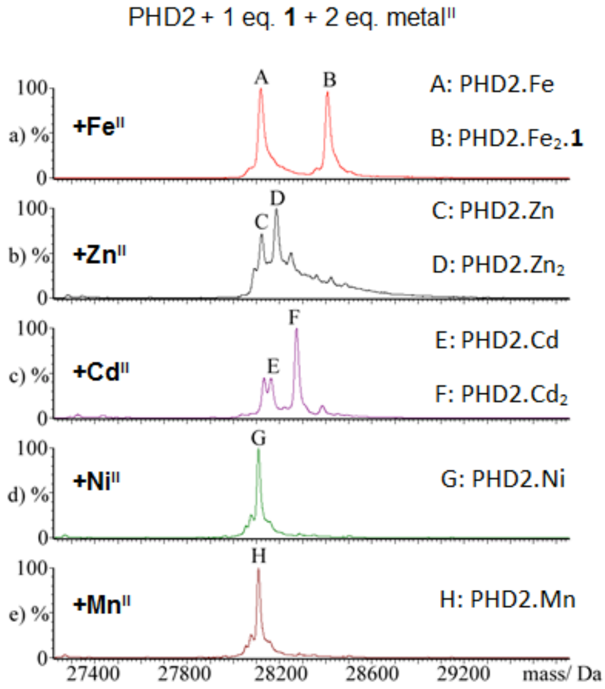
1. ESI-MS studies



Supplementary Figure 1: (**A**, **B**) Non-denaturing ESI-MS competitive experiments in the presence of equimolar amounts of PHD2, *N*-oxalylglycine (NOG), and diacylhydrazine compounds, and 2 equiv. of Fe(II) ions. (**B**, f) Equimolar amounts of PHD2, bicyclic isoquinoline inhibitor (BIQ), and diacylhydrazine **9** and 2 equiv. of Fe(II) ions.



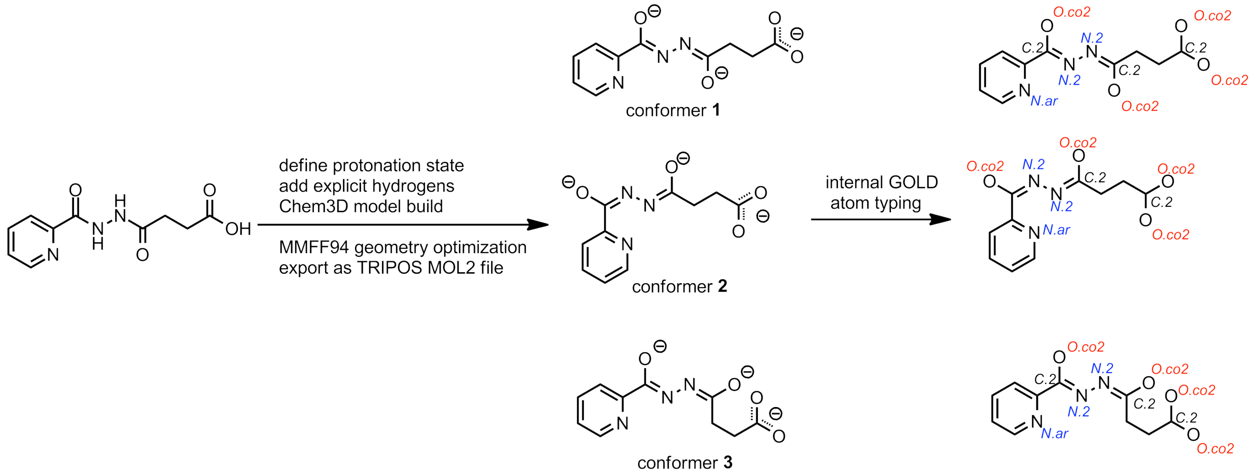
Supplementary Figure 2: Non-denaturing ESI-MS data on apo-PHD2 in the presence of 1 equiv. of hydrazide **1** and **2** equiv. of (a) Fe^{II}, (b) Zn^{II}, (c) Cd^{II}, (d) Ni^{II}, (e) Mn^{II} after 10 min incubation at room temperature (15 mM ammonium acetate, pH 7.5).

2. Docking studies

Docking studies were performed using the GOLD[1, 2] software suite (version 4.1). GOLD utilizes a genetic algorithm to generate diverse sets of ligand binding modes, which can be ranked using several scoring functions. GOLD uses an all-atom model based on SYBYL atom typing to describe ligand-receptor interactions. When docking to metalloproteins, coordination polyhedra are automatically inferred from the number and position of existing ligands in the coordination sphere of metal ions. Hydrogen bond acceptors in the ligand are then allowed to coordinate to metals in the protein.

Docking of diacylhydrazine compounds was performed using the crystal structure of human PHD2 in complex with a bicyclic inhibitor (PDB code: 2HBT). The PDB file was prepared in GOLD; hydrogen atoms were added and all water molecules and the small-molecule inhibitor were removed. The search space for ligand binding was defined as a 10 Å sphere centered at the catalytic Fe^{II} ion, which is coordinated by the histidine/aspartate catalytic triad. His^{313} and His^{374} were set to their monoprotonated states, such that the catalytic Fe^{II} ion is chelated by sp^2-hybridized imidazole nitrogen atoms.

Ligands were drawn in ChemDraw and loaded into Chem3D (ChemBioOffice 2010, CambridgeSoft Inc.). The diacylhydrazine moiety required particular attention due to the limited support of GOLD for this relatively uncommon chemical motif. Diacylhydrazines were drawn in their di-deprotonated form, which we expect to be relevant in the context of binding to two metal ions.[3] Carboxylic acids were assumed to be fully deprotonated and were modeled as carboxylates in all ligands, thus allowing salt bridge formation with positively charged residues in the PHD2 active site. Because the GOLD default settings cannot account for the geometric isomers of the diacylhydrazine moiety (**Supplementary Figure 3**), three different starting geometries were chosen per compound and subjected to the same docking protocol to facilitate a more comprehensive sampling of the conformation space. For each of the three starting conformations, geometry optimization was performed using the MMFF94 force field[4] as implemented in Chem3D. Three-dimensional ligand structures were then saved in the TRIPOS MOL2 format, including explicit hydrogen atoms. Atom typing of the protein receptor and ligands was performed automatically by GOLD and resulted in correct assignment of the nitrogen sp^2 atom type (“N.2”) to the diacylhydrazine nitrogen atoms, which is a prerequisite for adequate modeling of metal iron chelation.



Supplementary Figure 3. Definition of protonation states and atom types for docking experiments.

Docking runs were performed at the maximum docking accuracy setting using 10 rounds of "genetic" algorithm-based optimization per ligand conformer. Scoring was based on the GOLDScore fitness function. The "generate diverse solutions" option was enabled with an RMSD threshold between clusters of ligand poses of 1.5 Å; early termination was disabled. These settings enable the generation of a large set of different binding modes by deliberate assignment of lower docking scores to ligand poses in the population with high similarity to already identified binding modes.

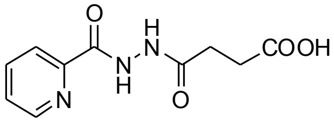
The GOLD software is particularly suitable for identification of diverse binding modes, rather than evaluation of ligand-receptor binding affinities, at least in part due to the limitations of currently available non-quantum mechanical scoring functions for evaluation of ligand-metalloprotein complexes.[5] Therefore, the obtained set of diverse ligand poses was manually inspected for presence of ligand-receptor interactions that are common to potent PHD2 inhibitors[6] and are likely to contribute substantially to the free energy of binding.

Ligand poses obtained from the three different starting conformations were combined and filtered for the presence of (i) chelation of the active site iron (ii) salt bridge formation with Arg^{383} and (iii) formation of hydrophobic and π-π stacking interactions between the ligand and hydrophobic and aromatic amino acids (Ile^{256}, Met^{299}, Tyr^{310} and Trp^{389}) at the opening of the PHD2 active site. Selected poses that pass the above criteria are shown in **Figure 5** (main text) and, in conjunction with SAR data, suggest possible chelation modes for the second iron ion.

3. Synthetic procedures

Reactions involving moisture sensitive reagents were carried out under a nitrogen atmosphere, and oven-dried glassware was used throughout. Anhydrous solvents were obtained from solvent stills and were activated by passing over a short column of activated alumina. Reagents were obtained from Acros, Aldrich, Avocado, Fluka, or Lancaster fine chemical suppliers and used as supplied. Thin layer chromatography (TLC) was performed on Merck DC-Kieselgel 60 F254 0.2 mm precoated plates with fluorescence indicator. Visualization of spots was achieved using UV light (254 nm) and by developing in a basic solution of $KMnO_4$ solution, followed by heating. Silica gel for flash column chromatography (40−63 µm particle size) was purchased from VWR. ^{1}H NMR spectra were recorded on a Bruker AC200 (200 MHz), Bruker AV400 (400 MHz) or Bruker Avance AV500 (500 MHz) spectrometer and referenced to residual solvent peaks. Chemical shifts are quoted in parts per million (ppm). Assignments were made on the basis of chemical shifts, coupling constants (*J*), ^{13}C, DEPT, COSY, HMQC data and comparison with spectra of related compounds. Resonances are described as s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), and brs (broad singlet). Coupling constants are given in Hz and are reported to the nearest 0.5 Hz. ^{13}C NMR spectra were recorded on a Bruker AV400 spectrometer at 100 MHz or a Bruker Avance AV500 spectrometer (fitted with an inverse cryoprobe for ^{13}C observation) at 125 MHz and referenced to $CDCl_3$ or DMSO-d_6. Infra-red (IR) spectra were recorded on a Bruker Tensor 27 FT-IR spectrophotometer as KBr disks or as thin films on sodium chloride plates. Selected absorption maxima (ν_{max}) are given in wavenumbers (cm^{-1}) and are uncorrected. High resolution mass spectra (HRMS) were recorded under the conditions of electrospray ionization on a Bruker MicroTOF. Melting points were recorded on a Leica VMTG heated-stage microscope melting point apparatus. Elemental analyses were performed at London Metropolitan University, London, U.K. Analytical HPLC was performed on an Agilent Technologies 1200 Series HPLC system. A 10 µL aliquot of a ~1 mg/mL solution of each compound was resolved on a Grace Vydac HPLC column (Cat. No. 218TP5415) using a gradient of 0-100% of water (incl. 0.1% formic acid) in acetonitrile (incl 0.1% formic acid) over 15 min (flow rate 1 mL/min). Unless stated otherwise, all tested compounds were >95% pure as judged by the area under the curve (AUC) of the absorbance trace at 254 nm.

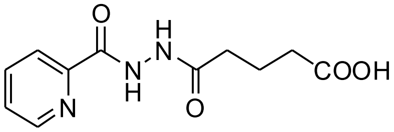
4-Oxo-4-(2-picolinoylhydrazinyl)butanoic acid (1)



To a stirred solution of 2-picolinyl hydrazide (274 mg, 2.0 mmol) in EtOAc (10 mL) was added a solution of succinic anhydride (200 mg, 2.0 mmol) in EtOAc (10 mL). The mixture was allowed to stand at room temperature for 5 h. The precipitate was collected and recrystallised from EtOH to afford 368 mg (78%) of **1** as a white solid.

mp 180-182 °C; ***R*** R_f 0.35 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=4.28 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3324, 3284, 2993 (br), 1727, 1697, 1647, 1528, 1494, 1152; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 2.47 (2H, t, *J*=7.0 Hz, CH$_2$), 2.51 (2H, t, *J*=7.0 Hz, CH$_2$), 7.65 (1H, dd , *J*=2.0 Hz, 4.5 Hz, ArCH), 8.02-8.04 (2H, m, 2×ArCH), 8.67-8.69 (1H, m, ArCH), 10.07 (1H, broad s, NH), 10.40 (1H, broad s, NH), 12.14 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 28.1 (CH$_2$), 28.8 (CH$_2$), 122.3 (ArCH), 127.0 (ArCH), 137.8 (ArCH), 148.6 (ArCH), 149.1 (ArC), 162.5 (CO), 170.0 (CO), 173.5 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{10}H_{10}N_3O_4$ (M-H$^+$), 236.0666; found, 236.0673.

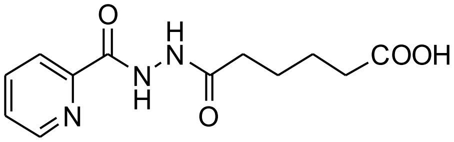
5-Oxo-5-(2-picolinoylhydrazinyl)pentanoic acid (2)



To a stirred solution of 2-picolinyl hydrazide (274 mg, 2.0 mmol) in EtOAc (10 mL) was added a solution of glutaric anhydride (228 mg, 2.0 mmol) in EtOAc (10 mL). The mixture was allowed to stand at room temperature for 5 h. The precipitate was collected and recrystallised from EtOH to afford 375 mg (75%) of **2** as a white solid.

mp 139-140 °C; **R_f** 0.40 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=4.58 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3333, 3287, 2934 (br), 1731, 1692, 1643, 1538, 1502, 1204; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 1.78 (2H, m, CH$_2$), 2.23 (2H, t, *J*=7.5 Hz, C**H**$_2$CONH), 2.31 (2H, t, *J*=7.5 Hz, C**H**$_2$COOH), 7.64-7.66 (1H, m, ArCH), 8.03-8.05 (2H, m, 2×ArCH), 8.67-8.68 (1H, m, ArCH), 9.97 (1H, broad s, N**H**COCH$_2$), 10.40 (1H, broad s, NHCOAr), 12.07 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 20.5 (CH$_2$**C**H$_2$CH$_2$), 32.4 (CH$_2$), 32.8 (CH$_2$), 122.3 (ArCH), 127.0 (ArCH), 137.9 (ArCH), 148.6 (ArCH), 149.1 (ArC), 162.7 (ArCO), 170.6 (CO), 174.2 (COOH); **HRMS** (ESI$^-$) calcd. for $C_{11}H_{12}N_3O_4$ (M-H$^+$), 250.0822; found, 250.0829.

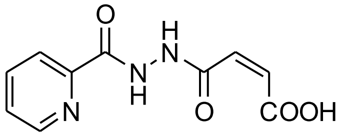
6-Oxo-6-(2-picolinoylhydrazinyl)hexanoic acid (3)



To a solution of monomethyl adipate (352 mg, 2.2 mmol) in THF (20 mL) were added 1-hydroxybenzotriazole (HOBt, 324 mg, 2.4 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI, 460 mg, 3.0 mmol), Et_3N (0.3 mL, 2.4 mmol) and 2-picolinyl hydrazide (274 mg, 2 mmol) and the resulting solution was stirred at room temperature for 16 h. Then THF was evaporated, the residue was dissolved in $CHCl_3$ (20 mL) and washed with satd. aq. citric acid (2×10 mL) and satd. aq. $NaHCO_3$ (2×10 mL), dried over $MgSO_4$ and evaporated. Purification by flash chromatography using petrol ether 40-60 °C/EtOAc (50:50) afforded 303 mg (55%) of a colourless oil. Thereof, 90 mg (0.32 mmol) was dissolved in a mixture of THF (5 mL) and 1N NaOH (5 mL). After stirring for 1 h at room temperature, solvents were evaporated and water (5 mL) was added. After acidification to pH 3 with 1N HCl, the aqueous phase was extracted with EtOAc (6×10 mL). Organic phases were collected, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was recrystallised from EtOH to yield 64 mg (42% over 2 steps) of **3** as a white solid.

mp 100-105 °C; ***R*$_f$** 0.40 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=4.96 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3374, 3241 (br), 1737, 1695, 1626, 1591, 1500, 1169; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 1.48-1.52 (2H, m, CH_2), 1.55-1.58 (2H, m, CH_2), 2.18-2.26 (4H, m, 2×CH_2CO), 7.63-7.66 (1H, m, ArCH), 8.02-8.03 (2H, m, 2×ArCH), 8.67-8.68 (1H, m, ArCH), 9.98 (1H, d, *J*=3.5 Hz, NH), 10.40 (1H, d, *J*=3.5 Hz, NH), 12.02 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 24.1 (CH_2), 24.6 (CH_2), 33.0 (**C**H_2CO), 33.4 (**C**H_2CO), 122.3 (ArCH), 126.9 (ArCH), 137.8 (ArCH), 148.2 (ArCH), 149.2 (ArC), 170.8 (Ar**C**OCH_2), 174.4 (CH_2**C**ONH), 175.9 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{12}H_{14}N_3O_4$ (M-H$^+$), 264.0979; found, 264.0979.

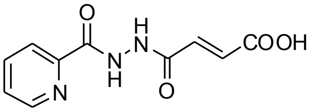
(*Z*)-4-Oxo-4-(2-picolinoylhydrazinyl)but-2-enoic acid (4)



To a stirred solution of 2-picolinyl hydrazide (137 mg, 1.0 mmol) in EtOAc (10 mL) was added a solution of maleic anhydride (98 mg, 1.0 mmol) in EtOAc (5 mL). The mixture was allowed to stand at room temperature for 24 h. The precipitate was collected and recrystallised from EtOH to afford 212 mg (90%) of **4** as a white solid.

mp 188-189 °C; ***R*** R_f 0.15 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=4.57 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3350, 3251, 1761, 1687, 1644, 1592, 1533, 1493, 1459, 1330, 1239; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 6.33 (1H, d, *J*=12.0 Hz, olefinic CH), 6.45 (1H, d, *J*=12.0 Hz, olefinic CH), 7.66-7.68 (1H, m, ArCH), 8.04-8.06 (2H, m, 2×ArCH), 8.69-8.71 (1H, m, ArCH), 10.69 (2H, broad s, 2×NH), 13.34 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 122.4 (ArCH), 126.3 (olefinic C), 127.1 (ArCH), 133.5 (olefinic C), 138.0 (ArCH), 148.7 (ArCH), 148.9 (ArC), 162.3 (CO), 162.7 (CO), 167.0 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{10}H_8N_3O_4$ (M-H$^+$), 234.0509; found, 234.0511.

(*E*)-4-Oxo-4-(2-picolinoylhydrazinyl)but-2-enoic acid (5)

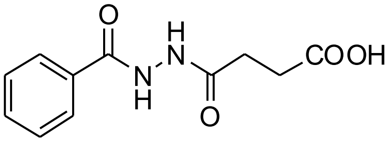


Compound **30** (150 mg, 0.6 mmol) was added to a solution of LiOH·H$_2$O (75 mg, 1.8 mmol) in water:THF (1:2) (15 ml) and the mixture was stirred at room temperature. TLC analysis (EtOAc: MeOH, 4:1) indicated complete consumption of starting material (R_f 0.6) after 3 h. The reaction mixture was then washed with EtOAc (3×10 mL). The aqueous layer was acidified to pH 3 with 1 N HCl. A white precipitate was obtained, filtered off, washed with water and dried *in vacuo* to afford 130 mg (98%) of **5** as a white solid.

mp 272-275 °C; **HPLC** t_R=4.81 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3400-3100 (br), 3317, 3213, 1724, 1630, 1153; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 6.66 (1H, d, *J*=15.5 Hz, olefinic CH), 7.04 (1H, d, *J*=15.5 Hz, olefinic CH), 7.65-7.68 (1H, m, ArCH), 8.02-8.06 (2H, m, 2×ArCH), 8.69-8.70 (1H, m, ArCH), 10.73 (1H, broad s, NH), 10.75 (1H, broad s, NH); **^{13}C-NMR**

(125 MHz, DMSO-d_6) δ_C 122.4 (ArCH), 127.1 (ArCH), 131.2 (olefinic CH), 134.2 (olefinic CH), 138.0 (ArCH), 148.7 (ArCH), 148.8 (ArC), 161.8 (C=O), 162.4 (C=O), 166.1 (COOH); **HRMS** (ESI$^-$) calcd. for $C_{10}H_8N_3O_4$ (M-H$^+$) 234.0520; found 234.0512.

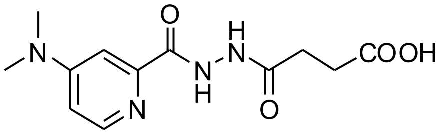
4-(2-Benzoylhydrazinyl)-4-oxobutanoic acid (6)



To a stirred solution of benzhydrazide (136 mg, 1.0 mmol) in EtOAc (10 mL) was added a solution of succinic anhydride (100 mg, 1.0 mmol) in EtOAc (5 mL). The mixture was allowed to stand at room temperature for 24 h. The precipitate was collected and recrystallised from EtOH to afford 193 mg (81%) of **6** as a white solid.

mp 169-172 °C; ***R*$_f$** 0.75 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=4.72 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3194 (br), 1724, 1674, 1614, 1575, 1483, 1211; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 2.46-2.49 (4H, m, CH_2CH_2), 7.48-7.51 (2H, m, 2×ArCH), 7.56-7.59 (1H, m, ArCH), 7.86-7.88 (2H, m, 2×ArCH), 9.93 (1H, broad s, NH), 10.32 (1H, broad s, NH), 12.15 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 27.9 (CH_2), 28.8 (CH_2), 127.4 (ArCH), 128.4 (ArCH), 131.8 (ArCH), 132.5 (ArC), 165.4 (ArCO), 170.5 (CO), 173.6 (CO) ppm; **HRMS** (ESI$^-$) calcd. for $C_{11}H_{11}N_2O_4$ (M-H$^+$), 235.0713; found, 235.0715.

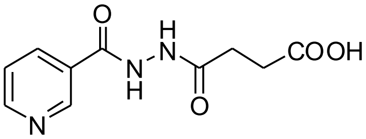
4-(2-(4-Dimethylamino)picolinoyl)hidrazinyl)-4-oxobutanoic acid (7)



To a stirred solution of compound **30** (45 mg, 0.25 mmol) in EtOAc (5 mL) was added a solution of succinic anhydride (25 mg, 0.25 mmol) in EtOAc (5 mL). The mixture was allowed to stand at room temperature for 16 h. Solids were collected and recrystallised from EtOH to afford 60 mg (86%) of **7** as a white solid.

mp 155-158 ºC; **HPLC** t_R=3.95 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3431 (bs), 1723, 1636, 1606, 1512; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 2.45-2.48 (4H, m, 2×CH_2), 3.03 (6H, s, NMe_2), 6.78 (1H, dd, *J*=2.5 Hz, 6.0 Hz, ArCH), 7.23 (1H, d, *J*=2.5 Hz, ArCH), 8.17 (1H, d, *J*=6.0 Hz, ArCH), 10.10 (1H, broad s, NH), 10.19 (1H, broad s, NH), 12.14 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 28.1 (CH_2), 28.9 (CH_2), 38.8 (NMe_2), 104.6 (ArCH), 108.7 (ArCH), 148.4 (ArCH), 149.3 (ArC), 154.8 (ArC), 162.9 (ArCO), 169.7 (CO), 173.6 (COOH) ppm; **HRMS** (ESI$^+$) calcd. for $C_{12}H_{17}N_4O_4$ (M+H$^+$), 281.1244; found, 281.1247.

4-(2-Nicotinoylhydrazinyl)-4-oxobutanoic acid (8)

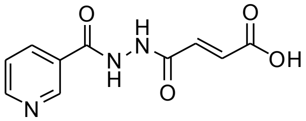


To a stirred solution of nicotinic hydrazide (274 mg, 2.0 mmol) in EtOAc (15 mL) was added a solution of succinic anhydride (200 mg, 2.0 mmol) in EtOAc (10 mL). The mixture was then heated at 70 °C for 3 h. Solids were collected and recrystallised from EtOH to afford 398 mg (84%) of **8** as a white solid.

mp 209-210 °C; ***R***

(ArCO), 170.5 (CO), 173.5 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{10}H_{10}N_3O_4$ (M-H$^+$), 236.0666; found, 236.0670.

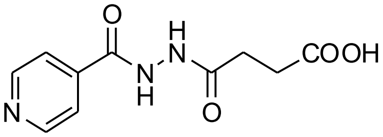
(*E*)-4-(2-Nicolinoylhydrazinyl)-4-oxobut-2-enoic acid (9)



Compound **31** (150 mg, 0.57 mmol) was added to a stirred solution of LiOH·H_2O (75 mg, 1.8 mmol) in H_2O:THF (1:2 v/v) (15 ml) at room temperature. TLC analysis (EtOAc:MeOH, 4:1 v/v) indicated complete consumption of starting material (R_f 0.6) after 3 h. The reaction mixture was then washed with EtOAc (3×10 mL). The aqueous layer was acidified with to pH 3with 1 N HCl. The obtained precipitate was filtered off, washed with water, and dried under *vacuo* to afford 87 mg (65%) of **9** as a white solid.

mp 274-276 ^oC; **HPLC** t_R=3.77 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3400-3100 (br), 3206, 3076, 1702, 1619, 1596, 1153; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 6.68 (1H, d, *J*=15.5 Hz, olefinic CH), 7.05 (1H, d, *J*=15.5 Hz, olefinic CH), 7.68-7.71 (1H, m, ArCH), 8.36-8.40 (1H, m, ArCH), 8.83-8.85 (1H, m, ArCH), 9.10 (1H, d, *J*=2.0 Hz, ArCH), 10.76 (1H, broad s, NH), 10.99 (1H, broad s, NH); **^{13}C-NMR** (125 MHz, DMSO-d_6) δ_C 124.3 (ArCH), 128.5 (ArC), 131.4 (olefinic CH), 134.1 (olefinic CH), 137.0 (ArCH), 147.2 (ArCH), 151.1 (ArCH), 162.3 (C=O), 163.3 (C=O), 166.1 (COOH); **HRMS** (ESI$^-$) calcd. for $C_{10}H_8N_3O_4$ (M-H$^+$), 234.0520; found, 234.0514.

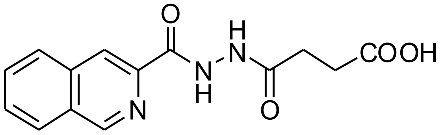
4-(2-Isonicotinoylhydrazinyl)-4-oxobutanoic acid (10)



To a stirred solution of isonicotinic hydrazide (274 mg, 2.0 mmol) in MeOH (10 mL) was added a solution of succinic anhydride (200 mg, 2.0 mmol) in 10 mL of MeOH. The mixture was refluxed for 2 h. The precipitate was collected and recrystallised from EtOH to afford 323 mg (68%) of **10** as a white solid.

mp 221-223 °C; ***R*$_f$** 0.25 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=2.18 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3201, 2514 (br), 1710, 1614, 1556, 1476, 1342, 1204; **^{1}H-NMR** (500MHz, DMSO-d$_6$) δ_H 7.79 (2H, d, 8.0 Hz, 2×ArCH), 8.77 (2H, d, 8.0 Hz, 2×ArCH), 10.06 (1H, broad s, NH), 10.66 (1H, broad s, ArCONH), 12.06 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 28.1 (CH$_2$), 28.7 (CH$_2$), 121.3 (ArCH), 139.5 (ArC), 150.4 (ArCH), 163.9 (ArCO), 170.5 (CO), 173.5 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{10}H_{10}N_3O_4$ (M-H$^+$), 236.0666; found, 236.0666.

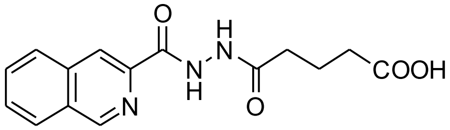
4-(2-Isochinoline-3-carbonyl)hydrazinyl)-4-oxobutanoic acid (11)



To a stirred solution of **51** (94 mg, 0.5 mmol) in CHCl$_3$ (10 mL) was added a solution of succinic anhydride (50 mg, 0.5 mmol) in CHCl$_3$ (5 mL). The mixture was allowed to stand at room temperature for 5 h. The precipitate was collected and recrystallised from EtOH to afford 125 mg (87%) of **11** as a white solid.

mp 215-216 °C; ***R*$_f$** 0.45 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=6.20 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3350, 3176 (br), 1711, 1621, 1481, 1416, 1326, 1190; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 2.41-2.44 (4H, m, CH$_2$CH$_2$), 7.84 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 7.91 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 8.22 (1H, d, *J*=8.0 Hz, ArCH), 8.28 (1H, d, *J*=8.0 Hz, ArCH), 8.58 (1H, s, ArCH), 9.41 (1H, s, ArCH), 10.20 (1H, broad s, NH), 10.52 (1H, broad s, NH), 12.16 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 28.2 (CH$_2$), 28.8 (CH$_2$), 120.3 (ArCH), 127.9 (ArCH), 128.0 (ArCH), 129.4 (ArCH), 129.4 (ArC), 131.5 (ArCH), 135.2 (ArC), 142.8 (ArC), 151.8 (ArCH), 162.6 (ArCO), 169.9 (CO), 173.6 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{14}H_{12}N_3O_4$ (M-H$^+$), 286.0822; found, 286.0823.

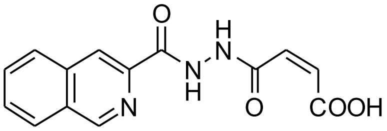
5-(2-(Isochinoline-3-carbonyl)hydrazinyl)-5-oxopentanoic acid (12)



To a stirred solution of **51** (56 mg, 0.30 mmol) in $CHCl_3$ (10 mL) was added a solution of glutaric anhydride (34 mg, 0.30 mmol) in $CHCl_3$ (5 mL). The mixture was allowed to stand at room temperature for 3 h. The precipitate was collected and recrystallised from EtOH to afford 44 mg (49%) of **12** as a white solid.

mp 176-179 °C; ***R*$_f$** 0.40 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=6.41 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3350, 3170 (br), 1703, 1638, 1561, 1490, 1439, 1221; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 1.78-1.81 (2H, m, $CH_2CH_2CH_2$), 2.26 (2H, t, *J*=7.0 Hz, CH_2CONH), 2.33 (2H, t, *J*=7.0 Hz, CH_2COOH), 7.84 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 7.91 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 8.23 (1H, d, *J*=8.0 Hz, ArCH), 8.28 (1H, d, *J*=8.0 Hz, ArCH), 8.59 (1H, s, ArCH), 9.42 (1H, s, ArCH), 10.08 (1H, s, NH), 10.52 (1H, s, NH), 12.07 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500MHz, DMSO-d_6) δ_C 20.5 ($CH_2\mathbf{C}H_2CH_2$), 32.4 (CH_2), 32.8 (CH_2), 120.3 (ArCH), 127.9 (ArCH), 128.0 (ArCH), 129.4 (ArCH), 129.4 (ArC),131.5 (ArCH), 135.3 (ArC), 142.9 (ArC), 151.7 (ArCH), 162.8 (ArCO), 170.6 (CO), 174.2 (COOH); **HRMS** (ESI$^-$) calcd. for $C_{15}H_{14}N_3O_4$ (M-H$^+$), 300.0979; found, 300.0982.

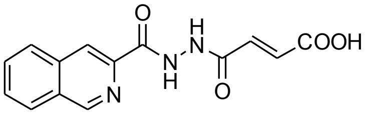
(*Z*)-4-(2-(Isochinoline-3-carbonyl)hydrazinyl)-4-oxobut-2-enoic acid (13)



To a stirred solution of **51** (56 mg, 0.3 mmol) in $CHCl_3$ (10 mL) was added a solution of maleic anhydride (29 mg, 0.3 mmol) in $CHCl_3$ (5 mL). The mixture was allowed to stand at room temperature for 20 h. The precipitate was collected and recrystallised from EtOH to afford 81 mg (95%) of **13** as a white solid.

mp 200-203 °C; ***R*$_f$** 0.40 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=6.77 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3190, 1735, 1678, 1587, 1488; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 6.36 (1H, d, *J*=12.0 Hz, olefinic CH), 6.45 (1H, d, *J*=12.0 Hz, olefinic CH), 7.83-7.87 (1H, m, ArCH), 7.89-7.93 (1H, m, ArCH), 8.24 (1H, d, *J*=8.0 Hz, ArCH), 8.29 (1H, d, *J*=8.0 Hz, ArCH), 8.62 (1H, s, ArCH), 9.43 (1H, s, ArCH), 10.83 (2H, broad s, 2×NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 120.5 (ArCH), 126.6 (olefinic CH), 127.9 (ArCH), 128.1 (ArCH), 129.4 (ArC), 129.5 (ArCH), 131.5 (ArCH), 133.5 (olefinic CH), 135.2 (ArC), 142.5 (ArC), 151.9 (ArCH), 162.3 (CO), 162.5 (CO), 167.0 (COOH) ppm; **HRMS** (ESI^+) calcd. for $C_{14}H_{12}N_3O_4$ ($M+H^+$), 286.0822; found, 286.0813.

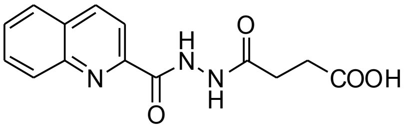
(*E*)-4-(2-(Isochinoline-3-carbonyl)hydrazinyl)-4-oxobut-2-enoic acid (14)



Compound **34** (150 mg, 0.5 mmol) was added to a stirred solution of $LiOH{\cdot}H_2O$ (63 mg, 1.5 mmol) in H_2O:THF (1:2 v/v) (15 ml) at room temperature. TLC analysis (EtOAc: MeOH, 4:1) indicated complete consumption of starting material (R_f 0.5) after 3 h. The reaction mixture was then washed with EtOAc (3×10 mL). The aqueous layer was acidified with to pH 3 with 1 N HCl and precipitates were filtered off, washed with water and dried *in vacuo* to afford 120 mg (88%) of **14** as a yellow solid.

mp >330 °C; **HPLC** t_R=6.84 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3209, 1714, 1605, 1486, 1300, 1186; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 6.67 (1H, d, *J*=15.5 Hz, olefinic CH), 7.05 (1H, d, *J*=15.5 Hz, olefinic CH), 7.84-7.87 (1H, m, ArCH), 7.90-7.93 (1H, m, ArCH) 8.24 (1H, d, *J*=8.0 Hz, ArCH), 8.29 (1H, d, *J*=8.0 Hz, ArCH), 8.61 (1H, s, ArCH), 9.43 (1H, s, ArCH), 10.85 (1H, broad s, NH), 10.89 (1H, broad s, NH); **^{13}C-NMR** (125 MHz, DMSO-d_6) δ_C 120.5 (ArCH), 127.9 (ArCH), 128.1 (ArCH), 129.4 (ArC), 129.5 (ArCH), 131.1 (olefinic CH), 131.6 (ArCH), 134.3 (olefinic CH), 135.2 (ArC), 142.8 (ArC), 151.9 (ArCH) 161.7 (C=O), 162.5 (C=O), 164.8 (COOH); **HRMS** (ESI^-) calcd. for $C_{14}H_{10}N_3O_4$ ($M-H^+$), 284.0677; found 284.0670.

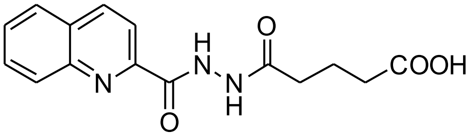
4-Oxo-4-(2-(chinoline-2-carbonyl)hydrazinyl)butanoic acid (15)



To a stirred solution of **52** (150 mg, 0.8 mmol) in EtOAc (10 mL) was added a solution of succinic anhydride (80 mg, 0.8 mmol) in EtOAc (10 mL). The mixture was allowed to stand at room temperature for 4 h. The precipitate was collected and recrystallised from EtOH to afford 191 mg (83%) of **15** as a white solid.

mp 181-185 °C; ***R*$_f$** 0.50 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=6.55 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3359, 3175 (br), 1724, 1682, 1638, 1565, 1489, 1428; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 2.50-2.51 (4H, m,CH_2CH_2), 7.75 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 7.90 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 8.09-8.16 (3H, m, 3×ArCH), 8.59 (1H, d, *J*=8.0 Hz, ArCH), 10.15 (1H, broad s, NH), 10.60 (1H, broad s, NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 28.1 (CH_2), 28.8 (CH_2), 118.7 (ArCH), 128.1 (ArCH), 128.4 (ArCH), 128.9 (ArC), 129.3 (ArCH), 130.7 (ArCH), 146.0 (ArC), 149.1 (ArC), 162.9 (ArCO), 170.3 (CO), 173.7 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{14}H_{12}N_3O_4$ (M-H$^+$), 286.0822; found, 286.0824.

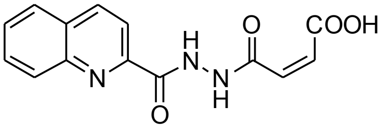
5-Oxo-5-(2-(chinoloine-2-carbonyl)hydrazinyl)pentanoic acid (16)



To a stirred solution of **52** (112 mg, 0.6 mmol) in 10 mL of EtOAc was added a solution of glutaric anhydride (69 mg, 0.6 mmol) in 10 mL of EtOAc. The mixture was allowed to stand at room temperature for 6 h. The precipitate was collected and recrystallised from EtOH to afford 114 mg (55%) of **16** as a white solid.

mp 153-154 °C; ***R*$_f$** 0.55 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=6.76 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3370, 3076, 1728, 1691, 1648, 1566, 1507, 1183; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 1.78-1.84 (2H, m, $CH_2C\mathbf{H_2}CH_2$), 2.27 (2H, t, *J*=7.5 Hz, CH_2), 2.33 (2H, t, *J*=7.5 Hz, CH_2), 7.76 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 7.91 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 8.10-8.16 (3H, m, 3×ArCH), 8.60 (1H, d, *J*=8.0 Hz, ArCH), 10.06 (1H, broad s, $CH_2CON\mathbf{H}$), 10.59 (1H, broad s, ArCONH), 12.10 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 20.5 ($CH_2CH_2CH_2$), 32.4 (CH_2), 32.8 (CH_2), 118.8 (ArCH), 128.2 (ArCH), 128.3 (ArCH), 128.9 (ArC), 129.3 (ArCH), 130.7 (ArCH), 138.0 (ArCH), 146.0 (ArC), 149.2 (ArC), 163.0 (ArCO), 170.8 (CO), 174.2 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{15}H_{14}N_3O_4$ (M-H$^+$), 300.0979; found, 300.0974.

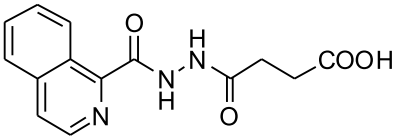
(*Z*)-4-Oxo-4-(2-(chinoline-2-carbonyl)hydrazinyl)but-2-enoic acid (17)



To a stirred solution of **52** (56 mg, 0.3 mmol) in EtOAc (10 mL) was added a solution of maleic anhydride (30 mg, 0.3 mmol) in EtOAc (5 mL). The mixture was allowed to stand at room temperature for 16 h. The precipitate was collected and recrystallised from EtOH to afford 63 mg (74%) of **17** as a white solid.

mp 202-205 °C; ***R*$_f$** 0.15 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=7.05 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3337, 3201, 2860 (br), 1734, 1685, 1591, 1488; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 6.37 (1H, d, *J*=12.0 Hz, =CH), 6.46 (1H, d, *J*=12.0 Hz, =CH), 7.77 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 7.92 (1H, ddd, *J*=1.0 Hz, 8.0 Hz, 8.0 Hz, ArCH), 8.12-8.18 (3H, m, 3×ArCH), 8.62 (1H, d, *J*=8.0 Hz, ArCH), 10.86 (2H, broad s, 2×NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 118.7 (ArCH), 126.5 (olefinic CH), 128.2 (ArCH), 128.4 (ArCH), 129.0 (ArC), 129.3 (ArCH), 130.8 (ArCH), 133.4 (olefinic CH), 138.1 (ArCH),146.0 (ArC), 149.0 (ArC), 162.5 (CO), 162.8 (CO), 167.0 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{14}H_{10}N_3O_4$ (M-H$^+$), 284.0666; found, 284.0670.

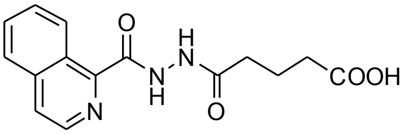
4-(2-(Isochinoline-1-carbonyl)hydrazinyl)4-oxobutanoic acid (18)



To a stirred solution of **53** (56 mg, 0.3 mmol) in EtOAc (10 mL) was added a solution of succinic anhydride (30 mg, 0.3 mmol) in EtOAc (5 mL). The mixture was allowed to stand at room temperature for 5 h. Solids were collected and recrystallised from EtOH to afford 62 mg (72%) of **18** as a white solid.

mp 205-207 °C; ***R*$_f$** 0.60 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=5.89 min; **IR** (KBr disk) ν_{max}/cm^{-1} 3202 (br), 1707, 1607, 1486; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 2.50-2.53 (4H, m, CH_2CH_2), 7.75-7.78 (1H, m, ArCH), 7.84-7.87 (1H, m, ArCH), 8.03 (1H, d, *J*=6.0 Hz, ArCH), 8.08 (1H, d, *J*=8.5 Hz, ArCH), 8.56 (1H, d, *J*=6.0 Hz, ArCH), 8.76 (1H, d, *J*=8.5 Hz, ArCH), 10.12 (1H, broad s, NH), 10.51 (1H, broad s, ArCONH), 12.16 (1H, broad s, COOH) ppm; ^{13}**C-NMR** (500 MHz, DMSO-d$_6$) δ_C 28.1 (CH$_2$), 28.8 (CH$_2$), 123.1 (ArCH), 125.5 (ArC), 126.2 (ArCH), 127.1 (ArCH), 128.4 (ArCH), 130.8 (ArCH), 136.2 (ArC), 141.1 (ArCH), 151.4 (ArC), 165.1 (ArCO), 170.3 (CO), 173.6 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{14}H_{12}N_3O_4$ (M-H$^+$), 286.0822; found, 286.0823.

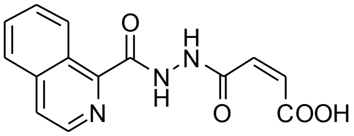
5-(2-(Isochinoline-1-carbonyl-hydrazinyl)-5-oxopentanoic acid (19)



To a stirred solution of **53** (112 mg, 0.6 mmol) in EtOAc (10 mL) was added a solution of glutaric anhydride (68 mg, 0.6 mmol) in EtOAc (5 mL). The mixture was allowed to stand at room temperature for 5 h. The precipitate was collected and recrystallised from EtOH to afford 91 mg (50%) of **19** as a white solid.

mp 169-170 °C; ***R*** R_f 0.55 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **HPLC** t_R=6.11 min; **IR** (KBr disk) ν_{max}/cm^{-1} 3265, 1710, 1608, 1491; ^{1}**H-NMR** (500 MHz, DMSO-d$_6$) δ_H 1.79-1.85 (2H, m, CH$_2$C**H**$_2$CH$_2$), 2.28 (2H, t, *J*=7.5 Hz, CH$_2$), 2.34 (2H, t, *J*=7.5 Hz, CH$_2$), 7.75-7.78 (1H, m, ArCH), 7.84-7.87 (1H, m, ArCH), 8.04 (1H, d, *J*=5.5 Hz, ArCH), 8.08 (1H, d, *J*=8.0 Hz, ArCH), 8.57 (1H, d, *J*=5.5 Hz, ArCH), 8.79 (1H, d, *J*=8.0 Hz, ArCH), 10.05 (1H, broad s, NH), 10.49 (1H, broad s, NH), 12.09 (1H, broad s, COOH); ^{13}**C-NMR** (500 MHz, DMSO-d$_6$) δ_C 20.5 (CH$_2$**C**H$_2$CH$_2$), 32.4 (CH$_2$), 32.8 (CH$_2$), 123.2 (ArCH), 125.5 (ArC), 126.2 (ArCH), 127.1 (ArCH), 128.5 (ArCH), 130.8 (ArCH), 136.3 (ArC), 141.1 (ArCH), 151.2 (ArC), 165.2 (ArCO), 170.9 (CO), 174.2 (COOH); **HRMS** (ESI$^-$) calcd. for $C_{15}H_{14}N_3O_4$ (M-H$^+$), 300.0979; found, 300.0979.

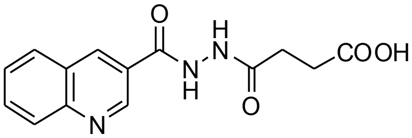
(*Z*)-4-(2-(Isochinoline-1-carbonyl)hydrazinyl)-4-oxobut-2-enoic acid (20)



To a stirred solution of **53** (187 mg, 1.0 mmol) in 15 mL of EtOAc was added a solution of maleic anhydride (98 mg, 1.0 mmol) in 10 mL of EtOAc. The mixture was allowed to stand at room temperature for 22 h. The precipitate was collected and recrystallised from MeOH to afford 214 mg (75%) of **20** as a white solid.

R$_f$ 0.25 (silica gel, EtOAc:MeOH:AcOH 80:20:2); **mp** 161-164 °C ; **IR** (KBr disk) ν_{max}/cm^{-1}: 3371, 3063 (br), 1713, 1607, 1558, 1493; **^{1}H-NMR** (500 MHz, 500 MHz) δ_H 6.37 (1H, d, *J*=12.0 Hz, olefinic CH), 6.47 (1H, d, *J*=12.0 Hz, olefinic CH), 7.76-7.80 (1H, m, ArCH), 7.85-7.88 (1H, m, ArCH), 8.07 (1H, d, *J*=5.5 Hz, ArCH), 8.09 (1H, d, *J*=8.0 Hz, ArCH), 8.59 (1H, d, *J*=5.5 Hz, ArCH), 8.79 (1H, d, *J*=8.0 Hz, ArCH), 10.82 (2H, broad s, 2×NH), 13.31 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 123.5 (ArCH), 125.5 (ArC), 126.1 (ArCH), 126.6 (olefinic CH), 127.2 (ArCH), 128.6 (ArCH), 130.9 (ArCH), 133.3 (olefinic CH), 136.3 (ArC), 141.1 (ArCH), 150.6 (ArC), 162.9 (CO), 164.7 (CO), 167.1 (CO) ppm; **HRMS** (ESI$^-$) calcd. for $C_{14}H_{10}N_3O_4$ (M-H$^+$), 284.0666; found, 284.0671.

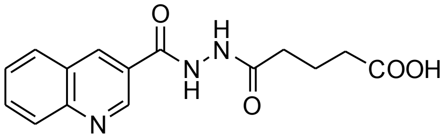
4-Oxo-4-(2-(chinoline-3-carbonyl)hydrazinyl)butanoic acid (21)



A suspension of hydrazide **54** (37 mg, 0.2 mmol) in EtOAc (10 mL) was heated to 70 °C to dissolve the starting material. Then, a solution of succinic anhydride (20 mg, 0.2 mmol) in EtOAc (2 mL) was added and the reaction mixture was stirred at 70 °C for 1 h, at 50 °C for 3 h and finally at room temperature for 3 h. Solids were collected and washed with EtOAc to afford 42 mg (74%) of **21** as a white solid.

mp 208-211 ºC; **HPLC** t_R=4.84 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3438 (bs), 3197, 1728, 1611, 1594, 1491; ^{1}H-NMR (500 MHz, DMSO-d$_6$) δ_H 2.47-2.51 (4H, m, 2×CH$_2$), 7.72 (1H, dd, *J*=7.0 Hz, 7.0 Hz, ArCH), 7.90 (1H, dd, *J*=7.0 Hz, 7.0 Hz, ArCH), 8.09-8.14 (2H, m, 2×ArCH), 8.88 (1H, d, *J*=2.0 Hz, ArCH), 9.28 (1H, d, *J*=2.0 Hz, ArCH), 10.11 (1H, broad s, NH), 10.72 (1H, broad s, NH), 12.11 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 28.1 (CH$_2$), 28.8 (CH$_2$), 125.3 (ArC), 126.4 (ArC), 127.6 (ArCH), 128.8 (ArCH), 129.2 (ArCH), 131.7 (ArCH), 136.1 (ArCH), 148.6 (ArC), 148.6 (ArCH), 164.1 (CO), 170.5 (CO), 173.6 (COOH) ppm; **HRMS** (ESI$^+$) calcd. for $C_{14}H_{13}N_3NaO_4$ (M+Na$^+$), 310.0798; found, 310.0803.

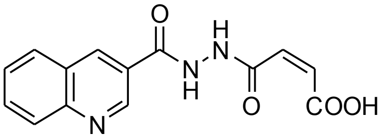
5-Oxo-4-(2-(chinoline-3-carbonyl)hydrazinyl)pentanoic acid (22)



A suspension of hydrazide **54** (37 mg, 0.2 mmol) in EtOAc (10 mL) was heated to 70 °C to dissolve the starting material. Then, a solution of glutaric anhydride (23 mg, 0.2 mmol) in EtOAc (2 mL) was added and the reaction mixture was stirred at 70 °C for 4 h. The mixture was cooled down, solids were collected and washed with EtOAc to afford 44 mg (74%) of **22** as a white solid.

mp 219-222 ºC; **HPLC** t_R=5.05 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3198 (bs), 1724, 1618, 1595, 1490, 1188; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 1.77-1.83 (2H, m, CH_2), 2.28 (2H, t, *J*=7.5 Hz, CH_2CO), 2.33 (2H, t, *J*=7.5 Hz, CH_2CO), 7.73 (1H, dd, *J*=7.5 Hz, 7.5 Hz, ArCH), 7.91 (1H, dd, *J*=7.5 Hz, 7.5 Hz, ArCH), 8.10-8.15 (2H, m, 2×ArCH), 8.88 (1H, d, *J*=2.0 Hz, ArCH), 9.28 (1H, d, *J*=2.0 Hz, ArCH), 10.04 (1H, broad s, NH), 10.68 (1H, broad s, NH), 12.08 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 20.5 (CH_2), 32.4 (**C**H_2CO), 32.8 (**C**H_2CO), 125.3 (ArC), 126.4 (ArC), 127.6 (ArCH), 128.8 (ArCH), 129.2 (ArCH), 131.5 (ArCH), 136.1 (ArCH), 148.6 (ArCH), 148.7 (ArC), 164.2 (CO), 171.2 (CO), 174.2 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{15}H_{15}N_3NaO_4$ (M+Na$^+$), 324.0955; found, 324.0951.

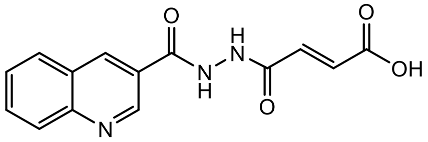
(*Z*)-4-Oxo-4-(2-(chinoline-3-carbonyl)hydrazinyl)but-2-enoic acid (23)



A suspension of hydrazide **54** (37 mg, 0.2 mmol) in EtOAc (10 mL) was heated to 70 °C to dissolve the starting material. Then, a solution of maleic anhydride (20 mg, 0.2 mmol) in EtOAc (2 mL) was added and the reaction mixture was stirred at 70 °C for 4 h. The mixture was cooled to room temperature, solids were collected and washed with EtOAc to afford 53 mg (94%) of **23** as a white solid.

mp 188-191 ºC; **HPLC** t_R=5.21 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3241 (bs), 1683, 1646, 1541, 1285; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 6.37 (1H, d, *J*=12.0 Hz, olefinic CH), 6.45 (1H, d, *J*=12.0 Hz, olefinic CH), 7.73 (1H, dd, *J*=7.5 Hz, 7.5 Hz, ArCH), 7.91 (1H, dd, *J*=7.5 Hz, 7.5 Hz, ArCH), 8.10-8.15 (2H, m, 2×ArCH), 8.91 (1H, d, *J*=2.0 Hz, ArCH), 9.30 (1H, d, *J*=2.0 Hz, ArCH), 10.72 (1H, broad s, NH), 10.99 (1H, broad s, NH), 13.14 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 125.1 (ArC), 126.4 (ArC), 126.9 (olefinic CH), 127.6 (ArCH), 127.6 (ArCH), 128.8 (ArCH), 129.3 (ArCH), 131.6 (ArCH), 132.9 (olefinic CH), 136.3 (ArCH), 148.6 (ArCH), 148.7 (ArC), 163.1 (CO), 163.9 (CO), 167.0 (COOH) ppm; **HRMS** (ESI$^-$) calcd. for $C_{14}H_{10}N_3O_4$ (M-H$^+$), 284.0677; found, 284.0671.

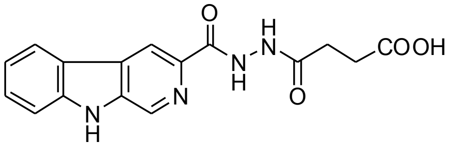
(*E*)- 4-Oxo-4-(2-(quinoline-3-carbonyl)hydrazinyl)but-2-enoic acid (24)



Compound **41** (90 mg, 0.3 mmol) was added to a stirring solution of LiOH.H$_2$O (38 mg, 0.9 mmol) in H$_2$O:THF (1:2) (15 ml) at room temperature. TLC analysis (EtOAc: MeOH, 4:1) indicated complete consumption of starting material (R$_f$ 0.5) after 3 h. The reaction mixture was then washed with EtOAc (3 x 10 mL). The aqueous layer was acidified with 1 N HCl to pH 3, and the white precipitate formed was filtered, washed with H$_2$O, and dried under *vacuo* to give (*E*)- 4-oxo-4-(2-(quinoline-3-carbonyl)hydrazinyl)but-2-enoic acid (**15**) as a yellow solid 50 mg (63%).

mp > 330°C; **HPLC** t$_R$=7.38 min; **IR** (KBr disk) 3450 (br), 3186, 1698, 1607, 1487 cm^{-1}; **^{1}H NMR** (500 MHz, DMSO-d$_6$) δ 6.70 (1H, d, J= 15.5 Hz, =C-H), 7.08 (1H, d, J 15.5 Hz, =C-H), 7.75 (1H, dd, J 7.5, 7.6 Hz, ArCH), 7.93 (1H, dd, J 7.5, 7.6, ArCH), 8.12-8.17 (2H, m, 2 x ArCH) 8.93 (1H, d, J 2.0, ArCH), 9.31 (1H, d, J 2.0, ArCH), 10.79 (H, bs, NH), 11.06 (H, bs, NH); **^{13}C-NMR** (125 MHz, DMSO-d$_6$) δ 125.0 (ArC), 126.4 (ArC), 127.7 (ArCH), 128.6 (ArCH), 129.3 (ArCH), 131.3 (=CH), 131.7 (ArCH), 134.1 (=CH), 136.5 (ArCH), 148.5 (ArCH), 148.6 (ArC) 162.4 (C=O), 163.9 (C=O), 166.1 (COOH); MS (ES$^-$) *m/z* 284.1 (100) [M-H]$^-$; **HMRS** (ESI) calcd. for $C_{14}H_{12}N_3O_4$ [M-H]$^-$ 286.0822; found 286.0822.

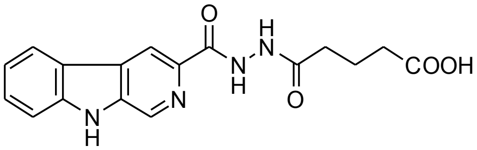
4-(2-(9*H*-Pyrido[3,4-*b*]indole-3-carbonyl)hydrazinyl)-4-oxobutanoic acid (25)



A suspension of compound **56** (45 mg, 0.2 mmol) in EtOAc (13 mL) was heated up to reflux to become mostly soluble. Then, a solution of succinic anhydride (20 mg, 0.2 mmol) in EtOAc (2 mL) was added and the mixture was refluxed for 12 h. The mixture was cooled down and precipitates were collected by filtration and washed with EtOAc to yield 52 mg (80%) of **25** as a white solid.

mp 248 ºC (decomp.); **HPLC** t_R=6.13 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3281, 1717, 1620, 1495, 1458; ^{1}**H-NMR** (500 MHz, DMSO-d$_6$) δ_H 2.49-2.52 (4H, m, 2×CH$_2$), 7.31-7.34 (1H, m, ArCH), 7.60-7.63 (1H, m, ArCH), 7.68 (1H, d, *J*=8.0 Hz, ArCH), 8.43 (1H, d, *J*=8.0 Hz, ArCH), 8.86 (1H, s, ArCH), 8.92 (1H, s, ArCH), 10.18 (1H, broad s, NH), 10.27 (1H, broad s, NH), 12.02 (1H, broad s, indole NH), 12.15 (1H, broad s, COOH) ppm; ^{13}**C-NMR** (500 MHz, DMSO-d$_6$) δ_C 28.2 (CH$_2$), 28.9 (CH$_2$), 112.3 (ArCH), 114.6 (ArCH), 120.1 (ArCH), 120.9 (ArC), 122.4 (ArCH), 128.0 (ArC), 128.7 (ArCH), 132.6 (ArCH), 137.3 (ArC), 138.5 (ArC), 141.0 (ArC), 163.2 (ArCO), 169.8 (CO), 173.6 (COOH) ppm; **HRMS** (ESI$^+$) calcd. for $C_{16}H_{15}N_4O_4$ (M+H$^+$) 327.1088; found 327.1087.

