Synthesis and Biological Evaluation of 2-Aminoisonicotinic Acid Analogues as HIF-1α Inhibitors

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Synthetic Procedure and Spectral Data

General synthetic procedures. Proton NMR spectra were recorded on a Varian 300 (300 MHz) spectrometer at ambient temperature. Chemical shifts are reported as ppm (δ) downfield from an internal standard tetramethylsilane. Mass spectrometric data were determined by use of Agilent 6890 series and JEOL, JMS-600M Gas Chromatograph/Mass Spectrometer. Solvents and reagents were used as received from commercial sources. All reactions were carried out under an atmosphere of dried argon in flame-dried glassware. Products were purified to a minimum purity of 95% as determined by HPLC, either by flash column chromatography using silica gel 60 (230 - 400 mesh Kieselgel 60). Additionally, thin-layer chromatography on 0.25 mm silica plates (E. Merck, silica gel 60 F254) was used to monitor reactions. The chromatograms were visualized using ultraviolet illumination, exposure to iodine vapors, dipping in PMA or Hanessian's solution. The purity of the products was checked by reversed phase high-pressure liquid chromatography (RP-HPLC), which was performed either on Dionex Corp. HPLC or on Waters Corp. HPLC system equipped with a UV detector set at 254 nm. The mobile phases used were A: H_2O containing 0.05% TFA, and B: CH_3CN. The HPLC employed an YMC Hydrosphere C18 (HS-302) column (5μ particle size, 12 nm pore size), 4.6 mm dia. × 150 mm with a flow rate of 1.0 mL/min. Compound purity was assessed using one of the following methods, Method A: gradient 50% B to 100% B in 30 min; Method B: gradient 25% B to 100% B in 30 min; Method C: gradient 60% B to 100% B in 30 min.

General procedure for synthesis amides (12a-g): The compound **11** (100 mg, 1 equvi) was suspended in the acetonitrile and Et_3N (4.0 equvi), 50% PPAA (1.2 equvi) were added at room temperature. The reaction solution was stirred at room temperature for 30 min and amine (1.2 equvi) was added. The reaction solution was stirred overnight and evaporated under reduced pressure. The residue was purified by column chromatography to obtain compound.

2-[2-(4-adamantan-1-yl-phenoxy)acetamido]-*N*,*N*-dimethylisonicotinamide (12a): Afforded as a colorless foam (82 mg, yield 77%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.01 (s, 1H), 8.38 (d, J = 5.1 Hz, 1H), 8.31 (s, 1H), 7.33 (d, J = 8.7 Hz, 2H), 7.11 (dd, J = 1.2 Hz, 5.1 Hz, 1H), 6.94 (d, J = 9.0 Hz, 2H), 4.62 (s, 2H), 3.13 (s, 3H), 2.98 (s, 3H), 2.10 (b,3H), 1.88-1.89 (m, 6H), 1.71-1.81 (m, 6H); GC/MS(EI) [M+] 433; Purity = 100 % (as determined by RP-HPLC, Method B, t_R = 20.66 min).

2-[2-(4-adamantan-1-yl-phenoxy)acetamido]-N-isopropylisonicotinamide (12b): Afforded as a white solid (103 mg, yield 94%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.05 (s, 1H), 8.48 (s, 1H), 8.42 (d, J = 5.1 Hz, 1H), 7.54 (dd, J = 1.5 Hz, 5.1 Hz, 1H), 7.33 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 6.14 (d, J = 7.5 Hz, 1H), 4.63 (s, 2H), 4.29 (m, 1H), 2.09 (b, 3H), 1.88-1.89 (m, 6H), 1.71-1.81 (m, 6H), 1.29 (d, J = 6.6 Hz, 6H); GC/MS(EI) [M+] 447; Purity = 100% (as determined by RP-HPLC, Method B, t_R = 22.31 min).

2-(4-adamantan-1-yl-phenoxy)-*N*-[4-(morpholine-4-carbonyl)pyridin-2-yl]acetamide (12c): Afforded as a white foam (93 mg, yield 80%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.03 (s, 1H), 8.40 (d, J = 4.8 Hz, 1H), 8.31 (s, 1H), 7.33 (d, J = 9.3 Hz, 2H), 7.11 (dd, J = 1.8 Hz, 5.1 Hz, 1H), 6.95 (d, J = 9.3 Hz, 2H), 4.62 (s, 2H), 3.80 (b, 4H), 3.66 (b, 2H), 3.43 (b, 2H), 2.10 (b, 3H), 1.88-1.89 (m, 6H), 1.72-1.82 (m, 6H); GC/MS(EI) [M+] 475; Purity = 100% (as determined by RP-HPLC, Method B, t_R = 20.49 min).

2-[2-(4-adamantan-1-yl-phenoxy)acetamido]-*N*-[2-(dimethylamino)ethyl]isonicotinamide (12d): Afforded as a white foam (101 mg, yield 86%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.04 (s, 1H), 8.57 (s, 1H), 8.42 (d, J = 5.1 Hz, 1H), 7.55 (dd, J = 1.5 Hz, 5.1 Hz, 1H), 7.33 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 4.63 (s, 2H), 3.59 (dd, J = 5.7 Hz, 2H), 2.65 (t, J = 5.7 Hz, 2H), 2.37 (s, 6H), 2.09 (b, 3H), 1.88-1.89 (m, 6H), 1.71-1.81 (m, 6H); GC/MS(EI) [M+] 476; Purity = 100% (as determined by RP-HPLC, Method B, t_R = 11.95 min).

2-[2-(4-adamantan-1-yl-phenoxy)acetamido]-*N*-(2-morpholinoethyl)isonicotinamide (12e): Afforded as a pale yellow foam (106 mg, yield 83%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.06 (s, 1H), 8.57 (s, 1H), 8.44 (d, J = 5.1 Hz, 1H), 7.54 (dd, J = 1.5 Hz, 5.1 Hz, 1H), 7.34 (d, J = 8.7 Hz, 2H), 7.02 (b, 1H), 6.95 (d, J = 8.7 Hz, 2H), 4.64 (s, 2H), 3.77 (t, J = 4.5 Hz, 4H), 3.57 (dd, J = 5.7 Hz, 2H), 2.62 (t, J = 6.3 Hz, 2H), 2.53 (t, J = 5.1 Hz, 4H), 2.10 (b, 3H), 1.88-1.89 (m, 6H), 1.71-1.82 