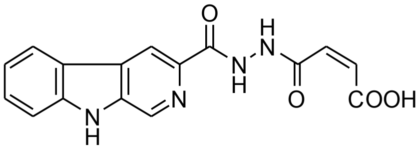
5-(2-(9*H*-Pyrido[3,4-*b*]indole-3-carbonyl)hydrazinyl)-5-oxopentanoic acid (26)



A suspension of **56** (74 mg, 0.3 mmol) in EtOAc (13 mL) was heated up to reflux to become mostly soluble. Then, glutaric anhydride (34 mg, 0.3 mmol) in EtOAc (2 mL) was added and the reaction mixture was refluxed for 12 h. The mixture was cooled, precipitates were collected by filtration and washed with EtOAc to afford 95 mg (88%) of **26** as a white solid.

mp 274-278ºC; **HPLC** t_R=6.30 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3248, 1710, 1605, 1491, 1458, 1253; ^{1}**H-NMR** (500 MHz, DMSO-d$_6$) δ_H 1.79-1.82 (2H, m, CH$_2$), 2.26 (2H, t, *J*=7.5 Hz, CH$_2$CO), 2.34 (2H, t, *J*=7.5 Hz, CH$_2$CO), 7.32 (1H, dd, *J*=7.5 Hz, *J*=8.0 Hz, ArCH), 7.61 (1H, dd, *J*=7.5 Hz, *J*=8.5 Hz, ArCH), 7.68 (1H, d, *J*=8.5 Hz, ArCH), 7.42 (1H, d, *J*=8.0 Hz, ArCH), 8.87 (1H, s, ArCH), 8.93 (1H, s, ArCH), 10.05 (1H, broad s, NH), 10.28 (1H, broad s, NH), 12.01 (1H, s, indole NH), 12.12 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 20.5 (CH$_2$), 32.4 (CH$_2$), 32.8 (CH$_2$), 112.3 (ArCH), 114.6 (ArCH), 120.1 (ArCH), 120.9 (ArC), 122.3 (ArCH), 128.0 (ArC), 128.7 (ArCH), 132.5 (ArCH), 137.3 (ArC), 138.6 (ArC), 141.0 (ArC), 163.5 (CO), 170.6 (CO), 174.2 (COOH) ppm; **HRMS** (ESI$^+$) calcd. for $C_{17}H_{16}N_4NaO_4$ (M+Na$^+$), 363.1064; found 363.1065.

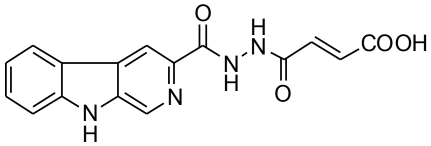
(*Z*)-4-(2-(9*H*-Pyrido[3,4-*b*]indole-3-carbonyl)hydrazinyl)-4-oxobut-2-enoic acid (27)



A suspension of **56** (45 mg, 0.2 mmol) in EtOAc (13 mL) was heated to reflux, a solution of maleic anhydride (19 mg, 0.2 mmol) in EtOAc (2 mL) was added and the reaction mixture was refluxed for 12 h. The mixture was then cooled and precipitates were collected by filtration and washed with EtOAc to yield 56 mg (87%) of **27** as a white solid.

mp 216-220 ºC; **HPLC** t$_R$=6.82 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3375 (bs), 1709, 1543, 1503, 1455, 1258; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 6.38 (1H, d, *J*=12.0 Hz, olefinic CH), 6.44 (1H, d, *J*=12.0 Hz, olefinic CH), 7.32 (1H, dd, *J*=7.5 Hz, *J*=8.0 Hz, ArCH), 7.61 (1H, dd, *J*=7.5 Hz, *J*=8.0 Hz, ArCH), 7.68 (1H, d, *J*=8.0 Hz, ArCH), 8.43 (1H, d, *J*=8.0 Hz, ArCH), 8.90 (1H, s, ArCH), 8.95 (1H, s, ArCH), 10.60 (1H, broad s, NH), 11.02 (1H, broad s, NH), 12.05 (1H, broad s, indole NH), 13.55 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 112.3 (ArCH), 114.8 (ArCH), 120.2 (ArCH), 120.9 (ArC), 122.4 (ArCH), 126.8 (olefinic CH), 128.1 (ArC), 128.8 (ArCH), 132.6 (ArCH), 133.5 (olefinic CH), 137.4 (ArC), 138.1 (ArC), 141.0 (ArC), 162.6 (CO), 162.9 (CO), 166.8 (COOH) ppm; **HRMS** (ESI$^+$) calcd. for $C_{16}H_{12}N_4NaO_4$ (M+Na$^+$) 347.0751; found 347.0749.

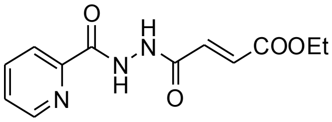
(*E*)-4-(2-(9*H*-Pyrido[3,4-*b*]indole-3-carbonyl)hydrazinyl)-4-oxobut-2-enoic acid (28)



Compound **44** (340 mg, 1.0 mmol) was added to a stirred solution of LiOH·H_2O (125 mg, 3.0 mmol) in H_2O:THF (1:2 v/v) (30 ml) at room temperature. TLC analysis (EtOAc:MeOH, 4:1 v/v) indicated complete consumption of starting material (R_f 0.5) after 3 h. The reaction mixture was then washed with EtOAc (3×10 mL). The aqueous layer was acidified to pH 3with 1 N HCl and the obtained precipitate was washed with water and dried *in vacuo* to afford 240 mg (76%) of **28** as a yellow solid.

mp >330 ^{o}C; $\boldsymbol{R_f}$ (EtOAc:MeOH, 4:1 v/v) 0.5; **IR** (KBr disk) ν_{max}/cm^{-1}: 3425 (br), 1688, 1611, 1493, 1458; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 6.67 (1H, d, *J*=15.5 Hz, olefinic CH), 7.10 (1H, d, *J*=15.5 Hz, olefinic CH), 7.33 (1H, dd, *J*=7.5 Hz, 8.0 Hz, ArCH), 7.62 (1H, dd, *J*=7.5 Hz, 8.0 Hz, ArCH), 7.69 (1H, d, *J*=8.0 Hz, ArCH), 8.44 (1H, d, *J*=8.0 Hz, ArCH), 8.89 (1H, s, ArCH), 8.95 (1H, s, ArCH), 10.62 (1H, d, *J*=2.0 Hz, NH), 10.91 (1H, d, *J*=2.0 Hz, NH), 12.09 (1H, broad s, indole NH); **^{13}C-NMR** (125 MHz, DMSO-d$_6$) δ_C 112.3 (ArCH), 114.8 (ArCH), 120.2 (ArCH), 120.9 (ArC), 122.4 (ArCH), 128.0 (ArC), 128.8 (ArCH), 130.9 (olefinic CH), 132.7 (ArCH), 134.5 (olefinic CH), 137.4 (ArC), 138.1 (ArC), 141.0 (ArC) 161.5 (C=O), 163.0 (C=O), 166.1 (COOH); **HRMS** (ESI$^-$) calcd. for $C_{18}H_{16}N_4O_4Na$ (M-H$^+$), 323.0786; found, 323.0794.

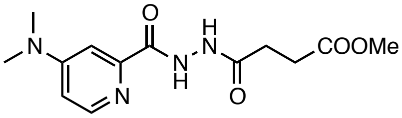
(*E*)-Ethyl 4-oxo-4-(2-picolinoylhydrazinyl)but-2-enoate (29)



Compound **45** (1.24 g, 4.0 mmol) was added to a stirred suspension of 2-picolinyl hydrazide (500 mg, 3.6 mmol) in THF: EtOAc (1:1) (50 ml) and the reaction mixture was stirred at room temperature overnight. The organic solvents were removed in *vacuo* and the resulting solid residue was washed with EtOAc (50 mL), filtered, and co-evaporated with diethyl ether to afford 411 mg (43%) of **29** as a white solid.

mp 188-192 °C; ***R*$_f$** (EtOAc:MeOH, 4:1) 0.6; **HPLC** t_R=6.47 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3357, 3188, 3115, 1717, 1665, 1570, 1368, 749; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 1.27 (3H, t, *J*=7.0 Hz, CH_3), 4.22 (2H, q, *J*=7.0 Hz, OCH_2), 6.71 (1H, d, *J*=15.5 Hz, olefinic CH), 7.12 (1H, d, *J*=15.5 Hz, olefinic CH), 7.66-7.68 (1H, m, ArCH), 8.04-8.05 (2H, m, 2×ArCH), 8.69-8.70 (1H, m, ArCH), 10.75 (1H, broad s, NH), 10.79 (1H, broad s, NH); **^{13}C-NMR** (125 MHz, DMSO-d_6) δ_C 14.1 (CH_3), 60.8 (OCH_2), 122.4 (ArCH), 127.1 (ArCH), 129.8 (olefinic CH), 134.8 (olefinic CH), 137.9 (ArCH), 148.8 (ArCH), 149.0 (ArC), 161.5 (C=O), 162.3 (C=O), 164.7 (C=O); **HRMS** (ESI$^+$) calcd. for $C_{12}H_{13}N_3O_4Na$ (M+Na$^+$), 286.0798; found, 286.0802. **Microanalysis** for $C_{12}H_{13}N_3O_4$ requires C, 54.75; H, 4.98; N, 15.96%; found C, 54.69, H, 4.98, N, 15.87%.

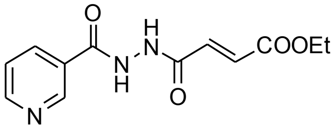
Methyl 4-(2-(4-dimethylamino)picolinoyl)hydrazinyl)-4-oxobutanoate (30)



To a solution of **48** (54 mg, 0.3 mmol) in THF (15 mL) were added 3-carbomethoxy propionylchloride (50 mg, 0.33 mmol) and Et_3N (47 µL, 0.33 mmol) at 0 °C. After 5 min, the reaction mixture was warmed up and stirred for 1 h at room temperature. Solids were removed by filtration and the filtrate was evaporated and purified by column chromatography (CH_2Cl_2 : MeOH 9:1) to afford 72 mg (82%) of **30** as a white solid.

mp 130-132 °C; **HPLC** t_R=4.55 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3230, 1738, 1670, 1609, 1518; **^{1}H-NMR** (500 MHz, $CDCl_3$) δ_H 2.67 (2H, t, *J*=7.0 Hz, CH_2), 2.75 (2H, t, *J*=7.0 Hz, CH_2), 3.08 (6H, s, $N(CH_3)_2$), 3.68 (3H, s, OCH_3), 6.56 (1H, dd, *J*=2.5 Hz, *J*=6.0 Hz, ArCH), 7.38 (1H, dd, *J*=2.5 Hz, ArCH), 8.14 (1H, dd, *J*=6.0 Hz, ArCH), 8.85 (1H, broad s, NH) ppm; **^{13}C-NMR** (500 MHz, $CDCl_3$) δ_C 28.9 (CH_2), 29.1 (CH_2), 39.2 ($N(CH_3)_2$), 51.9 (OCH_3), 105.4 (ArCH), 108.5 (ArCH), 148.3 (ArC), 148.6 (ArCH), 155.1 (ArC), 161.9 (CO), 168.4 (CO), 173.1 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{13}H_{19}N_4O_4$ (M+H$^+$), 295.1401; found, 295.1407.

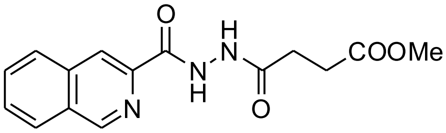
(*E*)-Ethyl 4-(2-nicolinoylhydrazinyl)-4-oxobut-2-enoate (31)



Compound **45** (1.24 g, 4.0 mmol) was added to a stirred suspension of nicotinic hydrazide (500 mg, 3.6 mmol) in THF: EtOAc (1:1) (50 ml) and the reaction mixture was stirred at room temperature overnight. Solvents were removed in *vacuo* and the resulting solid residue was washed with EtOAc (50 mL), filtered, and co-evaporated with diethyl ether to afford 600 mg (63%) of **31** as a white solid.

mp 177-180 °C; ***R*$_f$** (EtOAc:MeOH, 4:1) 0.6; **HPLC** t_R=5.19 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3256, 2992, 1724, 1636, 1506, 1367, 700; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 1.27 (3H, t, *J*=7.0 Hz, CH$_3$), 4.23 (2H, q, *J*=7.0 Hz, OCH$_2$), 6.73 (1H, d, *J*=15.5 Hz, olefinic CH), 7.12 (1H, d, *J*=15.5 Hz, olefinic CH), 7.56-7.58 (1H, m, ArCH), 8.23 (1H, dt, *J*=2.0, 8.0 Hz, ArCH), 8.78 (1H, dd, *J*=2.0 Hz, 5.0 Hz, ArCH), 9.04 (1H, d, J=2.0 Hz, ArCH), 10.74 (1H, broad s, NH), 10.90 (1H, broad s, NH); **^{13}C-NMR** (125 MHz, DMSO-d$_6$) δ_C 14.1 (CH$_3$), 61.0 (OCH$_2$), 123.8 (ArCH), 128.0 (ArC), 130.0 (olefinic CH), 134.6 (olefinic CH), 135.3 (ArCH), 148.5 (ArCH), 152.6 (ArCH), 162.0 (C=O), 163.9 (C=O), 164.7 (C=O); **HRMS** (ESI$^-$) calcd. for $C_{12}H_{12}N_3O_4$ (M-H$^+$), 262.0833; found, 262.0827. **Microanalysis** for $C_{12}H_{13}N_3O_4$ requires C, 54.75; H, 4.98; N, 15.96%; found C, 54.79, H, 4.97, N, 15.95%.

Methyl 4-(2-(isochinoline-3-carbonyl)hydrazinyl)-4-oxobutanoate (32)

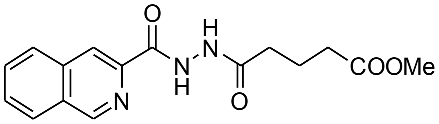


To a solution of **51** (84 mg, 0.45 mmol) in THF (15 mL) were added 3-carbomethoxy propionylchloride (75 mg, 0.5 mmol) and Et$_3$N (70 µL, 0.5 mmol) at 0 °C. After 5 min, the reaction mixture was warmed to room temperature and stirred for 1 h. Solids were removed by filteration and the filtrate was evaporated and recrystallised from EtOH to afford 130 mg (96%) of **32** as a white solid.

mp 214-218 °C; ***R*$_f$** 0.15 (silica gel, CH$_2$Cl$_2$:MeOH 98:2); **HPLC** t_R=6.98 min; **IR** (thin film) ν_{max}/cm^{-1}: 3365, 1730, 1639, 1625, 1502, 1484, 1439; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 2.54-

2.58 (4H, m, CH_2CH_2), 3.61 (3H, s, $COOCH_3$), 7.82-7.86 (1H, m, ArCH), 7.88-7.92 (1H, m, ArCH), 8.23 (1H, d, *J*=8.0 Hz, ArCH), 8.28 (1H, d, *J*=8.0 Hz, ArCH), 8.58 (1H, s, ArCH), 8.41 (1H, s, ArCH), 10.22 (1H, broad s, NH), 10.54 (1H, broad s, NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 28.0 (CH_2), 28.6 (CH_2), 51.4 (CH_3), 120.3 (ArCH), 127.9 (ArCH), 128.0 (ArCH), 129.4 (ArC), 129.5 (ArCH), 131.5 (ArCH), 135.2 (ArC), 142.8 (ArC), 151.8 (ArCH), 162.6 (CO), 169.6 (CO), 172.6 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{15}H_{15}N_3NaO_4$ (M+Na$^+$), 324.0955; found, 324.0957.

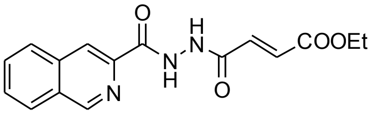
Methyl 5-(2-(isochinoline-3-carbonyl)hydrazinyl)-5-oxopentanoate (33)



To a solution of **51** (94 mg, 0.5 mmol) in THF (15 mL) were added methyl 4-(chloroformyl)butyrate (91 mg, 0.55 mmol) and Et_3N (77 µL, 0.55 mmol) at 0 °C. After 5 min, the reaction mixture was warmed up and stirred for 1 h at room temperature. After filtration, filtrate was evaporated and recrystallised from EtOH to afford 142 mg (90%) of **33** as a white solid.

mp 160-162 °C; ***R*~f~** 0.25 (silica gel, CH_2Cl_2:MeOH 98:2); **HPLC** t_R=7.30 min; **IR** (thin film) ν_{max}/cm^{-1}: 3382, 1729, 1637, 1625, 1500, 1476, 1439; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 1.79-1.86 (2H, m, $CH_2C\mathbf{H}_2CH_2$), 2.26 (2H, t, *J*=7.0 Hz, $COCH_2$), 2.43 (2H, t, *J*=7.0 Hz, CH_2COO), 3.62 (3H, s, $COOCH_3$), 6.82-6.86 (1H, m, ArCH), 7.89-7.92 (1H, m, ArCH), 8.22 (1H, d, *J*=8.0 Hz, ArCH), 8.28 (1H, d, *J*=8.0 Hz, ArCH), 8.59 (1H, s, ArCH), 9.42 (1H, s, ArCH), 10.08 (1H, broad s, NH), 10.54 (1H, broad s, ArCONH) ppm; **^{13}C-NMR** (500 MHz, DMSO-$_6$) δ_C 20.5 ($CH_2\mathbf{CH_2}CH_2$), 32.3 (CH_2), 32.3 (CH_2), 51.3 (CH_3), 120.3 (ArCH), 127.9 (ArCH), 128.0 (ArCH), 129.4 (ArC), 129.5 (ArCH), 131.5 (ArCH), 135.3 (ArC), 142.9 (ArC), 151.7 (ArCH), 162.8 (CO), 170.4 (CO), 173.1 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{16}H_{18}N_3O_4$ (M+H$^+$), 316.1292; found, 316.1292.

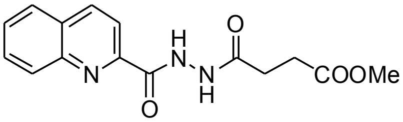
(*E*)-Ethyl 4-(2-(isochinoline-3-carbonyl)hydrazinyl)-4-oxobut-2-enoate (34)



Compound **45** (726 mg, 2.3 mmol) was added to a stirred suspension of compound **51** (400 mg, 2.1 mmol) in THF: EtOAc (1:1) (50 ml) and the reaction mixture was stirred at room temperature overnight. Solvents were removed in *vacuo* and the resulting residue was washed with EtOAc (50 mL), filtered, and co-evaporated with diethyl ether to afford 410 mg (62%) of **34** as an off-white solid.

mp 230-233 °C; ***R*$_\mathbf{f}$** (EtOAc:MeOH, 4:1) 0.5; **HPLC** t_R=8.50 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3359, 3215, 1712, 1663, 1503, 1304; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 1.27 (3H, t, *J*=7.0 Hz, CH_3), 4.23 (2H, q, *J*=7.0 Hz, OCH_2), 6.73 (1H, d, *J*=15.5 Hz, olefinic CH), 7.15 (1H, d, *J*=15.5 Hz, olefinic CH), 7.84-7.87 (1H, m, ArCH), 7.90-7.93 (1H, m, ArCH) 8.24 (1H, d, *J*=8.0 Hz, ArCH), 8.29 (1H, d, *J*=8.0 Hz, ArCH), 8.61 (1H, s, ArCH), 9.43 (1H, s, ArCH), 10.87 (1H, broad s, NH), 10.93 (1H, broad s, NH); **^{13}C-NMR** (125 MHz, DMSO-d$_6$) δ_C 14.1 (CH_3), 61.0 (OCH_2), 120.5 (ArCH), 127.9 (ArCH), 128.1 (ArCH), 129.4 (ArC), 129.5 (ArCH), 130.0 (olefinic CH), 131.5 (ArCH), 135.0 (olefinic CH), 135.2 (ArC), 142.5 (ArC), 151.9 (ArCH) 161.3 (C=O), 162.4 (C=O), 164.8 (C=O); **HRMS** (ESI$^+$) calcd. for $C_{16}H_{15}N_3O_4Na$ (M+Na$^+$), 336.0955; found, 336.0960. **Microanalysis** for $C_{16}H_{15}N_3O_4$ requires C, 58.95; H, 3.89; N, 14.73%; found C, 59.03, H, 3.88, N, 14.70%.

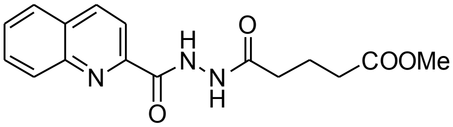
Methyl 4-oxo-4-(2-(chinoline-2-carbonyl)hydrazinyl)butanoate (35)



To a solution of **52** (94 mg, 0.5 mmol) in 10 mL of THF were added 3-carbomethoxy propionylchloride (83 mg, 0.55 mmol) and Et_3N (77 µL, 0.55 mmol) at 0 °C. After 5 min, the reaction mixture was warmed up and stirred for 1 h at room temperature. Solids were removed by filtration and the filtrate was concentrated and recrystallised from EtOH to afford 124 mg (82%) of **36** as a white solid.

mp 176-179 °C; ***R*$_f$** 0.20 (silica gel, CH_2Cl_2:MeOH 98:2); **HPLC** t_R=7.32 min; **IR** (thin film) ν_{max}/cm^{-1}: 3370, 1728, 1633, 1479, 1426; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 2.55-2.59 (4H, m, CH_2CH_2), 3.62 (3H, s, $COOCH_3$), 7.74-7.77 (1H, m, ArCH), 7.89-7.92 (1H, m, ArCH), 8.11-8.16 (3H, m, 3×ArCH), 8.60 (1H, d, *J*=7.0 Hz, ArCH), 10.18 (1H, broad s, NH), 10.60 (1H, broad s, ArCONH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 28.0 (CH_2), 28.5 (CH_2), 51.4 (CH_3), 118.8 (ArCH), 128.2 (ArCH), 128.3 (ArCH), 129.0 (ArC), 129.3 (ArCH), 130.7 (ArCH), 138.0 (ArCH), 146.0 (ArC), 149.2 (ArC), 162.8 (CO), 169.9 (CO), 172.6 (COO) ppm; **HRMS** (ESI$^-$) calcd. for $C_{15}H_{14}N_3O_4$ (M-H$^+$), 300.0979; found, 300.0980.

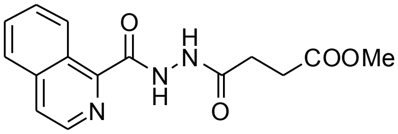
Methyl 5-oxo-5-(2-(chinoline-2-carbonyl)hydrazinyl)pentanoate (36)



To a solution of **52** (94 mg, 0.5 mmol) in THF (10 mL) were added methyl 4-(chloroformyl)butyrate (91 mg, 0.55 mmol) and Et_3N (77 µL, 0.55 mmol) at 0 °C. After 5 min, the reaction mixture was warmed up and stirred for 1 h at room temperature. After filtration, thefiltrate was evaporated and recrystallised from EtOH to afford 104 mg (66%) of **36** as a white solid.

mp 118-119 °C; ***R*$_f$** 0.20 (silica gel, CH_2Cl_2:MeOH 98:2); **HPLC** t_R=7.64 min; **IR** (thin film) ν_{max}/cm^{-1}: 3380, 1730, 1639, 1481; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 1.81-1.87 (2H, m, $CH_2C\mathbf{H_2}CH_2$), 2.27 (2H, t, *J*=7.5 Hz, CH_2CON), 2.44 (2H, t, *J*=7.5 Hz, CH_2COO), 3.63 (3H, s, $COOCH_3$), 7.74-7.78 (1H, m, ArCH), 7.89-7.93 (1H, m, ArCH), 8.10-8.16 (3H, m, 3×ArCH), 8.60 (1H, d, *J*=8.5 Hz, ArCH), 10.06 (1H, broad s, NH), 10.60 (1H, broad s, ArCONH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 20.5 ($CH_2\mathbf{C}H_2CH_2$), 32.3 (CH_2), 32.4 (CH_2), 51.3 (CH_3), 118.8 (ArCH), 128.2 (ArCH), 128.3 (ArCH), 128.9 (ArC), 129.3 (ArCH), 130.7 (ArCH), 138.0 (ArCH), 146.0 (ArC), 149.2 (ArC), 162.9 (CO), 170.7 (CO), 173.1 (COO) ppm; **HRMS** (ESI$^-$) calcd. for $C_{16}H_{16}N_3O_4$ (M-H$^+$), 314.1135; found, 314.1135.

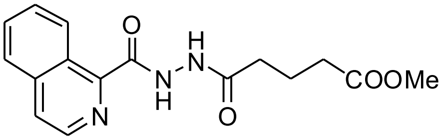
Methyl 4-(2-(isochinoline -1-carbonyl)hydrazinyl)-4-oxobutanoate (37)



To a solution of **53** (94 mg, 0.5 mmol) in THF (10 mL) were added 3-carbomethoxy propionylchloride (83 mg, 0.55 mmol) and Et_3N (77 µL, 0.55 mmol) at 0 °C. After 5 min, the reaction mixture was warmed up and stirred for 1 h at room temperature. After filtration, the filtrate was evaporated and recrystallised from EtOH to afford 129 mg (85%) of **37** as a white solid (Note: purity ~94% as judged by HPLC analysis).

***R*$_f$** 0.30 (silica gel, CH_2Cl_2:MeOH 98:2); **mp** 97-99 °C; **HPLC** t_R=4.28 min; **IR** (thin film) ν_{max}/cm^{-1}: 3363, 1734, 1678, 1641, 1477; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 2.55-2.61 (4H, m, CH_2CH_2), 3.62 (3H, s, $COOCH_3$), 7.74-7.78 (1H, m, ArCH), 7.83-7.87 (1H, m, ArCH), 8.03-8.09 (2H, m, 2×ArCH), 8.56 (1H, d, *J*=6.0 Hz, ArCH), 8.75 (1H, d, *J*=8.0 Hz, ArCH), 10.16 (1H, broad s, NH), 10.53 (1H, broad s, ArCONH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 28.0 (CH_2), 28.5 (CH_2), 51.4 (CH_3), 123.2 (ArCH), 124.3 (ArC), 125.5 (ArCH), 127.1 (ArCH), 128.5 (ArCH), 130.9 (ArCH), 136.3 (ArC), 141.0 (ArCH), 151.2 (ArC), 165.0 (ArCO), 170.0 (CONH), 172.6 (COO) ppm; **HRMS** (ESI$^-$) calcd. for $C_{15}H_{16}N_3O_4$ (M+H$^+$), 302.1135; found, 302.1124.

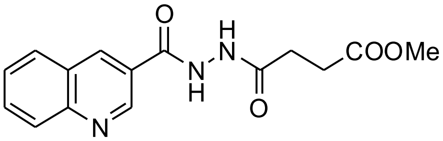
Methyl 5-(2-(isochinoline-1-carbonyl)hydrazinyl)-5-oxopentanoate (38)



To a solution of **53** (94 mg, 0.5 mmol) in THF (10 mL) were added methyl 4-(chloroformyl)butyrate (91 mg, 0.55 mmol) and Et_3N (77 µL, 0.55 mmol) at 0 °C. After 5 min, the reaction mixture was warmed up and stirred for 1 h at room temperature. Solids were removed by filtration and the filtrate was evaporated and purified by column chromatography (CH_2Cl_2:MeOH 19:1) to afford 140 mg (88%) of **38** as a colourless oil (Note: estimated purity by HPLC ~75%).

mp 97-99 °C; ***R*$_f$** 0.30 (silica gel, CH_2Cl_2:MeOH 98:2); **HPLC** t_R=7.02 min; **IR** (thin film) ν_{max}/cm^{-1}: 3365 (br), 1730, 1674, 1504, 1478, 1460; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 1.80-1.88 (2H, m, $CH_2\mathbf{CH_2}CH_2$), 2.28 (2H, t, *J*=7.0 Hz, CH_2), 2.43 (2H, t, *J*=7.0 Hz, CH_2), 3.63 (3H, s, $COOCH_3$), 7.74-7.78 (1H, m, ArCH), 7.83-7.87 (1H, m, ArCH), 8.04-8.09 (2H, m, 2×ArCH), 8.56 (1H, d, *J*=6.0 Hz, ArCH), 8.79 (1H, d, *J*=8.0 Hz, ArCH), 10.06 (1H, broad s, NH), 10.50 (1H, broad s, ArCONH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 20.5 ($CH_2\mathbf{C}H_2CH_2$), 32.5 (CH_2), 32.6 (CH_2), 51.4 (CH_3), 123.3 (ArCH), 125.6 (ArC), 126.3 (ArCH), 127.4 (ArCH), 128.6 (ArCH), 131.0 (ArCH), 136.4 (ArC), 141.2 (ArCH), 151.3 (ArC), 165.3 (CO), 170.9 (CO), 173.2 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{16}H_{18}N_3O_4$ (M+H$^+$), 316.1292; found, 316.1286.

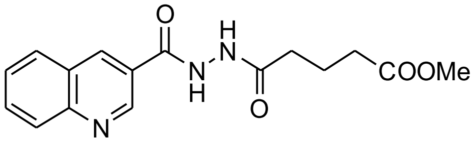
Methyl 4-oxo-4-(2-(chinoline-3-carbonyl)hydrazinyl)butanoate (39)



To a solution of **54** (56 mg, 0.3 mmol) in THF (15 mL) were added 3-carbomethoxy propionylchloride (50 mg, 0.33 mmol) and Et_3N (47 µL, 0.33 mmol) at 0 °C. After 5 min, the reaction mixture was warmed up and stirred for 1 h at room temperature. Solids were filtered off and the filtrate was evaporated and purified by column chromatography (CH_2Cl_2:MeOH 19:1) to afford 72 mg (80%) of **39** as a white solid.

mp 156-157 ºC; **HPLC** t_R=5.64 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3195, 1730, 1611, 1591, 1486; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 2.72 (2H, t, *J*=6.0 Hz, CH_2), 2.77 (2H, t, *J*=6.0 Hz, CH_2), 3.78 (3H, s, OCH_3), 7.89 (1H, dd, *J*=7.5 Hz, 7.5 Hz, ArCH), 8.08 (1H, dd, *J*=7.5 Hz, 7.5 Hz, ArCH), 8.27-8.31 (2H, m, 2×ArCH), 9.05 (1H, d, *J*=1.5 Hz, ArCH), 9.45 (1H, d, *J*=1.5 Hz, ArCH), 10.32 (1H, broad s, NH), 10.90 (1H, broad s, NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 28.0 (CH_2), 28.5 (CH_2), 51.4 (OCH_3), 125.3 (ArC), 126.4 (ArC), 127.6 (ArCH), 128.8 (ArCH), 129.2 (ArCH), 131.5 (ArCH), 136.1 (ArCH), 148.6 (ArCH), 148.7 (ArC), 164.1 (CO), 170.3 (CO), 172.6 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{15}H_{15}N_3NaO_4$ (M+Na$^+$), 324.0955; found, 324.0946.

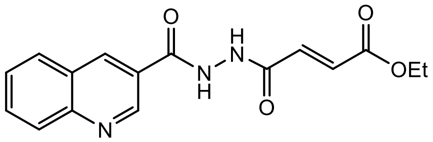
Methyl 5-oxo-5-(2-(chinoline-3-carbonyl)hydrazinyl)pentanoate (40)



To a solution of **54** (56 mg, 0.3 mmol) in THF (15 mL) were added methyl 4-(chloroformyl)butyrate (54 mg, 0.33 mmol) and Et_3N (47 µL, 0.33 mmol) at 0 °C. After 5 min, the reaction mixture was warmed up and stirred for 1 h at room temperature. After filtration, filtrate was evaporated and purified by column chromatography (CH_2Cl_2:MeOH, 19:1 v/v) to afford 76 mg (80%) of **40** as a white solid.

mp 152-154ºC; **HPLC** t_R=5.98 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3444 (bs), 3200, 1728, 1610, 1587, 1483; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 1.80-1.87 (2H, m, CH_2), 2.28 (2H, t, *J*=7.5 Hz, CH_2), 2.43 (2H, t, *J*=7.5 Hz, CH_2), 3.62 (3H, s, OCH_3), 7.73 (1H, dd, *J*=7.0 Hz, 8.0 Hz, ArCH), 7.91 (1H, dd, *J*=7.0 Hz, 8.0 Hz, ArCH), 8.10-8.15 (2H, m, 2×ArCH), 8.88 (1H, d, *J*=2.0 Hz, ArCH), 9.28 (1H, d, *J*=2.0 Hz, ArCH), 10.05 (1H, broad s, NH), 10.69 (1H, broad s, NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 20.5 (CH_2), 32.3 (**C**H_2CO), 32.4 (**C**H_2CO), 51.3 (OCH_3), 125.3 (ArC), 126.4 (ArC), 127.6 (ArCH), 128.8 (ArCH), 129.2 (ArCH), 131.5 (ArCH), 136.1 (ArCH), 148.6 (ArCH), 148.7 (ArC), 164.2 (CO), 171.0 (CO), 173.0 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{16}H_{17}N_3NaO_4$ (M+Na$^+$), 338.1111; found, 338.1104.

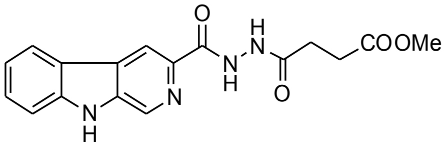
(*E*)-Ethyl 4-oxo-4-(2-(quinoline-3-carbonyl)hydrazinyl)but-2-enoate (41)



Compound **45** (520 mg, 1.6 mmol) was added to a stirred suspension of compound **54** (280 mg, 1.5 mmol) in THF: EtOAc (1:1) (50 ml) and the reaction mixture was stirred at room temperature overnight. The organic solvents were removed in *vacuo*, the resulting solid residue washed with EtOAc (50 mL), filtered, and co-evaporated with diethyl ether to give **41** as a off-white solid 240 mg (51%).

mp 223-226°C; **R$_f$** (EtOAc:MeOH, 4:1) 0.5; **IR** (KBr) 3209, 1714, 1605, 1486, 1300, 1186 cm^{-1}; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ 1.27 (3H, t, J 7.0, CH$_3$), 4.23 (2H, q, J 7.0, OCH$_2$), 6.75 (1H, d, J 16.0, =C-H), 7.16 (1H, d, J 16.0, =C-H), 7.74 (1H, dd, J 7.5, 7.6, ArCH), 7.92 (1H, dd, J 7.5, 7.6, ArCH), 8.10-8.16 (2H, m, 2 x ArCH) 8.91 (1H, d, J 2.0, ArCH), 9.30 (1H, d, J 2.0, ArCH), 10.93 (2H, bs, 2 x NH); **^{13}C-NMR** (125 MHz, DMSO-d$_6$) δ 14.1 (CH$_3$), 61.0 (OCH$_2$), 125.0 (ArC), 126.4 (ArC), 127.6 (ArCH), 128.8 (ArCH), 129.3 (ArCH), 130.1 (=CH), 131.7 (ArCH), 134.7 (=CH), 136.3 (ArCH), 148.6 (ArCH), 148.7 (ArC) 161.9 (C=O), 163.9 (C=O), 164.7 (C=O); MS (ES$^-$) *m/z* 312.1 (100) [M-H]$^-$; **HMRS** (ESI) calcd. for $C_{16}H_{15}N_3O_4Na$ [M+Na]$^+$ 336.0955; found 336.0949. Anal. ($C_{16}H_{15}N_3O_4$) requires: C, 61.34; H, 4.83; N, 13.41%; found C, 61.35, H, 4.81, N, 13.43%.

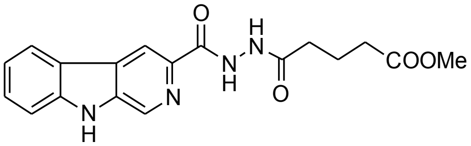
Methyl 4-(2-(9*H*-pyrido[3,4-*b*]indole-3-carbonyl)hydrazinyl)-4-oxobutanoate (42)



To a suspension of hydrazide **55** (90 mg, 0.4 mmol) in THF (10 mL) were added 3-carbomethoxy propionylchloride (66 mg, 0.44 mmol) and Et$_3$N (62 µL, 0.44 mmol) at 0 °C. After 5 min the reaction mixture was warmed up and stirred for 1 h at room temperature. A solid residue was collected and recrystallised from MeOH to afford 35 mg (26%) of **42** as a white solid.

mp 227-231 ºC; **HPLC** t$_R$=6.98 min; **IR** (KBr disk): 3375, 1731, 1618, 1493, 1460; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ$_H$ 2.54 (2H, t, *J*=6.0 Hz, CH$_2$), 2.59 (2H, t, *J*=6.0 Hz, CH$_2$), 3.61 (3H, s, OCH$_3$), 7.32 (1H, dd, *J*=7.5 Hz, *J*=8.0 Hz, ArCH), 7.61 (1H, dd, *J*=7.5 Hz, *J*=8.0 Hz, ArCH), 7.67 (1H, d, *J*=8.0 Hz, ArCH), 8.42 (1H, d, *J*=8.0 Hz, ArCH), 8.85 (1H, s, ArCH), 8.92 (1H, s, ArCH), 10.19 (1H, broad s, NH), 10.27 (1H, broad s, NH), 12.01 (1H, broad s, indole NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ$_C$ 28.0 (CH$_2$), 28.6 (CH$_2$), 51.4 (OCH$_3$), 112.3 (ArCH), 114.5 (ArCH), 120.1 (ArCH), 120.9 (ArC), 122.3 (ArCH), 128.0 (ArC), 128.7 (ArCH), 132.5 (ArCH), 137.3 (ArC), 138.5 (ArC), 141.0 (ArC), 163.2 (CO), 169.6 (CO), 172.6 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{17}H_{16}N_4NaO_4$ (M+Na$^+$), 363.1064; found, 363.1059.

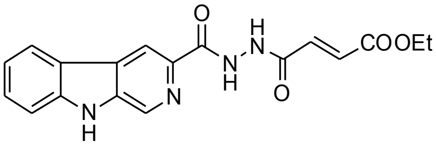
Methyl 5-(2-(9*H*-pyrido[3,4-*b*]indole-3-carbonyl)hydrazinyl)-5-oxopentanoate (43)



To a suspension of hydrazide **55** (68 mg, 0.3 mmol) in THF (10 mL) were added methyl 4-(chloroformyl)butyrate (54 mg, 0.33 mmol) and Et_3N (47 µL, 0.33 mmol) at 0 °C. After 5 min, the reaction mixture was warmed to room temperature and stirred for 1 h. Solids were filtered off and recrystallised from MeOH to afford 44 mg (41%) of **43** as a white solid.

mp 260-264°C; **HPLC** t_R=7.26 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3381, 1738, 1609, 1491, 1456, 1251; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 1.80-1.85 (2H, m, CH_2), 2.26 (2H, t, *J*=7.5 Hz, CH_2CO), 2.43 (2H, t, *J*=7.5 Hz, CH_2CO), 3.62 (3H, s, OCH_3), 7.32 (1H, dd, *J*=7.5 Hz, *J*=8.0 Hz, ArCH), 7.61 (1H, dd, *J*=7.5 Hz, *J*=8.0 Hz, ArCH), 7.67 (1H, d, *J*=8.0 Hz, ArCH), 8.42 (1H, d, *J*=8.0 Hz, ArCH), 8.86 (1H, s, ArCH), 8.92 (1H, s, ArCH), 10.05 (1H, broad s, NH), 10.27 (1H, broad s, NH), 12.01 (1H, broad s, indole NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 20.5 (CH_2), 32.3 (**C**H_2CO), 32.4 (**C**H_2CO), 51.3 (OCH_3), 112.3 (ArCH), 114.6 (ArCH), 120.1 (ArCH), 120.9 (ArC), 122.3 (ArCH), 128.0 (ArC), 128.7 (ArCH), 132.5 (ArCH), 137.3 (ArC), 138.6 (ArC), 141.0 (ArC), 163.5 (CO), 170.4 (CO), 173.1 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{18}H_{18}N_4NaO_4$ (M+Na$^+$), 377.1220; found 377.1211.

(E)-Ethyl 4-(2-(9H-pyrido[3,4-*b*]indole-3carbonyl)hydrazinyl)-4-oxobut-2-enoate (44)

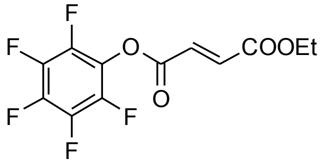


Compound **45** (465 mg, 1.5 mmol) was added to a stirred suspension of compound **55** (294 mg, 1.3 mmol) in THF: EtOAc (1:1) (50 ml) and the reaction mixture was stirred at room temperature overnight. Solvents were removed in *vacuo* and the resulting solid residue was washed with EtOAc (50 mL), filtered, and co-evaporated with diethyl ether to afford 447 mg (98%) of **44** as a white solid.

mp > 330 °C; ***R*$_f$** (EtOAc:MeOH, 4:1) 0.5; **HPLC** t_R=8.28 min; **IR** (KBr disk) ν_{max}/cm^{-1}: 3350, 3262, 1719, 1611, 1490, 1459, 741; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 1.28 (3H, t, *J*=7.0 Hz,

CH_3), 4.23 (2H, q, *J*=7.0 Hz, OCH_2), 6.73 (1H, d, *J*=15.5 Hz, olefinic CH), 7.17 (1H, d, *J*=15.5 Hz, olefinic CH), 7.33 (1H, dd, *J*=7.5 Hz, 8.0 Hz, ArCH), 7.62 (1H, dd, *J*=7.5 Hz, 8.0 Hz, ArCH), 7.69 (1H, d, *J*=8.0 Hz, ArCH), 8.44 (1H, d, *J*=8.0 Hz, ArCH), 8.89 (1H, s, ArCH), 8.95 (1H, s, ArCH), 10.64 (1H, broad s, NH), 10.94 (1H, broad s, NH), 12.05 (1H, broad s, indole NH); **^{13}C-NMR** (125 MHz, DMSO-d_6) δ_C 14.0 (CH_3), 61.0 (OCH_2), 112.3 (ArCH), 114.8 (ArCH), 120.2 (ArCH), 120.9 (ArC), 122.4 (ArCH), 128.0 (ArC), 128.8 (ArCH), 129.7 (olefinic CH), 132.6 (ArCH), 135.0 (olefinic CH), 137.4 (ArC), 138.1 (ArC), 141.0 (ArC), 161.2 (C=O), 163.0 (C=O), 164.8 (C=O); **HRMS** (ESI$^+$) calcd. for $C_{18}H_{16}N_4O_4Na$ (M+Na$^+$), 375.1064; found, 375.1071.

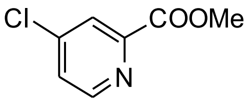
Ethyl perfluorophenyl fumarate (45)



To a stirred suspension of pentaflurophenol (3.87 g, 21.0 mmol) and ethyl fumaryl chloride (3.45 g, 21.2 mmol) in THF was added triethylamine (3.20 mL, 23.1 mmol) over 10 min. The resulting mixture was then stirred at room temperature overnight. The reaction mixture was filtered, concentrated in *vacuo*, and the residue was purified by flash chromatography (hexane: EtOAc, 4:1) to afford 4.5 g (69%) of **45** as colorless oil.

***R*f** (hexane: EtOAc, 4:1) 0.5; **IR** (thin film) ν_{max}/cm^{-1}: 2990, 2360, 1775, 1731, 1520, 1286, 1005, 762; **^{1}H-NMR** (500 MHz, $CDCl_3$) δ_H 1.36 (3H, t, *J*=7.0 Hz, CH_3), 4.33 (2H, q, *J*=7.0 Hz, OCH_2), 7.07 (1H, d, *J*=16.0 Hz, olefinic CH), 7.13 (1H, d, *J*=16.0 Hz, olefinic CH); **^{13}C-NMR** (125 MHz, $CDCl_3$) δ_C 14.0 (CH_3), 62.0 (OCH_2), 130.0 (olefinic C), 137.0 (ArC), 138.8 (olefinic C), 140.0 (ArC), 140.8 (ArCH), 142.0 (ArC), 160.7 (C=O), 164.2 (C=O) ; **^{19}F-NMR** (376 MHz, $CDCl_3$) δ_F -152.3 to -152.4 (2F, m, ArF), -157.1 to -157.2 (1F, m, ArF), -161.9 to -162.0 (2F, m, ArF).

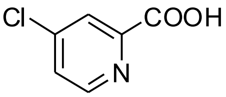
Methyl 4-chloropicolinate (46)



Picolinic acid (3.0 g, 24.4 mmol) was added to thionyl chloride (12 mL) and refluxed for 4 days. The reaction mixture was concentrated *in vacuo*, washed with Et_2O and cooled to 0 °C. The resulting oily product was dissolved in Et_2O, then MeOH (1.5 mL) was added cautiously. Solids were filtered off, dissolved in satd. aq. $NaHCO_3$ (25 mL), extracted with EtOAc (2×25 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was recrystallised from hexane to afford 1.71 g (41%) of **46** as white needles.

mp 45-47 ºC; **IR** (KBr disk) ν_{max}/cm^{-1}: 3417 (bs), 1719, 1577, 1558, 1441, 1304; **^{1}H-NMR** (500 MHz, $CDCl_3$) δ_H 4.03 (3H, s, $COOCH_3$), 7.51 (1H, dd, *J*=2.0 Hz, 5.0 Hz, ArCH), 8.15 (1H, d, *J*=2.0 Hz, ArCH), 8.66 (1H, d, *J*=5.0 Hz, ArCH) ppm; **^{13}C-NMR** (500 MHz, $CDCl_3$) δ_C 53.3 (OCH_3), 125.7 (ArCH), 127.2 (ArCH), 145.5 (ArC), 149.3 (ArC), 150.7 (ArCH), 164.7 (COO) ppm.

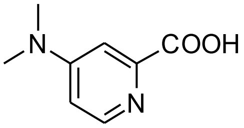
4-Chloropicolinic acid (47)



A solution of **46** (530 mg, 3.1 mmol) in 1 N NaOH (9.3 mL) was stirred for 1 h at room temperature. The solution was acidified with 10 N HCl to pH 4. The white precipitate was collected and washed with water to afford 424 mg (87%) of **47**.

mp 176ºC (decomp.); **IR** (KBr disk) ν_{max}/cm^{-1}: 3442, 1700, 1602, 1321; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 7.79 (1H, dd, *J*=2.0 Hz, 5.0 Hz, ArCH), 8.06 (1H, d, *J*=2.0 Hz, ArCH), 8.69 (1H, d, *J*=5.0 Hz, ArCH), 13.55 (1H, broad s, COOH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 125.1 (ArCH), 127.4 (ArCH), 144.5 (ArC), 150.8 (ArC), 151.4 (ArCH), 165.6 (COOH) ppm.

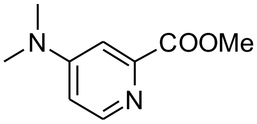
4-(Dimethylamino)picolinic acid (48)



A solution of compound **47** (390 mg, 2.48 mmol) in aqueous dimethylamine (40%, 4 mL) was stirred at 150 °C for 2 h in a sealed tube. The resulting solution was concentrated *in vacuo*, dissolved in EtOAc (30 mL) and washed with satd. aq. $NaHCO_3$ (30 mL). The organic phase was dried over Na_2SO_4 and evaporated *in vacuo* to afford 408 mg (99%) of **48** as a white solid.

mp >300ºC; **IR** (KBr disk) ν_{max}/cm^{-1}: 3384, 1724, 1639, 1583; **^{1}H-NMR** (500 MHz, DMSO-d_6) δ_H 3.27 (6H, s, NMe_2), 7.10 (1H, dd, *J*=3.0 Hz, 7.5 Hz, ArCH), 7.43 (1H, d, *J*=3.0 Hz, ArCH), 8.20 (1H, d, *J*=7.5 Hz, ArCH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d_6) δ_C 40.1 (NMe_2), 108.0 (ArCH), 108.2 (ArCH), 139.2 (ArC), 139.9 (ArCH), 157.5 (ArC), 161.2 (COOH); **HRMS** (ESI^+) calcd. for $C_8H_{10}N_2NaO_2$ ($M+Na^+$), 189.0634; found, 189.0638.

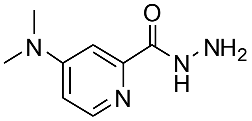
Methyl 4-(dimethylamino)picolinate (49)



Thionyl chloride (1.8 mL) was slowly added to an ice-cooled solution of compound **48** (353 mg, 2.13 mmol) in MeOH (15 mL). The solution was then heated to reflux for 16 h and concentrated *in vacuo*. The residue was dissolved in satd. $NaHCO_3$ (30 mL), extracted with EtOAc (3×30 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford 351 mg (92%) of **49** as a white solid.

mp 95-97 ºC; **IR** (KBr disk) ν_{max}/cm^{-1}: 3448, 1737, 1600, 1290, 1252; **^{1}H-NMR** (200 MHz, DMSO-d_6) δ_H 3.07 (6H, s, NMe_2), 3.98 (3H, s, $COOCH_3$), 6.60 (1H, dd, *J*=2.5 Hz, 6.0 Hz, ArCH), 7.41 (1H, d, *J*=2.5 Hz, ArCH), 8.32 (1H, d, *J*=6.0 Hz, ArCH) ppm; **^{13}C-NMR** (200 MHz, DMSO-d_6) δ_C 39.7 (NMe_2), 53.2 (OCH_3), 108.6 (ArCH), 109.3 (ArCH), 148.5 (ArC), 150.2 (ArCH), 155.3 (ArC), 167.3 (COO) ppm; **HRMS** (ESI^+) calcd. for $C_9H_{13}N_2O_2$ ($M+H^+$), 181.0972; found, 181.0971.

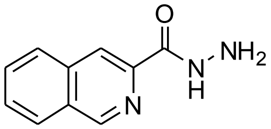
4-(Dimethylamino)picolinohydrazide (50)



To a solution of **49** (250 mg, 1.39 mmol) in MeOH (10 mL) was added hydrazine hydrate (278 mg, 5.56 mmol) and the mixture was refluxed for 4 h. The mixture was cooled, solvent evaporated and crude residue recrystallised from MeOH to afford 230 mg (92%) of **50** as a white solid.

mp 107-109 ºC; **IR** (KBr disk) ν_{max}/cm^{-1}: 3398, 1680, 1603, 1517, 1382, 985; **^{1}H-NMR** (200 MHz, $CDCl_3$) δ_H 3.04 (6H, s, NMe_2), 4.06 (2H, broad s, NH_2), 6.53 (1H, dd, *J*=2.5 Hz, 6.0 Hz, ArCH), 7.39 (1H, d, *J*=2.5 Hz, ArCH), 8.12 (1H, d, *J*=6.0 Hz, ArCH), 9.02 (1H, broad s, NH) ppm; **^{13}C-NMR** (200 MHz, $CDCl_3$) δ_C 39.7 (NMe_2), 105.6 (ArCH), 108.7 (ArCH), 148.9 (ArCH), 149.6 (ArC), 155.5 (ArC), 166.1 (CO) ppm; **HRMS** (ESI$^+$) calcd. for $C_8H_{13}N_4O$ (M+H$^+$), 181.1084; found, 181.1083.

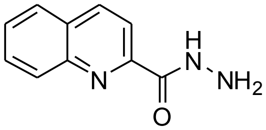
Isochinoline-3-carbohydrazide (51)



A solution of methyl 3-isochinolinecarboxylate (407 mg, 2.18 mmol) and hydrazine monohydrate (436 mg, 8.72 mmol) in MeOH (15 mL) was heated under reflux for 6 h and then cooled to room temperature. The MeOH was evaporated and the remaining white residue purified by flash chromatography (CH_2Cl_2:MeOH 9:1) to afford 343 mg (84%) of **51** as a white solid.

***R*$_f$** 0.30 (silica gel, CH_2Cl_2:MeOH 98:2); **mp** 114-117 °C; **IR** (thin film) ν_{max}/cm^{-1}: 3292, 1674, 1611, 1505; **^{1}H-NMR** (500 MHz, DMSO-d$_6$) δ_H 4.66 (2H, broad s, NH_2), 7.79-7.82 (1H, m, ArCH), 7.86-7.89 (1H, m, ArCH), 8.19 (1H, d, *J*=8.0 Hz, ArCH), 8.24 (1H, d, *J*=8.0 Hz, ArCH), 8.52 (1H, s, ArCH), 9.35 (1H, s, ArCH), 9.99 (1H, broad s, NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 119.3 (ArCH), 127.8 (ArCH), 127.9 (ArCH), 129.0 (ArCH), 129.1 (ArC), 131.3 (ArCH), 135.4 ArC), 143.6 (ArC), 151.6 (ArCH), 162.8 (ArCO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{10}H_{10}N_3O$ (M+H$^+$), 188.0818; found, 188.0820.

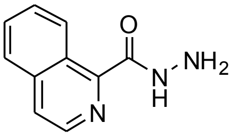
Chinoline -2-carbohydrazide (52)



A solution of methyl 2-chinolinecarboxylate (655 mg, 3.5 mmol) and hydrazine monohydrate (700 mg, 14 mmol) in MeOH (10 mL) was heated under reflux for 5 h and then cooled to room temperature. Solvents were evaporated and the remaining residue was purified by flash chromatography (CH_2Cl_2:MeOH, 9:1) to afford 630 mg (96%) of **52** as a white solid.

***R*$_f$** 0.40 (silica gel, CH_2Cl_2:MeOH 98:2); **mp** 136-137 °C; **IR** (thin film) ν_{max}/cm^{-1}: 1673, 1497, 1428; **^{1}H-NMR** (400 MHz, DMSO-d$_6$) δ_H 4.67 (2H, broad s, NH_2), 6.67-7.71 (1H, m, ArCH), 7.82-7.87 (1H, m, ArCH), 8.04-8.10 (1H, m, ArCH), 8.10 (1H, d, *J*=8.5 Hz, ArCH), 8.53 (1H, d, *J*=8.5 Hz, ArCH), 10.04 (1H, broad s, NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 119.5 (ArCH), 128.8 (ArCH), 128.9 (ArCH), 129.5 (ArC), 130.1 (ArCH), 131.3 (ArCH), 138.6 (ArCH), 146.9 (ArC), 150.9 (ArC), 163.8 (ArCO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{10}H_9N_3NaO$ (M+Na$^+$), 210.0638; found, 210.0637.

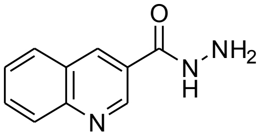
Isochinoline-1-carbohydrazide (53)



A solution of methyl 1-isochinolinecarboxylate (1.22 g, 6.5 mmol) and hydrazine monohydrate (1.3 g, 26 mmol) in MeOH (15 mL) was heated under reflux for 6 h and then cooled to room temperature. Solvents were evaporated and the residue was purified by flash chromatography (CH_2Cl_2:MeOH 9:1) to afford 1.00 g (82%) of **53** as a white solid.

***R*$_f$** 0.30 (silica gel, CH_2Cl_2:MeOH 98:2); **mp** 107-109 °C; **IR** (thin film) ν_{max}/cm^{-1}: 1670, 1623, 1505; **^{1}H-NMR** (400 MHz, DMSO-d$_6$) δ_H 4.69 (2H, broad s, NH_2), 7.69-7.73 (1H, m, ArCH), 7.79-7.83 (1H, m, ArCH), 7.96 (1H, d, *J*=5.5 Hz, ArCH), 8.02 (1H, d, *J*=8.5 Hz, ArCH), 8.51 (1H, d, *J*=5.5 Hz, ArCH), 8.74 (1H, d, *J*=8.5 Hz, ArCH), 9.95 (1H, broad s, NH) ppm; **^{13}C-NMR** (400 MHz, DMSO-d$_6$) δ_C 123.7 (ArCH), 126.3 (ArCH), 127.2 (ArCH), 127.9 (ArCH), 129.1 (ArCH), 131.5 (ArCH), 137.2 (ArC), 141.9 (ArCH), 152.6 (ArC), 166.2 (ArCO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{10}H_9N_3NaO$ (M+Na$^+$), 210.0638; found, 210.0637.

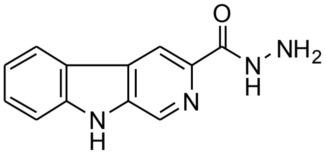
Chinoline-3-carbohydrazide (54)



A solution of methyl chinoline-3-carboxylate (374 mg, 2.00 mmol) and hydrazine monohydrate (400 mg, 8.00 mmol) in MeOH (15 mL) was heated under reflux for 6 h and then cooled to room temperature. Solvents were removed *in vacuo* and the resulting residue was purified by flash chromatography (CH_2Cl_2:MeOH, 9:1) to afford 302 mg (81%) of **54** as a white solid.

mp 176-178 ºC; **IR** (KBr disk) ν_{max}/cm^{-1}: 3307, 1654, 1618, 1315; ^{1}**H-NMR** (500 MHz, DMSO-d$_6$) δ_H 4.64 (2H, broad s, NH_2), 7.69 (1H, dd, *J*=1.0 Hz, 7.0 Hz, ArCH), 7.86 (1H, dd, *J*=7.0 Hz, 7.0 Hz, ArCH), 7.87 (1H, dd, *J*=7.0 Hz, 7.0 Hz, ArCH), 8.07-8.11 (2H, m, 2×ArCH), 8.80 (1H, d, *J*=2.0 Hz, ArCH), 9.26 (1H, d, *J*=2.0 Hz, ArCH), 10.12 (1H, broad s, NH) ppm; ^{13}**C-NMR** (500 MHz, DMSO-d$_6$) δ_C 126.1 (ArC), 126.6 (ArC), 127.4 (ArCH), 128.7 (ArCH), 129.1 (ArCH), 131.1 (ArCH), 135.3 (ArCH), 148.4 (ArC), 148.5 (ArCH), 164.5 (COO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{10}H_9N_3NaO$ (M+Na$^+$), 210.0638; found, 210.0645.

9*H*-Pyrido[3,4-*b*]indole-3-carbohydrazide (55)



55 was prepared following a previously described method.[7] To a solution of ethyl 9*H*-pyrido[3,4-*b*]indole-3-carboxylate (500 mg, 2.08 mmol) in EtOH (10 mL) was added hydrazine hydrate (2.2 mL). The mixture was refluxed for 6 h, cooled, and the resulting precipitate was collected and recrystallised from EtOH yielding 380 mg (76%) of **55** as a brown/white solid.

mp 272-276 ºC (lit. 289-291 ºC)[7]; **IR** (KBr disk) ν_{max}/cm^{-1}: 3245, 1648, 1618, 1496, 1457, 1341, 721; ^{1}**H-NMR** (500 MHz, DMSO-d$_6$) δ_H 4.55 (2H, broad s, NH_2), 7.29-7.32 (1H, m, ArCH), 7.58-7.61 (1H, m, ArCH), 7.66 (1H, d, *J*=8.0 Hz, ArCH), 8.41 (1H, d, *J*=8.0 Hz, ArCH), 8.82

(1H, s, ArCH), 8.88 (1H, s, ArCH), 9.65 (1H, broad s, NH), 11.93 (1H, broad s, indole NH) ppm; **^{13}C-NMR** (500 MHz, DMSO-d$_6$) δ_C 112.2 (ArCH), 113.8 (ArCH), 120.0 (ArCH), 120.9 (ArC), 122.3 (ArCH), 128.0 (ArC), 128.6 (ArCH), 132.5 (ArCH), 137.0 (ArC), 139.4 (ArC), 141.0 (ArC), 164.0 (CO) ppm; **HRMS** (ESI$^+$) calcd. for $C_{12}H_{11}N_4O$ (M+H$^+$), 227.0927; found 227.0927.

Supplementary References

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3. S.-X. Liu, S. Lin, B.-Z. Lin, C.-C. Lin and J.-Q. Huang, *Angew. Chem., Int. Ed.*, 2001, **40**, 1084-1087.
4. T. A. Halgren, *J. Comput. Chem.*, 1996, **17**, 490-519.
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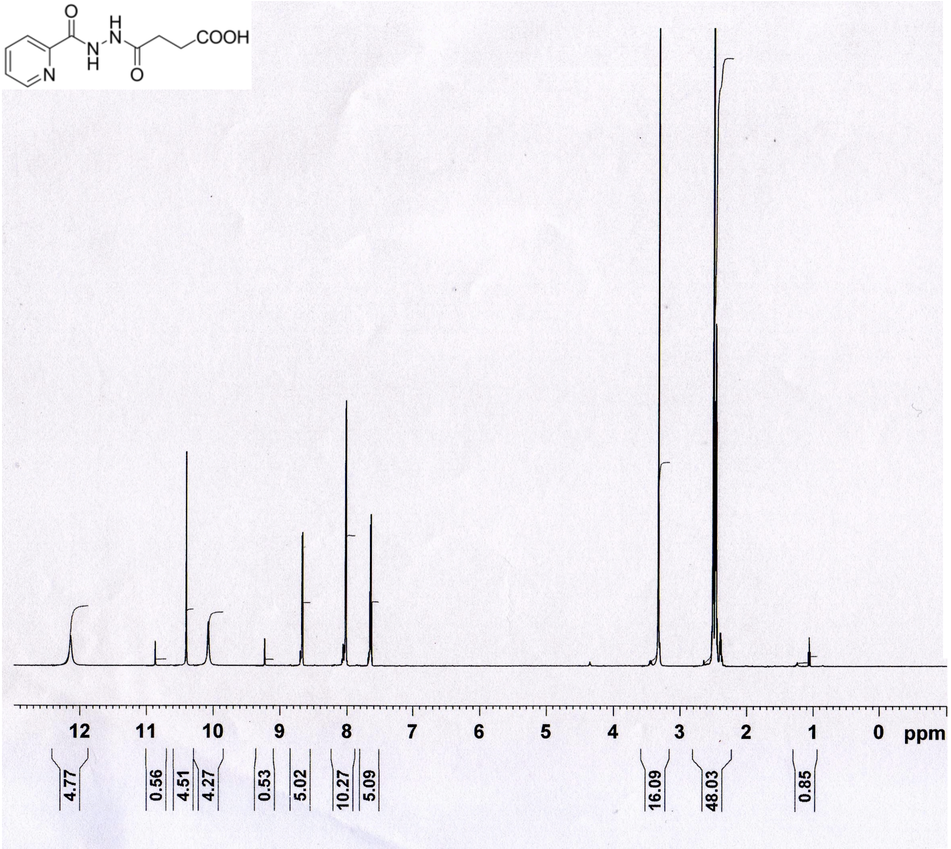
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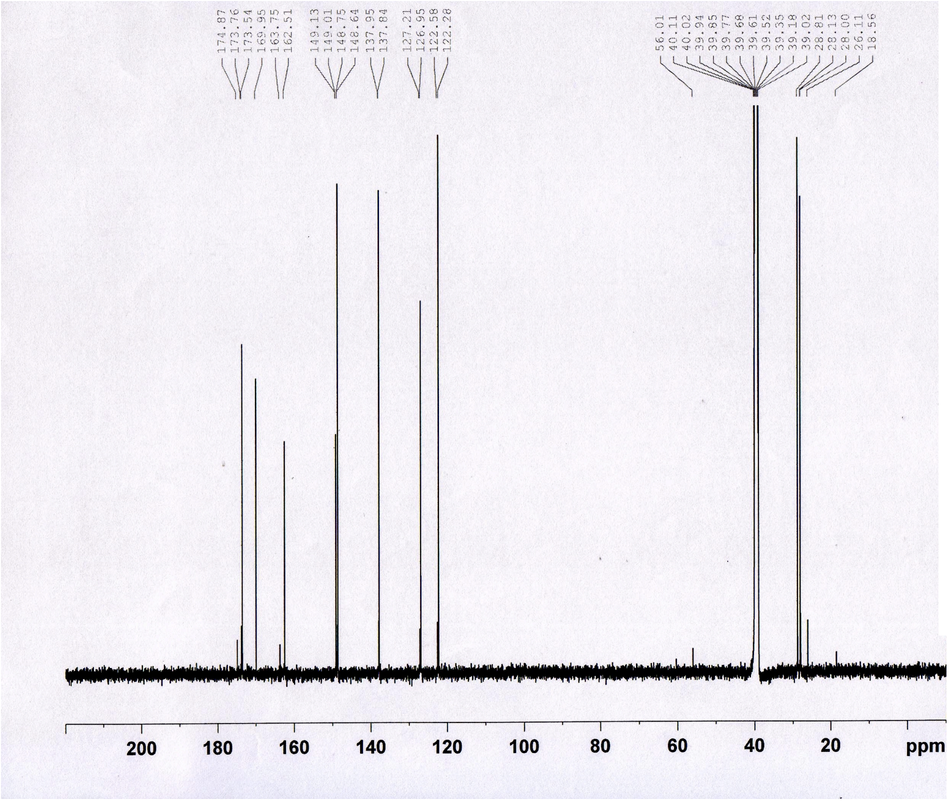
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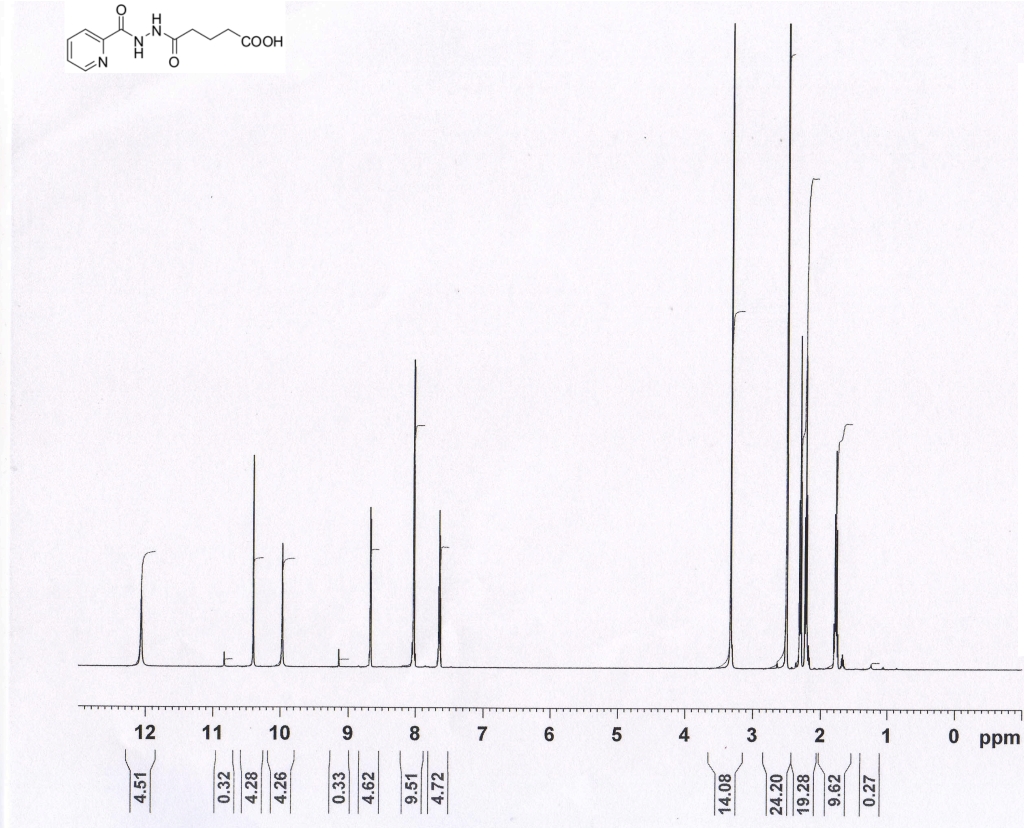
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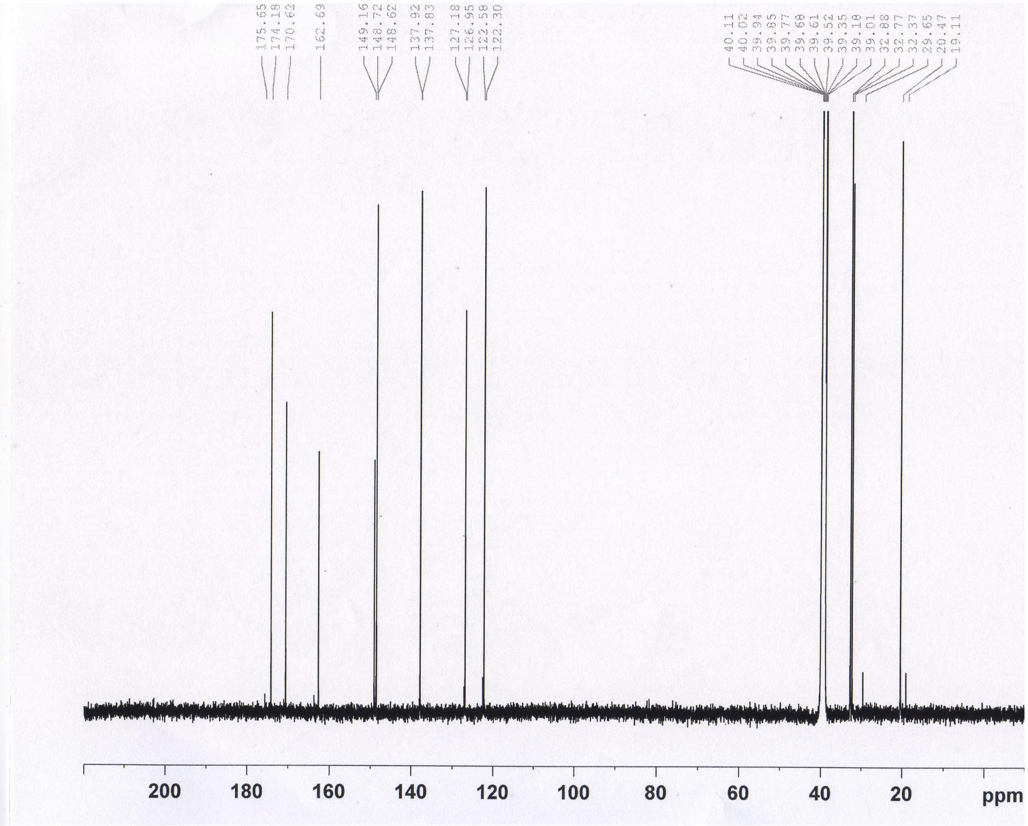
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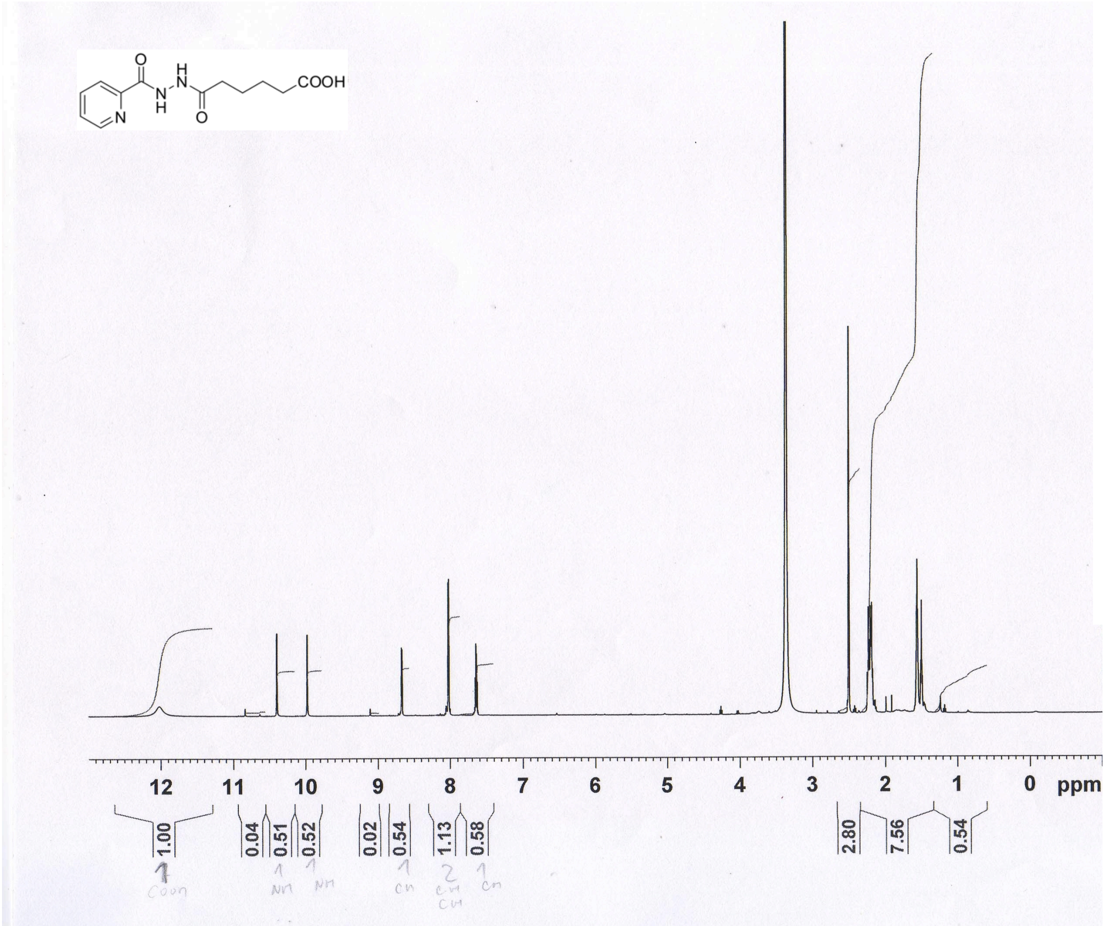
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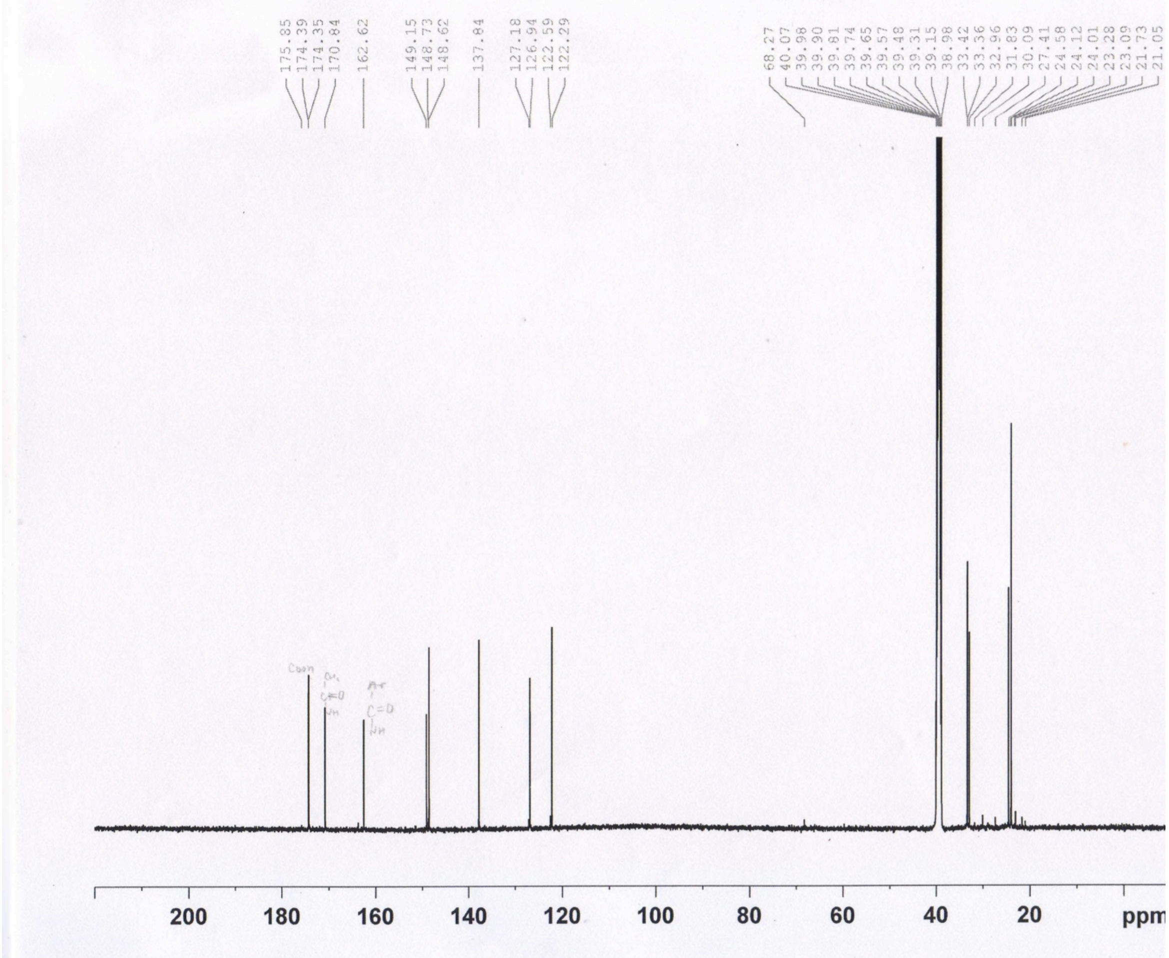
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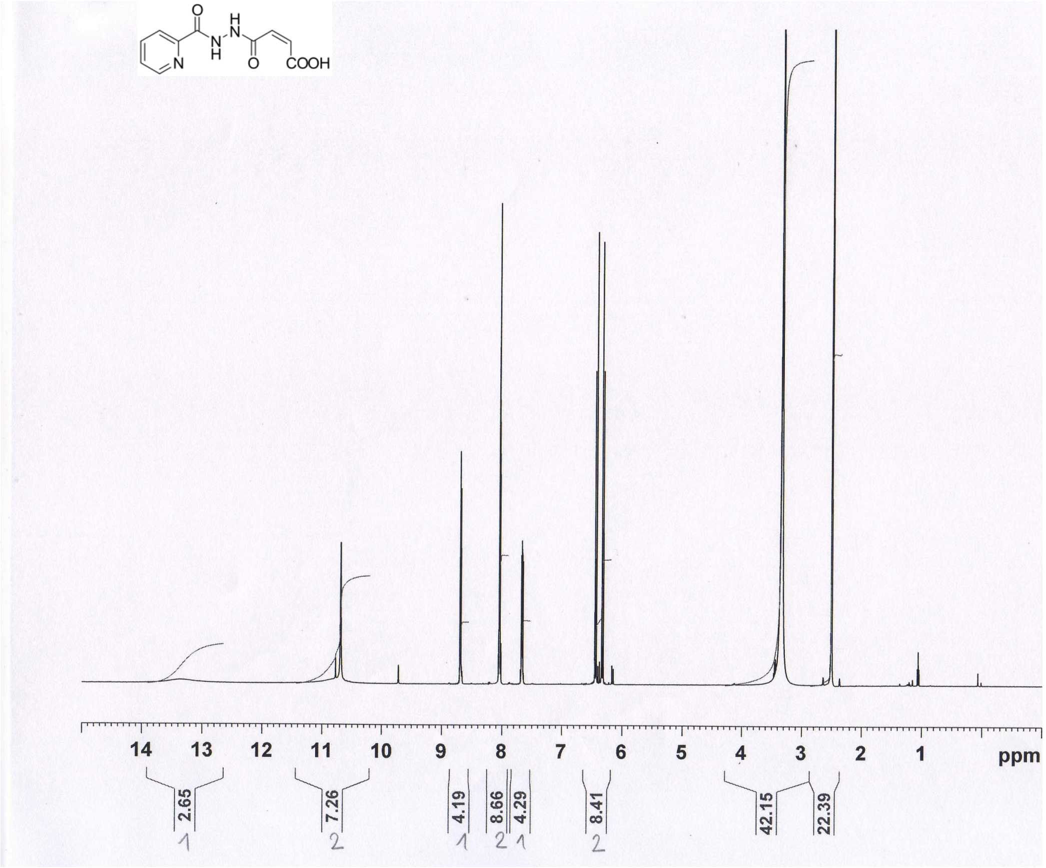
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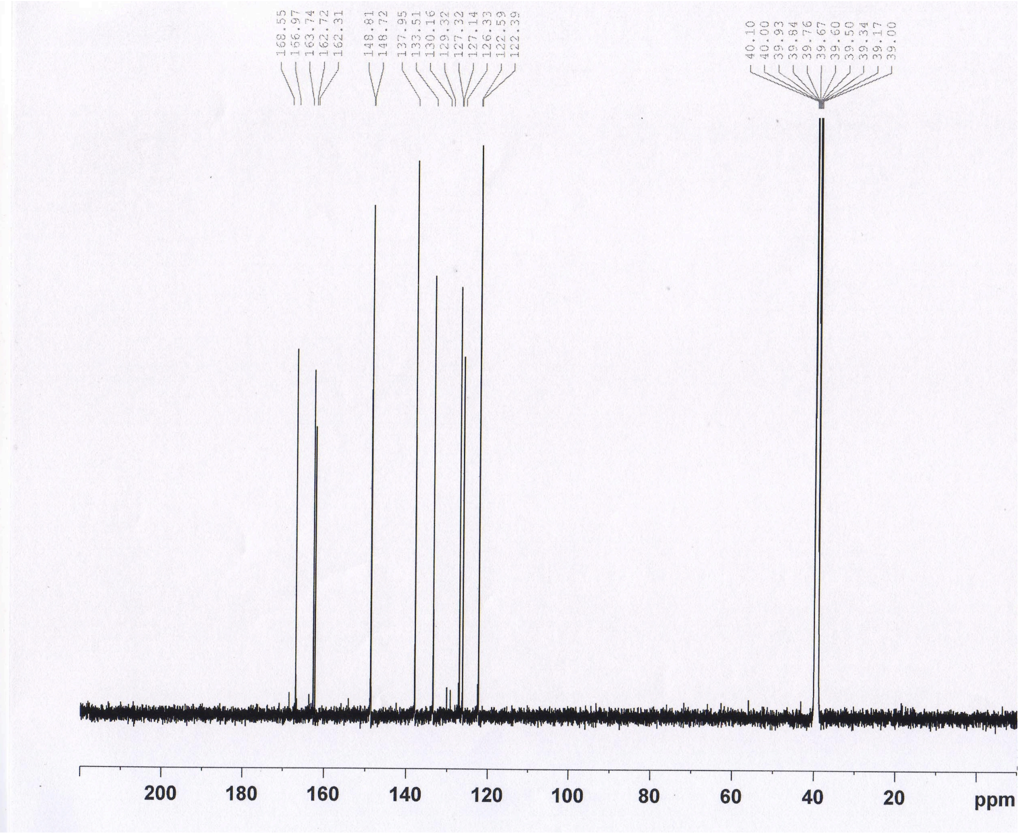
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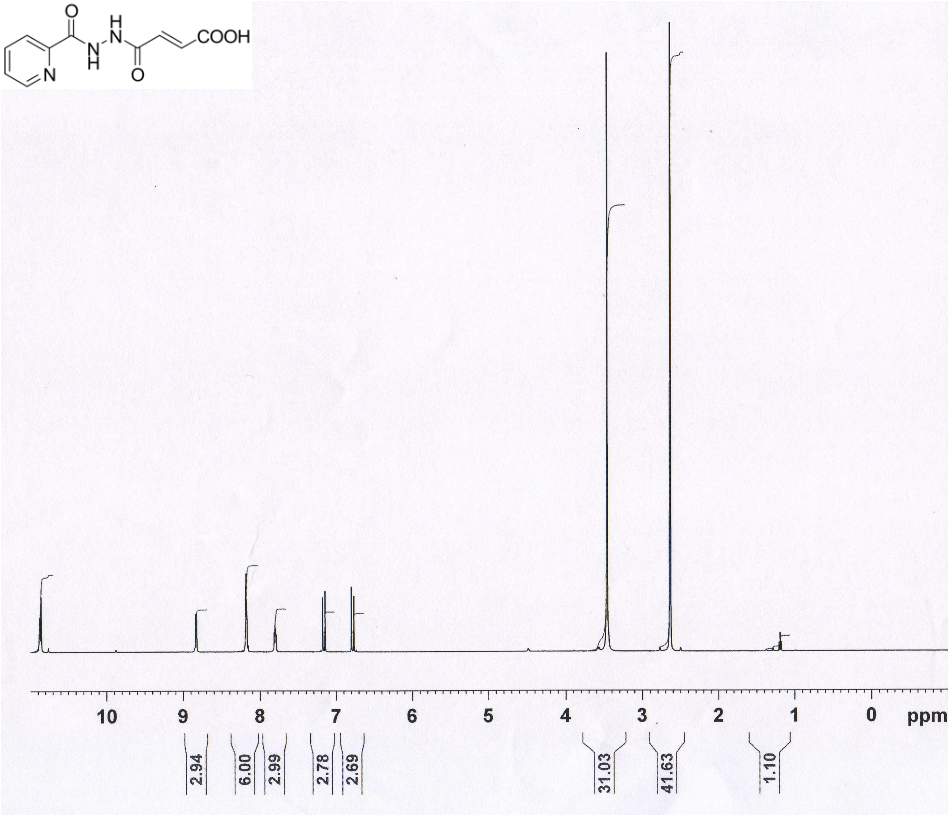
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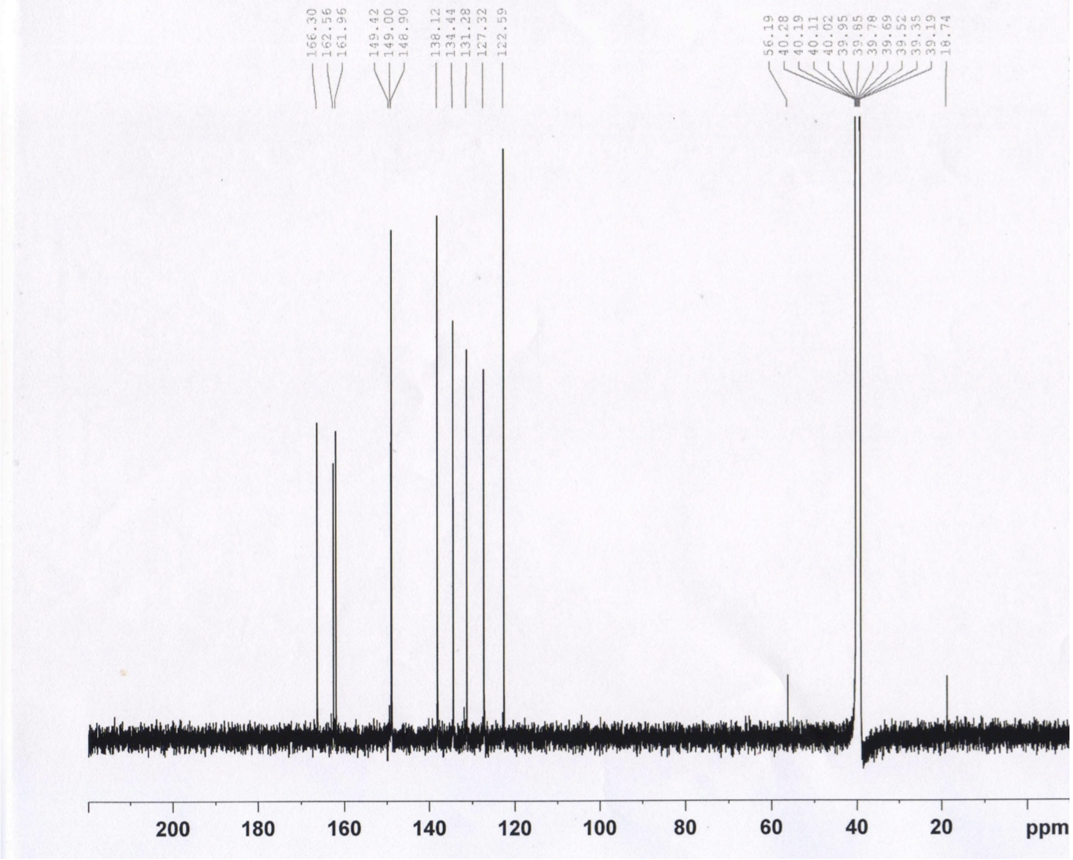
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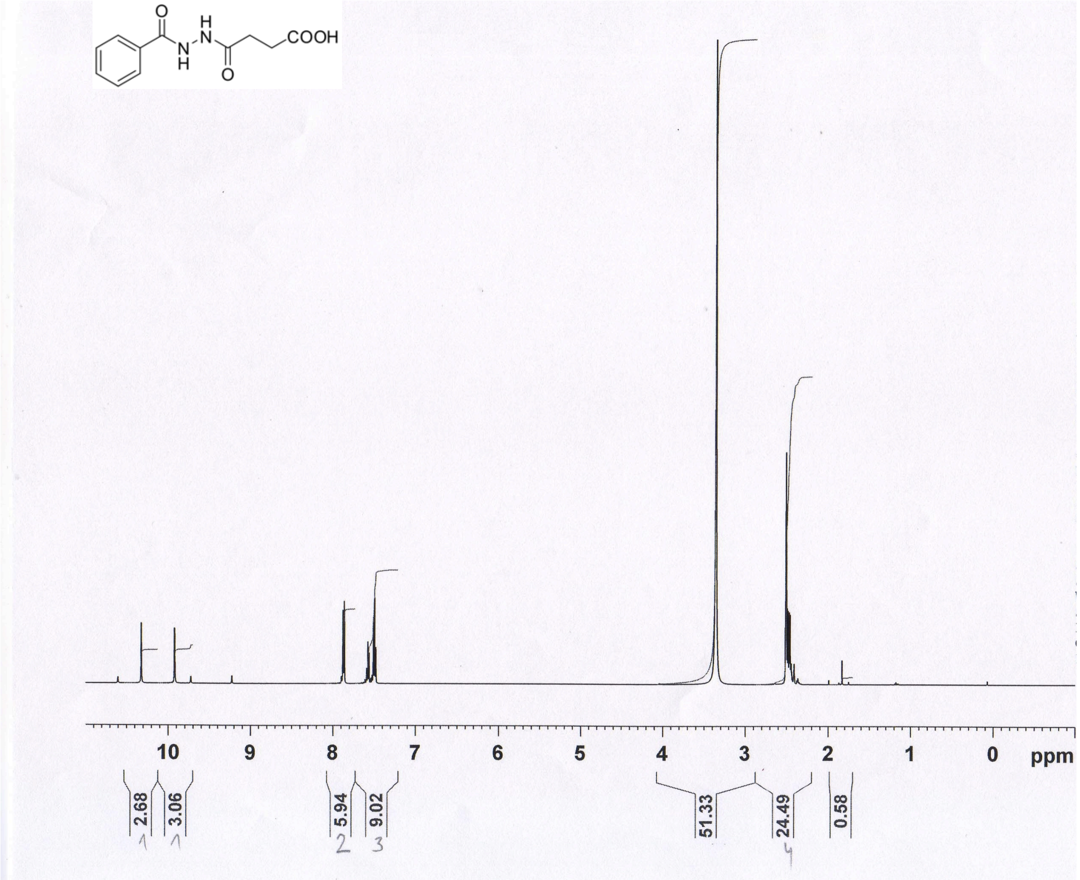
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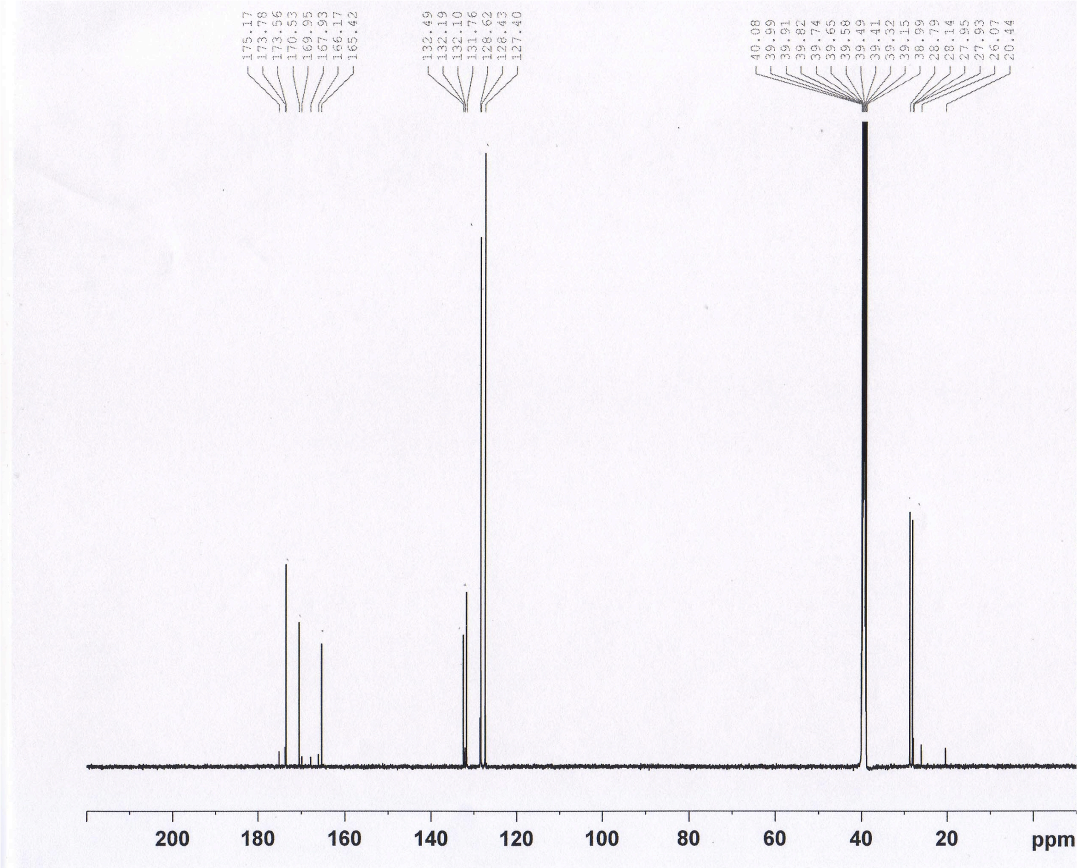
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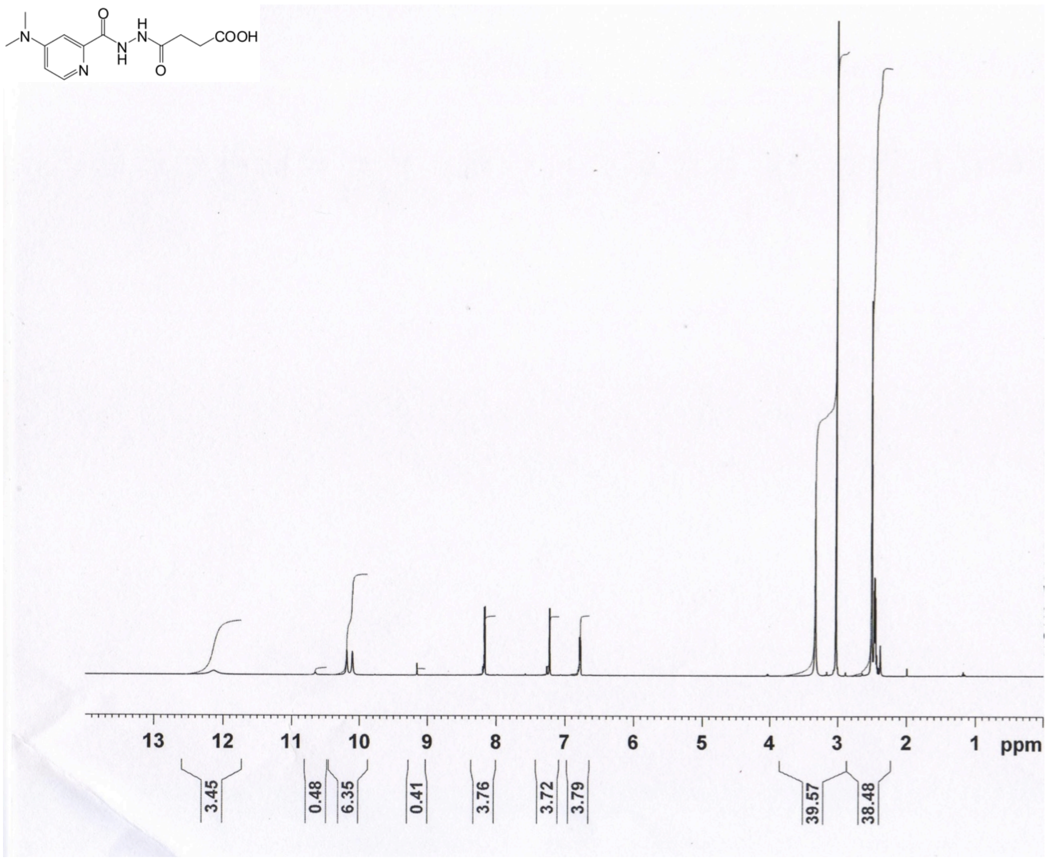
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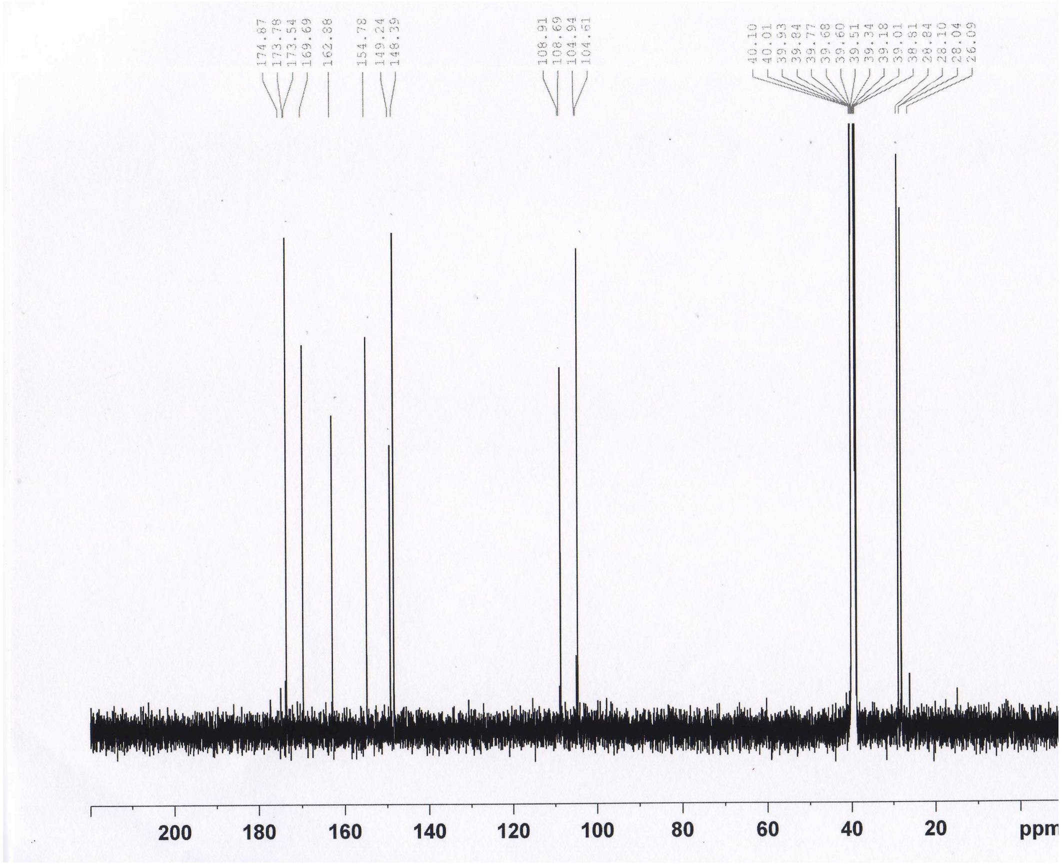
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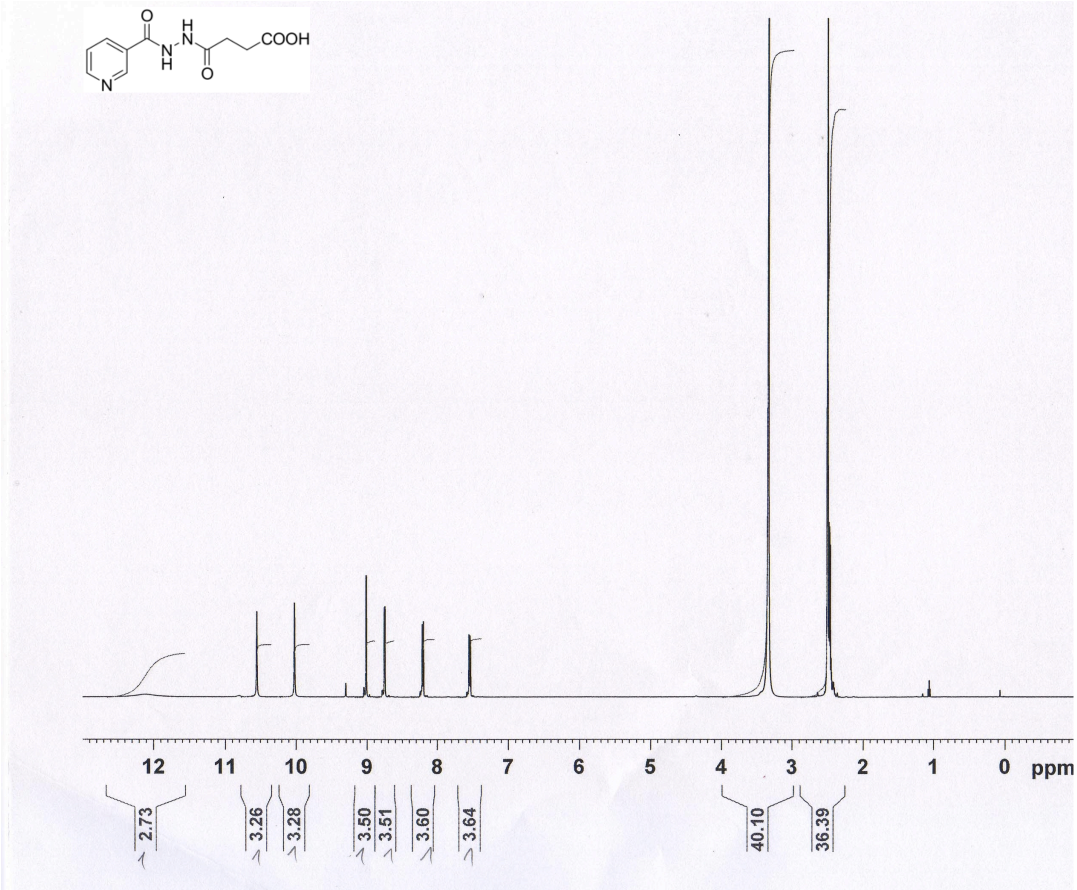
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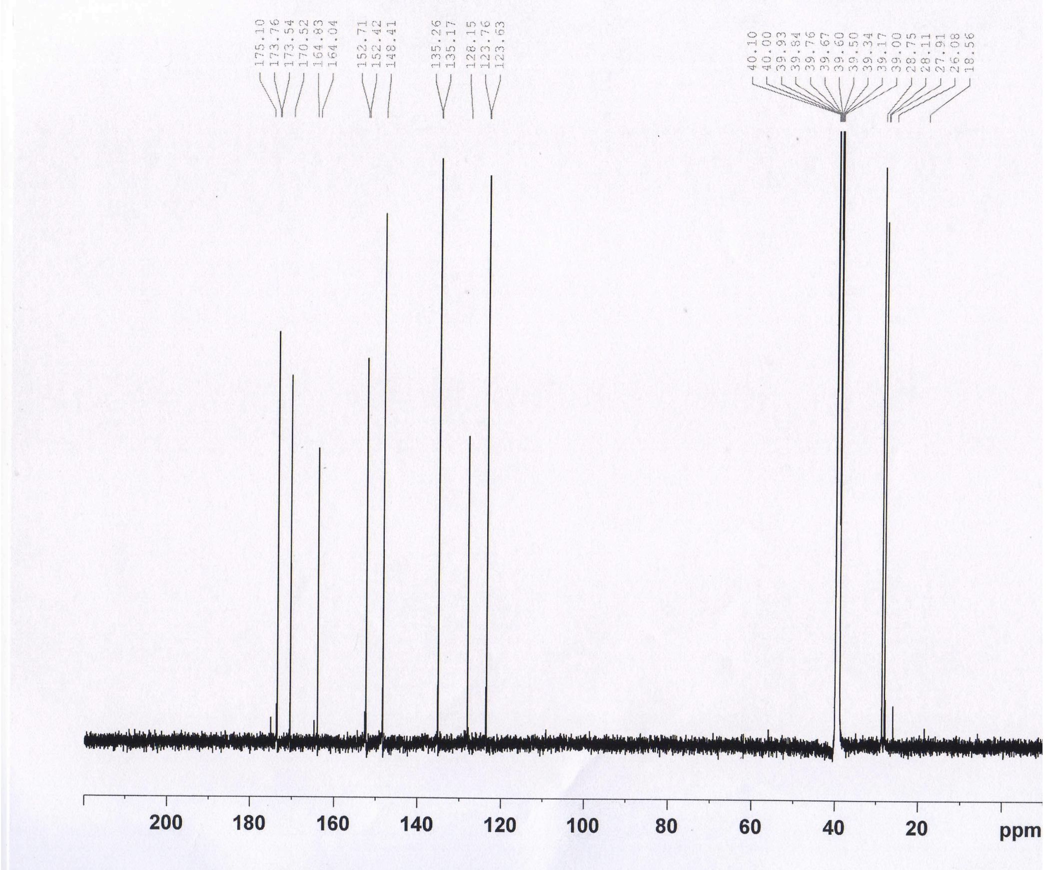
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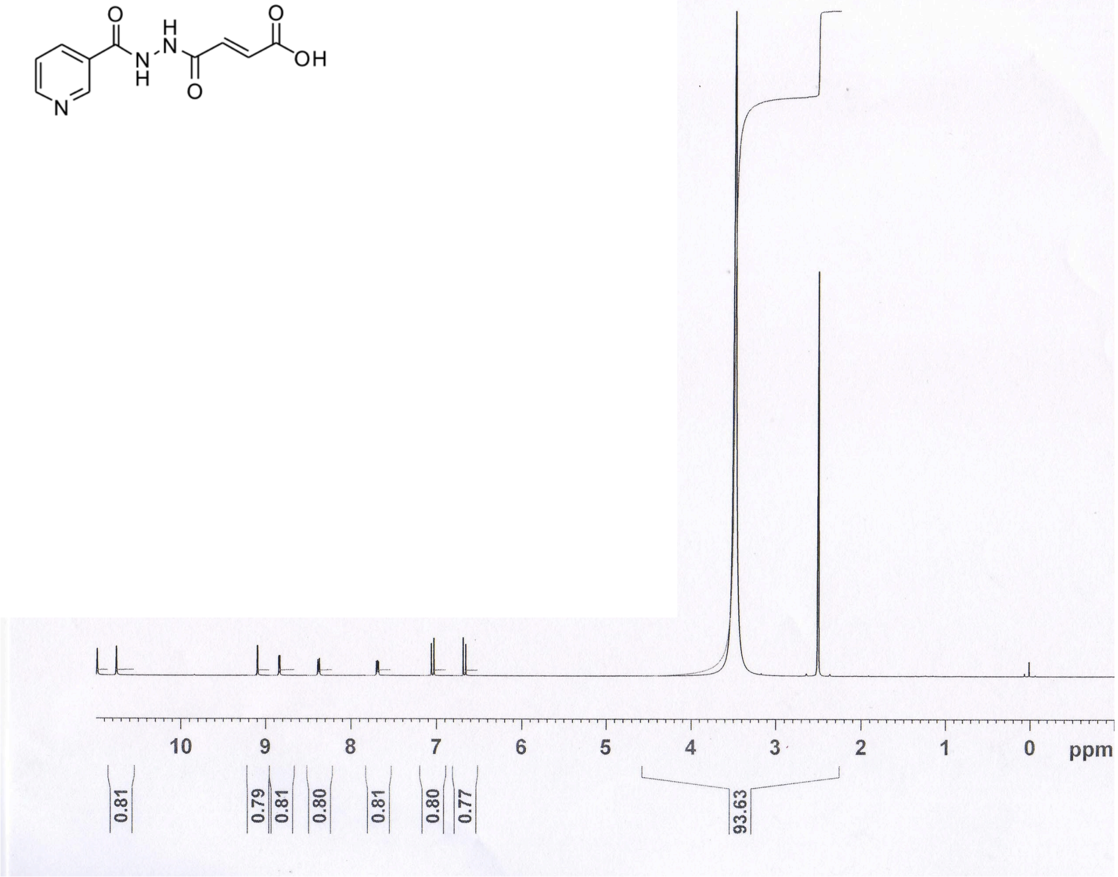
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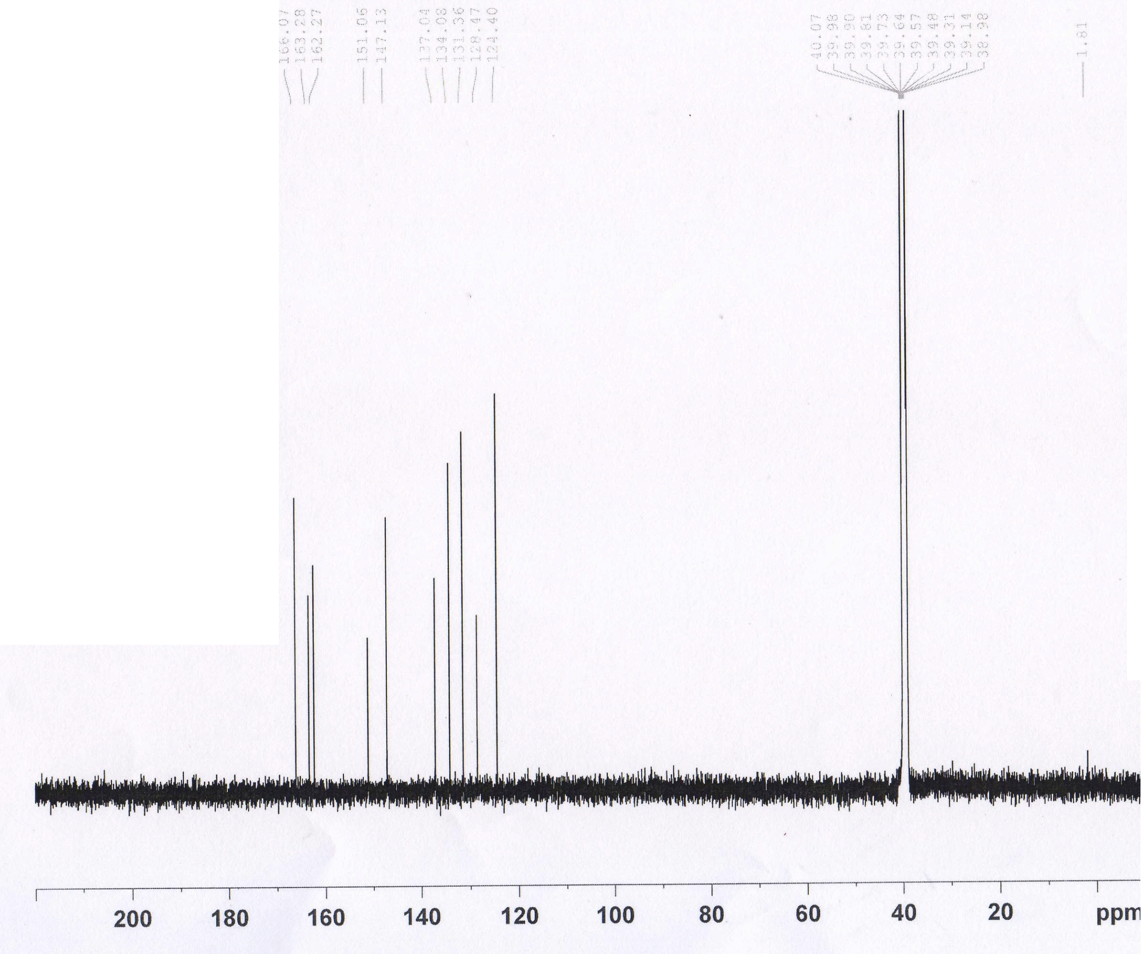
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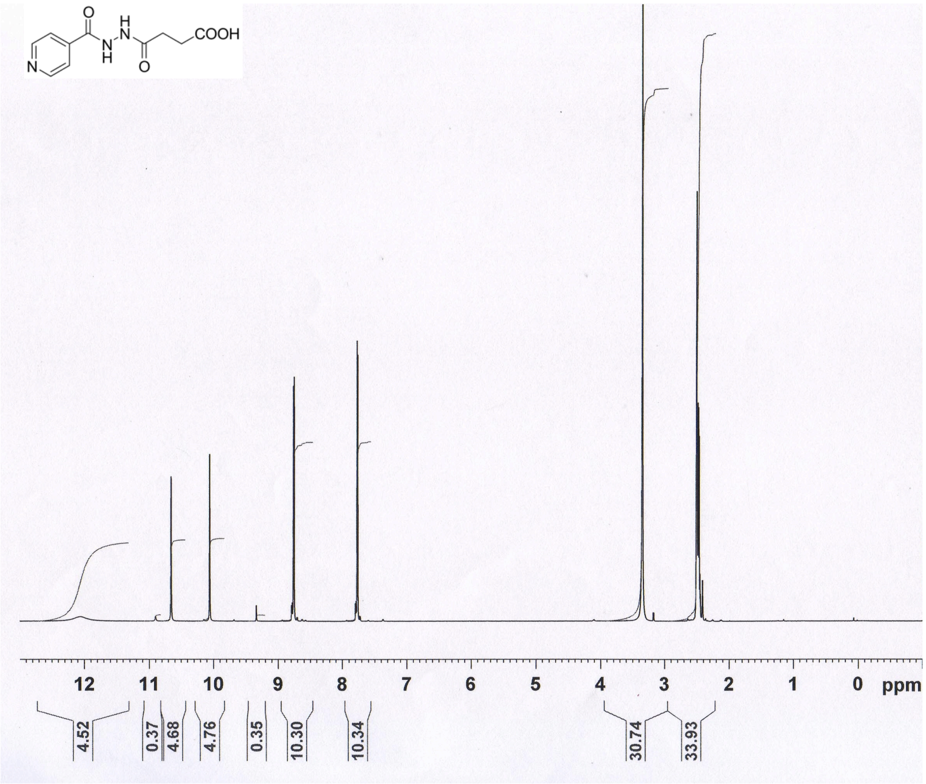
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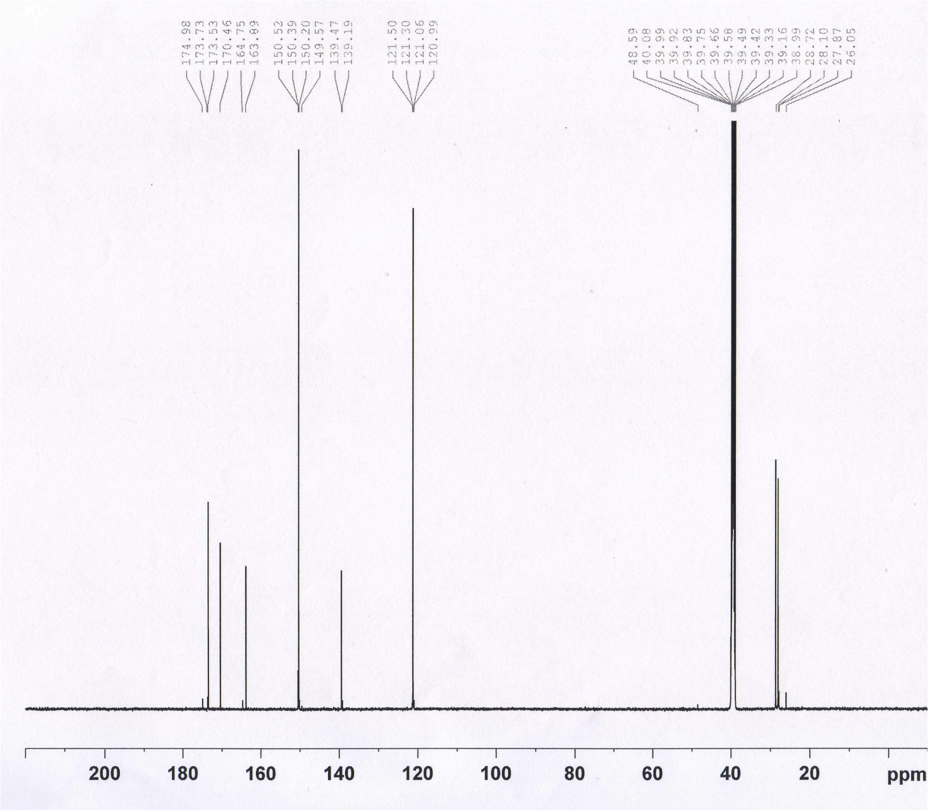
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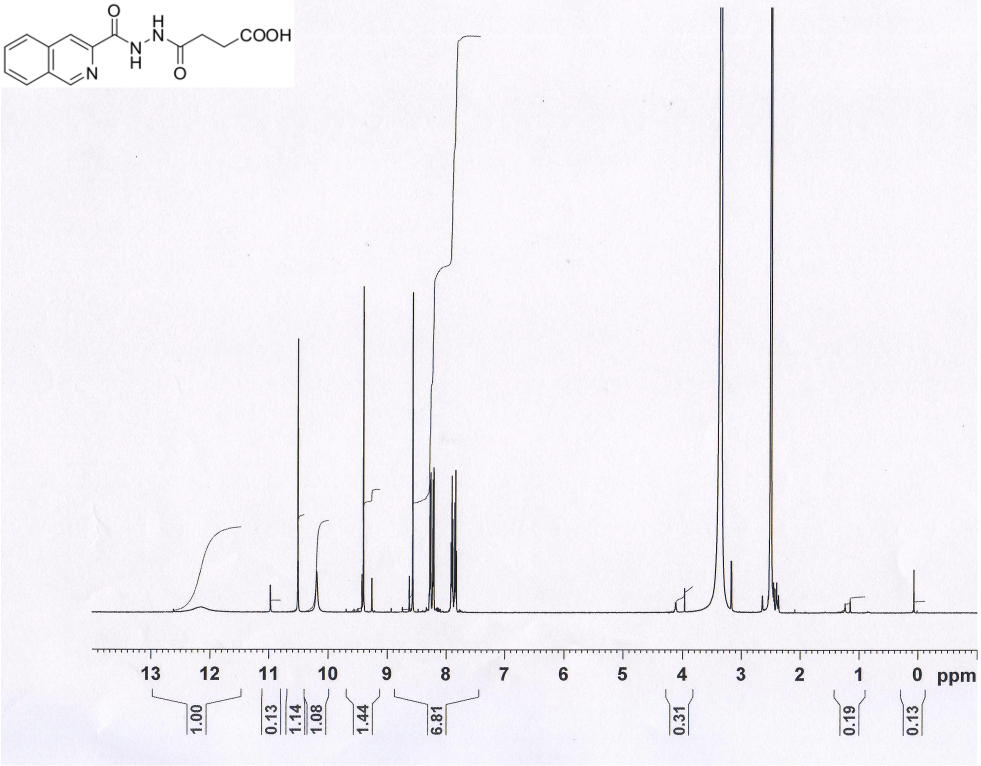
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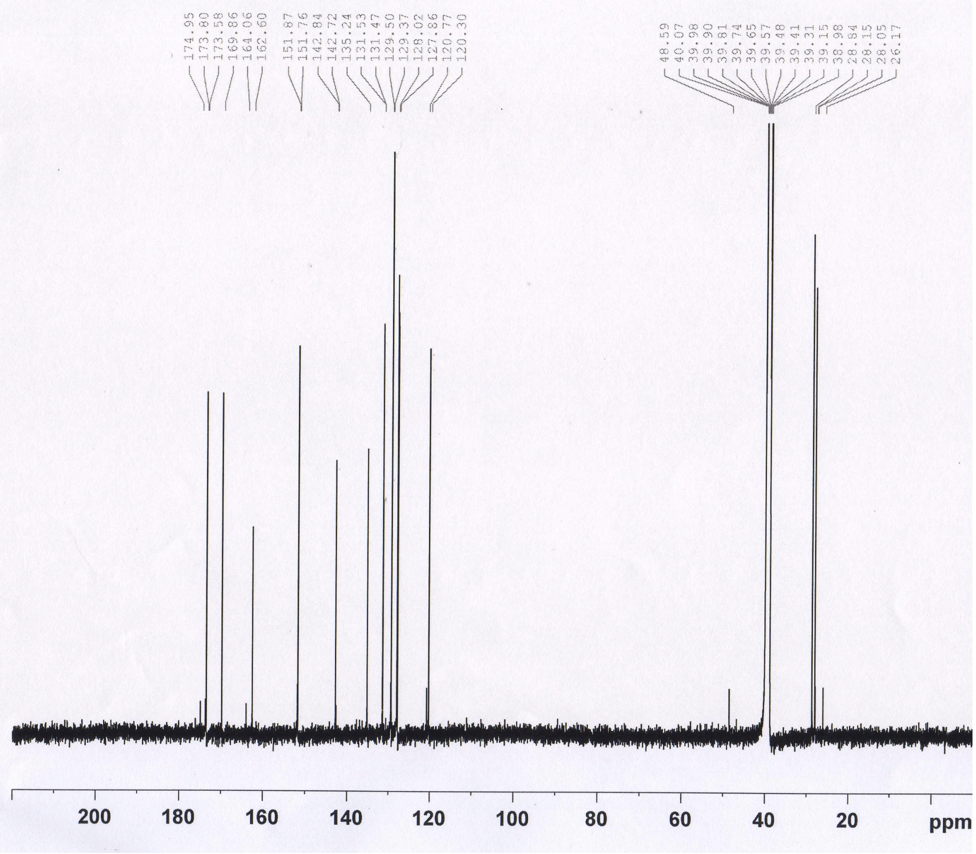
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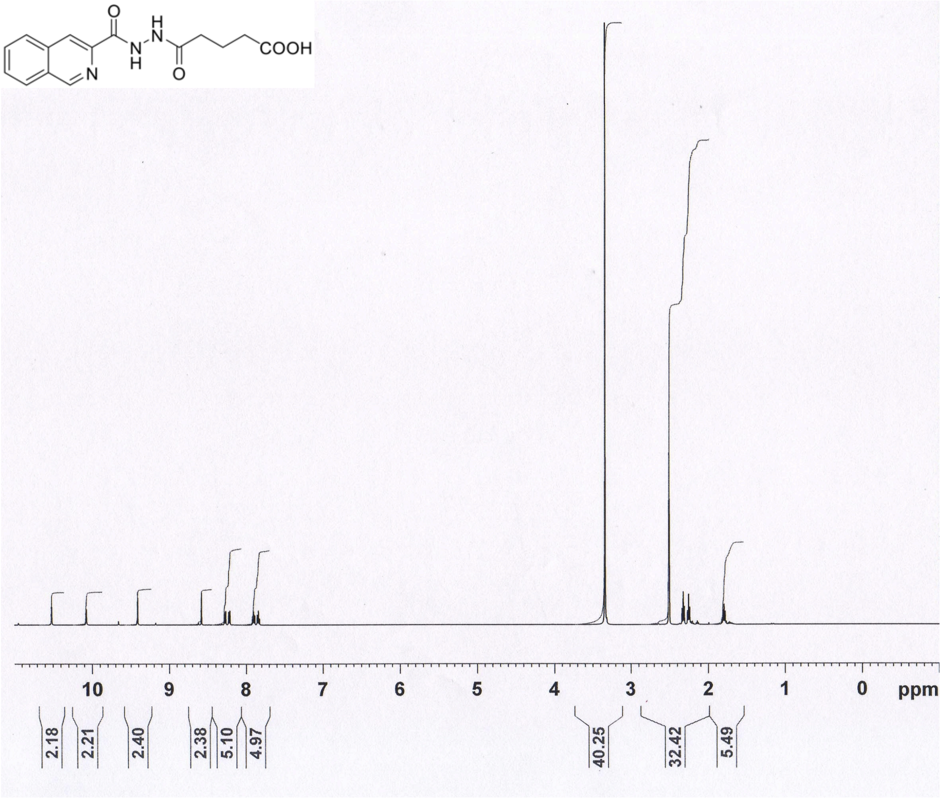
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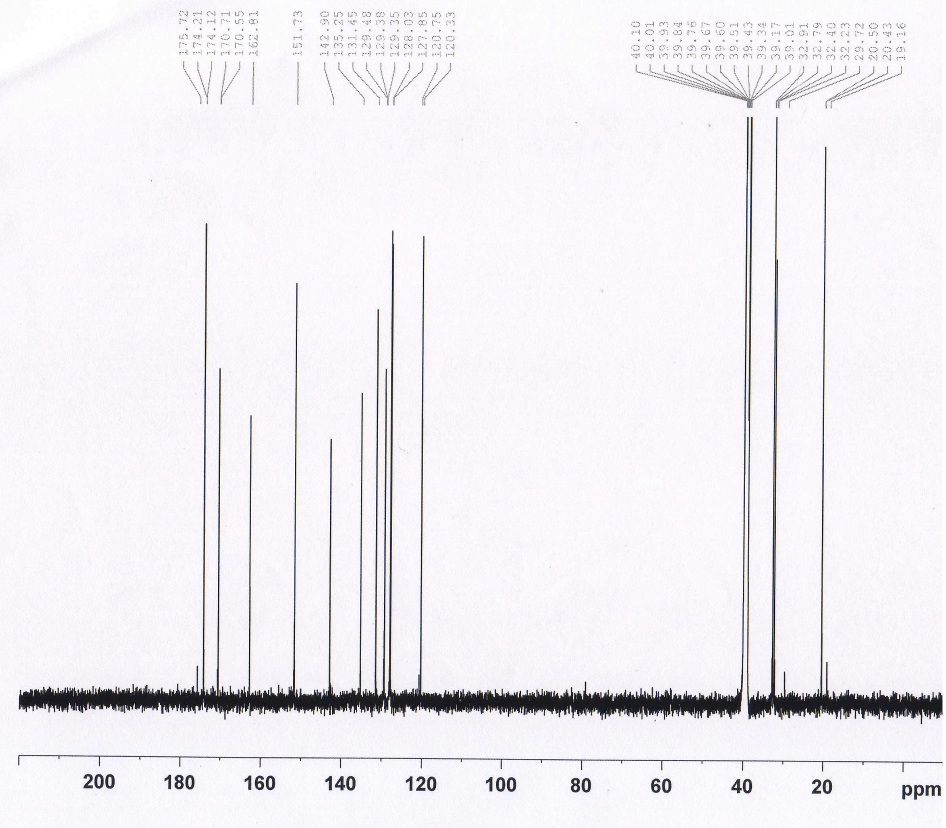
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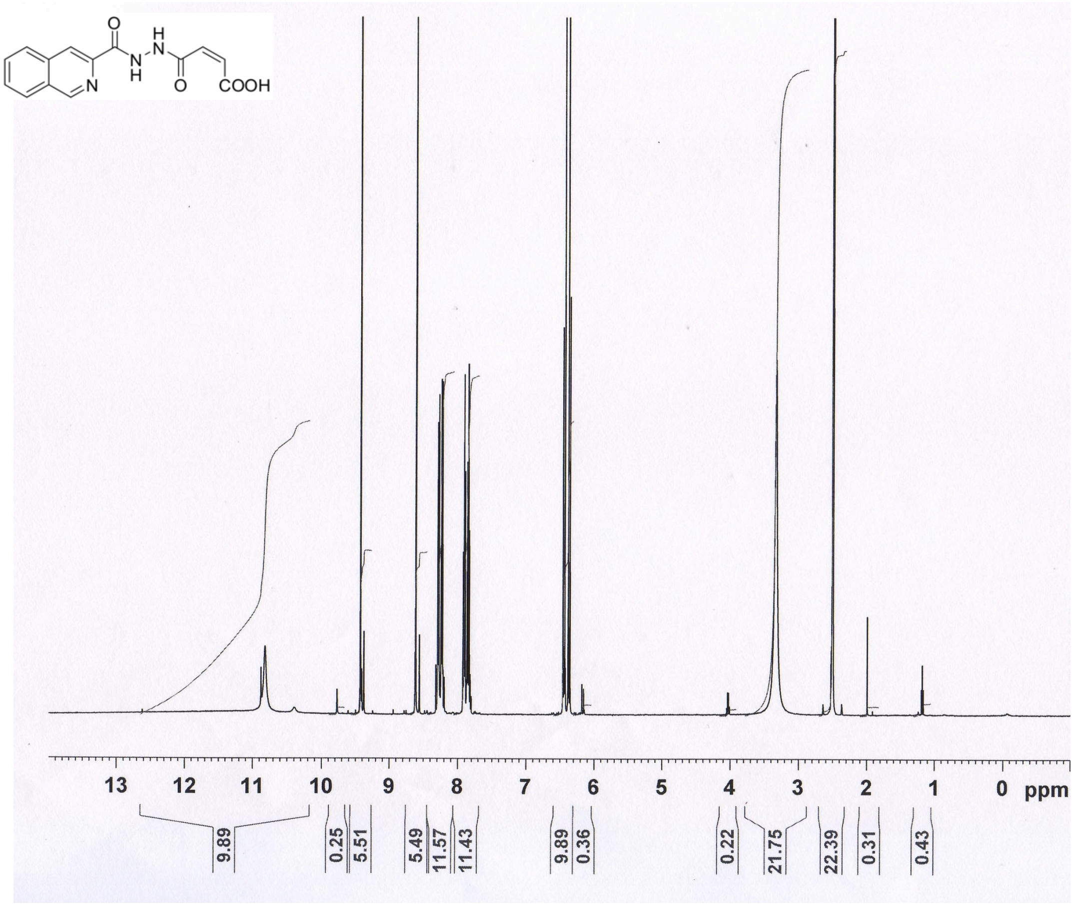
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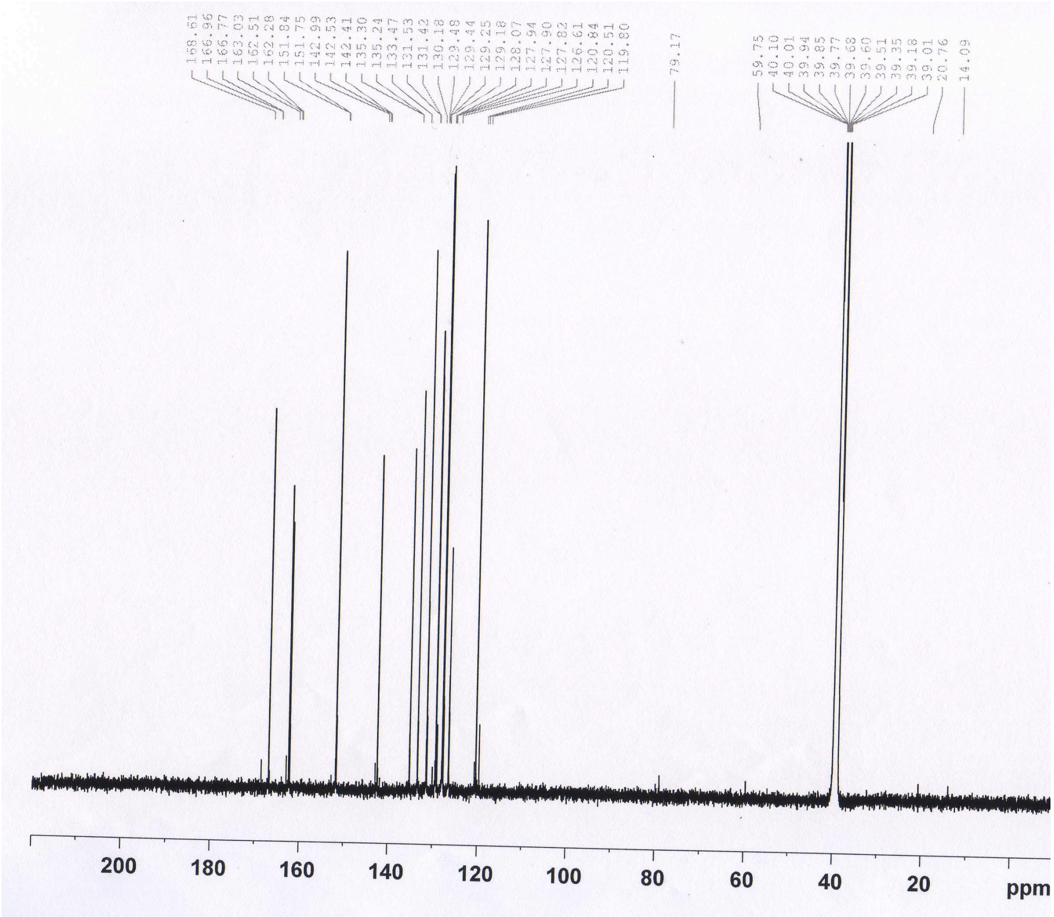
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127.90
127.82
126.61
120.84
120.51
119.80
79.17
59.75
40.10
40.01
39.94
39.85
39.77
39.68
39.60
39.51
39.35
39.18
39.01
20.76
14.09
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140
120
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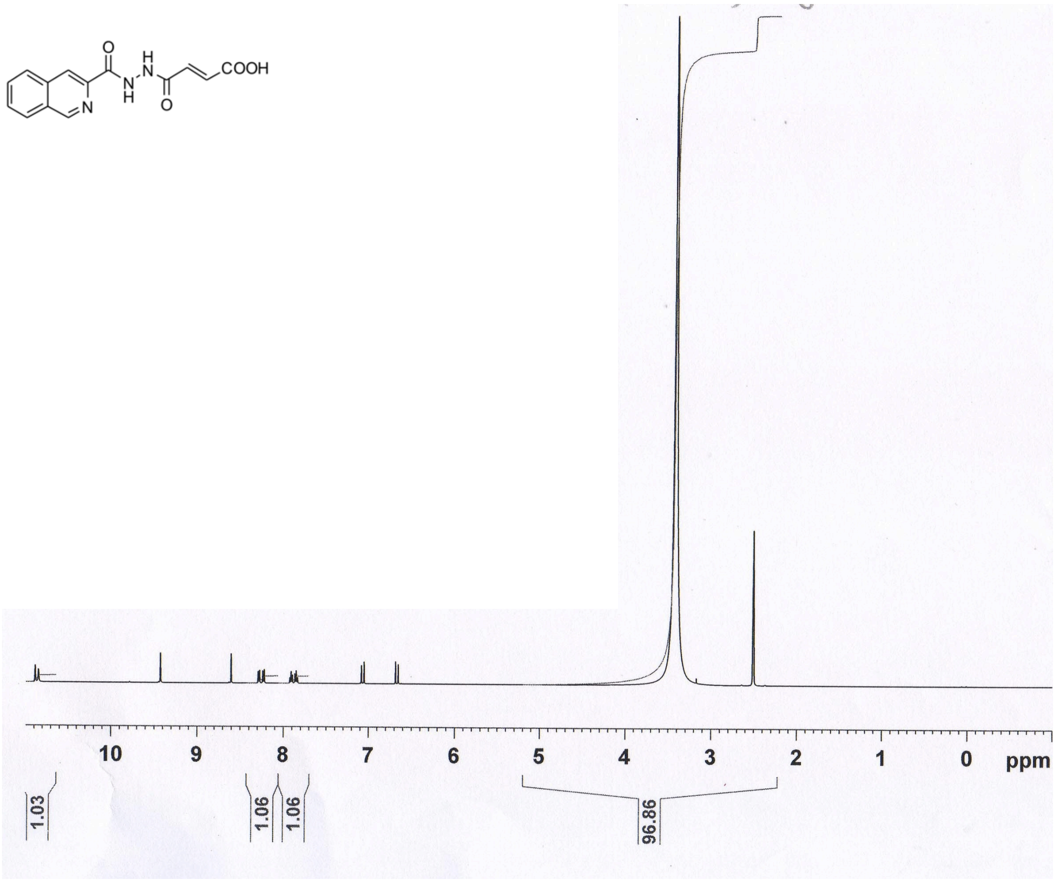
80
60
40
20
ppm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N
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4
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0
ppm
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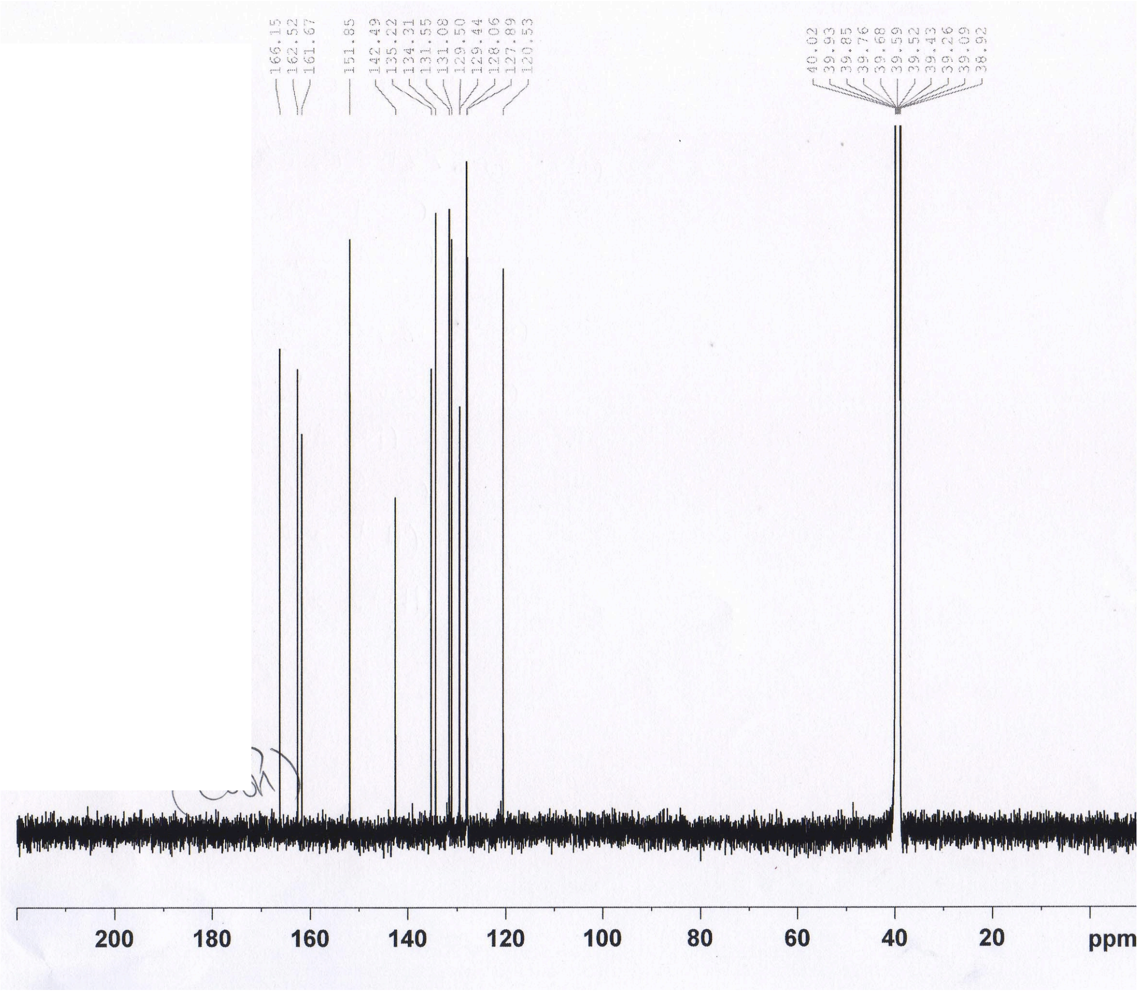
1.06
1.06
96.86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142.49
135.22
134.31
131.55
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129.50
129.44
128.06
127.89
120.53
40.02
39.93
39.85
39.76
39.68
39.59
39.52
39.43
39.26
39.09
38.92
(COOH)
200
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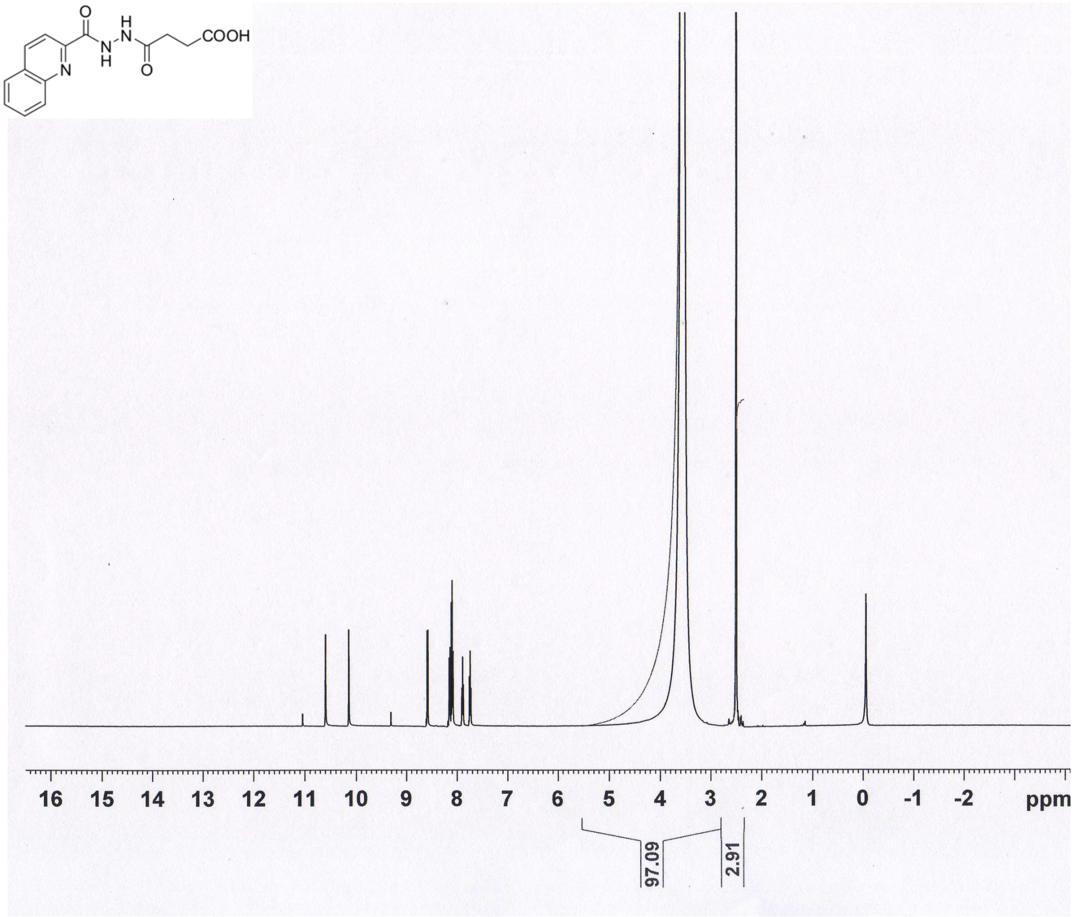
160
140
120
100
80
60
40
20
ppm


O
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N
N
H
O
COOH
N
16
15
14
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11
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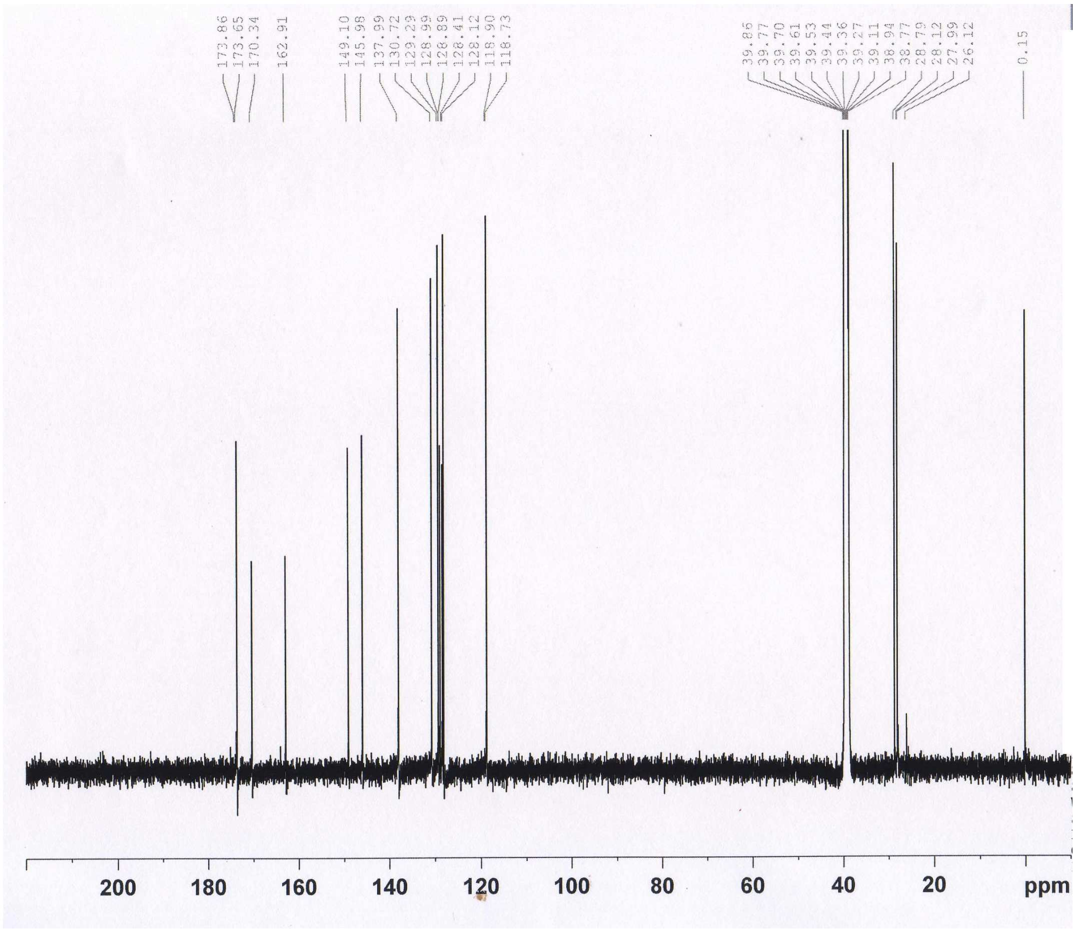
8
7
6
5
4
3
2
1
0
-1
-2
ppm
97.09
2.91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145.98
137.99
130.72
129.29
128.99
128.89
128.41
128.12
118.90
118.73
39.85
39.77
39.70
39.61
39.53
39.44
39.36
39.27
39.11
38.94
38.77
28.79
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27.99
26.12
0.15
200
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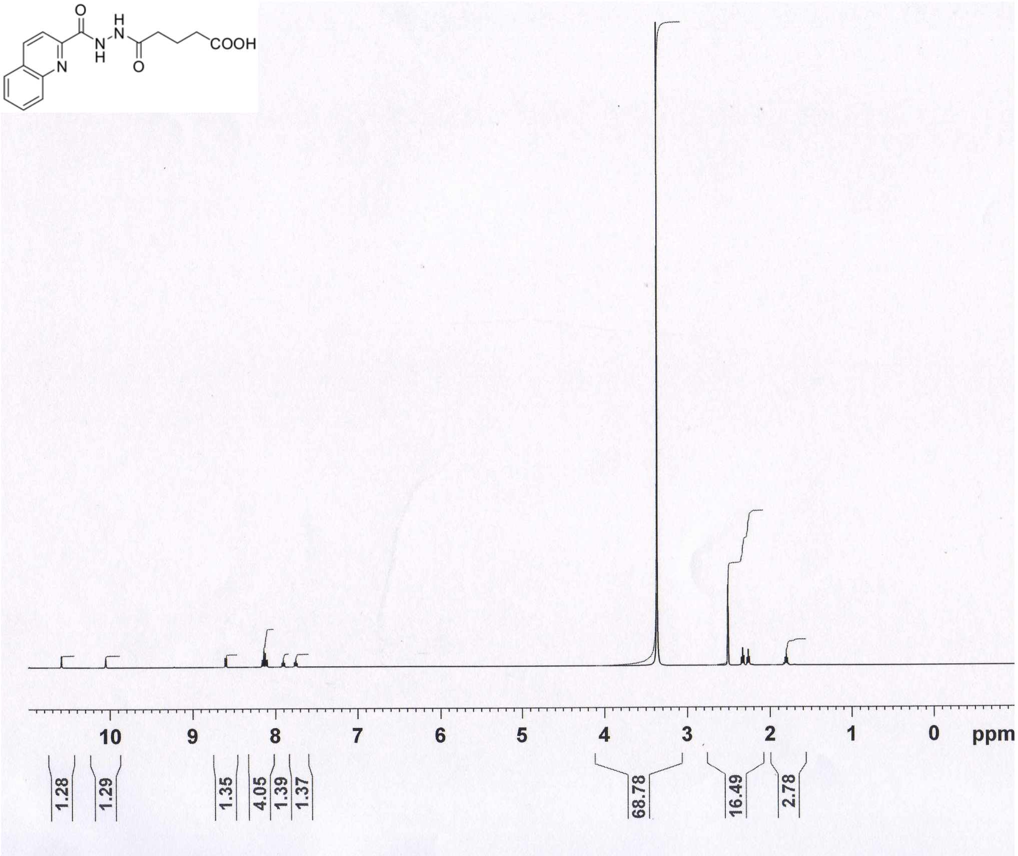
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
COOH
N
10
9
8
7
6
5
4
3
2
1
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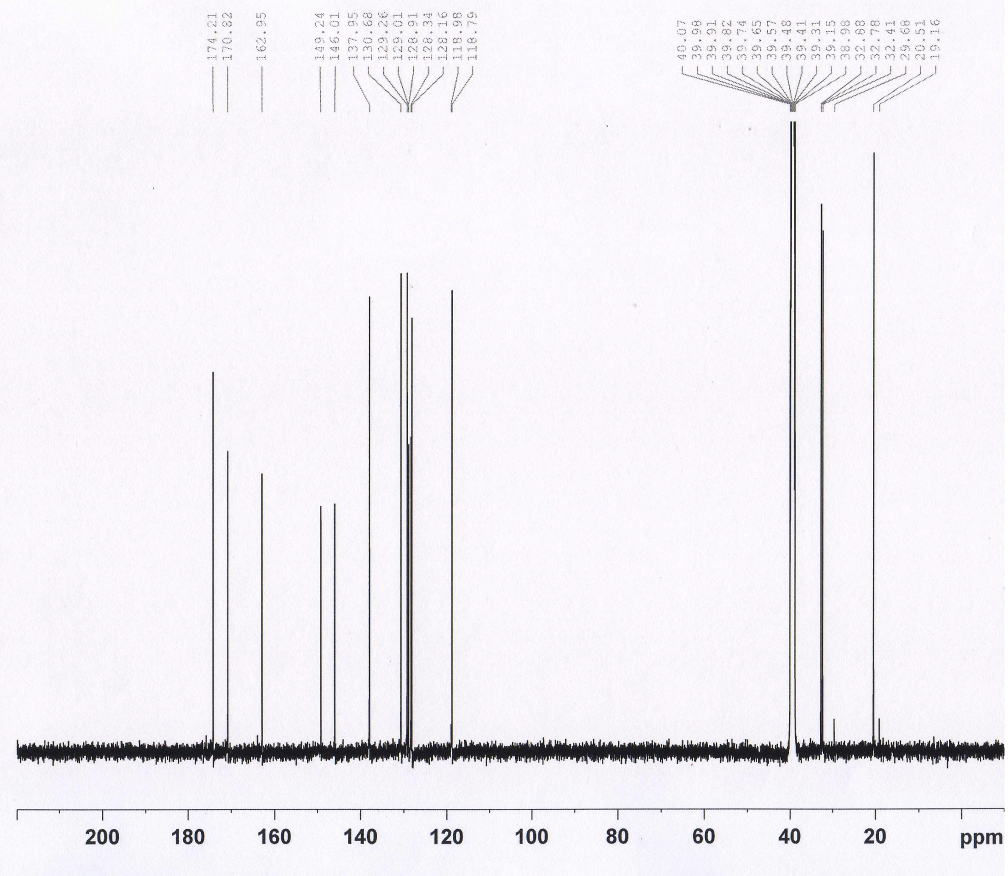
ppm
1.28
1.29
1.35
4.05
1.39
1.37
68.78
16.49
2.78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137.95
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129.26
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128.91
128.34
128.16
118.98
118.79
40.07
39.98
39.91
39.82
39.74
39.65
39.57
39.48
39.41
39.31
39.15
38.98
32.88
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32.41
29.68
20.51
19.16
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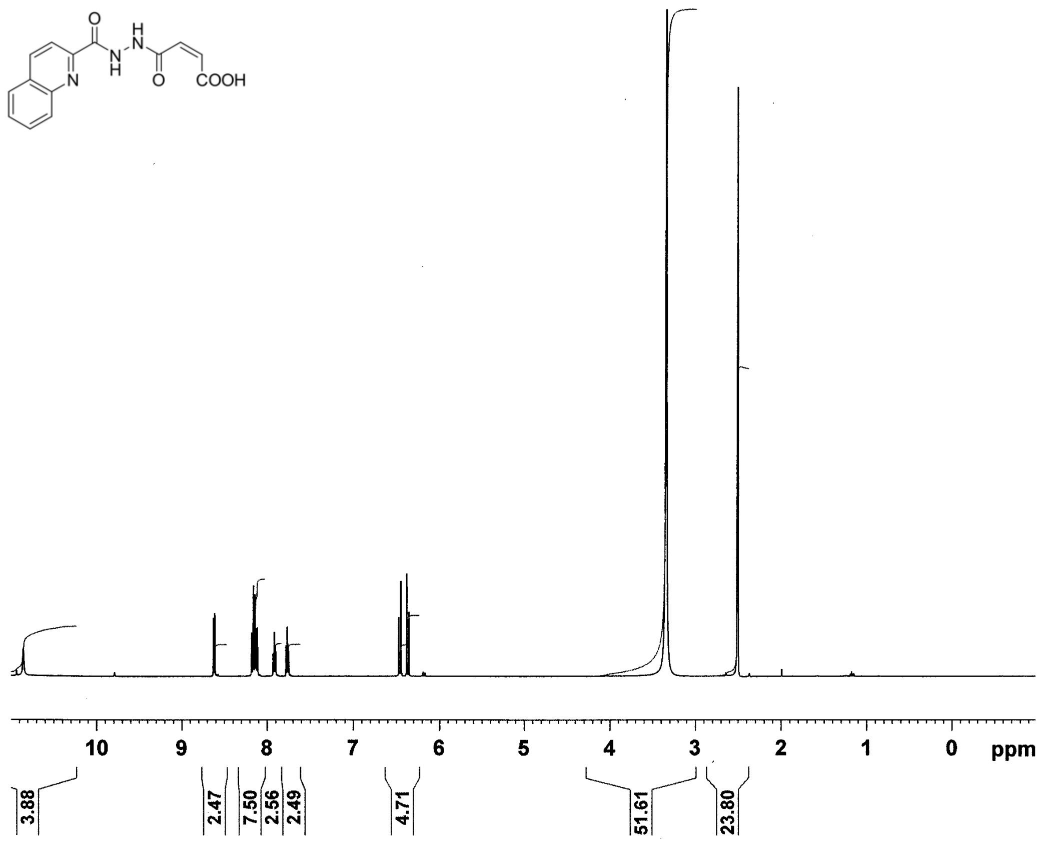
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
N
COOH
10
9
8
7
6
5
4
3
2
1
0
ppm
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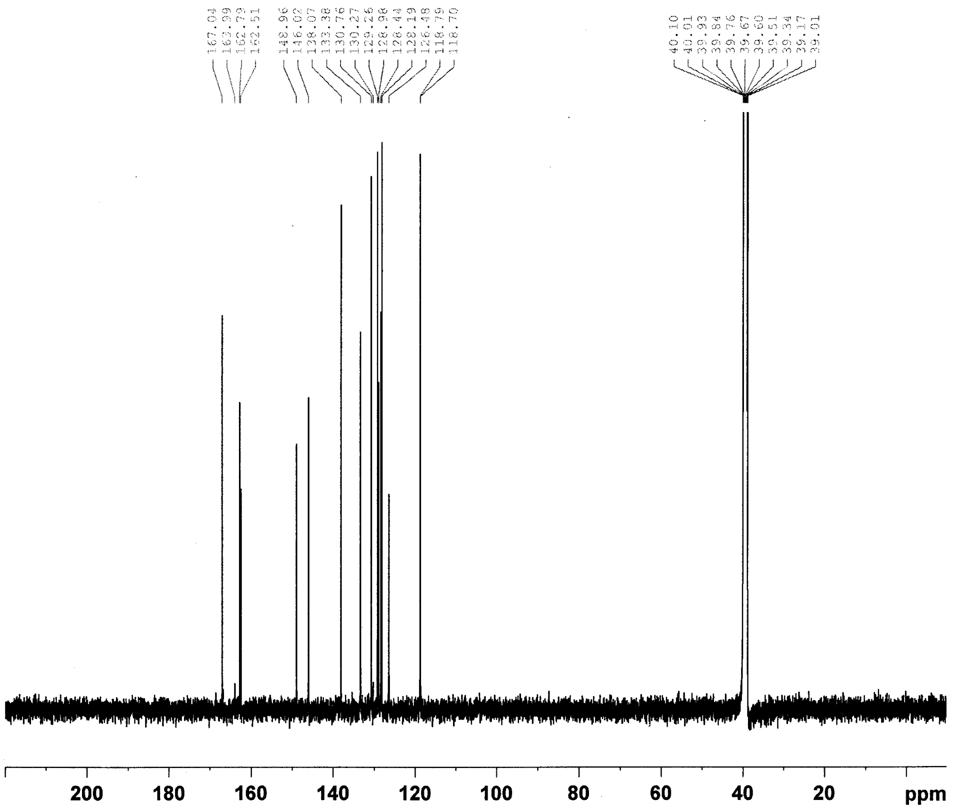
2.47
7.50
2.56
2.49
4.71
51.61
23.80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146.02
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133.38
130.76
130.27
129.26
128.98
128.44
128.19
126.48
118.79
118.70
40.10
40.01
39.93
39.84
39.76
39.67
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39.34
39.17
39.01
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140
120
100
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60
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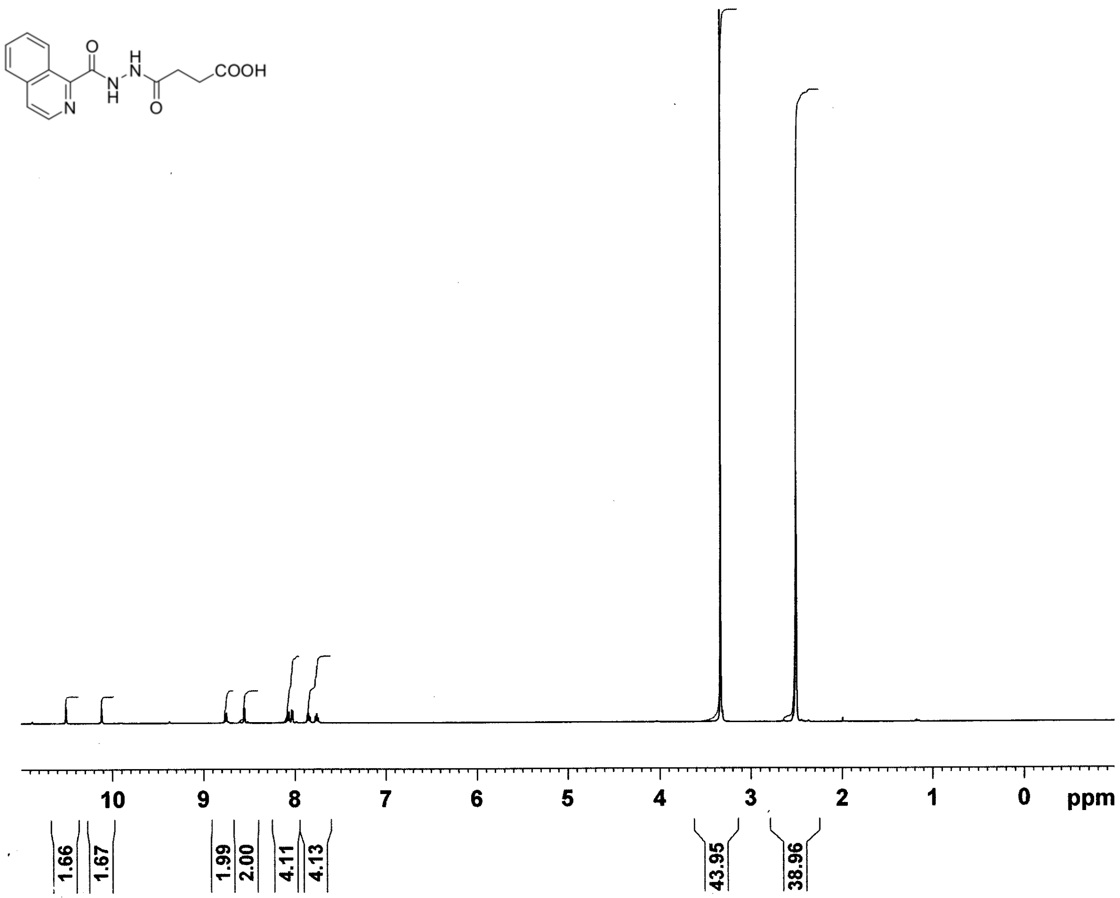
20
ppm


O
H
N
N
H
N
O
COOH
10
9
8
7
6
5
4
3
2
1
0
ppm
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1.67
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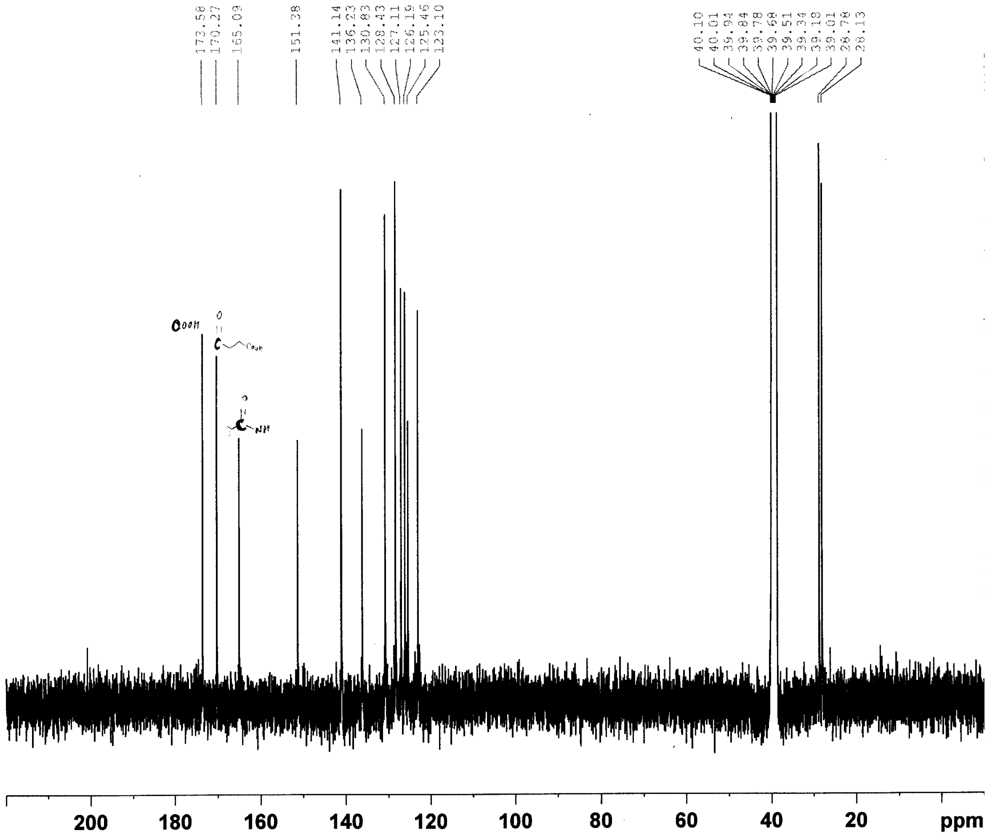
2.00
4.11
4.13
43.95
38.96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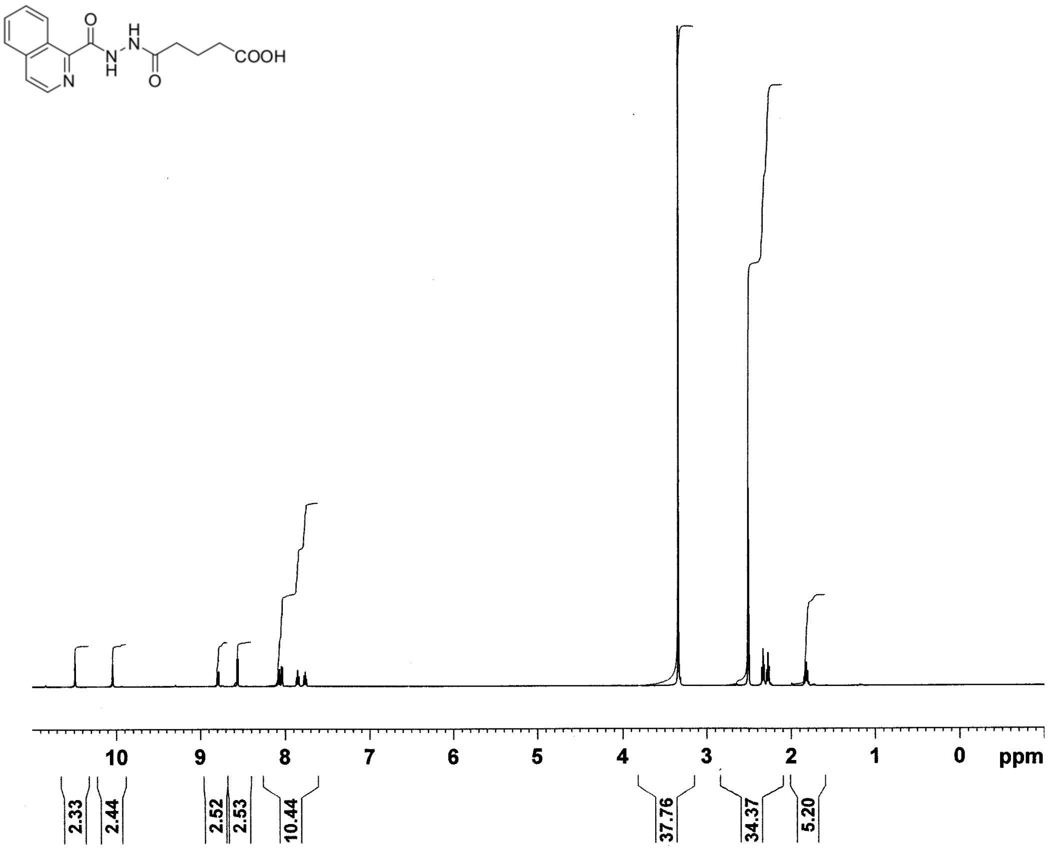
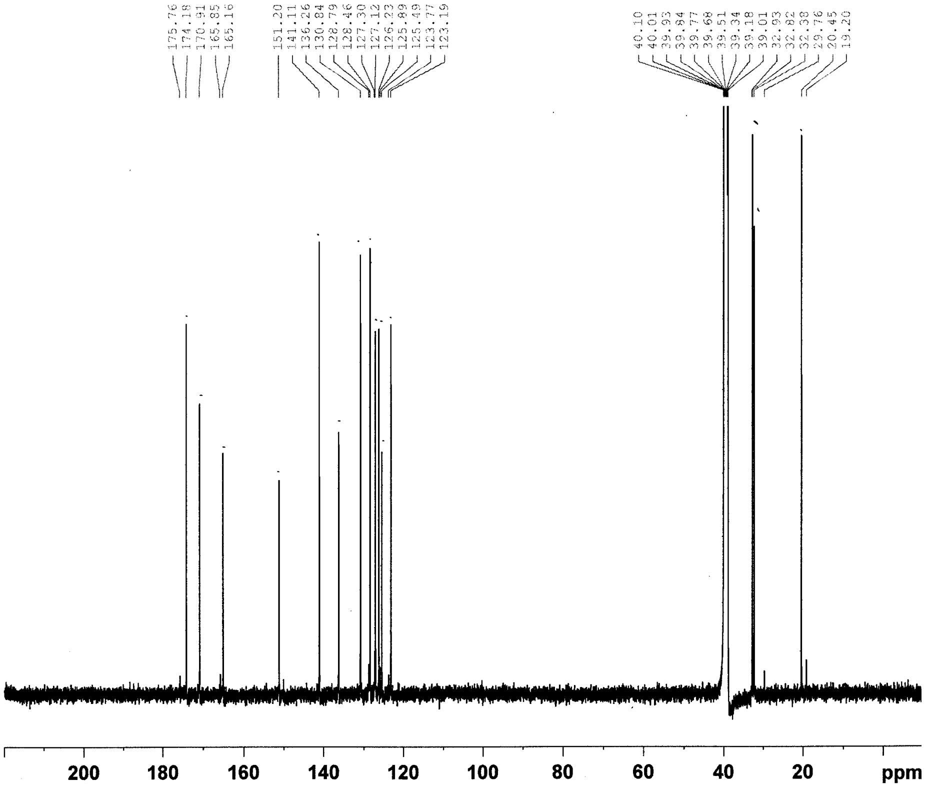
151.38
141.14
136.23
130.83
128.43
127.11
126.19
125.46
123.10
40.10
40.01
39.94
39.84
39.78
39.68
39.51
39.34
39.18
39.01
28.78
28.13
COOH
COOH
NH
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
COOH
N

10
9
8
7
6
5
4
3
2
1
0
ppm
2.33
2.44
2.52
2.53
10.44
37.76
34.37
5.20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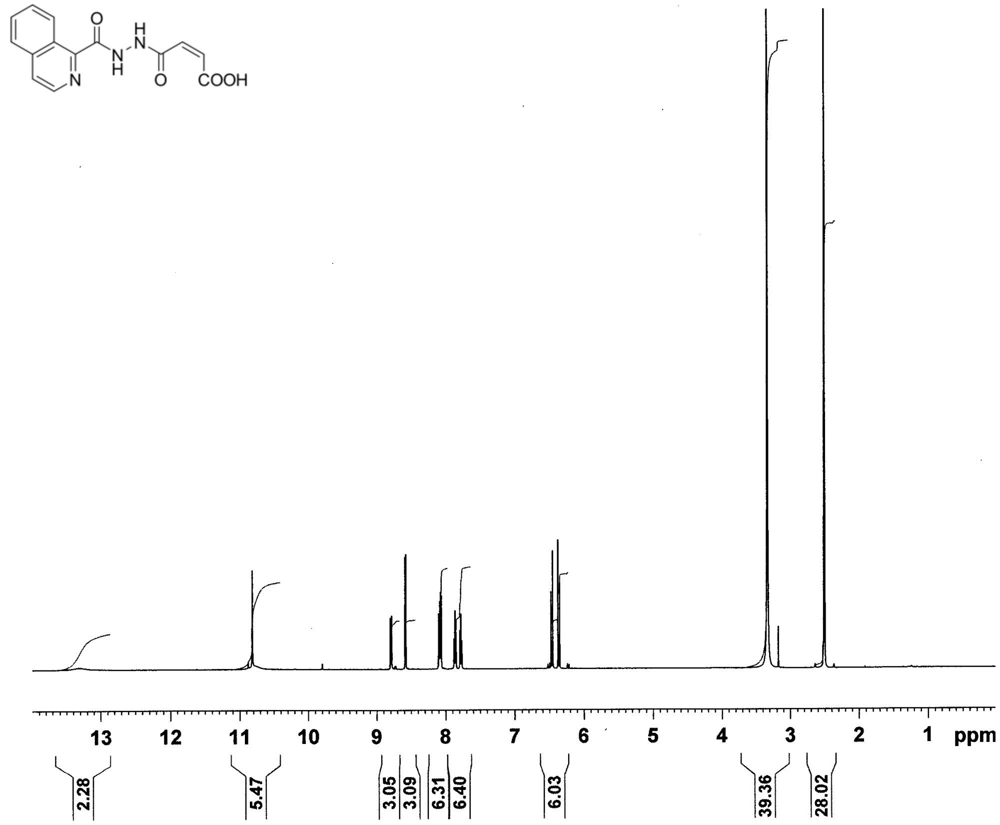
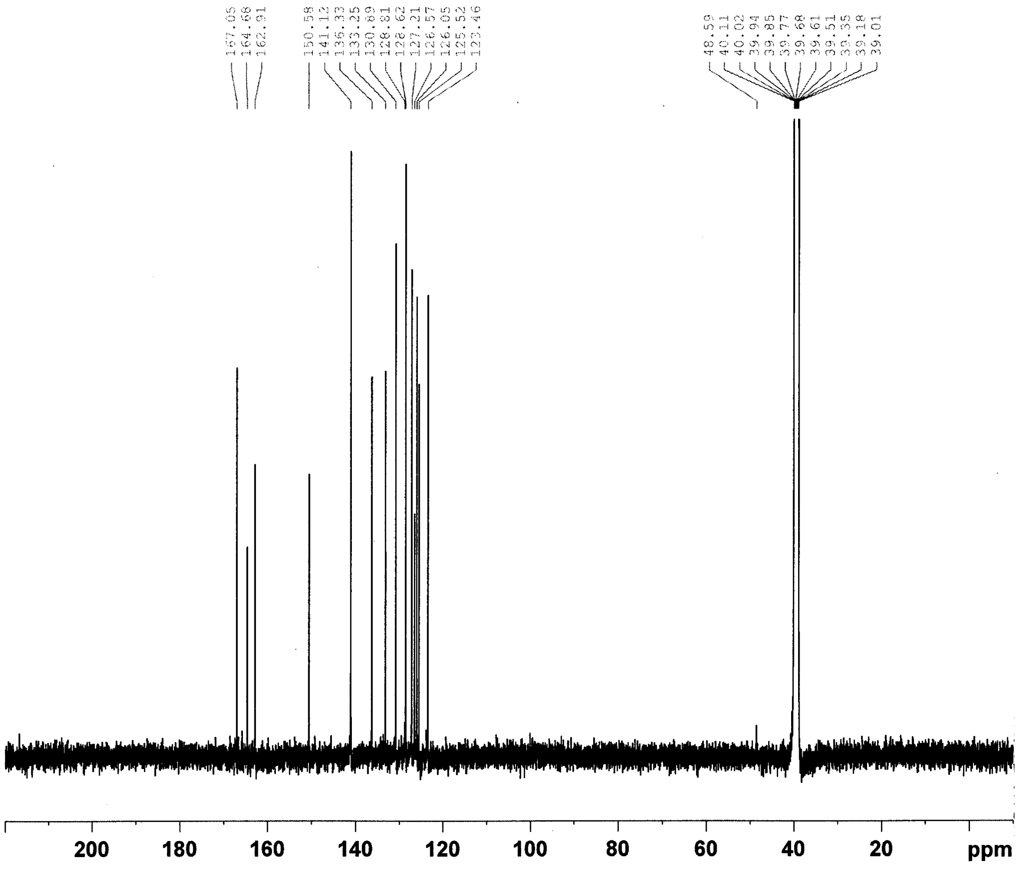
174.18
170.91
165.85
165.16
151.20
141.11
136.26
130.84
128.79
128.46
127.30
127.12
126.23
125.89
125.49
123.77
123.19
40.10
40.01
39.93
39.84
39.77
39.68
39.51
39.34
39.18
39.01
32.93
32.82
32.38
29.76
20.45
19.20
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
N
O
COOH
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
2.28
5.47
3.05
3.09
6.31
6.40
6.03
39.36
28.02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130.89
128.81
128.62
127.21
126.57
126.05
125.52
123.46
48.59
40.11
40.02
39.94
39.85
39.77
39.68
39.61
39.51
39.35
39.18
39.01
200
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140
120
100
80
60
40
20
ppm

O
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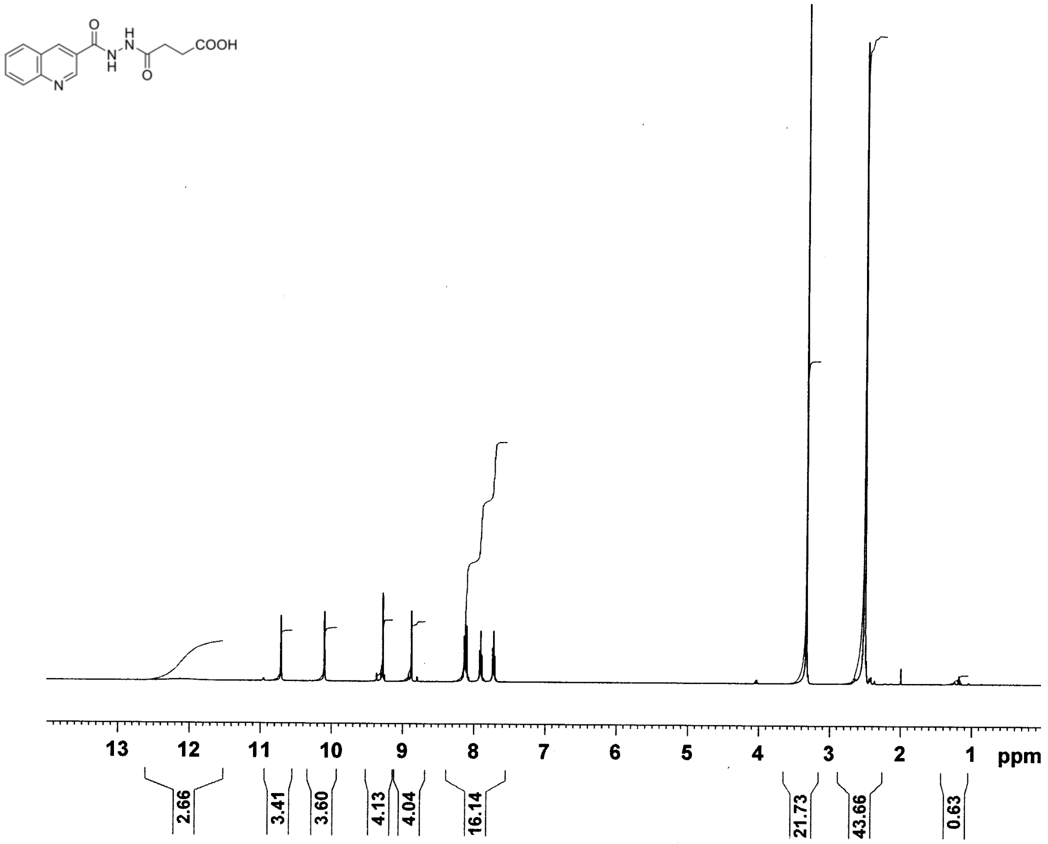
N
H
O
COOH
N


13
12
11
10
9
8
7
6
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3
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1
ppm
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3.41
3.60
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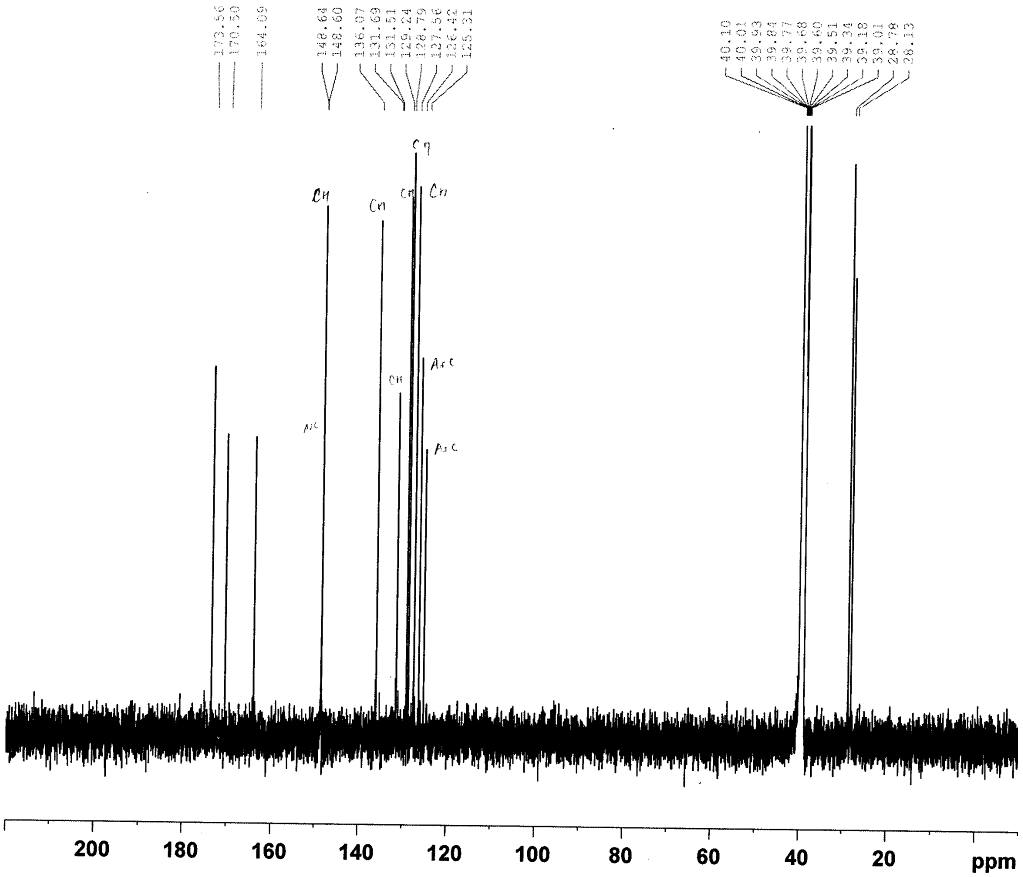
43.66
0.63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136.07
131.69
131.51
129.24
128.79
127.56
126.42
125.31
40.10
40.01
39.93
39.84
39.77
39.68
39.60
39.51
39.34
39.18
39.01
28.78
28.13
Cq
CH
CH
CH
CH
ArC
CH
ArC
ArC
200
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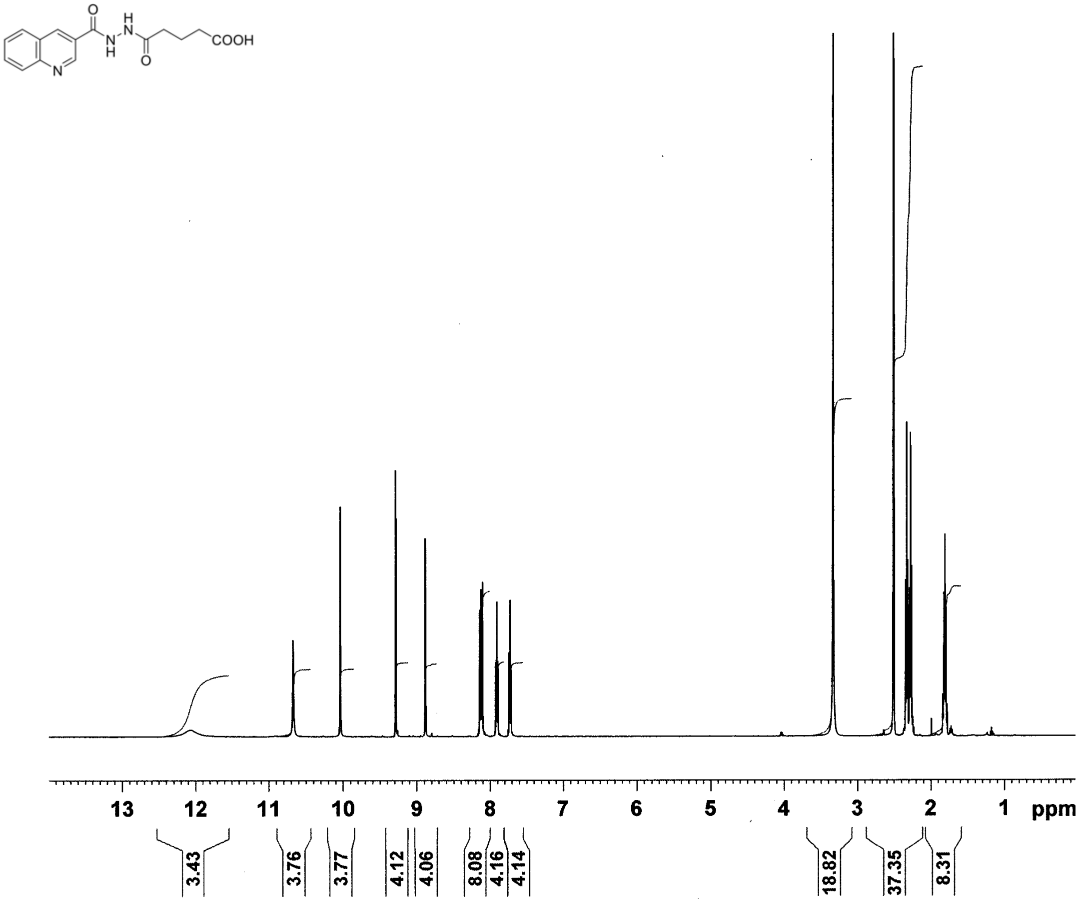
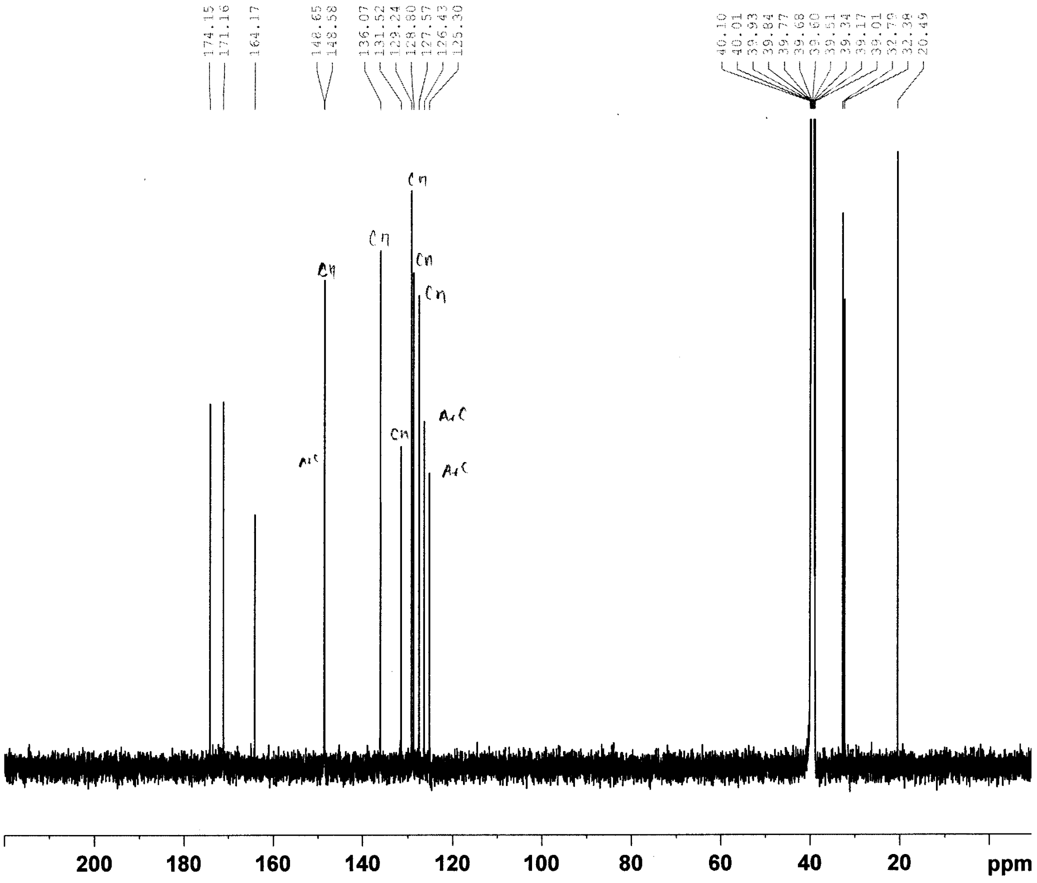
140
120
100
80
60
40
20
ppm


COOH
N
H
O
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
3.43
3.76
3.77
4.12
4.06
8.08
4.16
4.14
18.82
37.35
8.31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171.16
164.17
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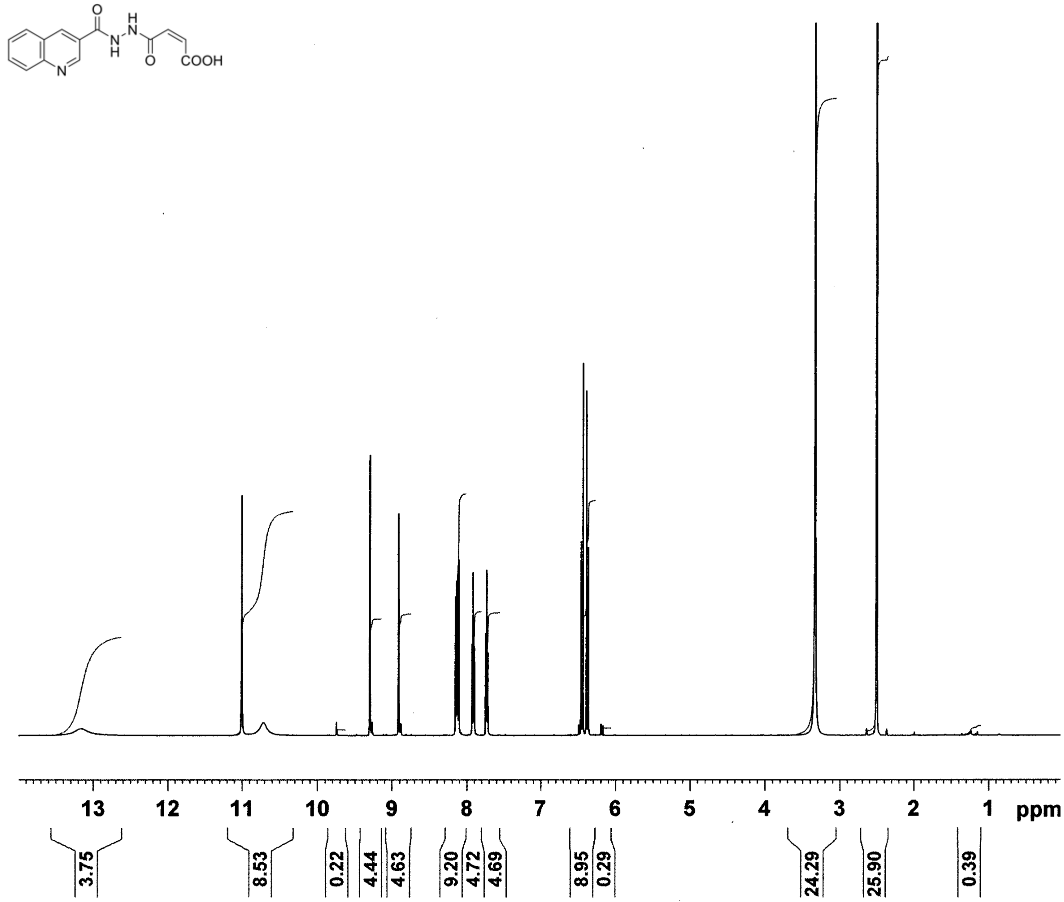
148.58
136.07
131.52
129.24
128.80
127.57
126.43
125.30
40.10
40.01
39.93
39.84
39.77
39.68
39.60
39.51
39.34
39.17
39.01
32.79
32.38
20.49
CH
CH
CH
CH
CH
CH
ArC
ArC
ArC
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
COOH
N
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
3.75
8.53
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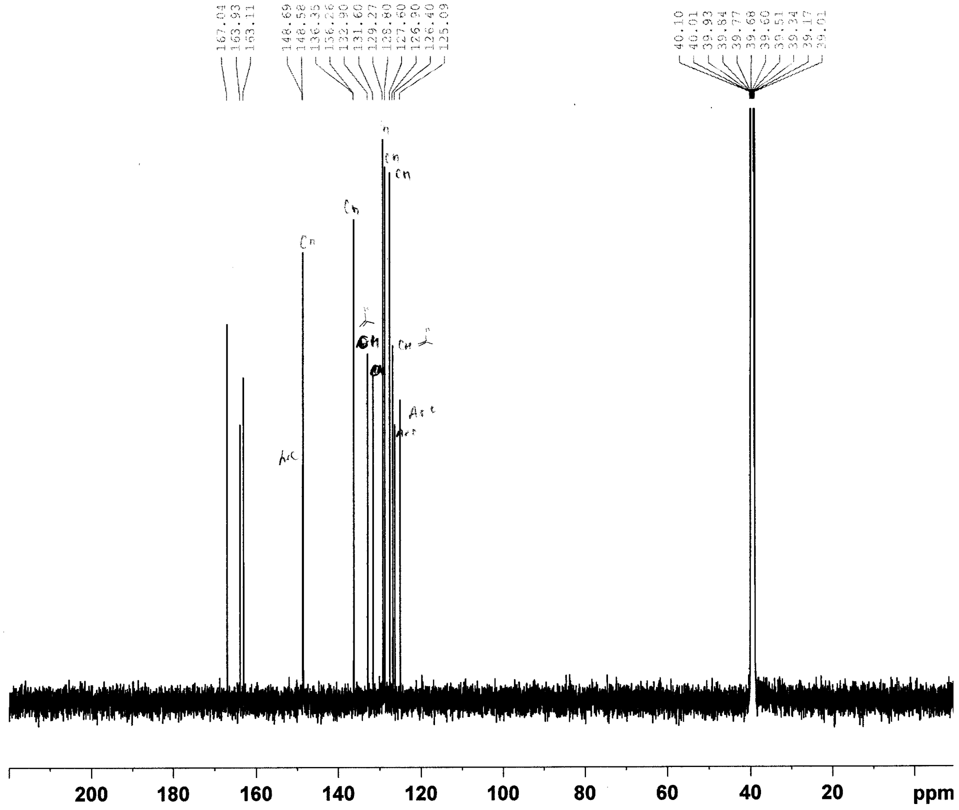
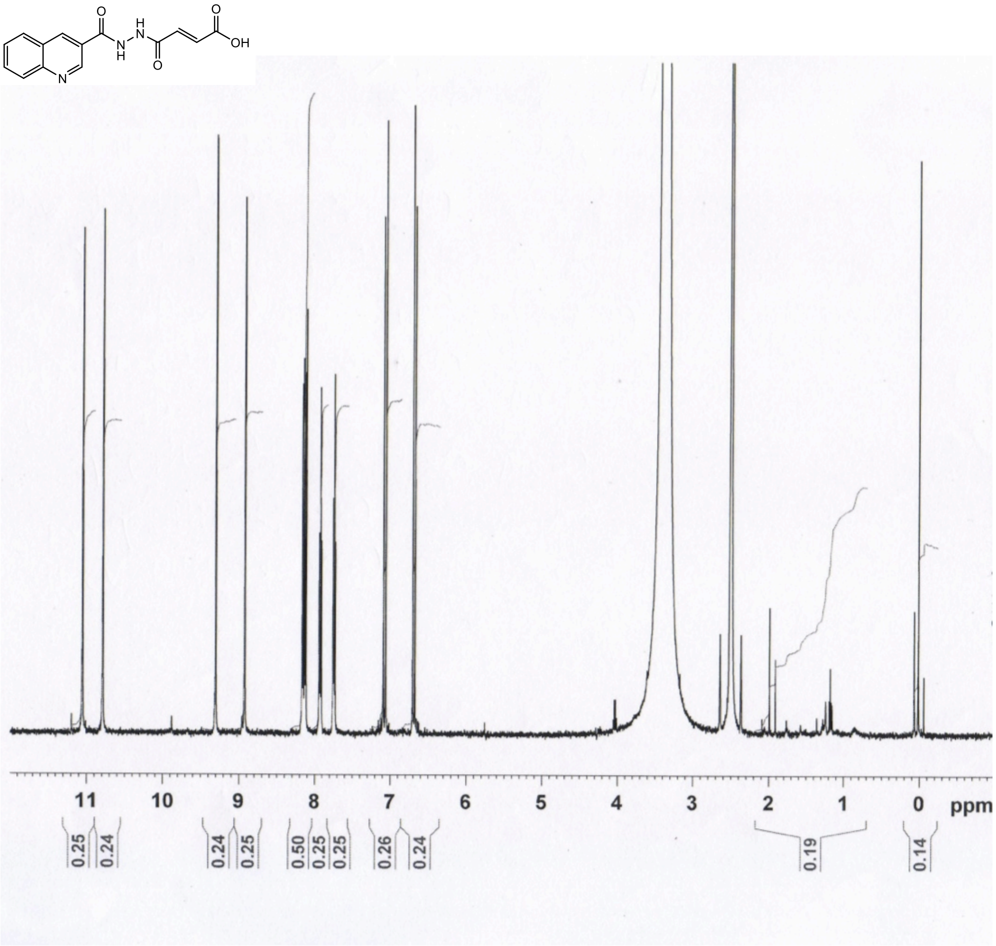
4.44
4.63
9.20
4.72
4.69
8.95
0.29
24.29
25.90
0.39


167.04
163.93
163.11
148.69
148.58
136.35
136.26
132.90
131.60
129.27
128.80
127.60
126.90
126.40
125.09
40.10
40.01
39.93
39.84
39.77
39.68
39.60
39.51
39.34
39.17
39.01
CH
CH
CH
CH
CH
CH
CH
Ar C
Ar C
Ar C
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
O
OH
N
11
10
9
8
7
6
5
4
3
2
1
0
ppm
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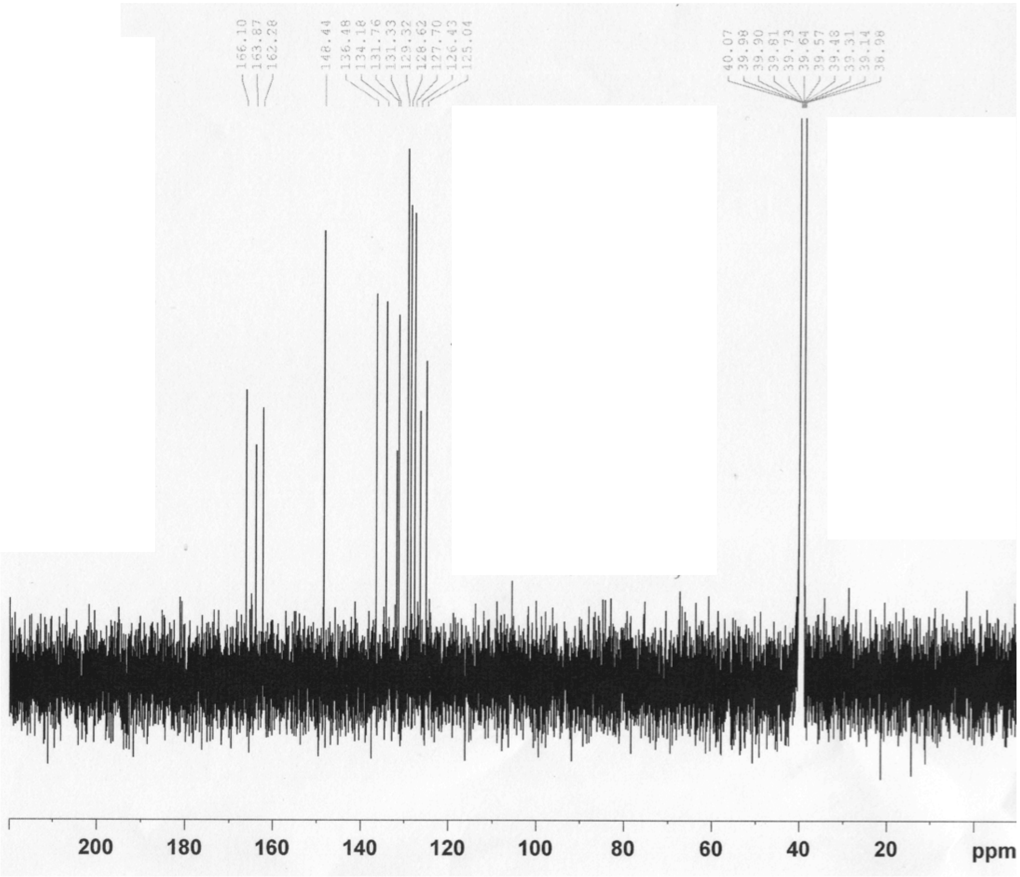
0.24
0.24
0.25
0.50
0.25
0.25
0.26
0.24
0.19
0.14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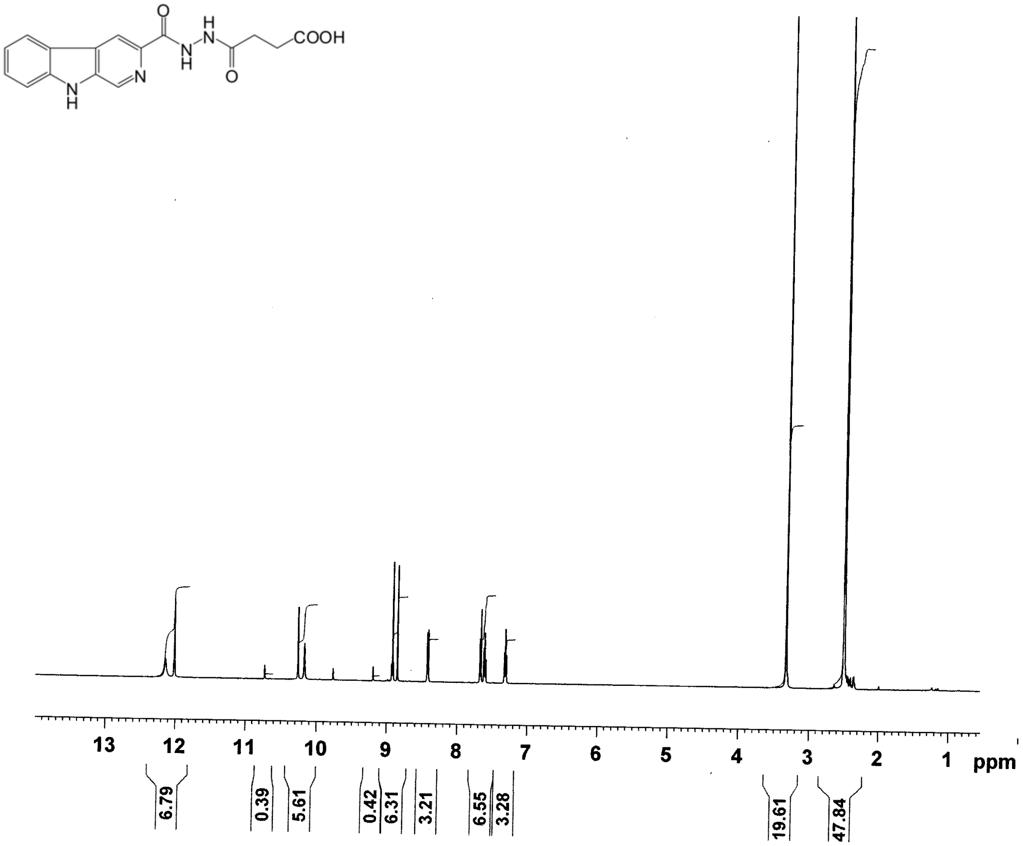
131.76
131.33
129.32
128.62
127.70
126.43
125.04
40.07
39.98
39.90
39.81
39.73
39.64
39.57
39.48
39.31
39.14
38.98
200
180
160
140
120
100
80
60
40
20
ppm


COOH
N
H
O
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
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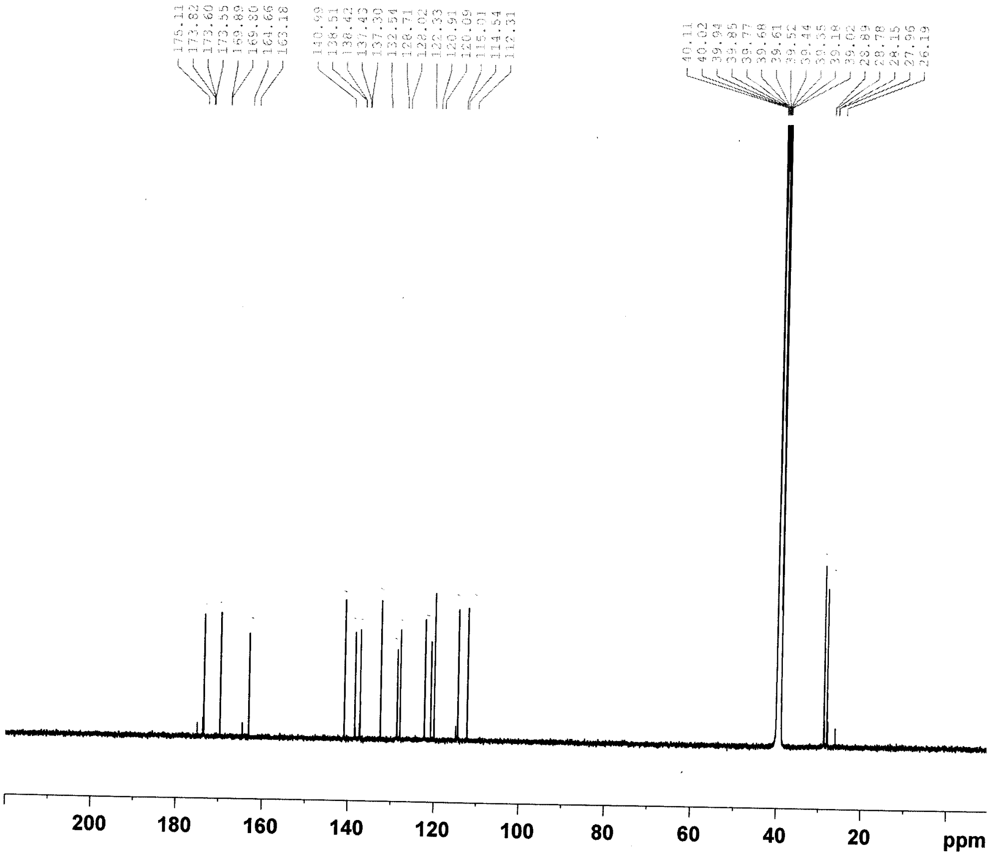
0.39
5.61
0.42
6.31
3.21
6.55
3.28
19.61
47.84


175.11
173.82
173.60
173.55
169.89
169.80
164.66
163.18
140.99
138.51
138.42
137.43
137.30
132.54
128.71
128.02
122.33
120.91
120.09
115.01
114.54
112.31
40.11
40.02
39.94
39.85
39.77
39.68
39.61
39.52
39.44
39.35
39.18
39.02
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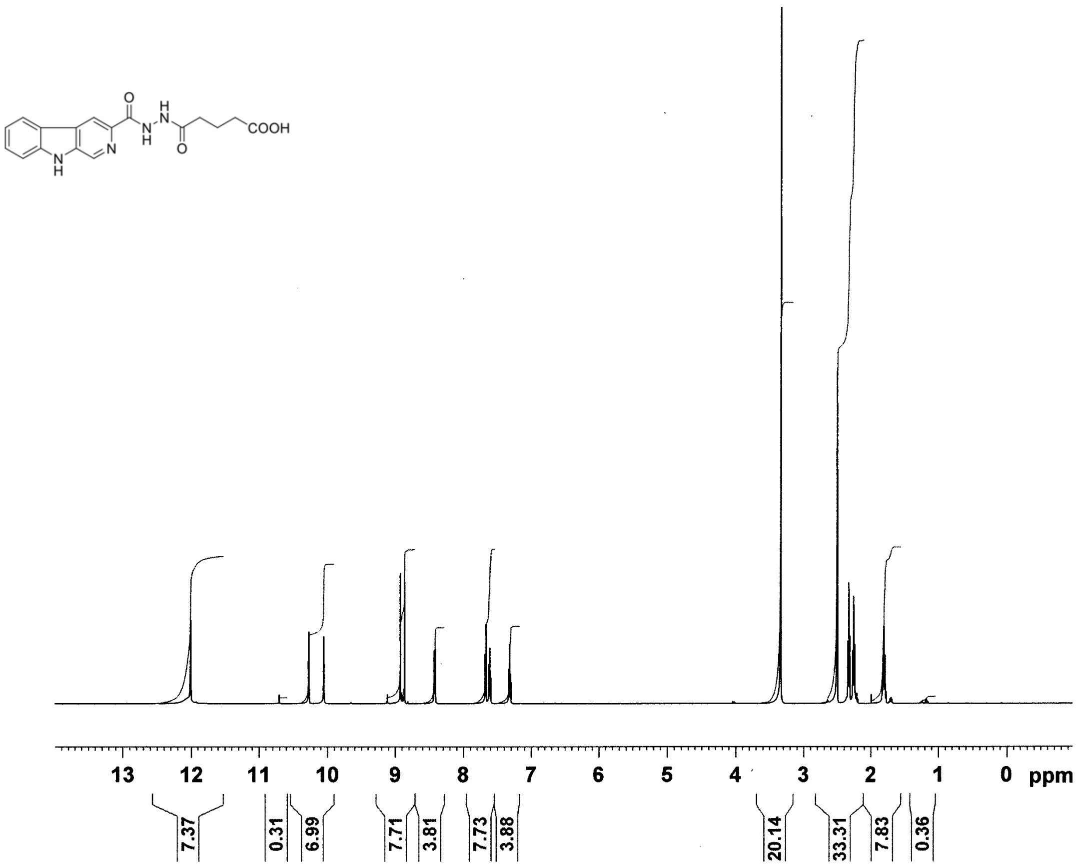
28.15
27.96
26.19
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
COOH
N
H
N
13
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
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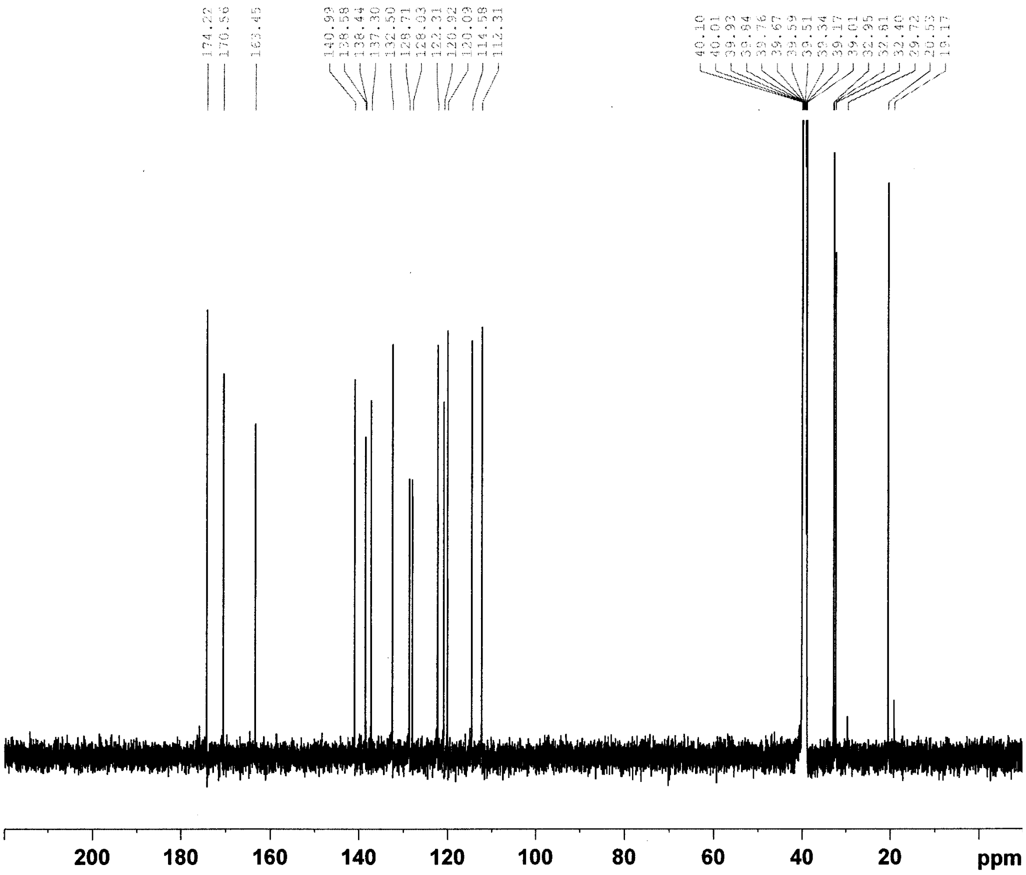
0.31
6.99
7.71
3.81
7.73
3.88
20.14
33.31
7.83
0.36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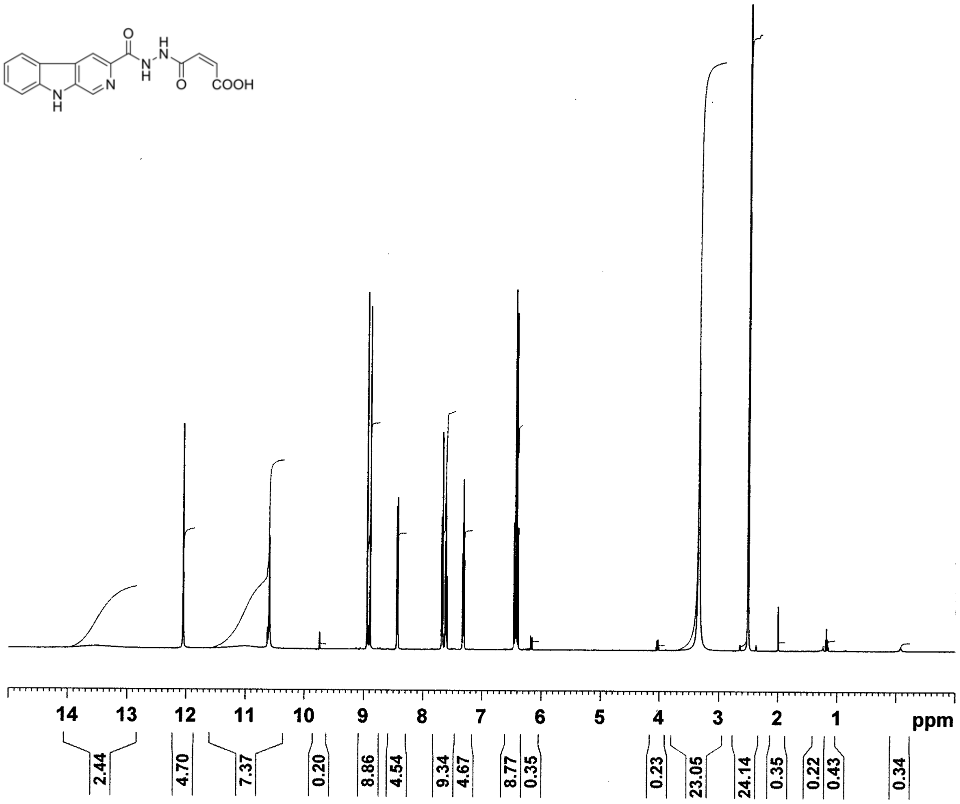
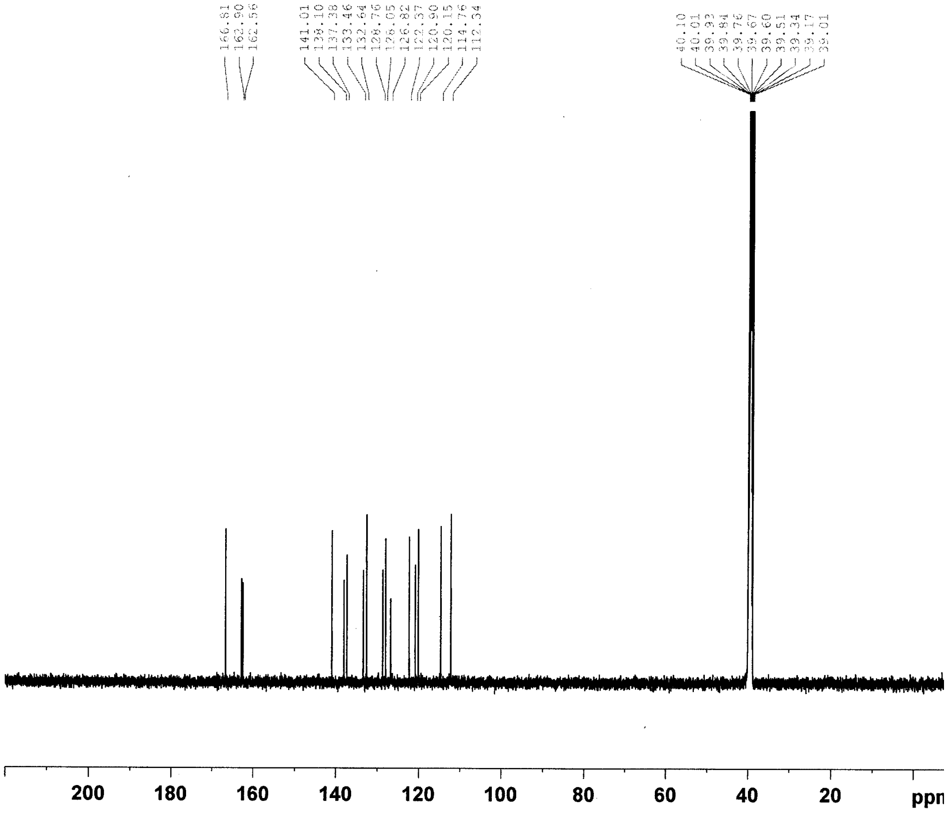
138.44
137.30
132.50
128.71
128.03
122.31
120.92
120.03
114.58
112.31
40.10
40.01
39.93
39.84
39.76
39.67
39.59
39.51
39.34
39.17
39.01
32.95
32.61
32.40
29.72
20.53
19.17
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
N
N
H
O
COOH
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
2.44
4.70
7.37
0.20
8.86
4.54
9.34
4.67
8.77
0.35
0.23
23.05
24.14
0.35
0.22
0.43
0.34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162.56
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137.38
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132.64
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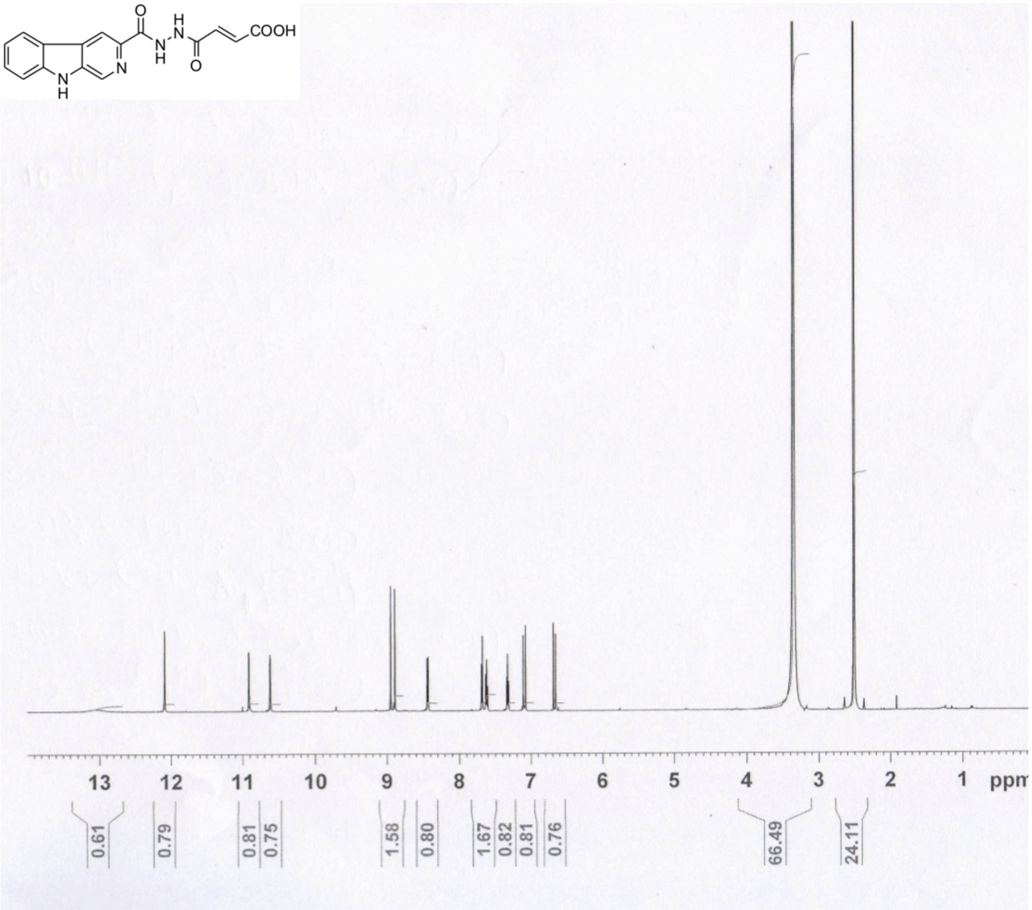
128.05
126.82
122.37
120.90
120.15
114.76
112.34
40.10
40.01
39.93
39.84
39.76
39.67
39.60
39.51
39.34
39.17
39.01
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
COOH
O
N
N
H
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
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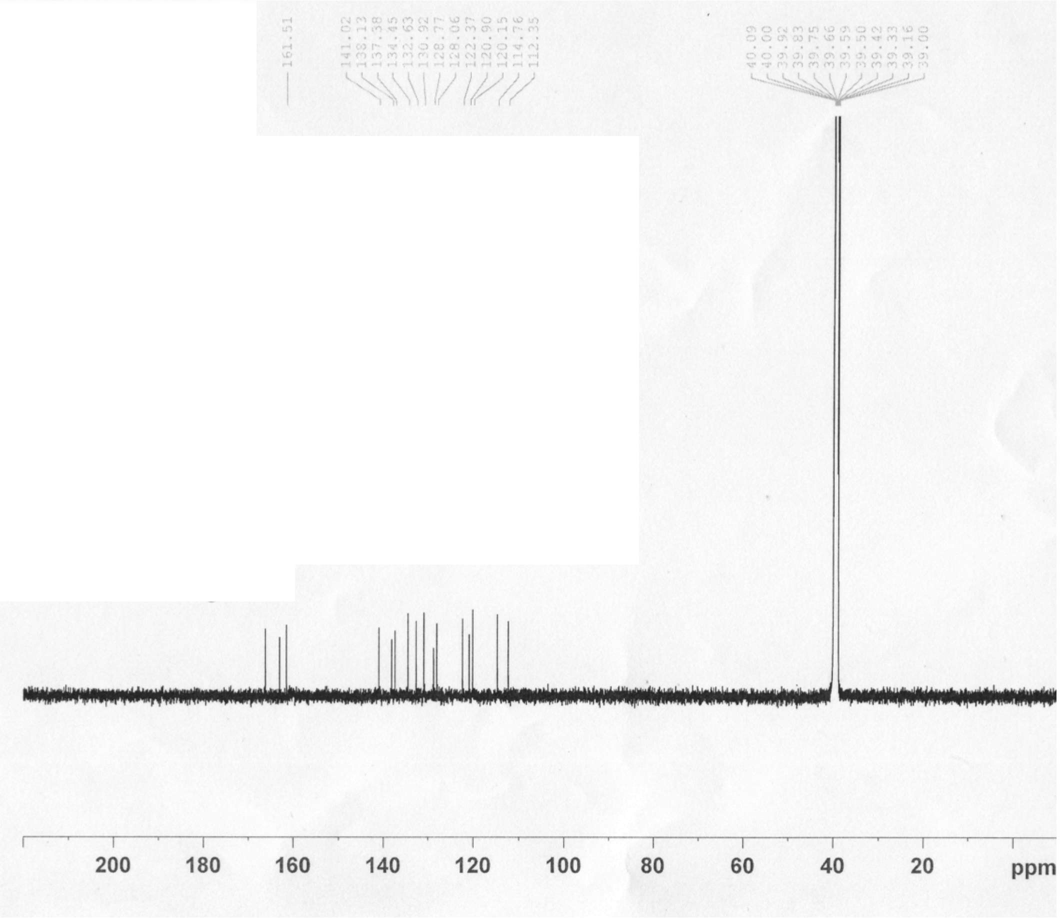
0.79
0.81
0.75
1.58
0.80
1.67
0.82
0.81
0.76
66.49
24.11


161.51
141.02
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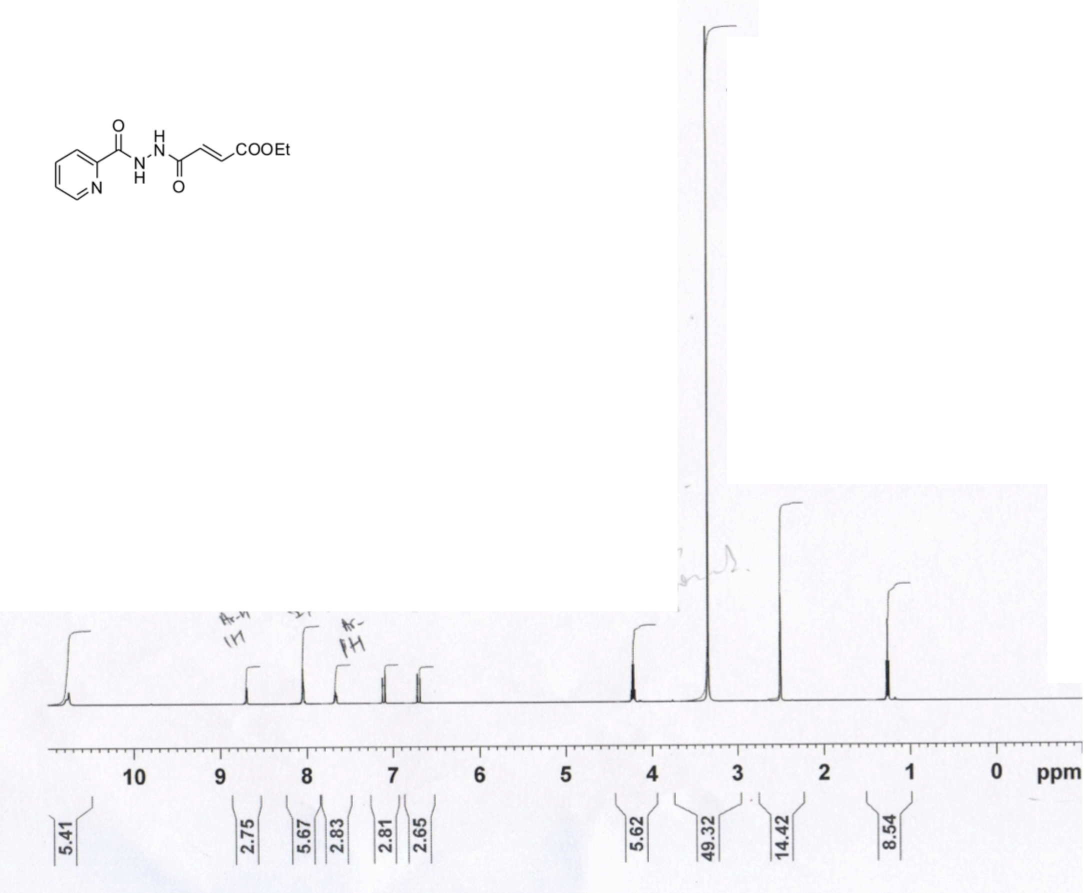
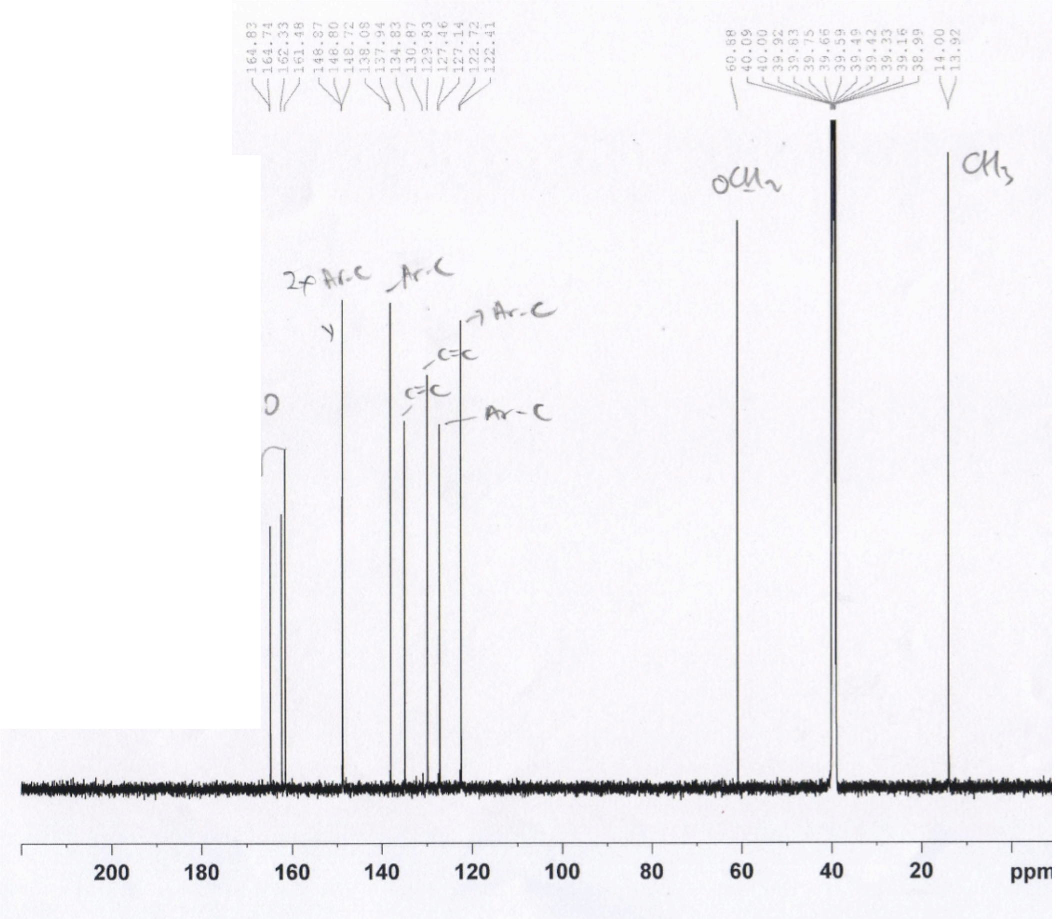
137.38
134.45
132.63
130.92
128.77
128.06
122.37
120.90
120.15
114.76
112.35
40.09
40.00
39.92
39.83
39.75
39.66
39.59
39.50
39.42
39.33
39.16
39.00
200
180
160
140
120
100
80
60
40
20
ppm

COOEt
N
H
O


Ar-H
1H
Ar-
1H
10
9
8
7
6
5
4
3
2
1
0
ppm
5.41
2.75
5.67
2.83
2.81
2.65
5.62
49.32
14.42
8.54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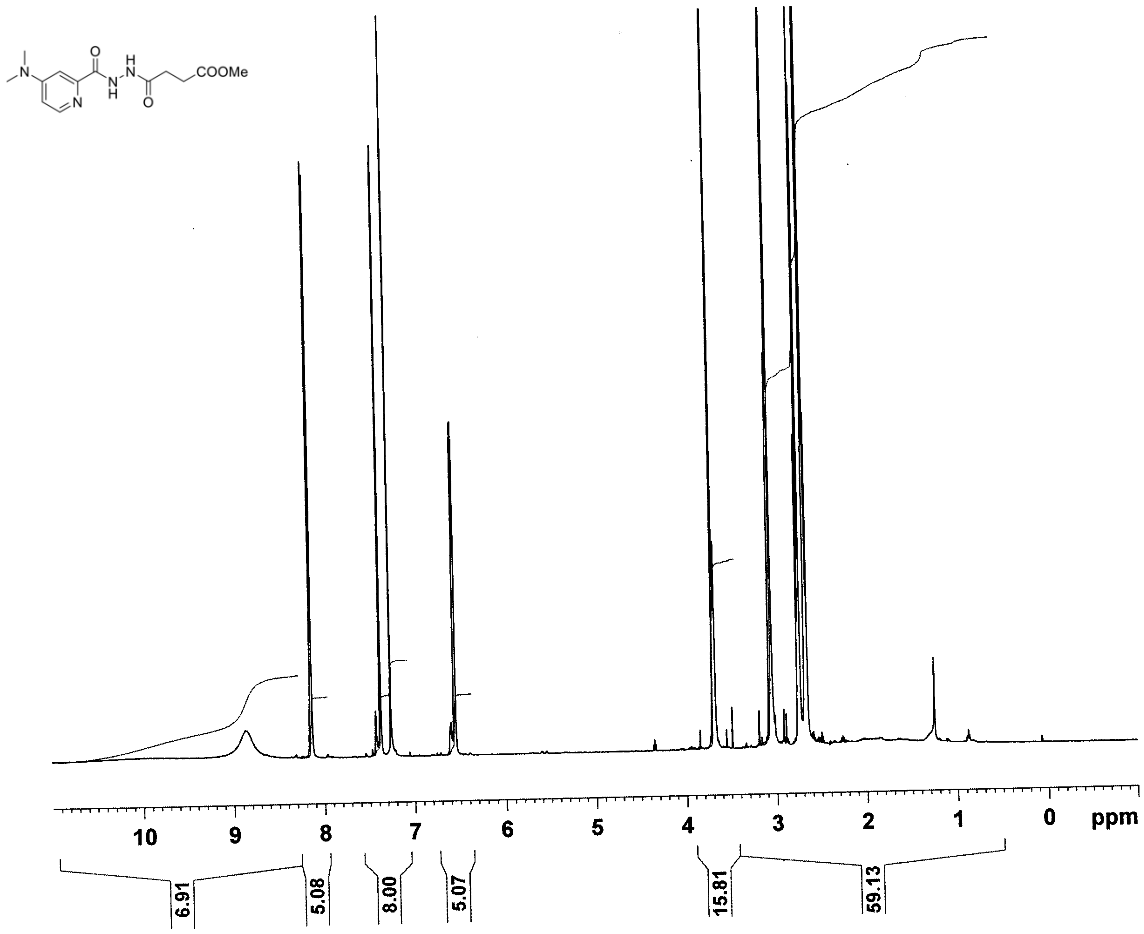
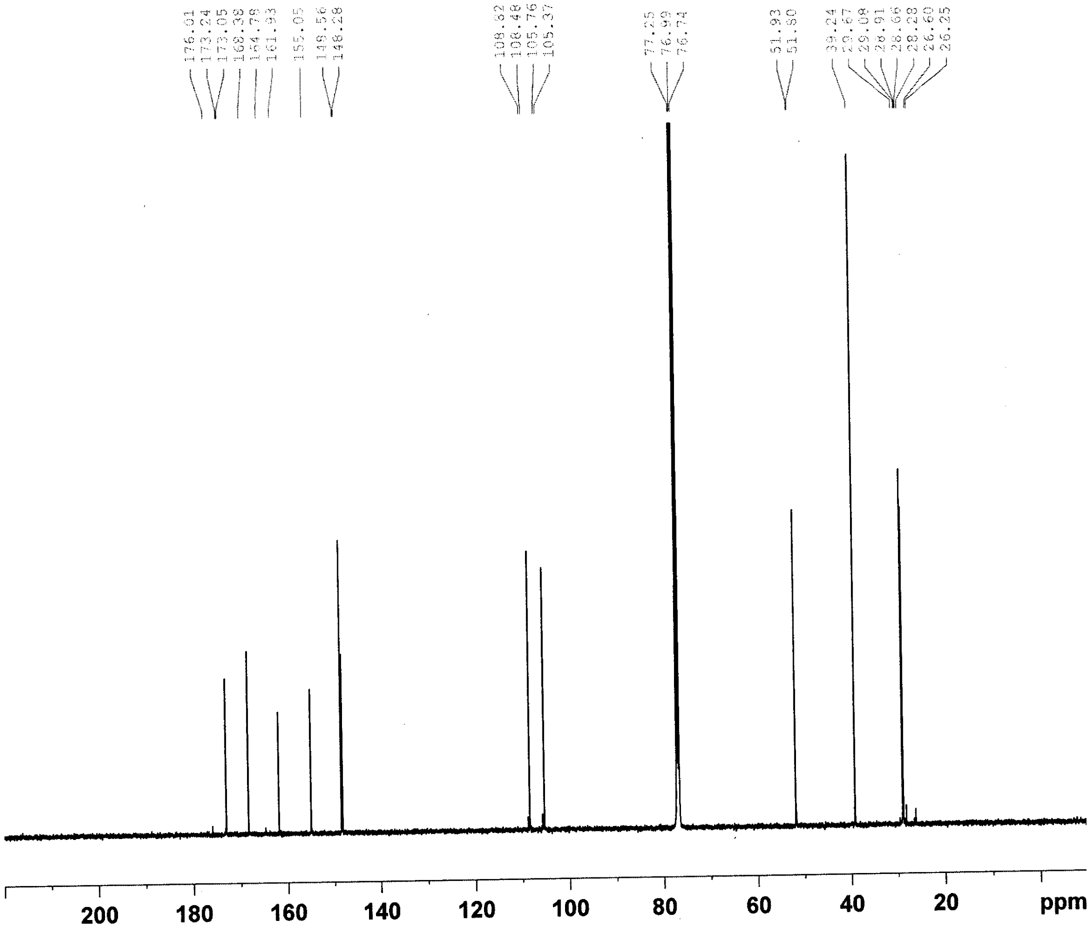
138.08
137.94
134.83
130.87
129.83
127.46
127.14
122.72
122.41
60.88
40.09
40.00
39.92
39.83
39.75
39.66
39.59
39.49
39.42
39.33
39.16
38.99
14.00
13.92
OCH2
CH3
2x Ar-C
Ar-C
Ar-C
C=C
C=C
Ar-C
200
180
160
140
120
100
80
60
40
20
ppm


N
O
H
N
N
H
O
COOMe
N
10
9
8
7
6
5
4
3
2
1
0
ppm
6.91
5.08
8.00
5.07
15.81
59.13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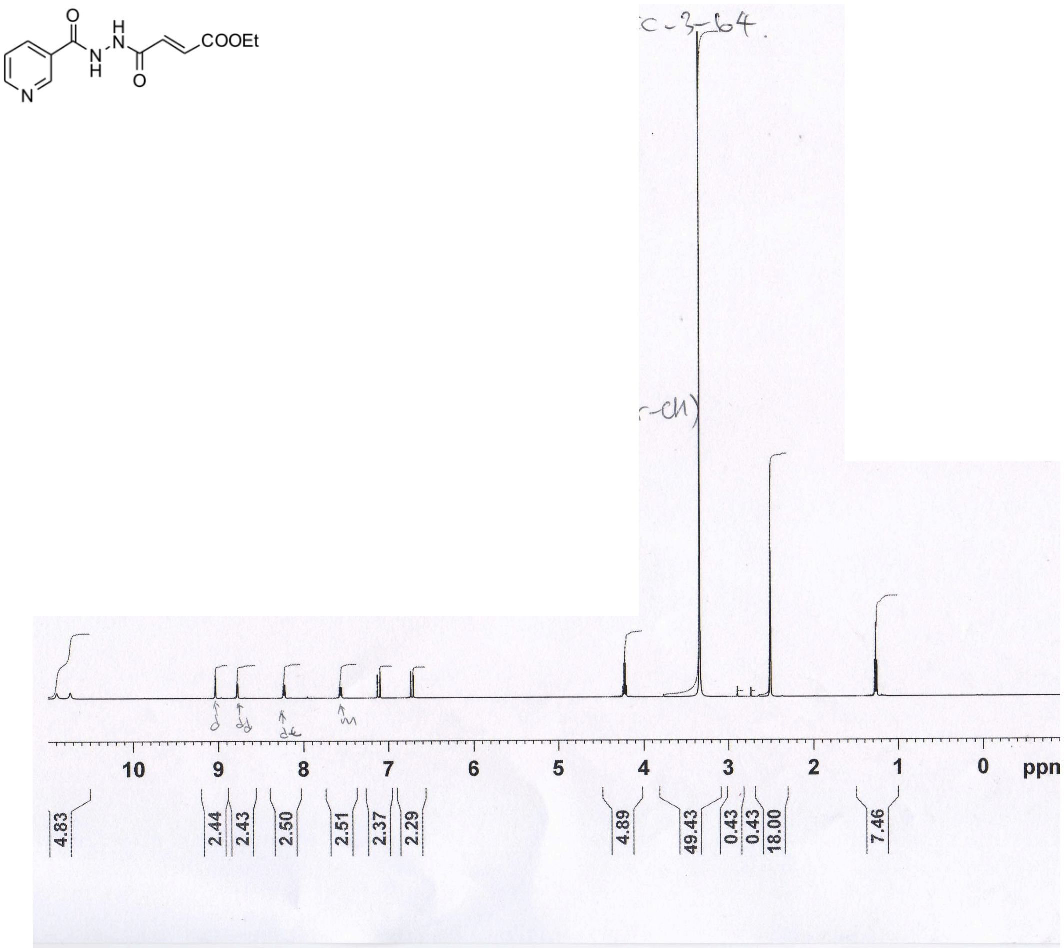
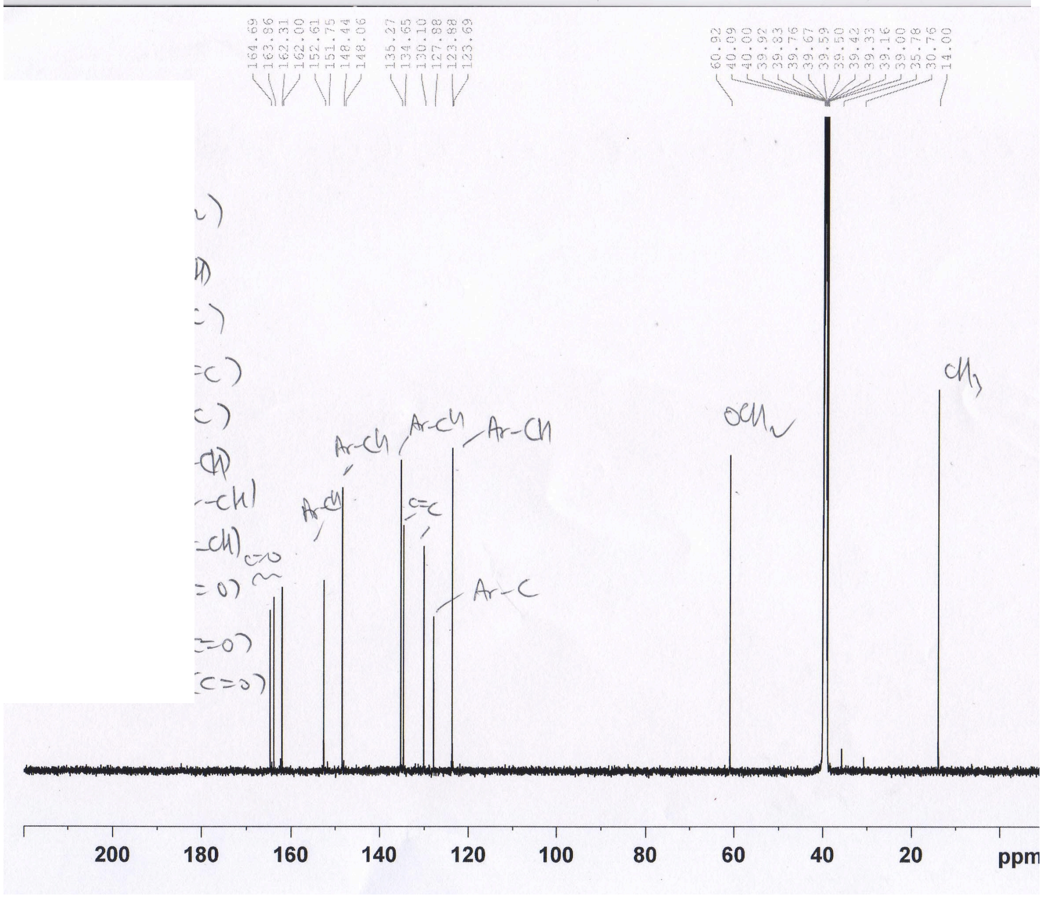
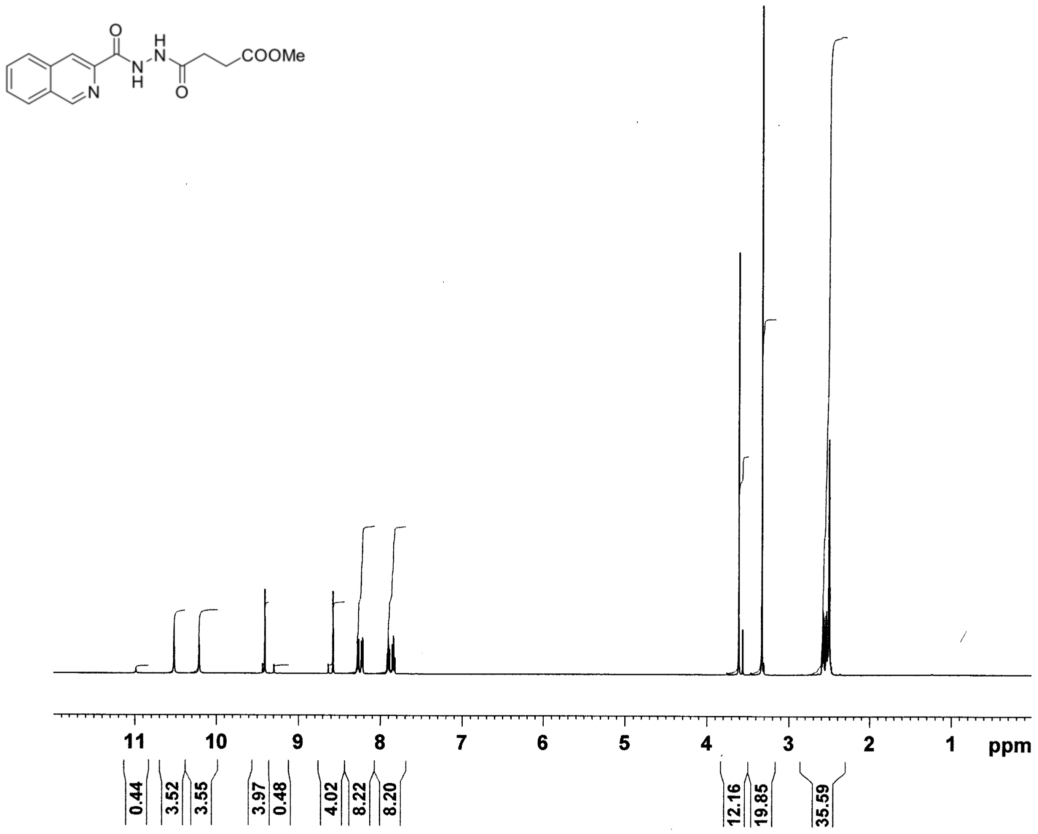
173.24
173.05
168.38
164.78
161.93
155.05
149.56
148.28
108.82
108.48
105.76
105.37
77.25
76.99
76.74
51.93
51.80
39.24
29.67
29.08
28.91
28.66
28.28
26.60
26.25
200
180
160
140
120
100
80
60
40
20
ppm


COOEt
N
H
O
c-3-64.
r-CH)
d
dd
dt
m
10
9
8
7
6
5
4
3
2
1
0
ppm
4.83
2.44
2.43
2.50
2.51
2.37
2.29
4.89
49.43
0.43
0.43
18.00
7.46


164.69
163.86
162.31
162.00
152.61
151.75
148.44
148.06
135.27
134.65
130.10
127.88
123.88
123.69
60.92
40.09
40.00
39.92
39.83
39.76
39.67
39.59
39.50
39.42
39.33
39.16
39.00
35.78
30.76
14.00
C=O
Ar-CH
Ar-CH
Ar-CH
Ar-CH
C=C
Ar-C
OCH2
CH3
200
180
160
140
120
100
80
60
40
20
ppm


COOMe
N
H
O
11
10
9
8
7
6
5
4
3
2
1
ppm
0.44
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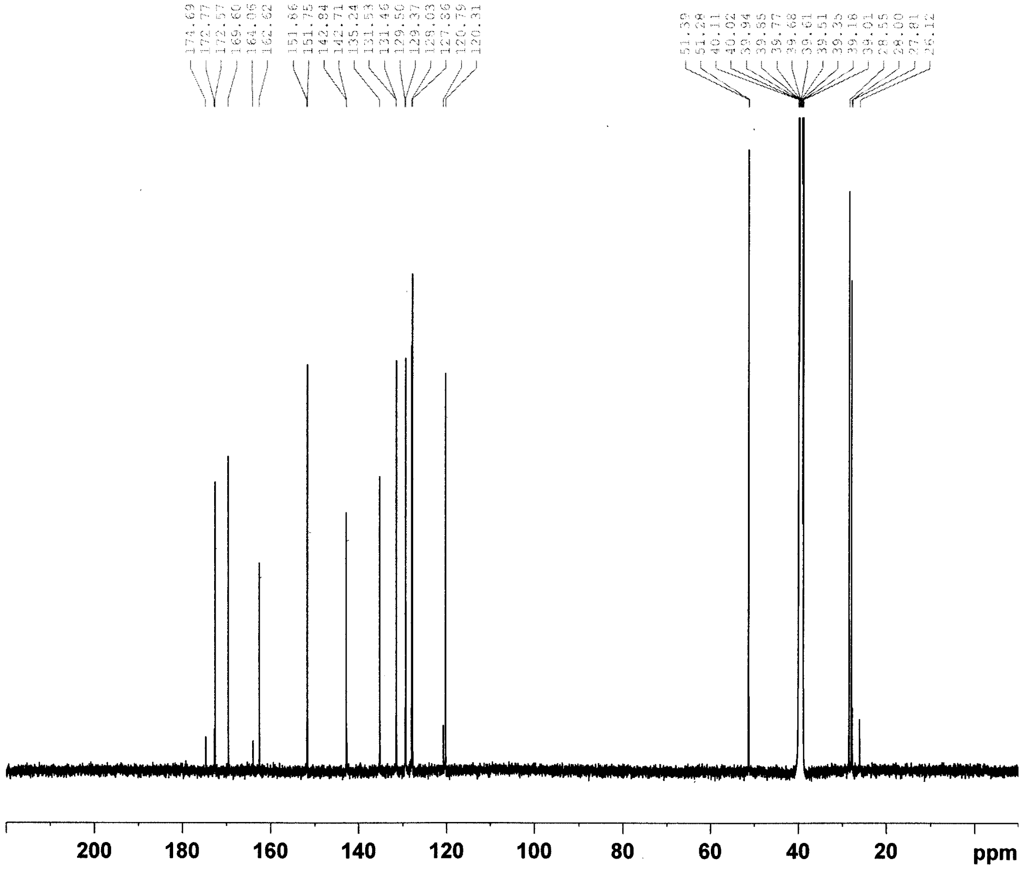
3.55
3.97
0.48
4.02
8.22
8.20
12.16
19.85
35.59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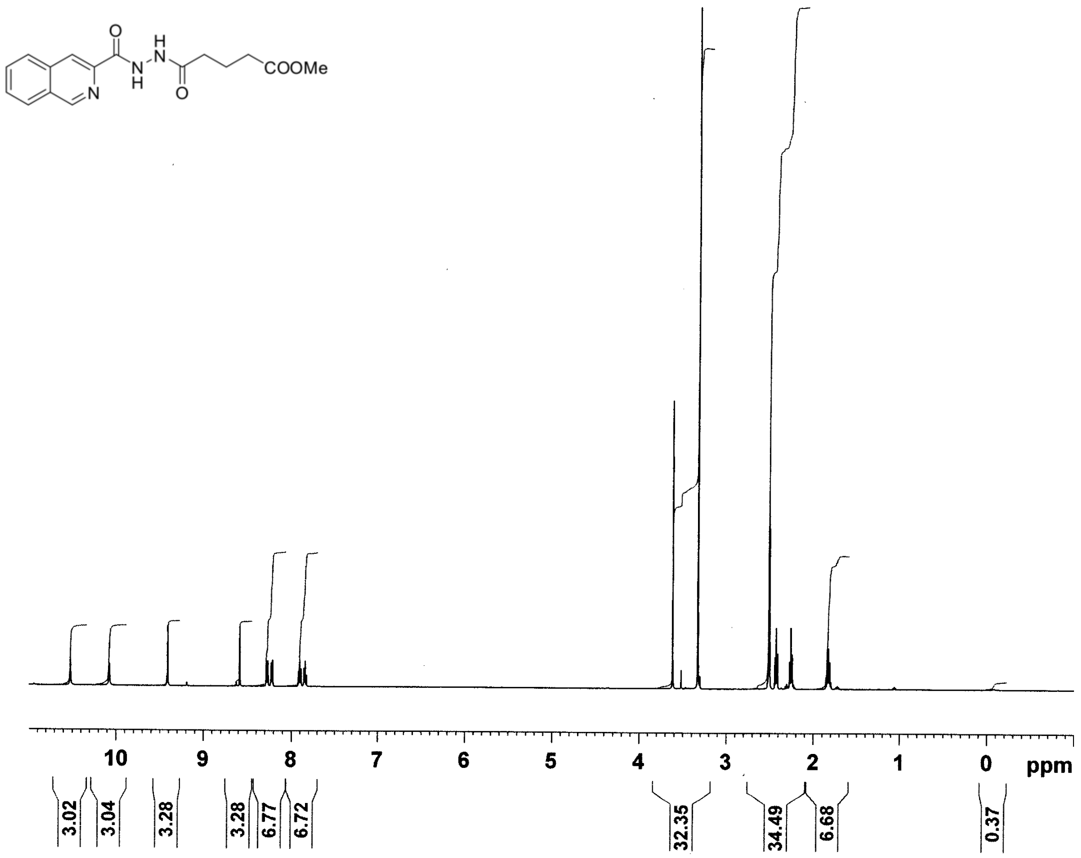
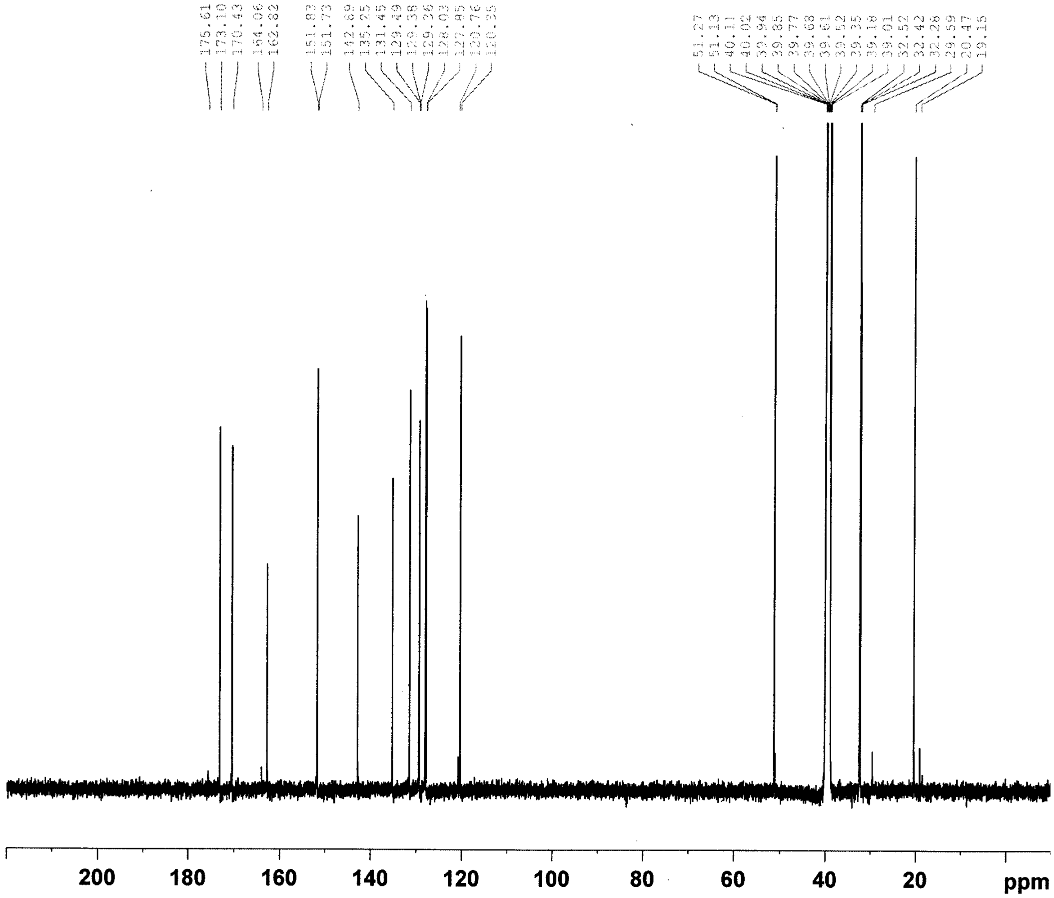
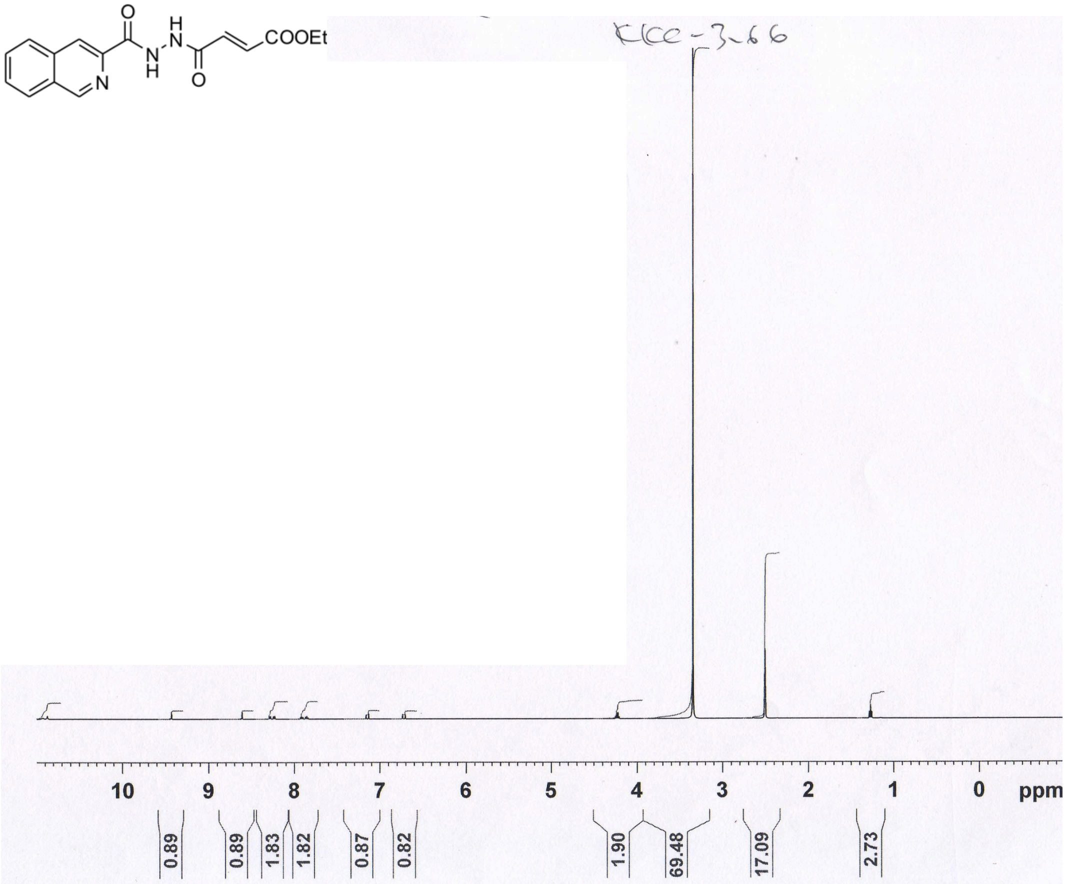
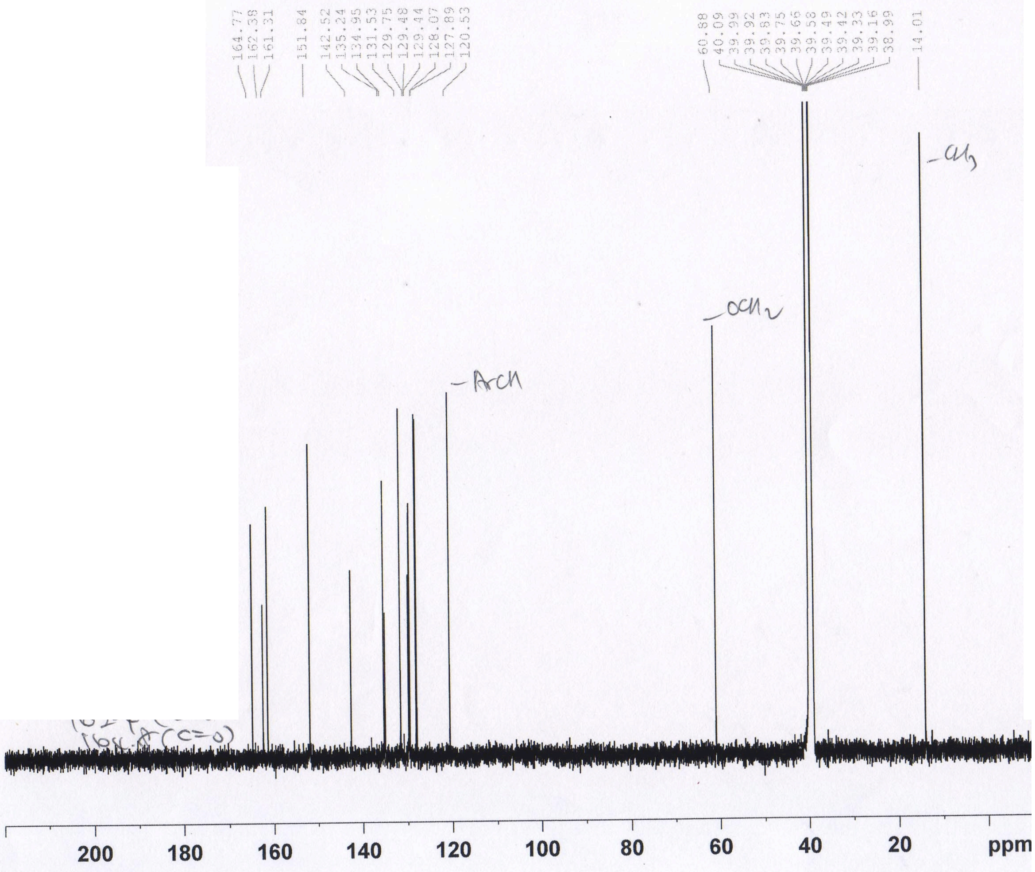
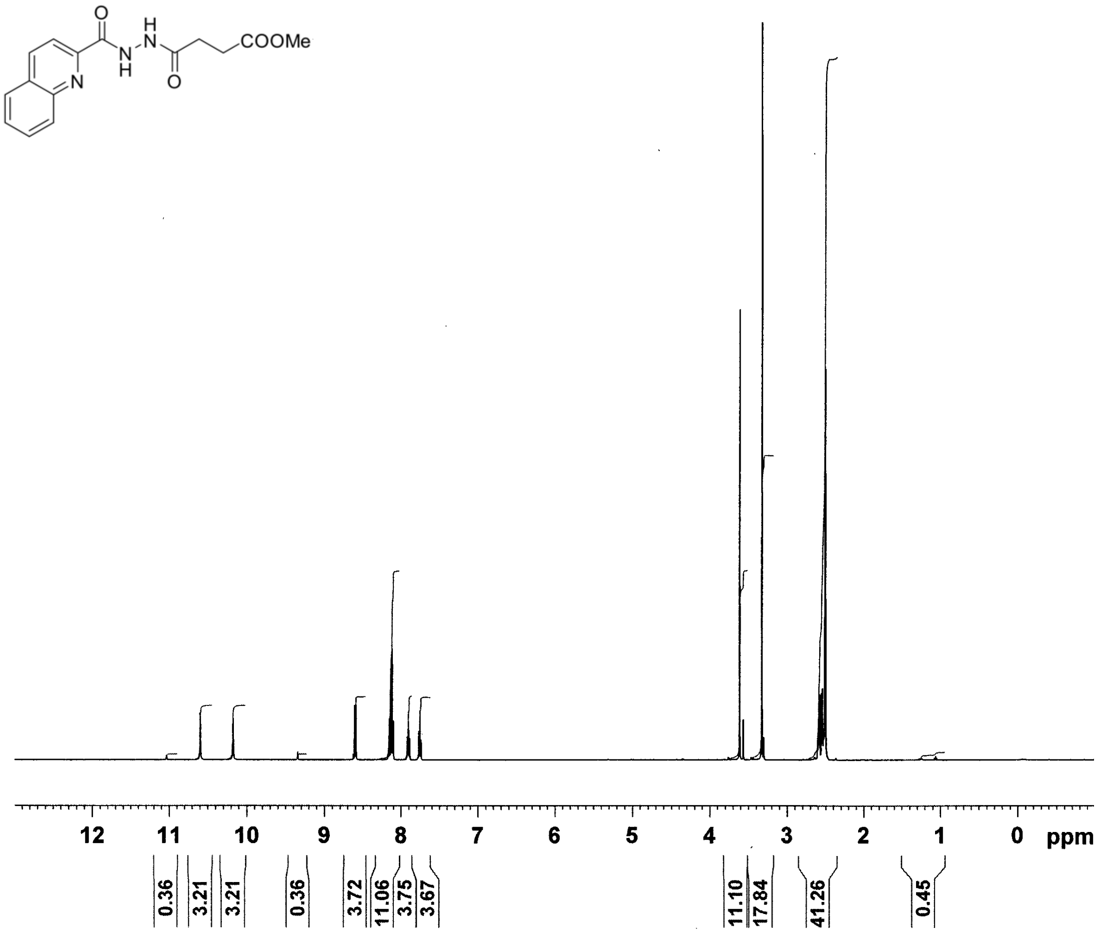
172.77
172.57
169.60
164.06
162.62
151.86
151.75
142.84
142.71
135.24
131.53
131.46
129.50
129.37
129.03
127.86
120.79
120.31
51.39
51.28
40.11
40.02
39.94
39.85
39.77
39.68
39.61
39.51
39.35
39.18
39.01
28.50
28.00
27.81
26.12
200
180
160
140
120
100
80
60
40
20
ppm

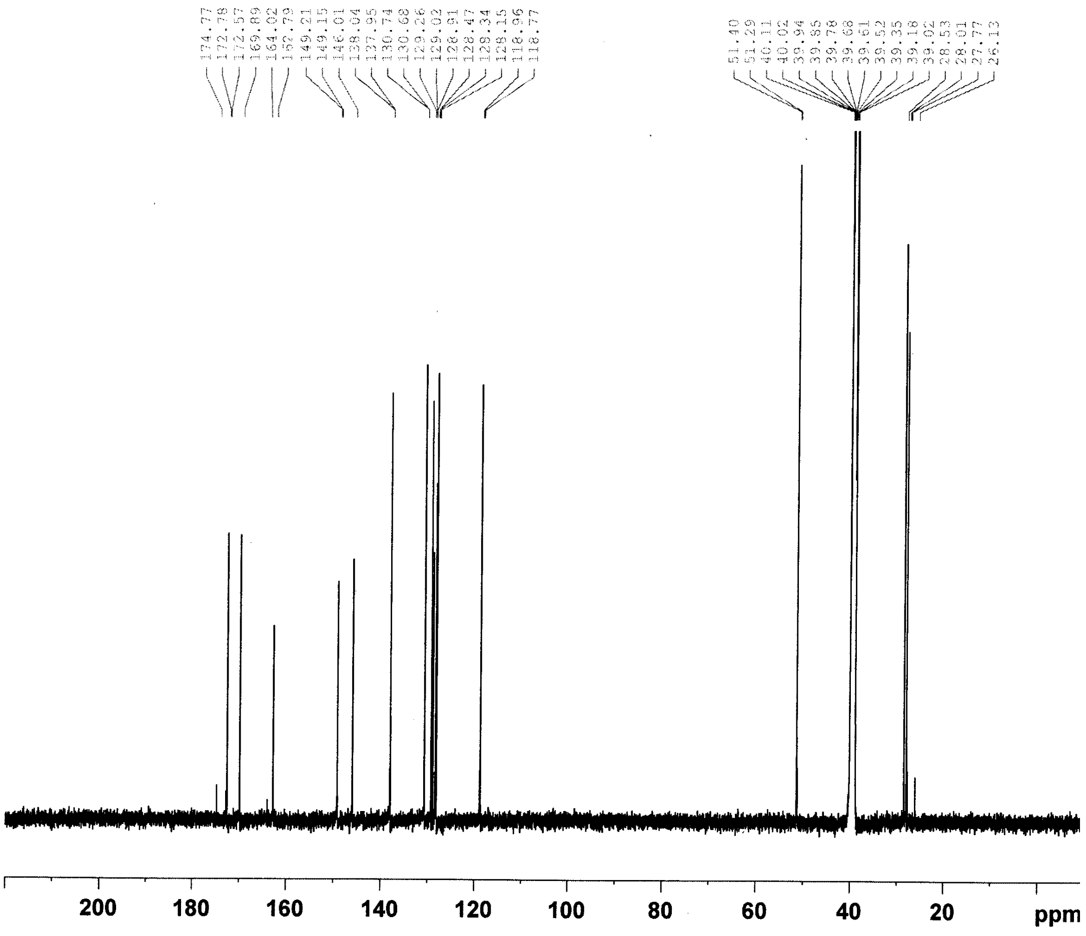

O
H
N
N
H
N
O
COOMe
10
9
8
7
6
5
4
3
2
1
0
ppm
3.02
3.04
3.28
3.28
6.77
6.72
32.35
34.49
6.68
0.37

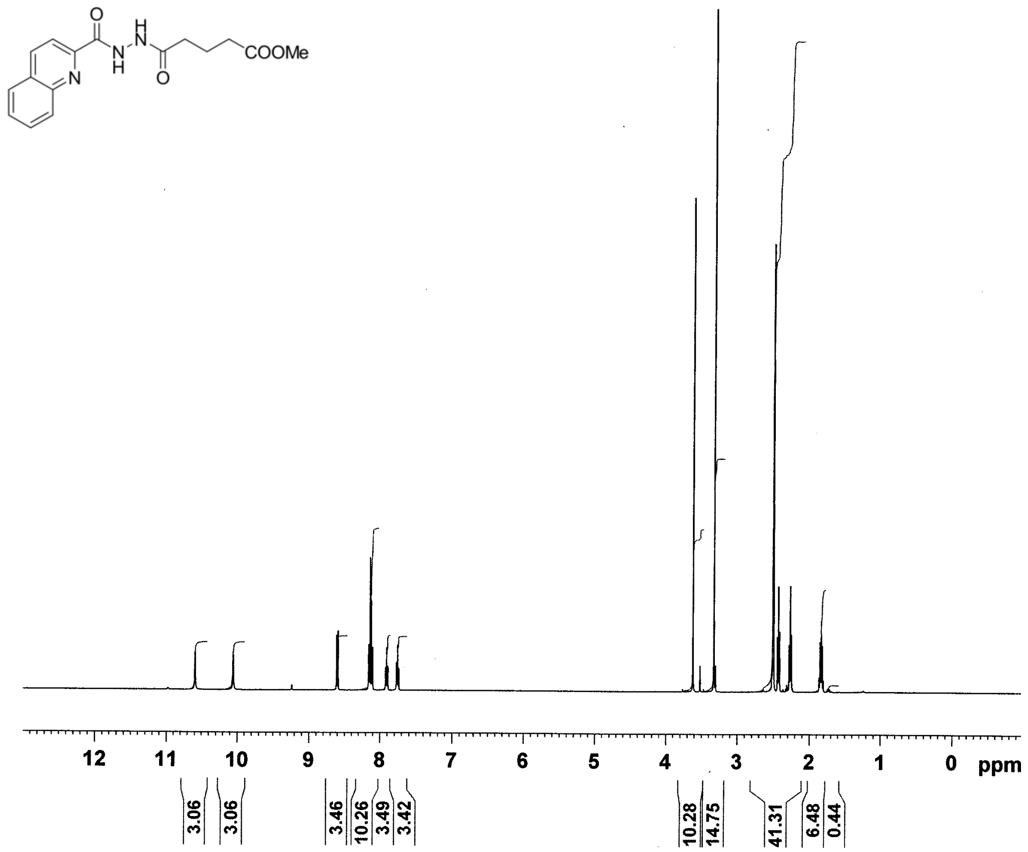

175.61
173.10
170.43
154.06
162.82
151.83
151.73
142.69
135.25
131.45
129.49
129.38
129.36
128.03
127.85
120.76
120.35
51.27
51.13
40.11
40.02
39.94
39.85
39.77
39.68
39.61
39.52
39.35
39.18
39.01
32.52
32.42
32.26
29.59
20.47
19.15
200
180
160
140
120
100
80
60
40
20
ppm

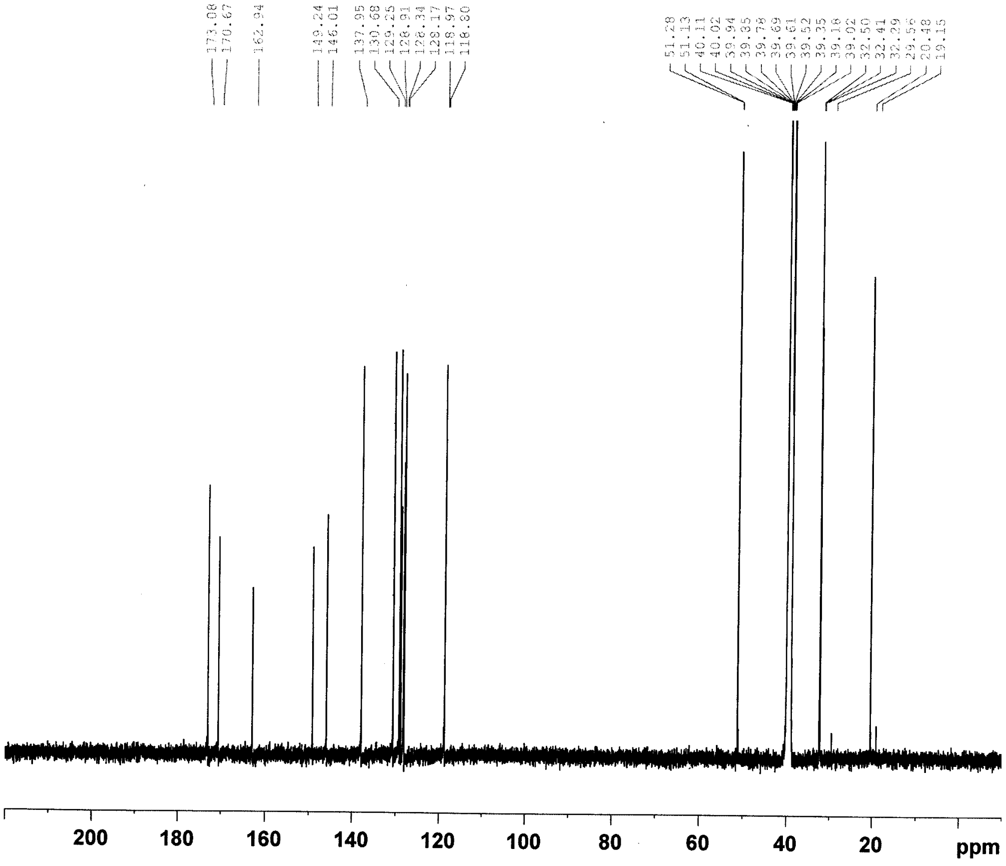

COOEt
N
H
O
KCC-3-66
10
9
8
7
6
5
4
3
2
1
0
ppm
0.89
0.89
1.83
1.82
0.87
0.82
1.90
69.48
17.09
2.73

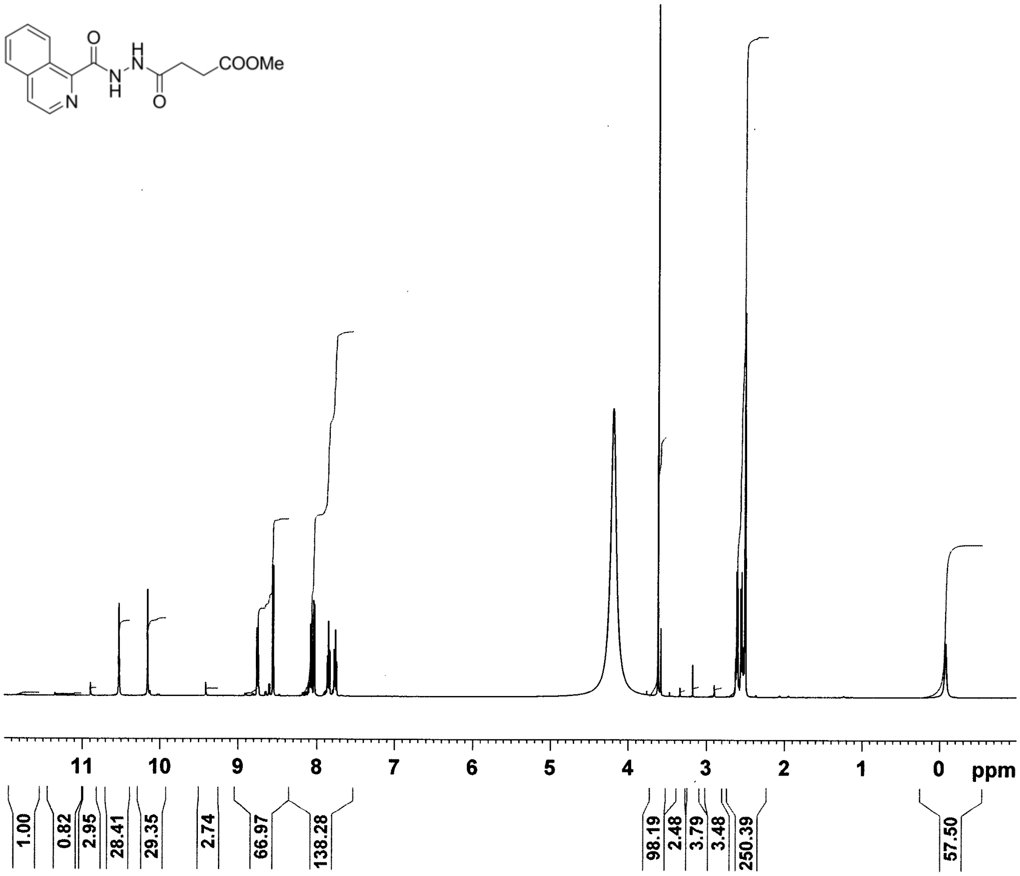

164.77
162.38
161.31
151.84
142.52
135.24
134.95
131.53
129.75
129.48
129.44
128.07
127.89
120.53
60.88
40.09
39.99
39.92
39.83
39.75
39.66
39.58
39.49
39.42
39.33
39.16
38.99
14.01
-CH3
-OCH2
-ArCH
164.8 (C=O)
200
180
160
140
120
100
80
60
40
20
ppm

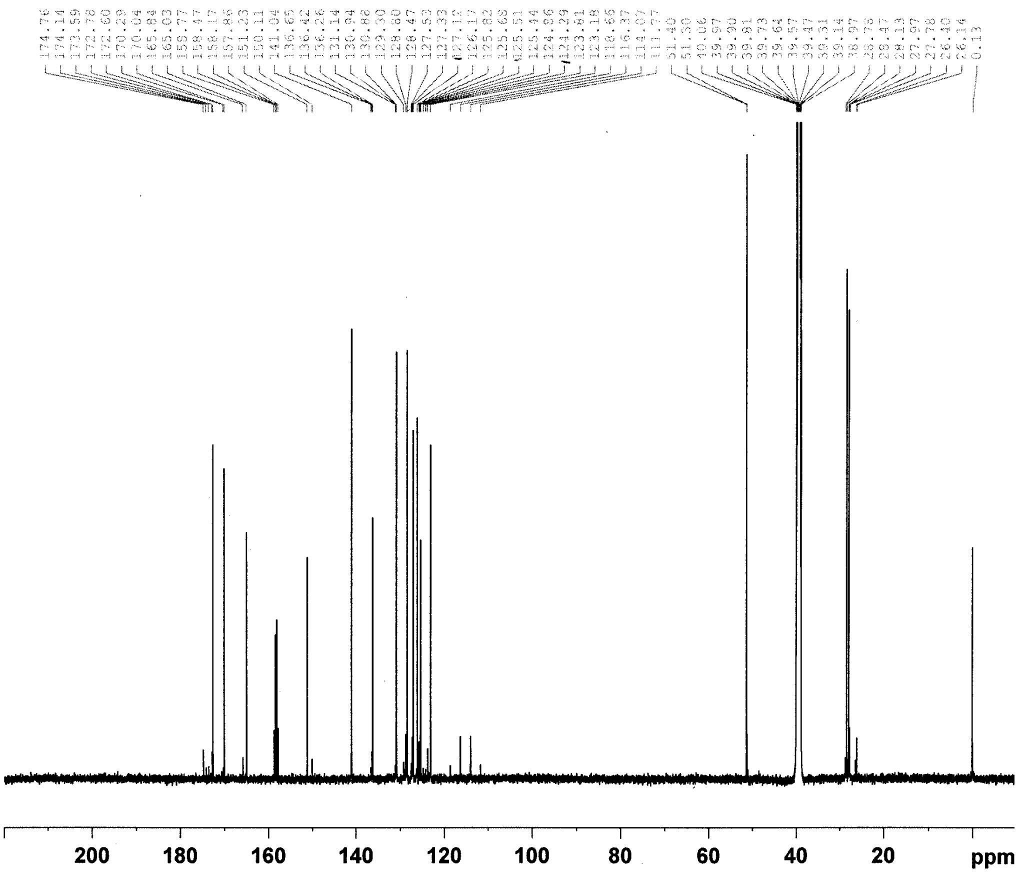

COOMe
N
H
O
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.36
3.21
3.21
0.36
3.72
11.06
3.75
3.67
11.10
17.84
41.26
0.45

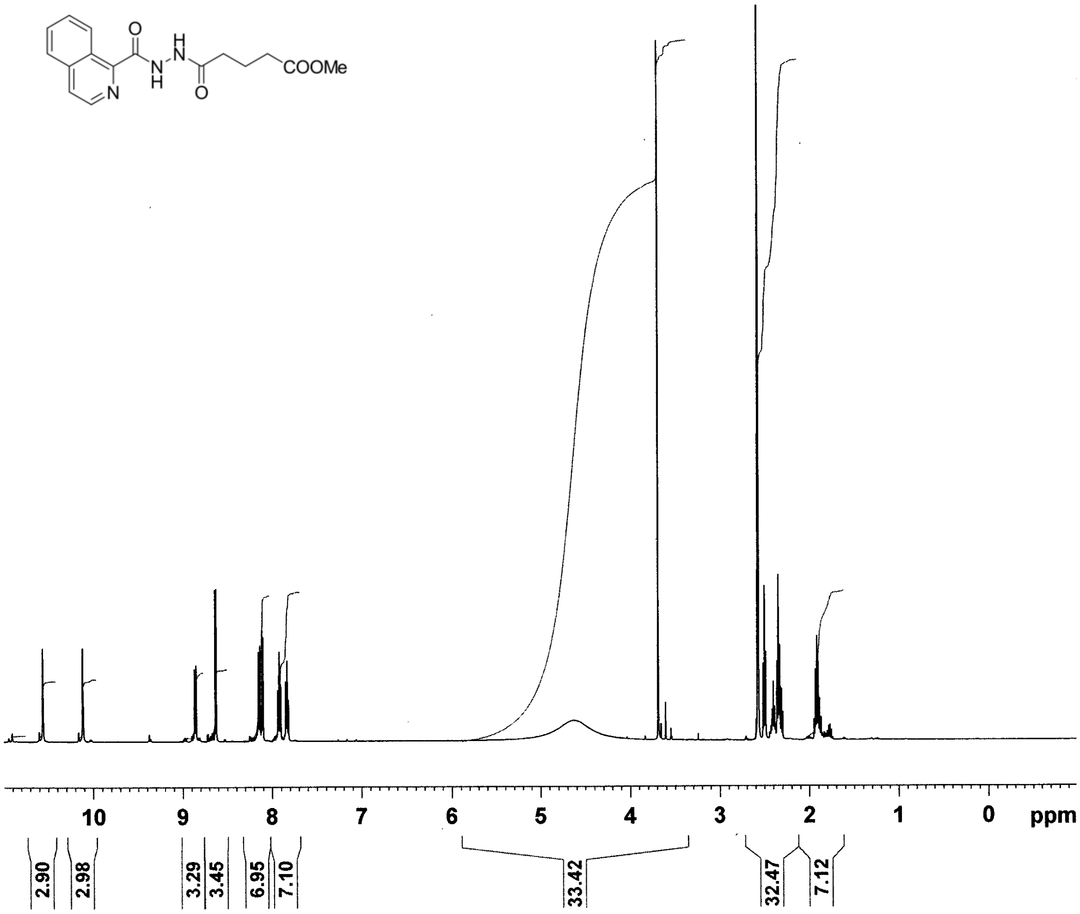

174.77
172.78
172.57
169.89
164.02
162.79
149.21
149.15
146.01
138.04
137.95
130.74
130.68
129.26
129.02
128.91
128.47
128.34
128.15
118.96
118.77
51.40
51.29
40.11
40.02
39.94
39.85
39.78
39.68
39.61
39.52
39.35
39.18
39.02
28.53
28.01
27.77
26.13
200
180
160
140
120
100
80
60
40
20
ppm

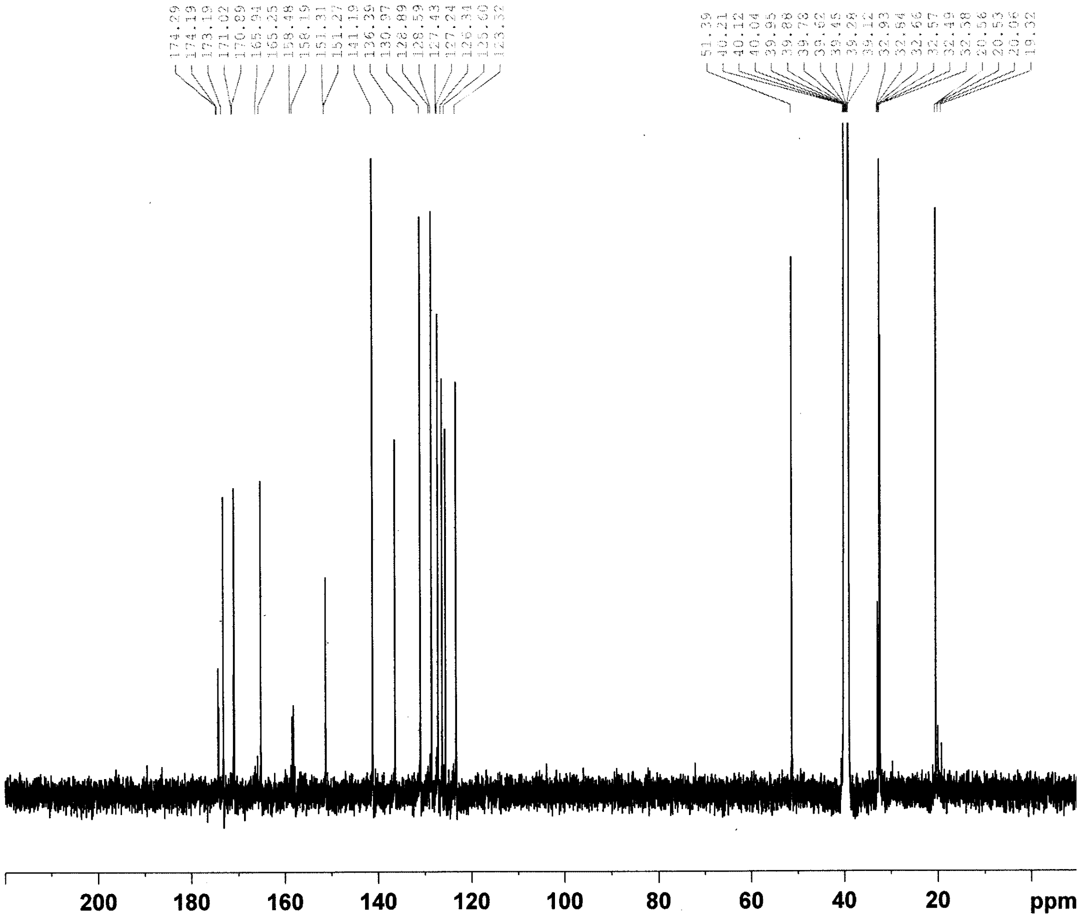

O
H
N
N
H
O
COOMe
N
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
3.06
3.06
3.46
10.26
3.49
3.42
10.28
14.75
41.31
6.48
0.44

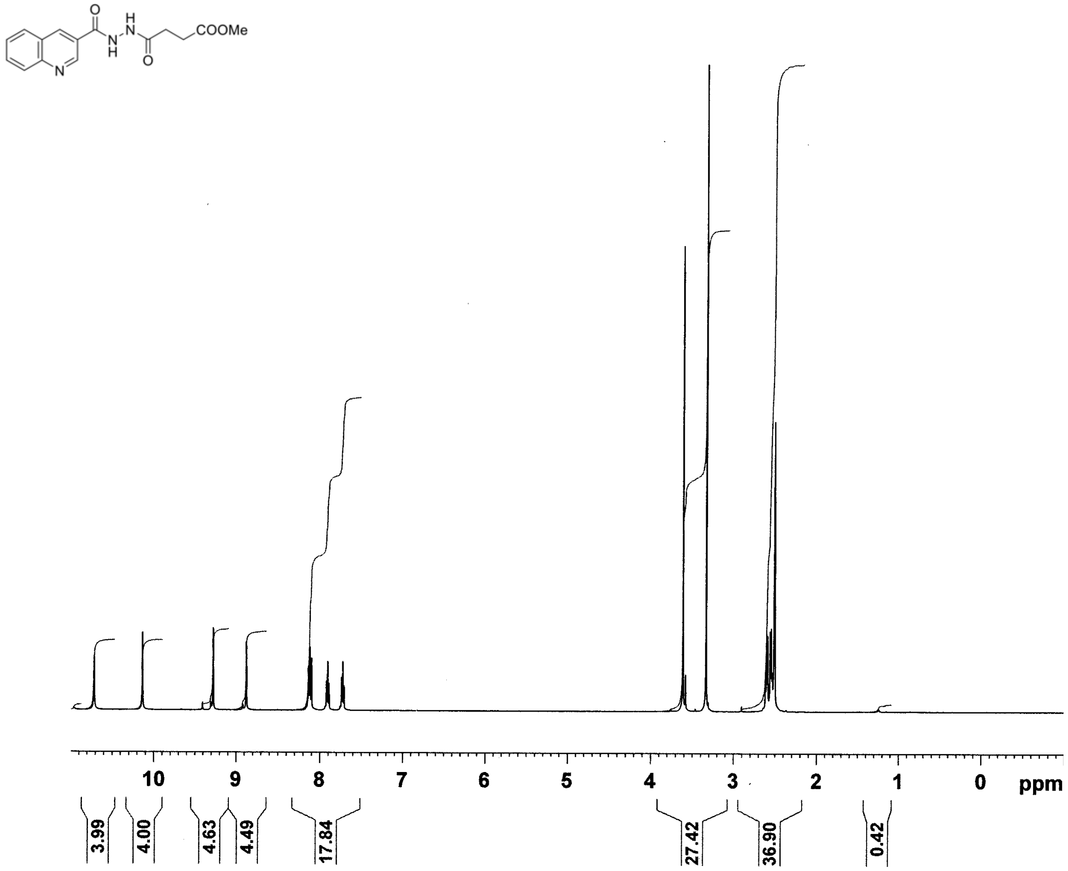

173.08
170.67
162.94
149.24
146.01
137.95
130.68
129.25
128.91
128.34
128.17
118.97
118.80
51.28
51.13
40.11
40.02
39.94
39.85
39.78
39.69
39.61
39.52
39.35
39.18
39.02
32.50
32.41
32.29
29.56
20.48
19.15
200
180
160
140
120
100
80
60
40
20
ppm

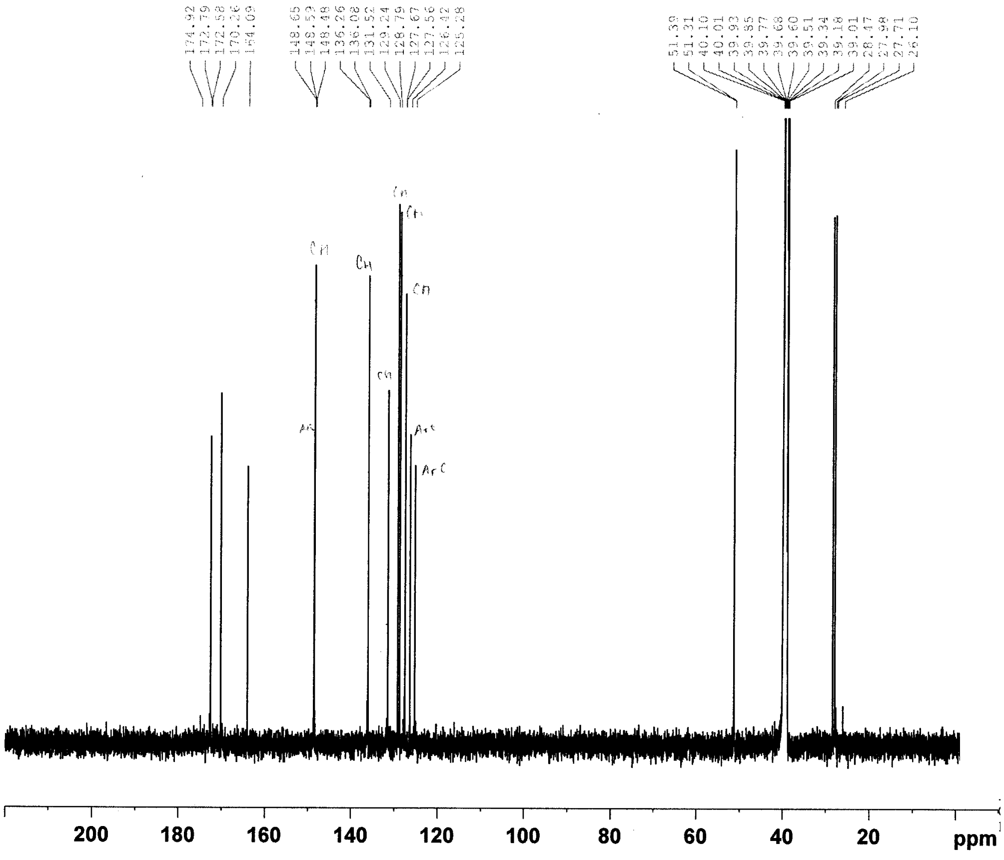

O
H
N
N
H
O
N
COOMe
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.82
2.95
28.41
29.35
2.74
66.97
138.28
98.19
2.48
3.79
3.48
250.39
57.50


174.76
174.14
173.59
172.78
172.60
170.29
170.04
165.84
165.03
158.77
158.47
158.17
157.86
151.23
150.11
141.04
136.65
136.42
136.26
131.14
130.94
130.88
129.30
128.80
128.47
127.53
127.33
127.12
126.17
125.82
125.68
125.51
125.44
124.86
124.29
123.81
123.18
118.66
116.37
114.07
111.77
51.40
51.30
40.06
39.97
39.90
39.81
39.73
39.64
39.57
39.47
39.31
39.14
38.97
28.76
28.47
28.13
27.97
27.78
26.40
26.14
0.13
200
180
160
140
120
100
80
60
40
20
ppm


COOMe
N
H
O
10
9
8
7
6
5
4
3
2
1
0
ppm
2.90
2.98
3.29
3.45
6.95
7.10
33.42
32.47
7.12


174.29
174.19
173.19
171.02
170.89
165.94
165.25
158.48
158.19
151.31
151.27
141.19
136.39
130.97
128.89
128.59
127.43
127.24
126.34
125.60
123.32
51.39
40.21
40.12
40.04
39.95
39.88
39.78
39.62
39.45
39.28
39.12
32.93
32.84
32.67
32.57
32.49
32.38
20.56
20.53
20.06
19.32
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
COOMe
N
10
9
8
7
6
5
4
3
2
1
0
ppm
3.99
4.00
4.63
4.49
17.84
27.42
36.90
0.42


174.92
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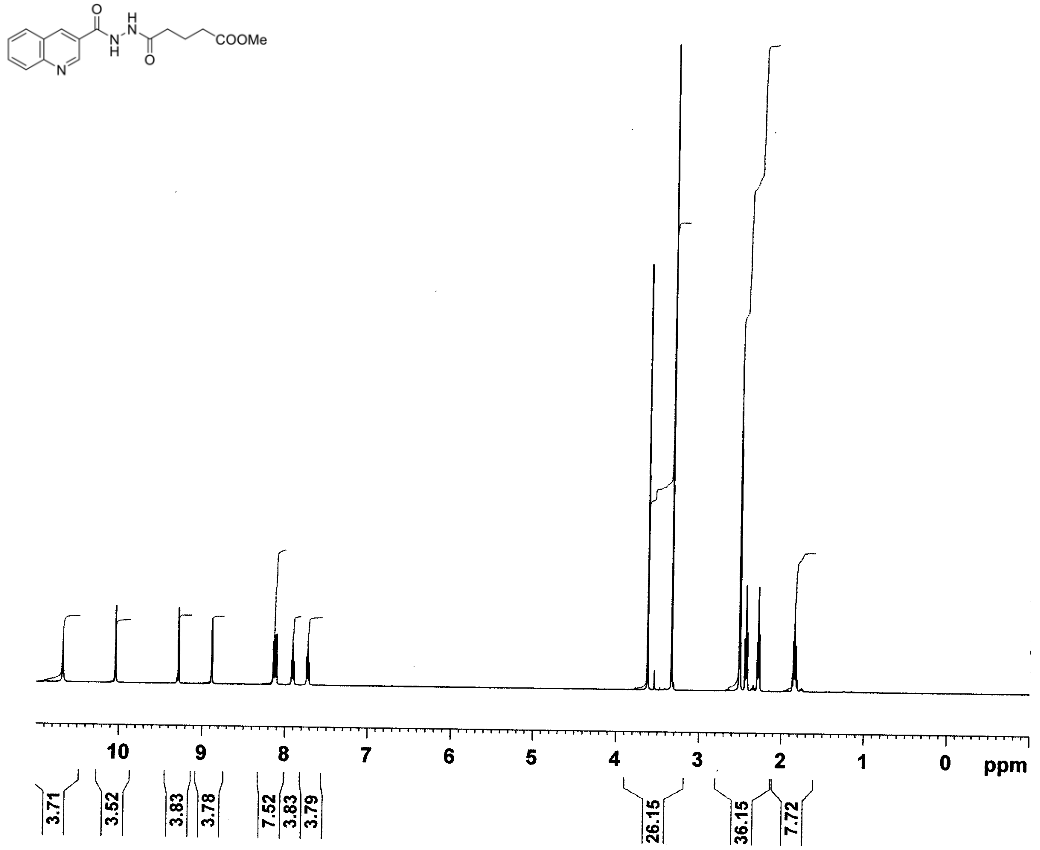
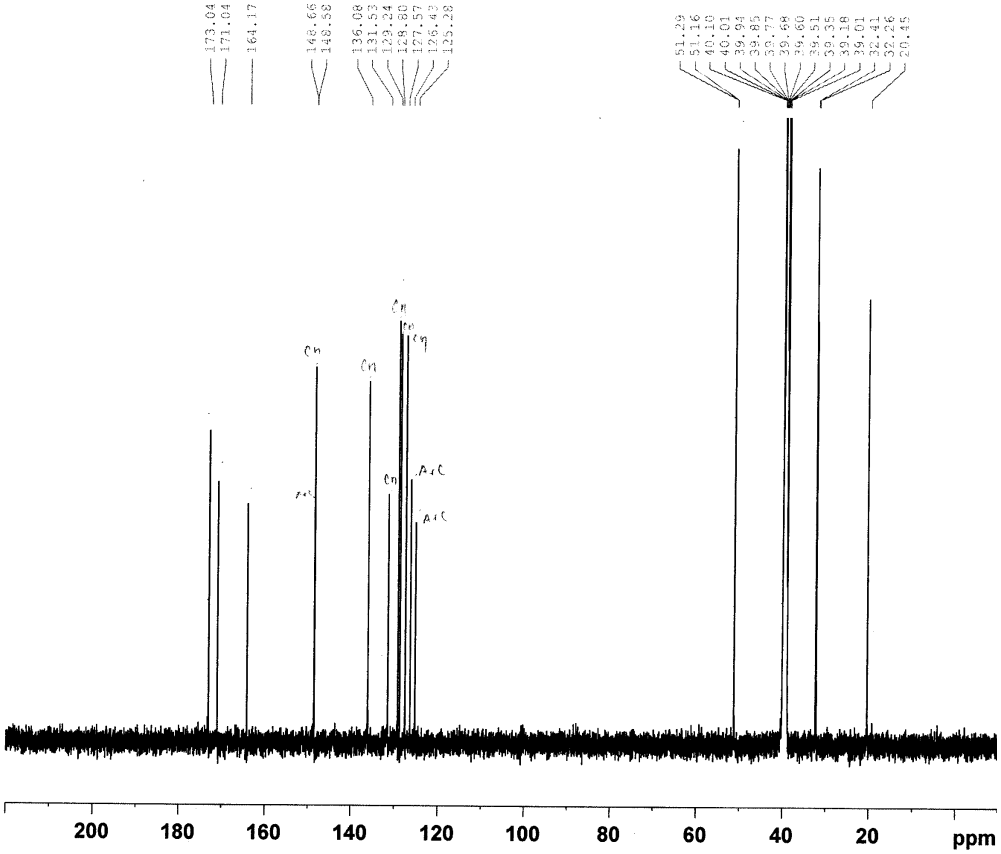
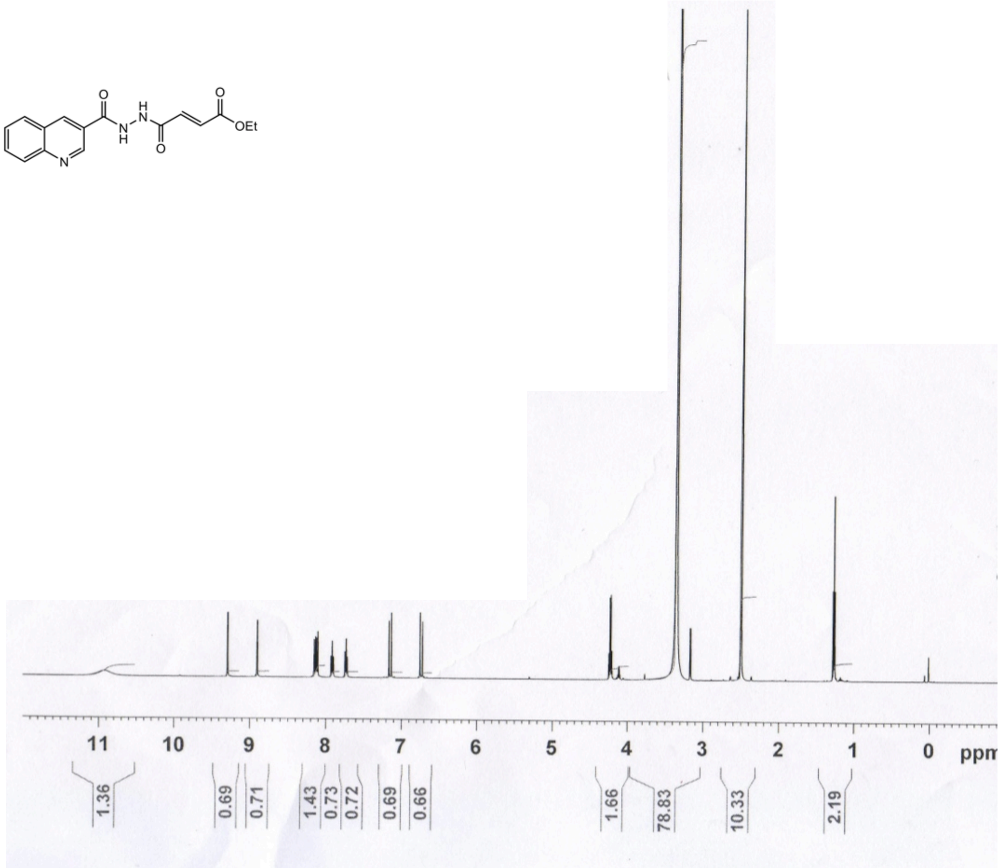
172.58
170.26
164.09
148.65
148.53
148.48
136.26
136.08
131.52
129.24
128.79
127.67
127.56
126.42
125.28
51.39
51.31
40.10
40.01
39.93
39.85
39.77
39.68
39.60
39.51
39.34
39.18
39.01
28.47
27.98
27.71
26.10
CH
CH
CH
CH
CH
CH
Ar
ArC
ArC
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
N
COOMe
10
9
8
7
6
5
4
3
2
1
0
ppm
3.71
3.52
3.83
3.78
7.52
3.83
3.79
26.15
36.15
7.72


173.04
171.04
164.17
148.66
148.58
136.08
131.53
129.24
128.80
127.57
126.43
125.28
51.29
51.16
40.10
40.01
39.94
39.85
39.77
39.68
39.60
39.51
39.35
39.18
39.01
32.41
32.26
20.45
CH
CH
CH
CH
CH
CH
ArC
ArC
ArC
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
O
OEt
N
11
10
9
8
7
6
5
4
3
2
1
0
ppm
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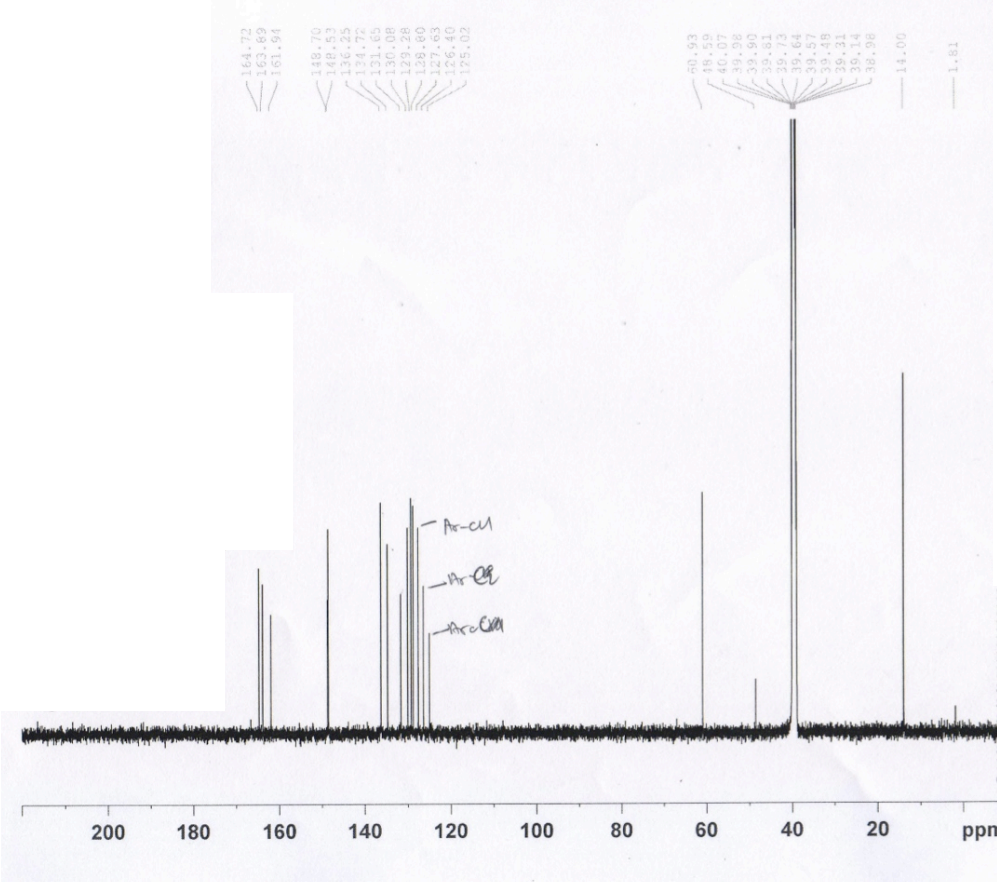
0.69
0.71
1.43
0.73
0.72
0.69
0.66
1.66
78.83
10.33
2.19


164.72
163.89
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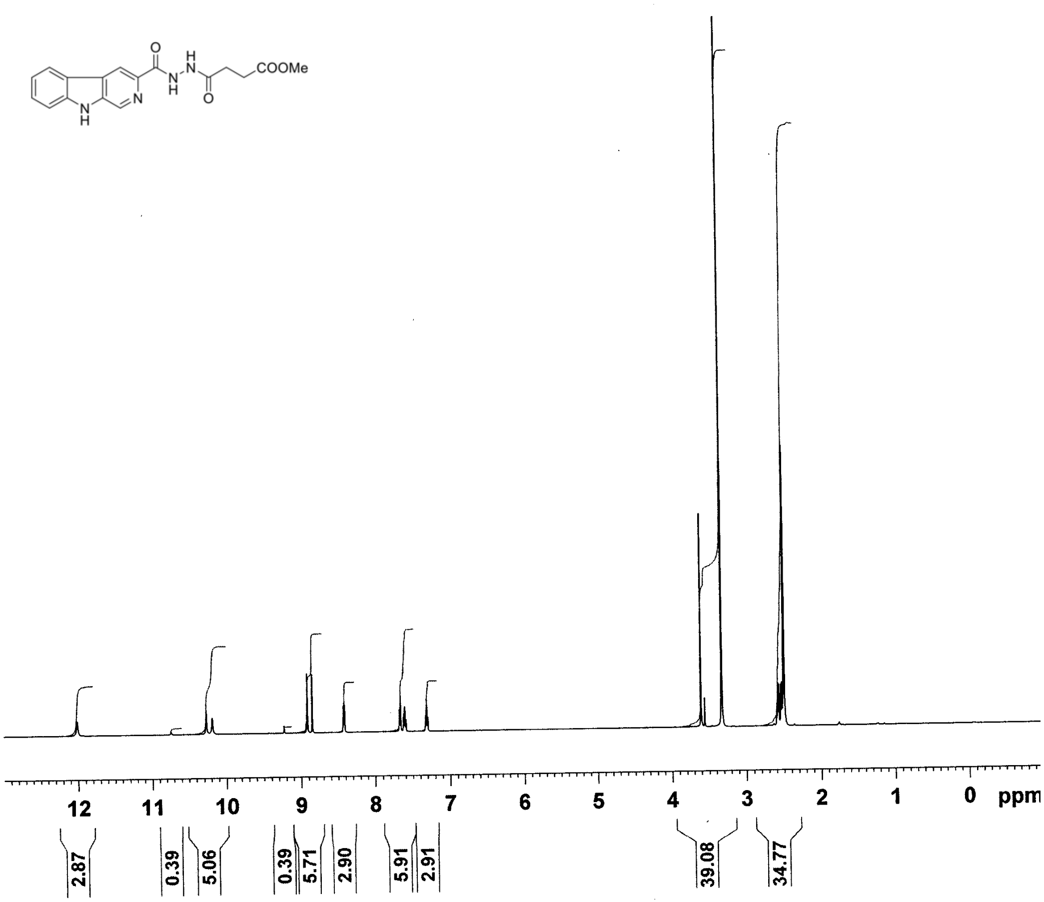
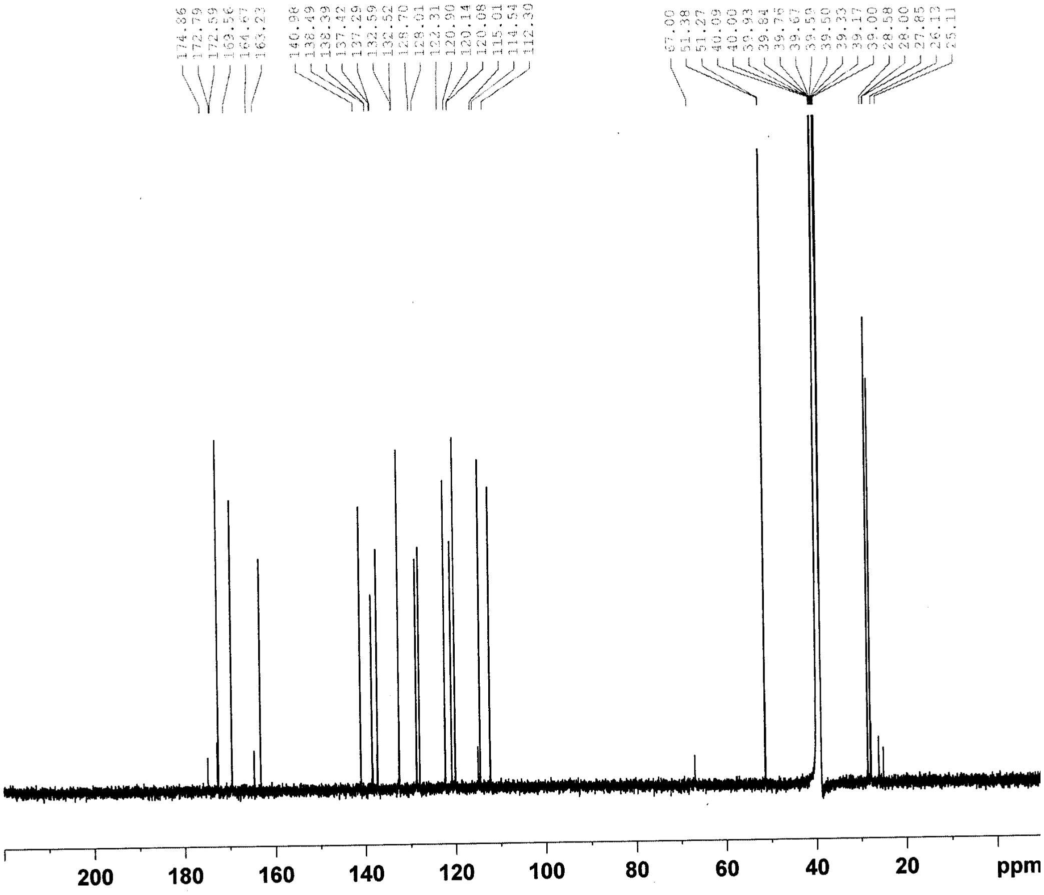
148.70
148.53
136.25
134.72
131.65
130.08
129.28
128.80
127.63
126.40
125.02
60.93
48.59
40.07
39.98
39.90
39.81
39.73
39.64
39.57
39.48
39.31
39.14
38.98
14.00
1.81
Ar-CH
Ar-CH
Ar-CH
200
180
160
140
120
100
80
60
40
20
ppm


O
H
N
N
H
O
COOMe
N
H
N
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
2.87
0.39
5.06
0.39
5.71
2.90
5.91
2.91
39.08
34.77


174.86
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169.56
164.67
163.23
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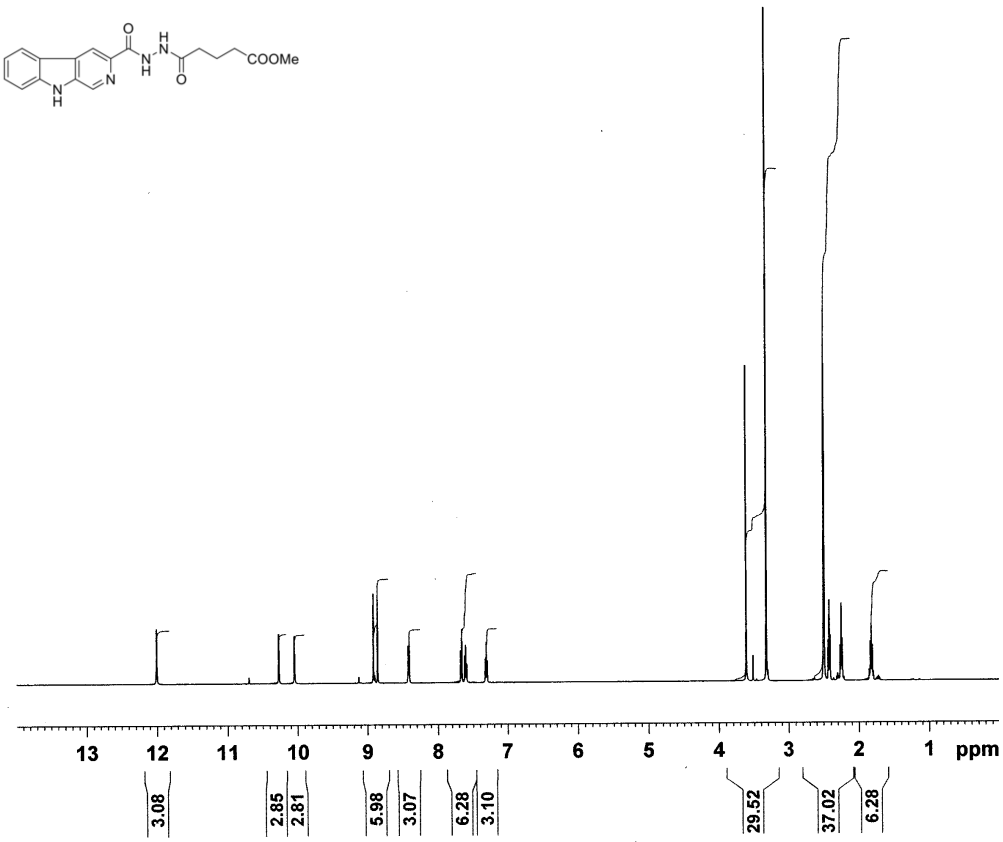
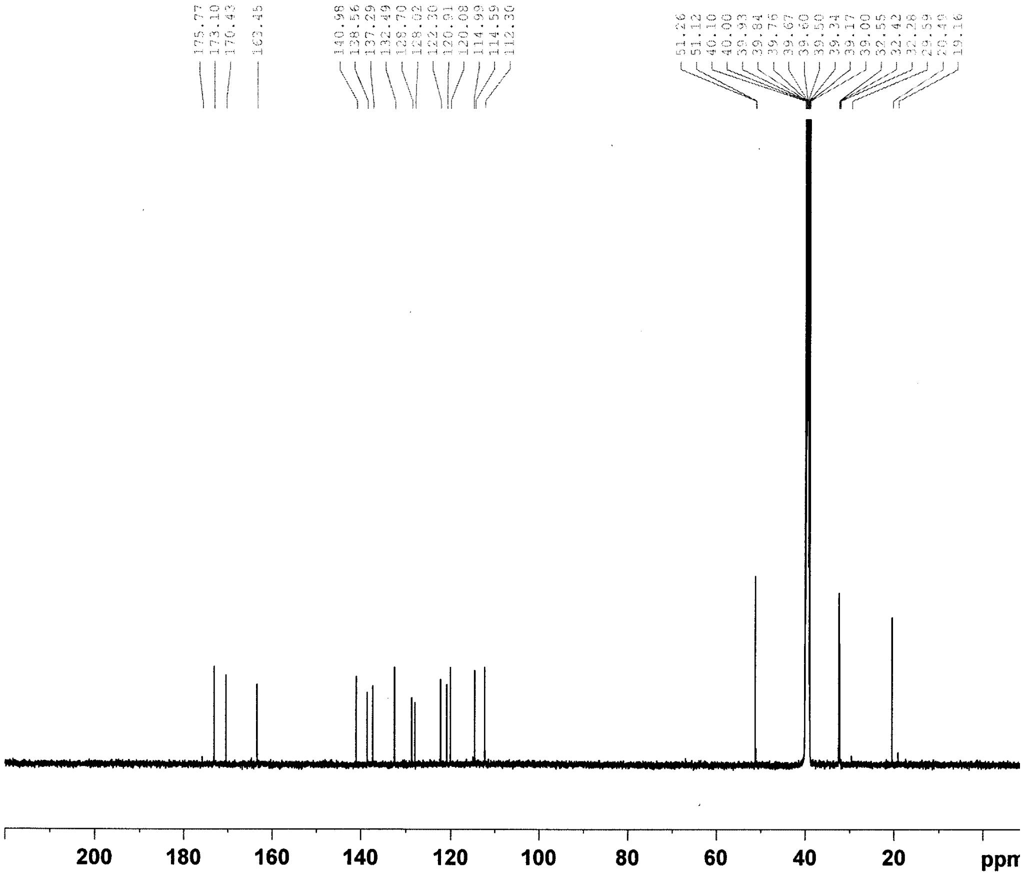
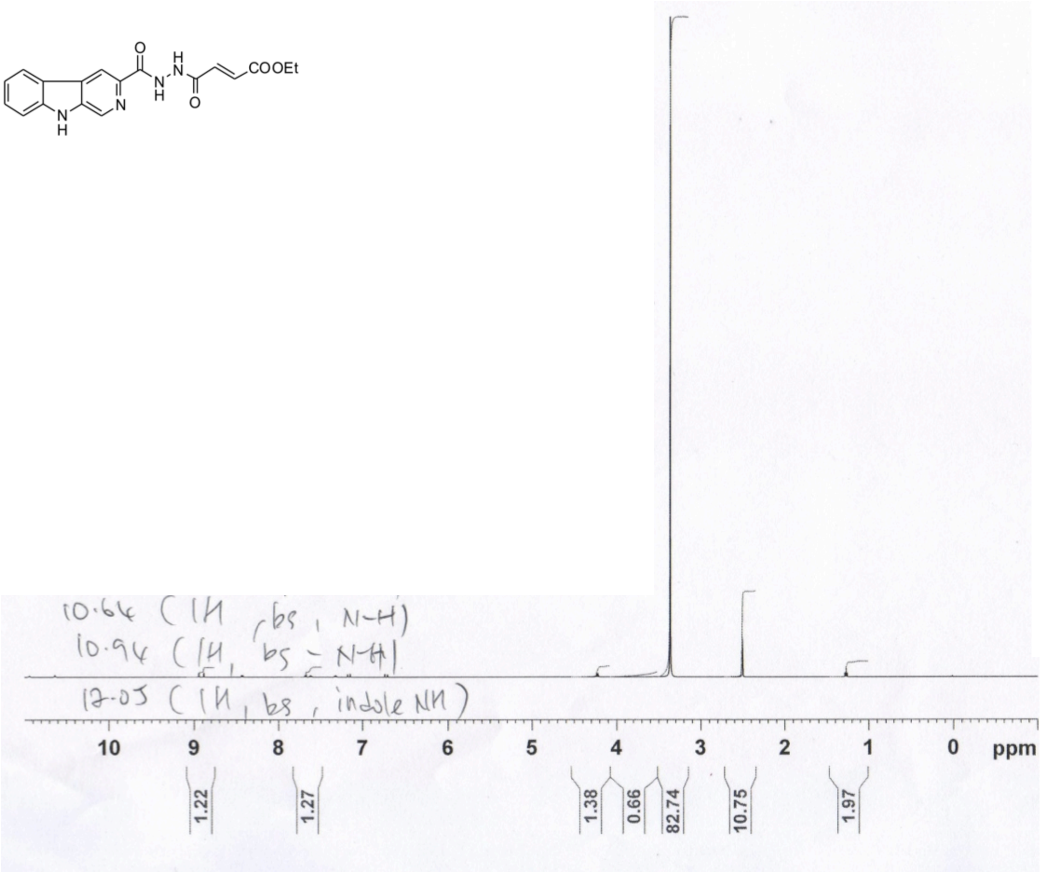
138.49
138.39
137.42
137.29
132.59
132.52
128.70
128.01
122.31
120.90
120.14
120.08
115.01
114.54
112.30
67.00
51.38
51.27
40.09
40.00
39.93
39.84
39.76
39.67
39.59
39.50
39.33
39.17
39.00
28.58
28.00
27.85
26.13
25.11
200
180
160
140
120
100
80
60
40
20
ppm

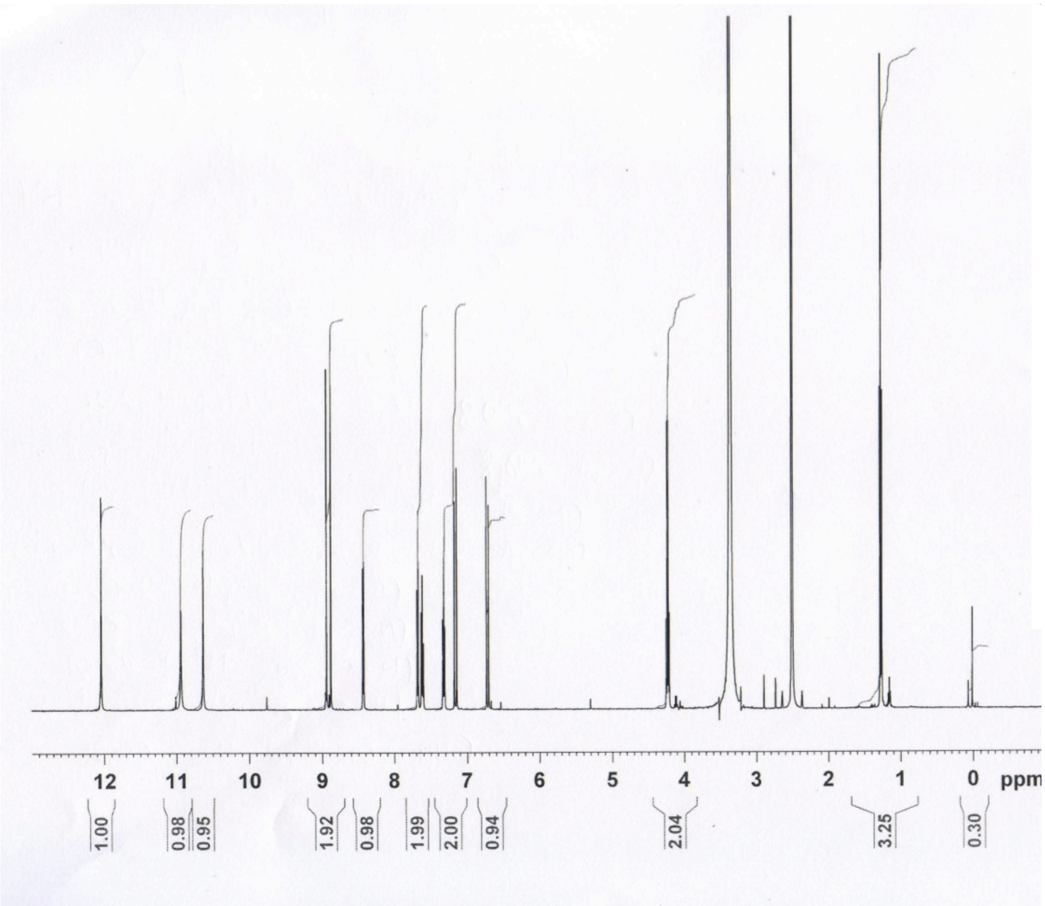

O
H
N
N
H
O
COOMe
N
N
H

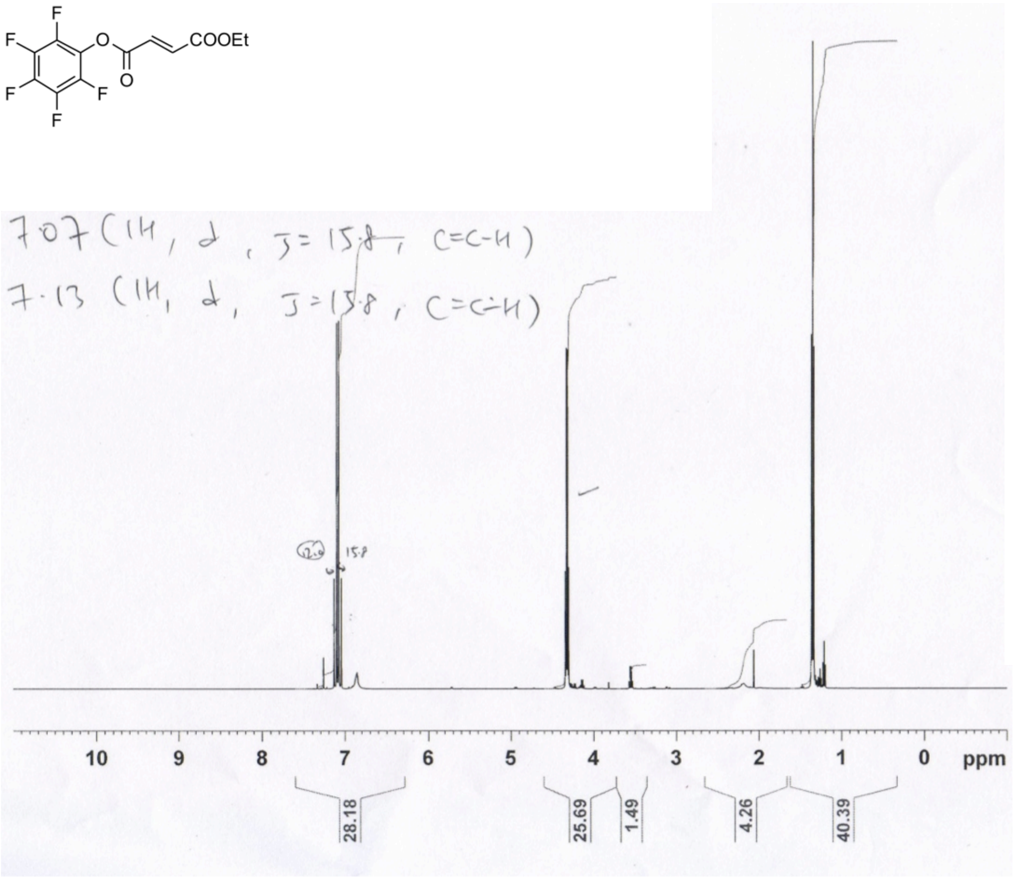
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
3.08
2.85
2.81
5.98
3.07
6.28
3.10
29.52
37.02
6.28


175.77
173.10
170.43
163.45
140.98
138.56
137.29
132.49
128.70
128.02
122.30
120.91
120.08
114.99
114.59
112.30
51.26
51.12
40.10
40.00
39.93
39.84
39.75
39.67
39.60
39.50
39.34
39.17
39.00
32.55
32.42
32.28
29.59
20.49
19.16
200
180
160
140
120
100
80
60
40
20
ppm

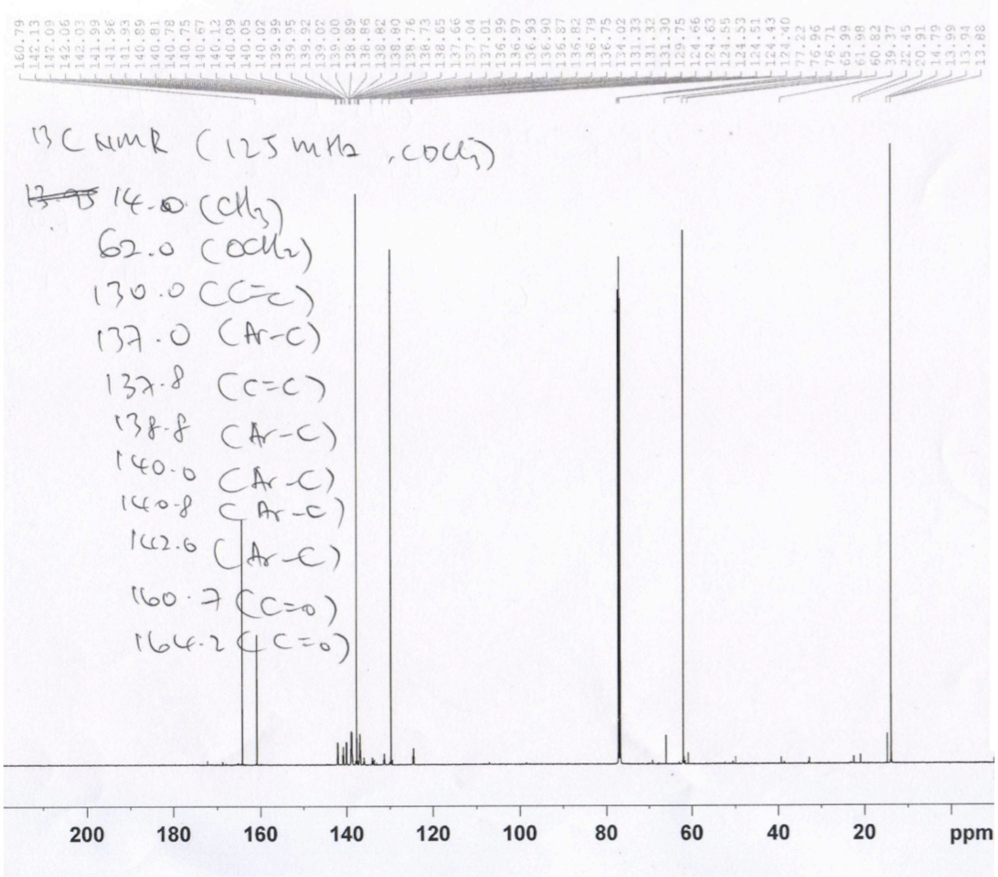


10.64 (1H, bs, N-H)
10.94 (1H, bs, N-H)
12.05 (1H, bs, indole NH)





7.07 (1H, d, J = 15.8, C=C-H)
7.13 (1H, d, J = 15.8, C=C-H)



^{13}C NMR (125 MHz, CDCl$_3$)

~~13.95~~ 14.0 (CH$_3$)

62.0 (OCH$_2$)

130.0 (C=C)

137.0 (Ar-C)

137.8 (C=C)

138.8 (Ar-C)

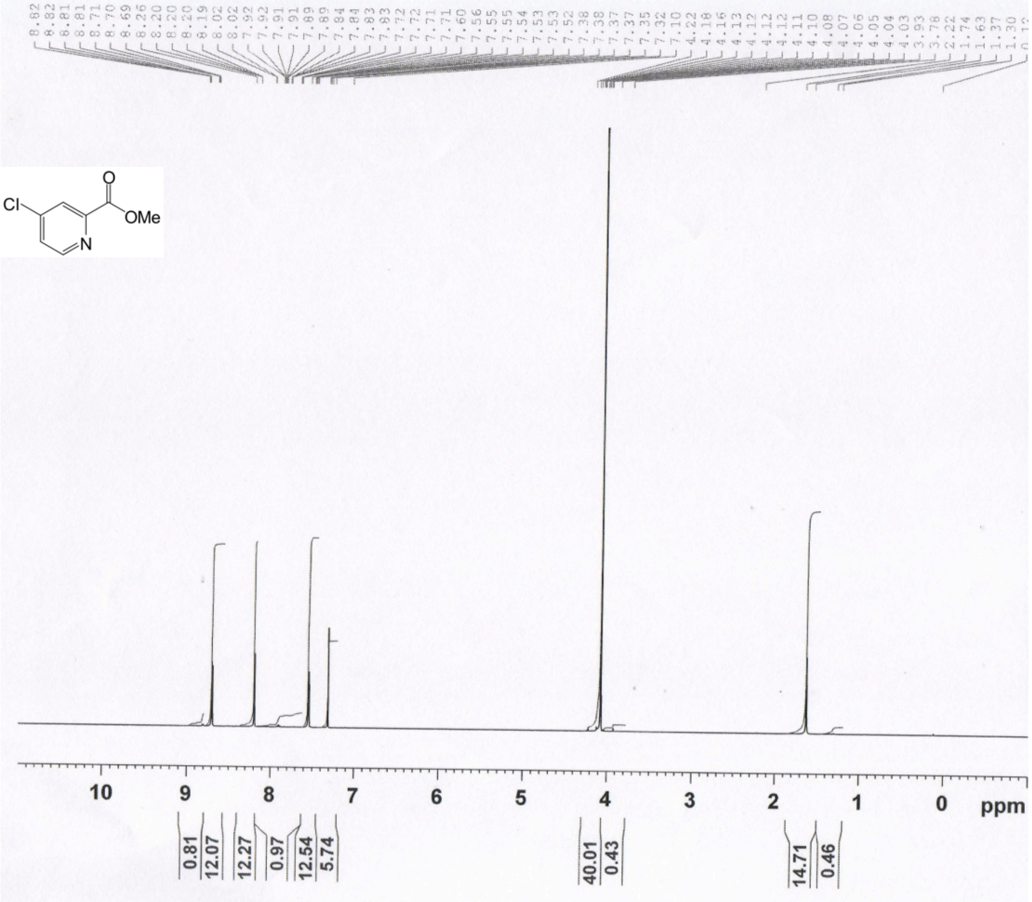
140.0 (Ar-C)

140.8 (Ar-C)

142.6 (Ar-C)

160.7 (C=O)

164.2 (C=O)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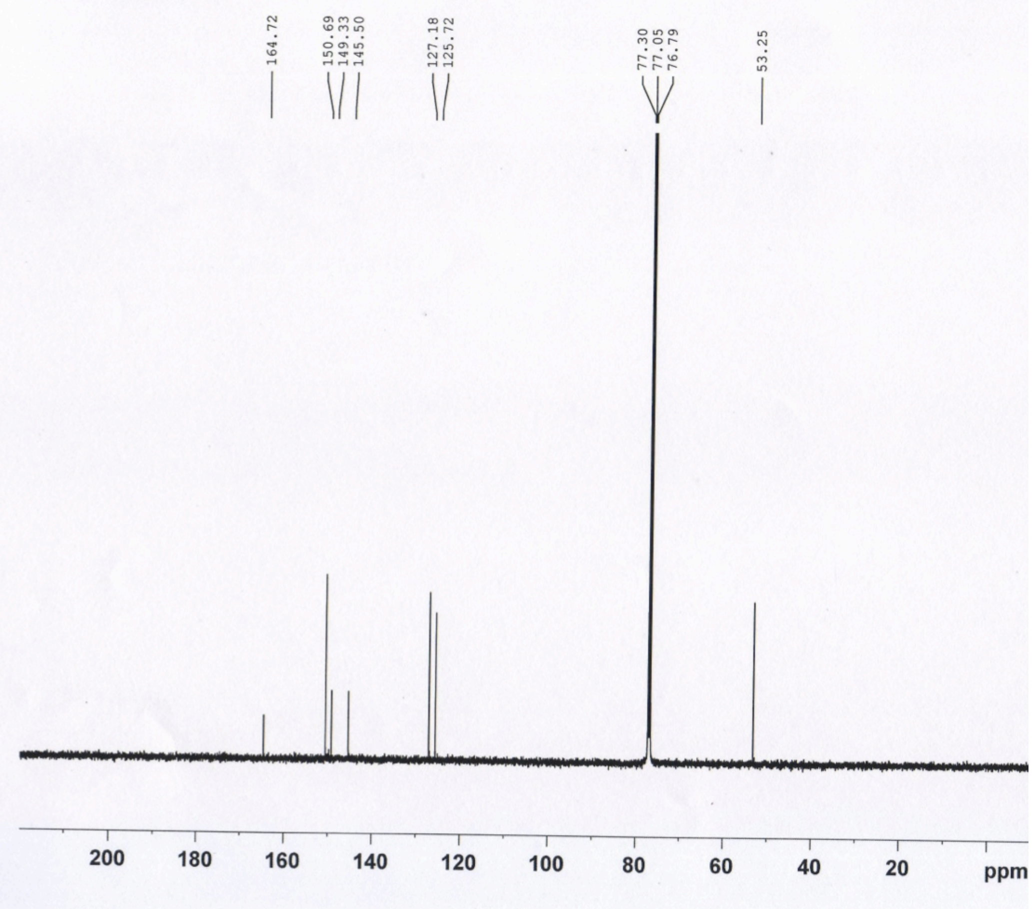
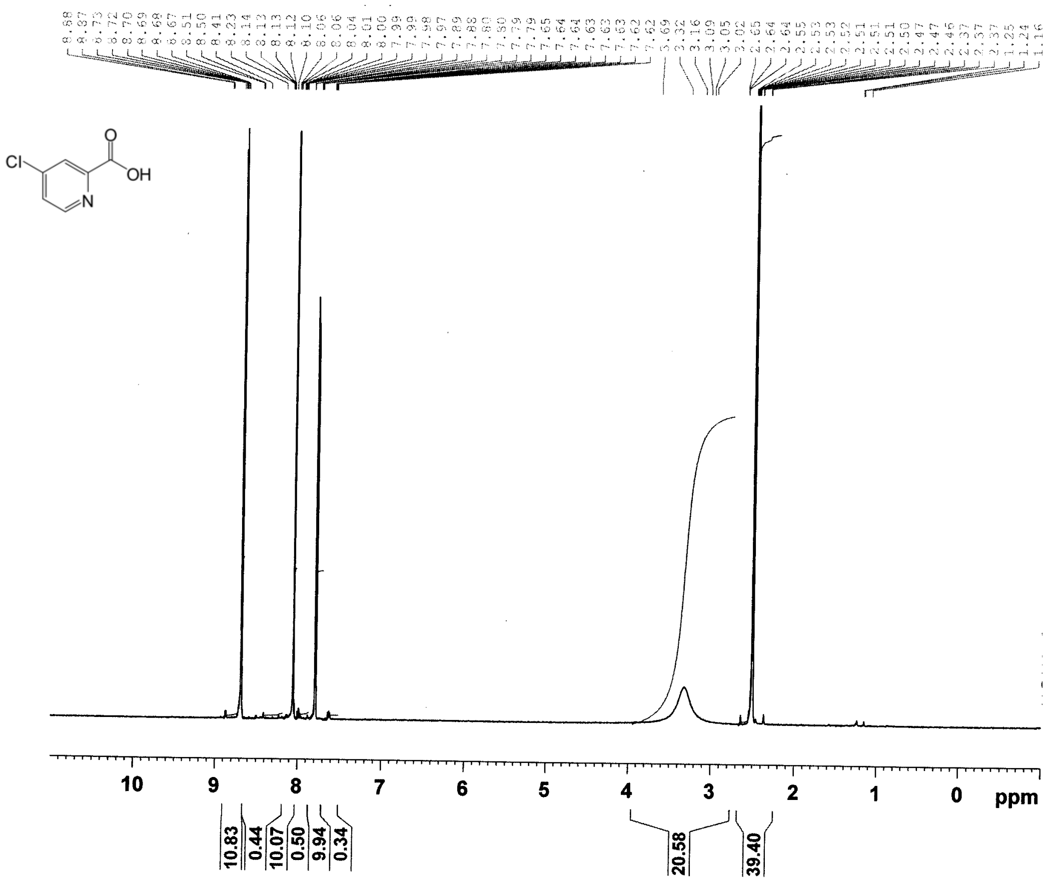
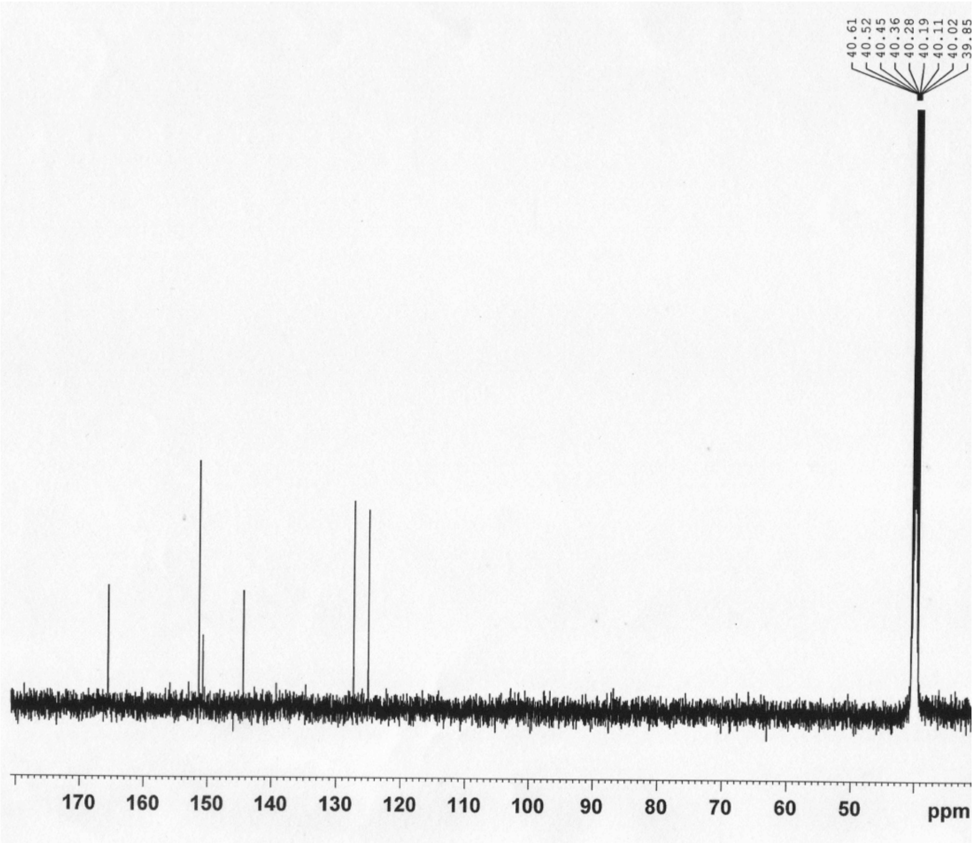
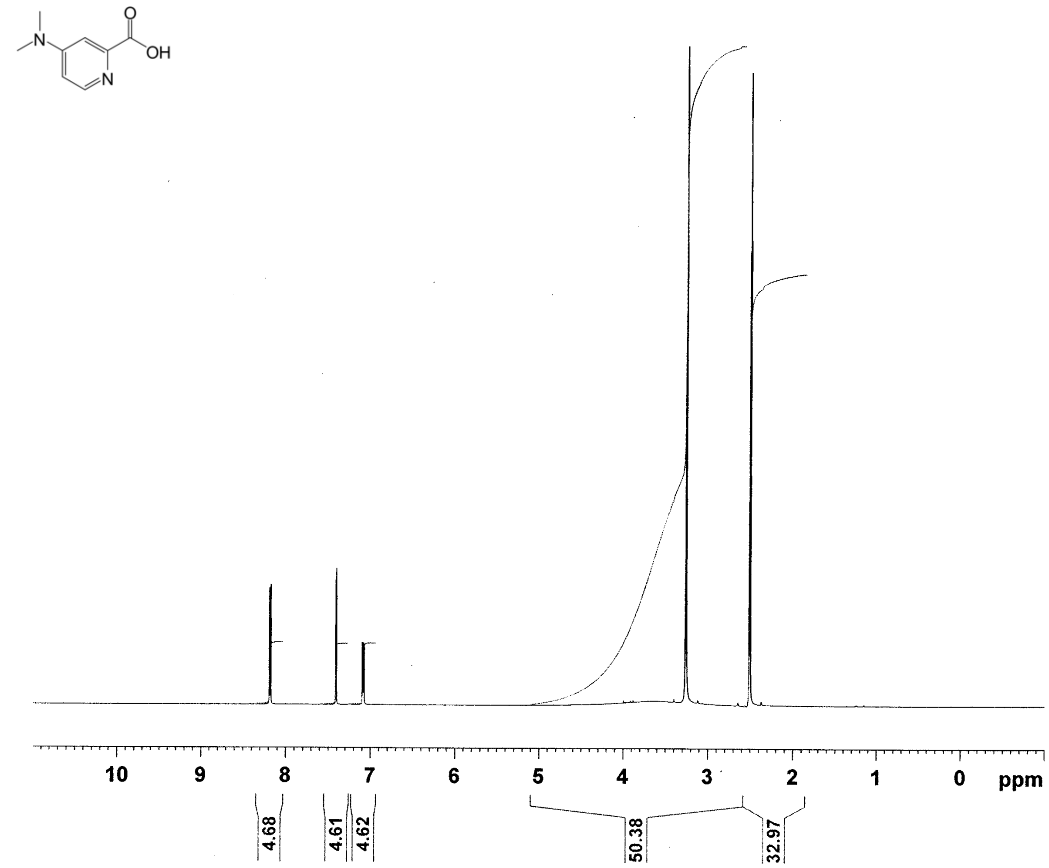
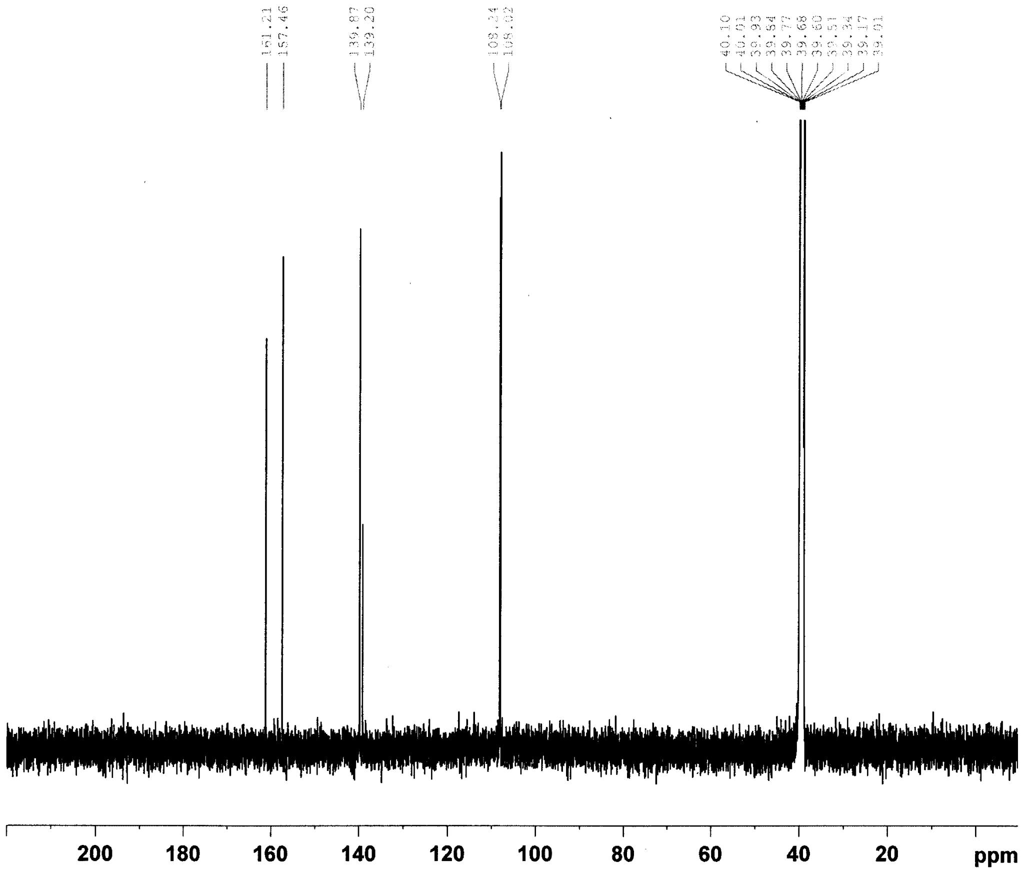
8.82
8.81
8.81
8.71
8.70
8.69
8.26
8.20
8.20
8.20
8.19
8.02
8.02
7.92
7.92
7.91
7.91
7.89
7.89
7.84
7.84
7.83
7.83
7.72
7.72
7.71
7.71
7.60
7.56
7.55
7.55
7.54
7.53
7.53
7.52
7.38
7.38
7.37
7.37
7.35
7.32
7.10
4.22
4.18
4.16
4.13
4.12
4.12
4.12
4.11
4.10
4.08
4.07
4.06
4.05
4.04
4.03
3.93
3.78
2.22
1.74
1.63
1.37
1.30
O
Cl
OMe
N
10
9
8
7
6
5
4
3
2
1
0
ppm
0.81
12.07
12.27
0.97
12.54
5.74
40.01
0.43
14.71
0.46

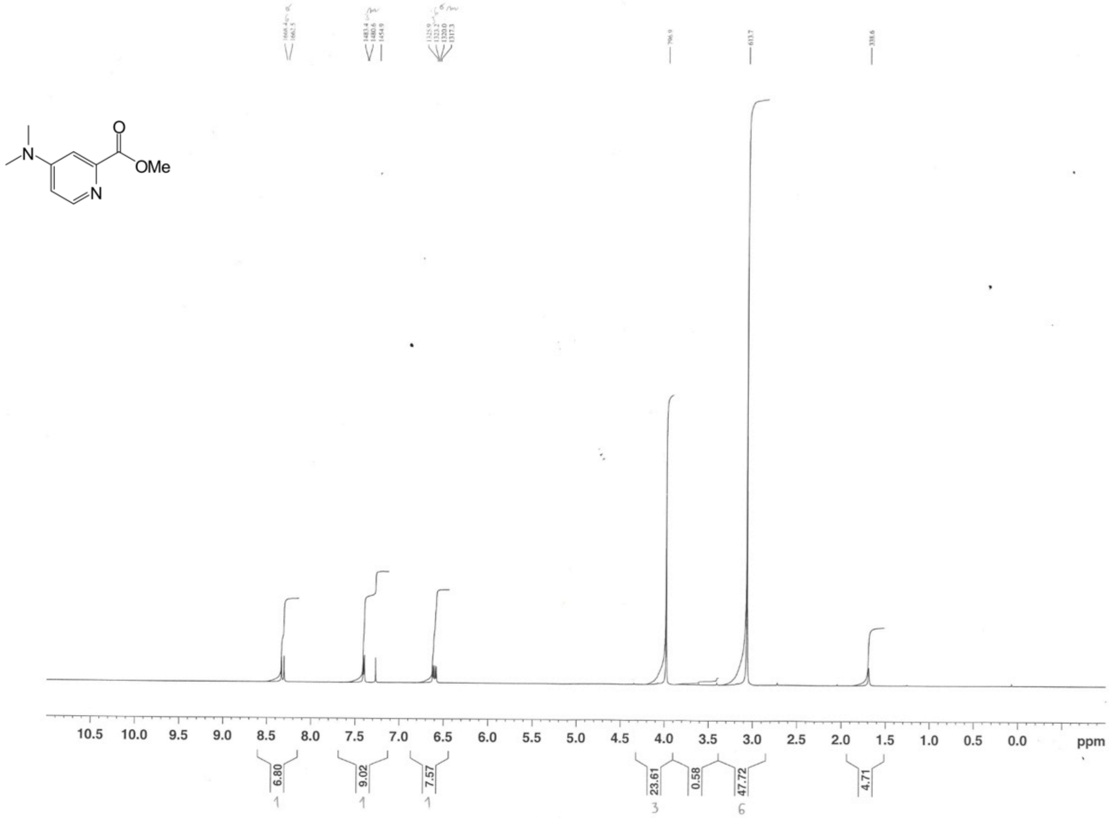

164.72
150.69
149.33
145.50
127.18
125.72
77.30
77.05
76.79
53.25
200
180
160
140
120
100
80
60
40
20
ppm

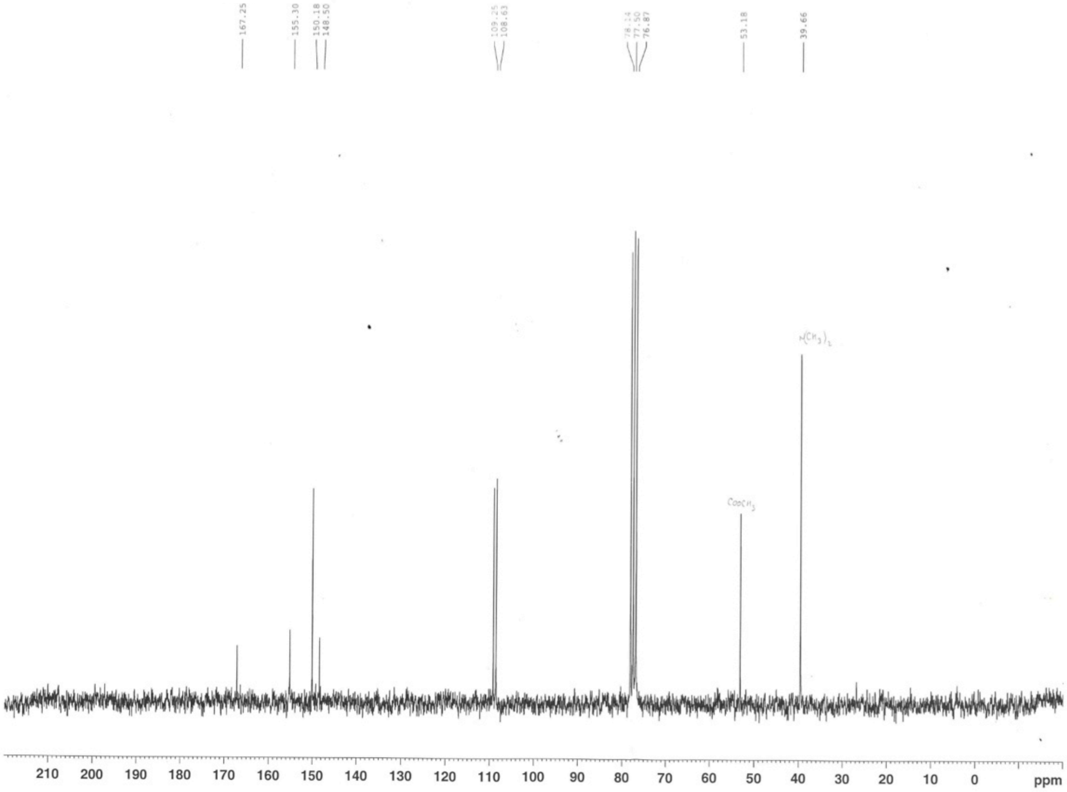

8.88
8.87
8.73
8.72
8.70
8.69
8.68
8.67
8.51
8.50
8.41
8.23
8.14
8.13
8.13
8.12
8.10
8.06
8.06
8.04
8.01
8.00
7.99
7.99
7.98
7.97
7.89
7.88
7.80
7.80
7.79
7.79
7.65
7.64
7.64
7.63
7.63
7.63
7.62
7.62
3.69
3.32
3.16
3.09
3.05
3.02
2.65
2.64
2.64
2.55
2.53
2.53
2.52
2.51
2.51
2.51
2.50
2.47
2.47
2.45
2.37
2.37
2.37
1.25
1.24
1.16
Cl
O
OH
N
10
9
8
7
6
5
4
3
2
1
0
ppm
10.83
0.44
10.07
0.50
9.94
0.34
20.58
39.40

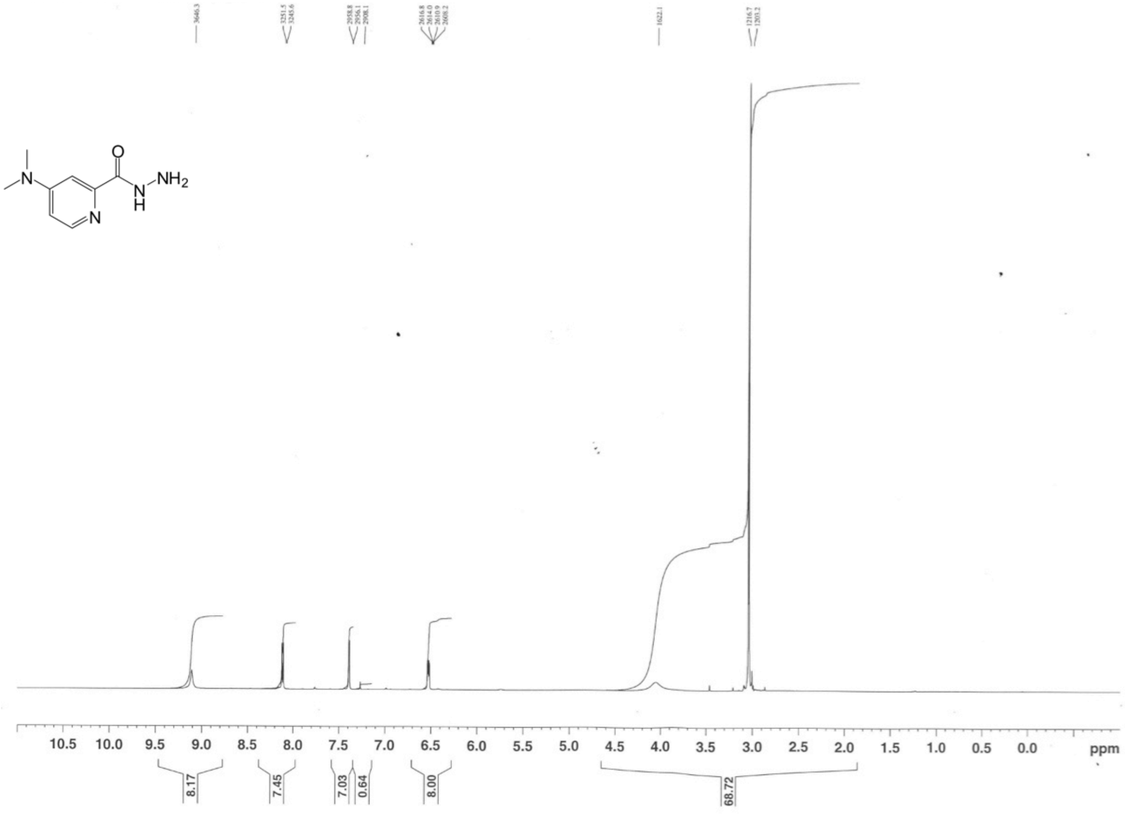

40.61
40.52
40.45
40.36
40.28
40.19
40.11
40.02
39.85
170
160
150
140
130
120
110
100
90
80
70
60
50
ppm

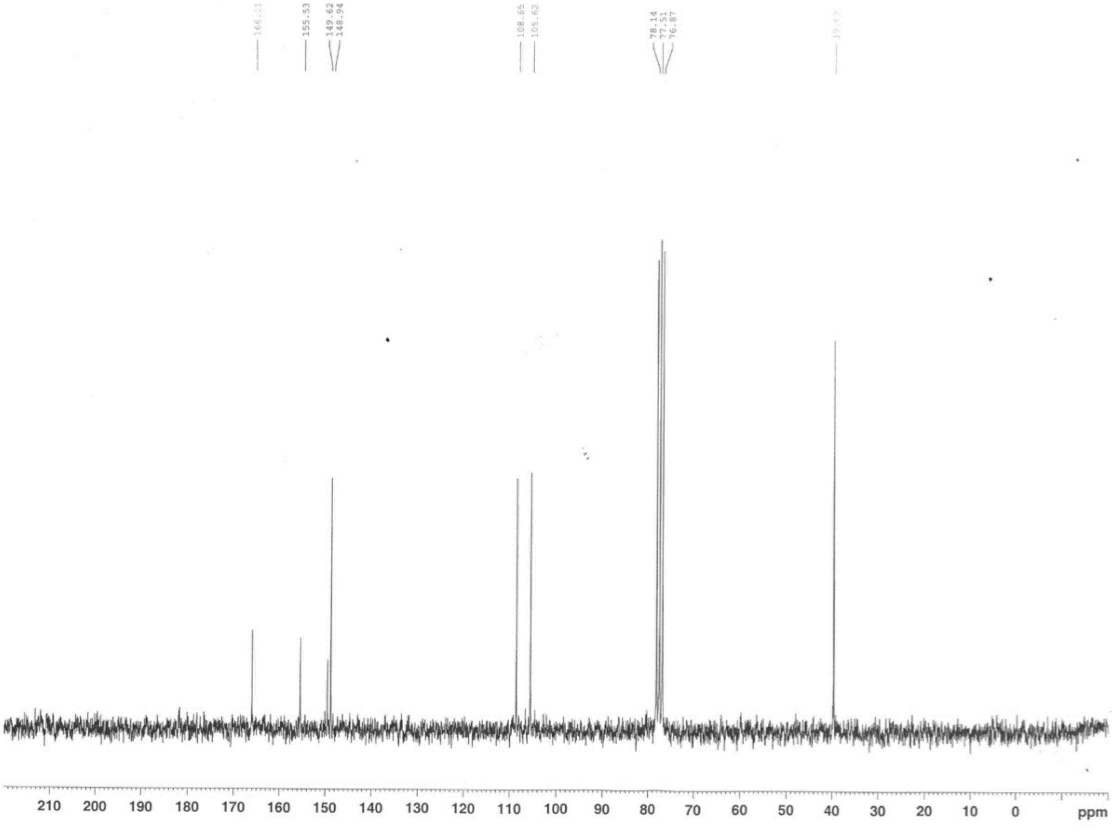

O
OH
N
N
10
9
8
7
6
5
4
3
2
1
0
ppm
4.68
4.61
4.62
50.38
32.97

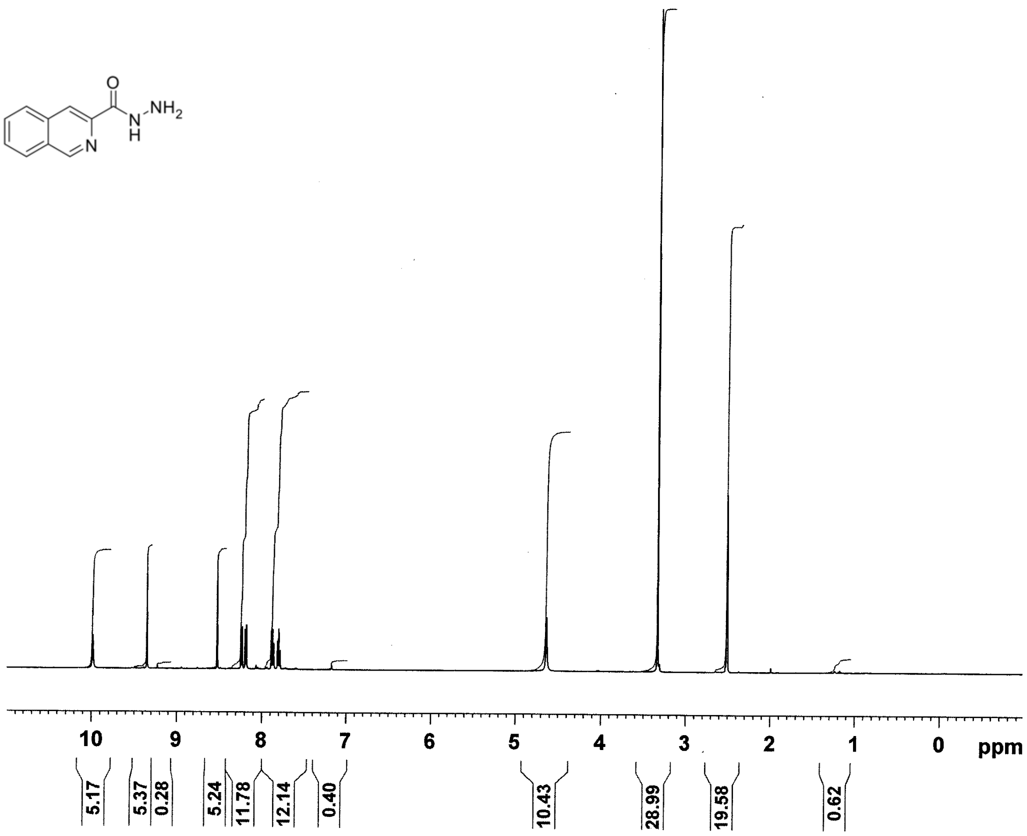

161.21
157.46
139.87
139.20
108.24
108.02
40.10
40.01
39.93
39.84
39.77
39.68
39.60
39.51
39.34
39.17
39.01
200
180
160
140
120
100
80
60
40
20
ppm

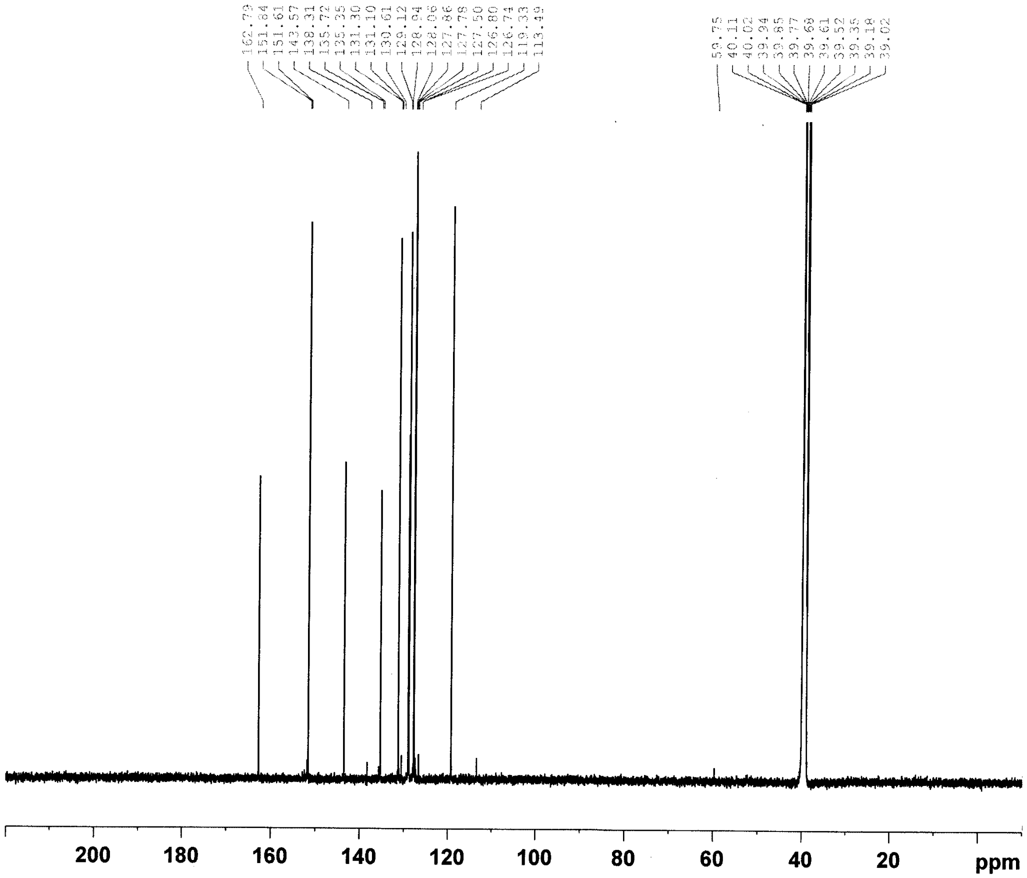

OMe
N
N
O
796.9
613.7
338.6
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
6.80
9.02
7.57
23.61
0.58
47.72
4.71
1
1
1
3
6

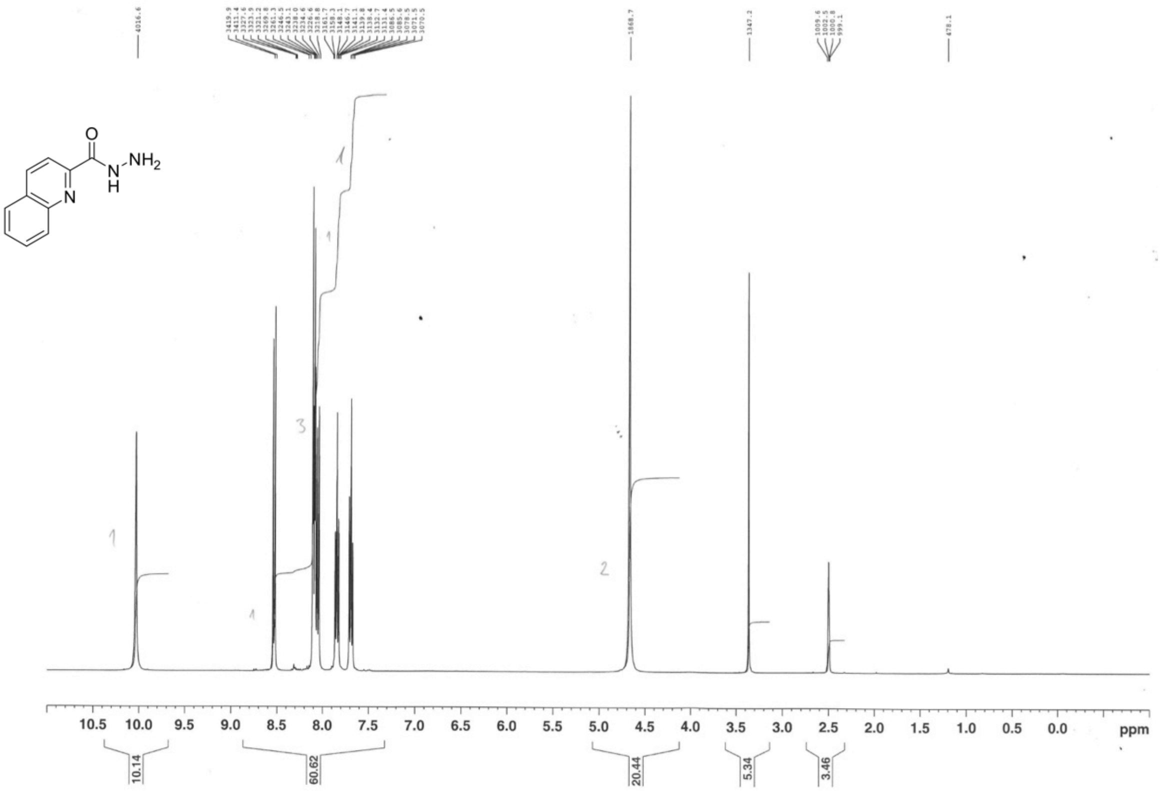

167.25
155.30
150.18
148.50
109.25
108.63
78.14
77.50
76.87
53.18
39.66
N(CH3)2
COOCH3
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

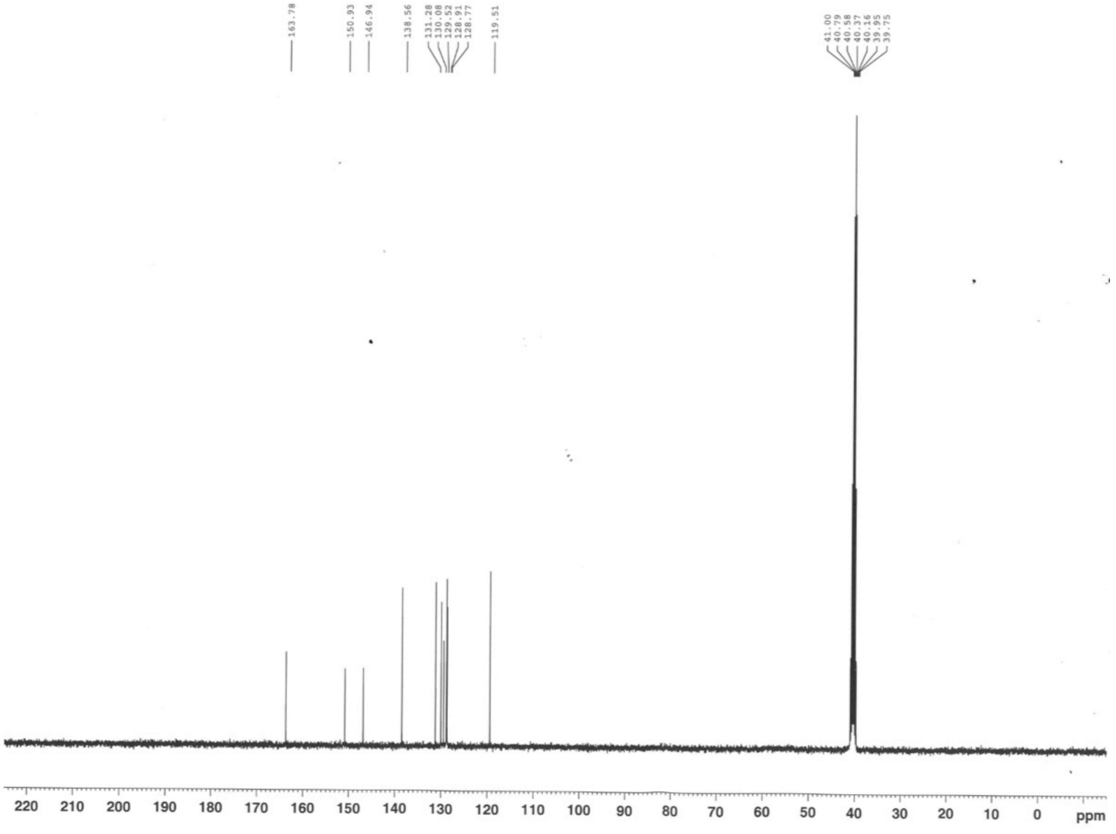

3646.3
3251.5
3245.6
2958.8
2956.1
2908.1
2616.8
2614.0
2610.9
2608.2
1622.1
1216.7
1203.2
N
O
N
H
NH2
N
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
8.17
7.45
7.03
0.64
8.00
68.72

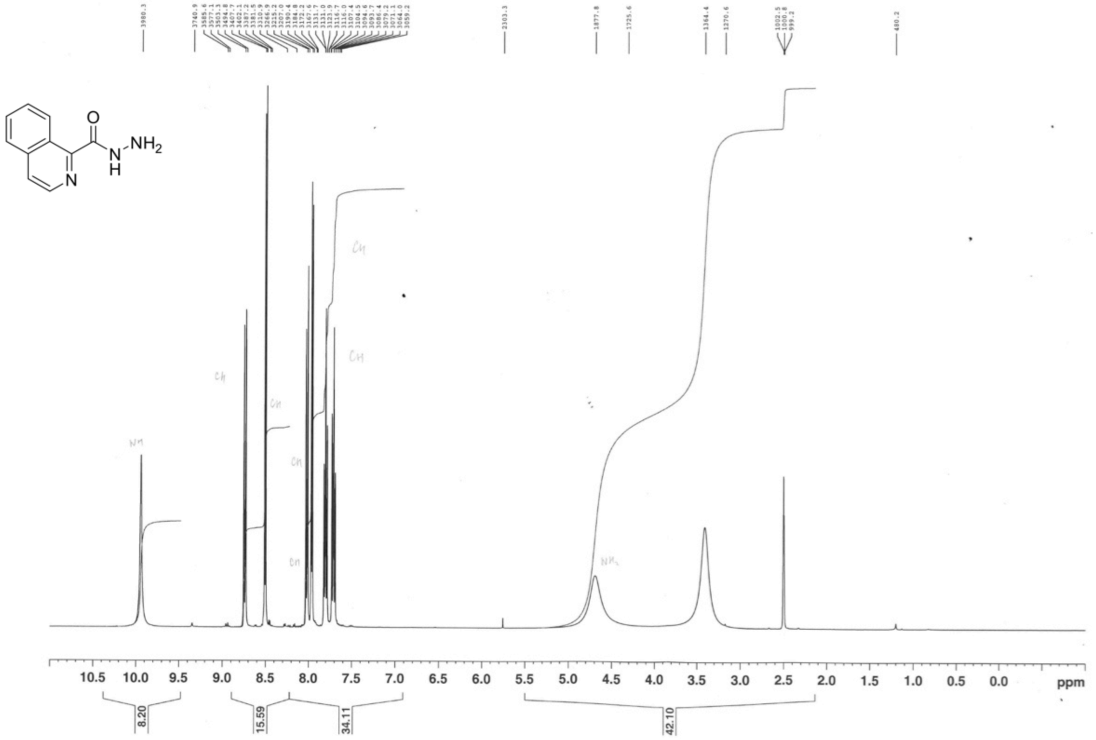

166.11
155.53
149.62
148.94
108.65
105.62
78.14
77.51
76.87
39.63
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


O
N
H
NH2
N
10
9
8
7
6
5
4
3
2
1
0
ppm
5.17
5.37
0.28
5.24
11.78
12.14
0.40
10.43
28.99
19.58
0.62


162.79
151.84
151.61
143.57
138.31
135.72
135.35
131.30
131.10
130.61
129.12
128.94
128.06
127.86
127.78
127.50
126.80
126.74
119.33
113.49
59.75
40.11
40.02
39.94
39.85
39.77
39.68
39.61
39.52
39.35
39.18
39.02
200
180
160
140
120
100
80
60
40
20
ppm


4016.6
3419.9
3411.4
3327.6
3323.9
3321.2
3269.8
3261.3
3246.5
3243.1
3238.0
3234.6
3226.6
3218.8
3161.7
3158.3
3148.1
3146.7
3141.1
3139.8
3138.4
3132.7
3131.4
3086.5
3085.6
3078.5
3071.5
3070.5
1868.7
1347.2
1009.6
1002.5
1000.8
999.1
478.1
O
NH2
N
H
N
1
1
1
3
1
2
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
10.14
60.62
20.44
5.34
3.46


163.78
150.93
146.94
138.56
131.28
130.08
129.52
128.91
128.77
119.51
41.00
40.79
40.58
40.37
40.16
39.95
39.75
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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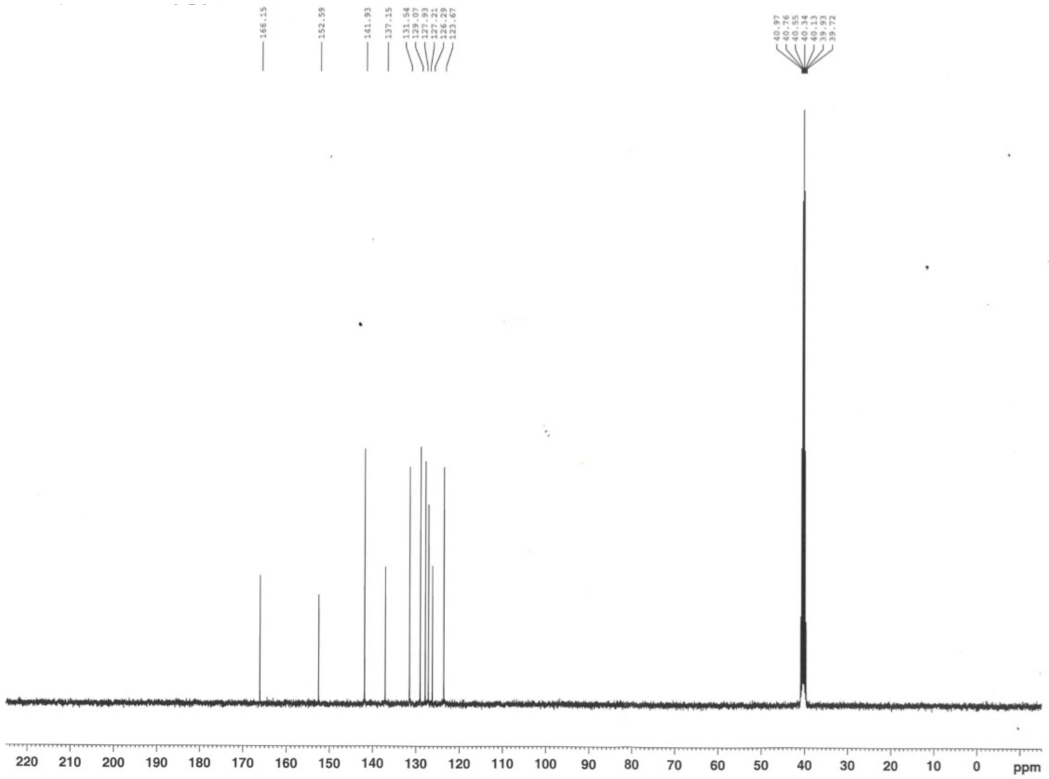
2303.3
1877.8
1725.6
1344.4
1270.6
1002.5
1000.8
999.2
480.2
O
NH2
N
H
N
NH
CH
CH
CH
CH
CH
NH2
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
8.20
15.59
34.11
42.10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137.15
131.54
129.07
127.93
127.21
126.29
123.67
40.97
40.76
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40.34
40.13
39.93
39.72
220
210
200
190
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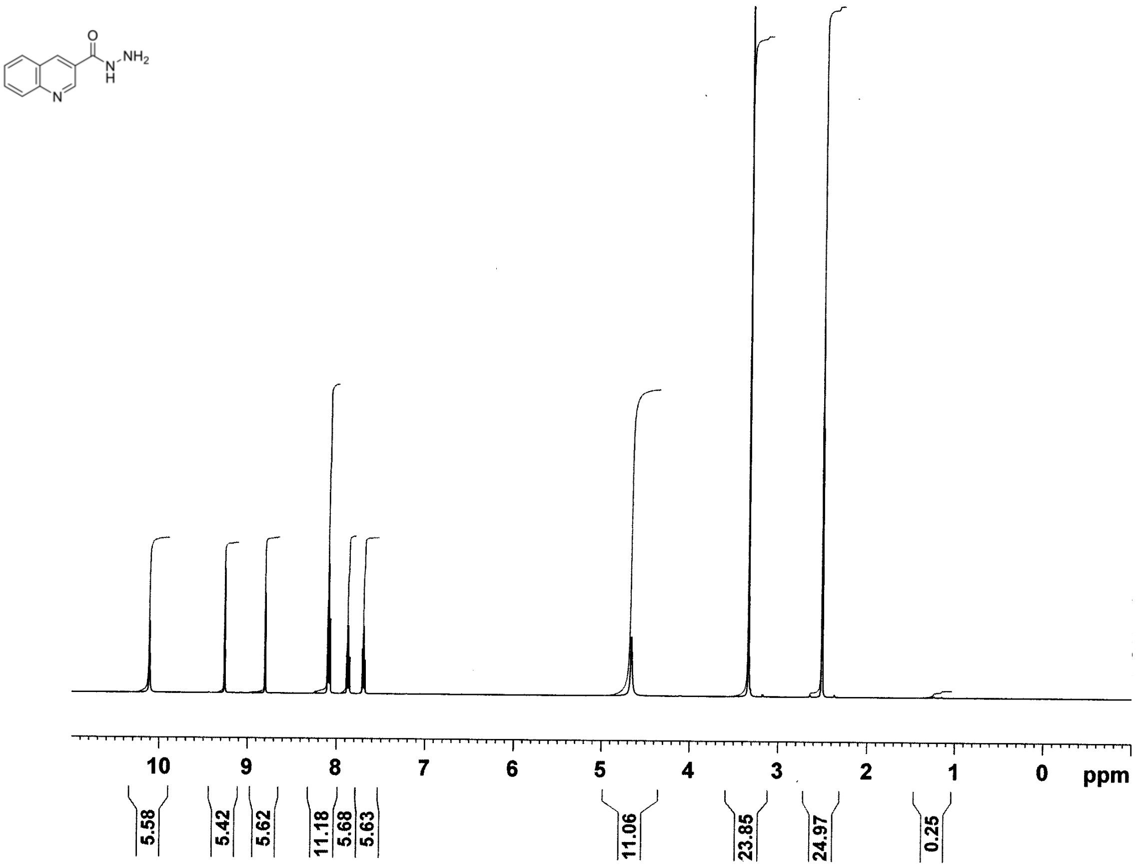
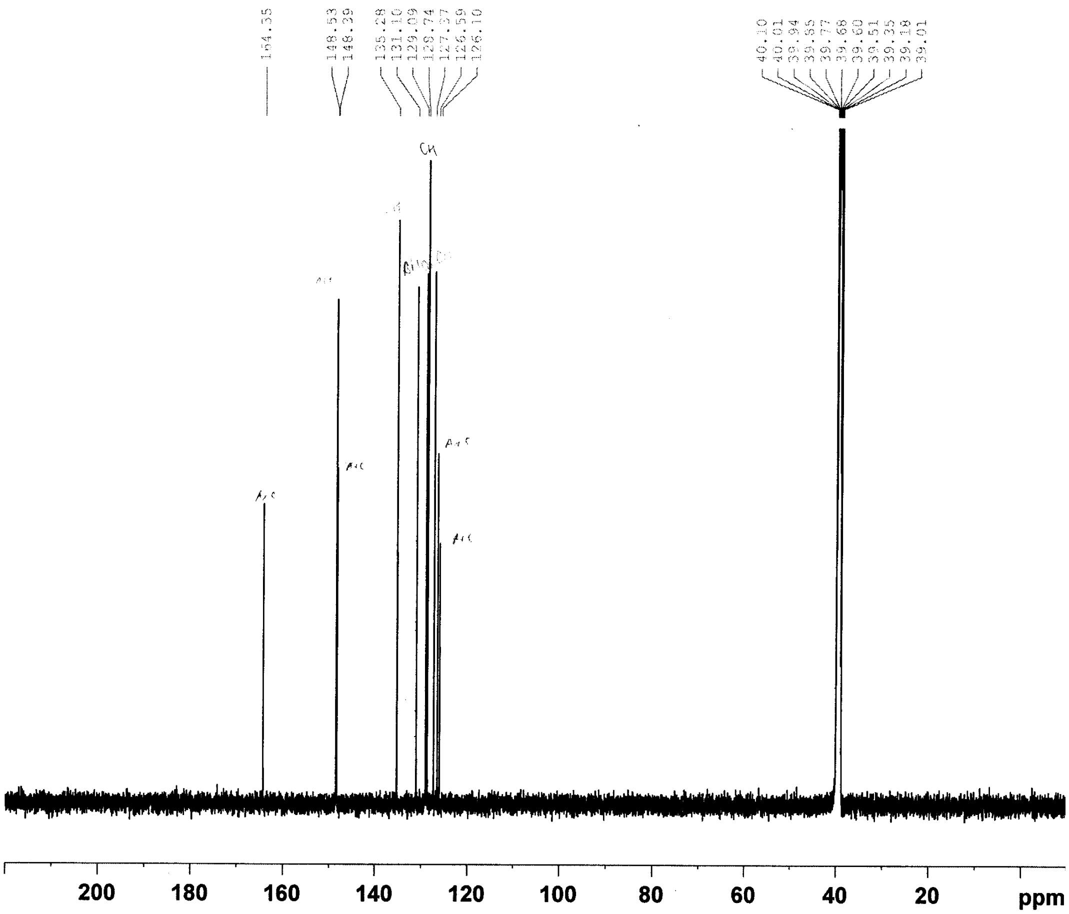
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


O
N
NH2
H
N

10
9
8
7
6
5
4
3
2
1
0
ppm
5.58
5.42
5.62
11.18
5.68
5.63
11.06
23.85
24.97
0.25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148.39
135.28
131.10
129.09
128.74
127.37
126.59
126.10
40.10
40.01
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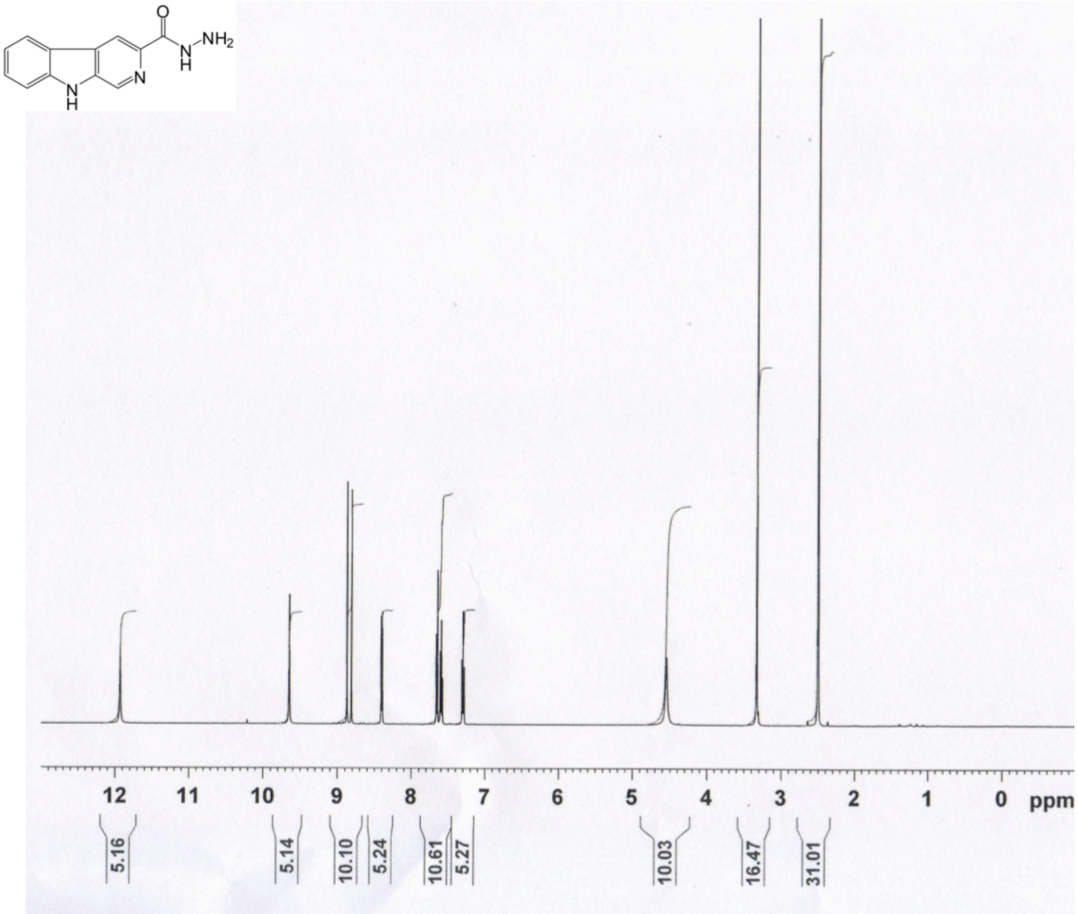
39.85
39.77
39.68
39.60
39.51
39.35
39.18
39.01
CH
ArC
ArC
ArC
ArC
ArC
200
180
160
140
120
100
80
60
40
20
ppm


O
N
H
NH2
N
N
H
12
11
10
9
8
7
6
5
4
3
2
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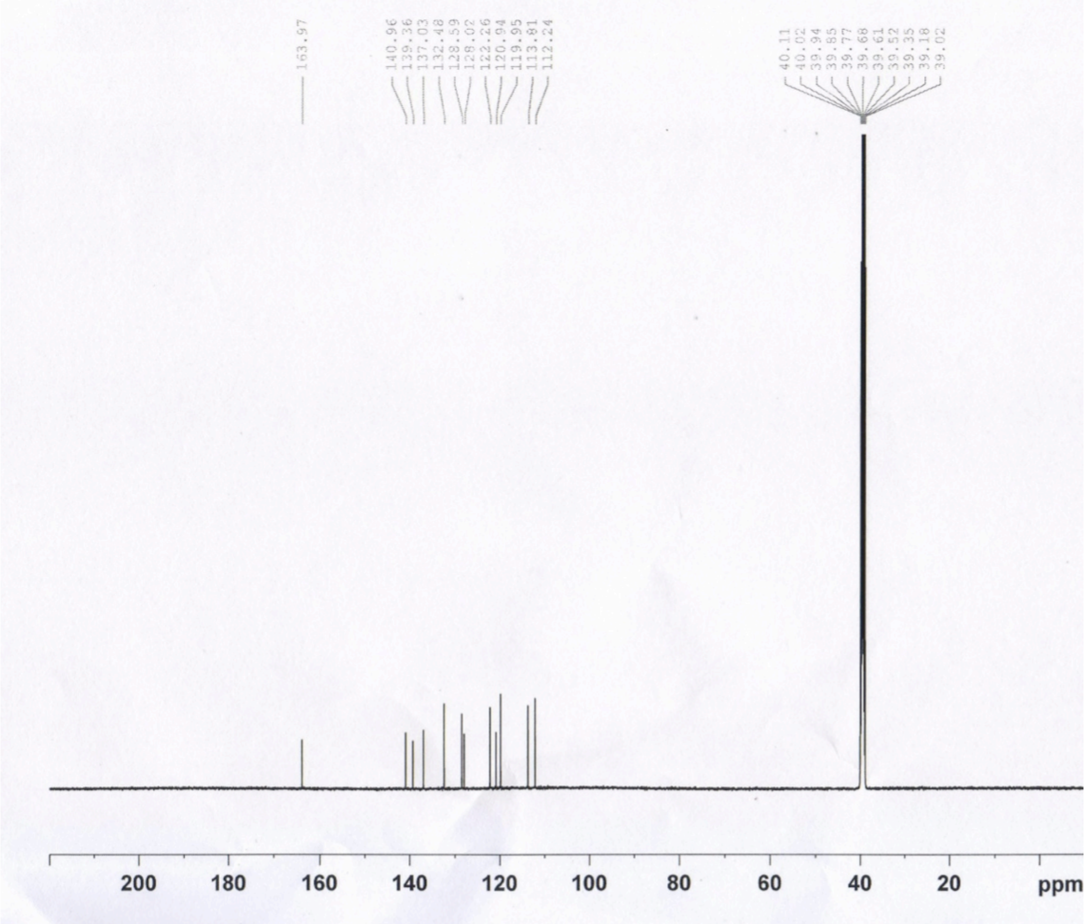
0
ppm
5.16
5.14
10.10
5.24
10.61
5.27
10.03
16.47
31.01


163.97
140.96
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137.03
132.48
128.59
128.02
122.26
120.94
119.95
113.81
112.24
40.11
40.02
39.94
39.85
39.77
39.68
39.61
39.52
39.35
39.18
39.02
200
180
160
140
120
100
80
60
40
20
ppm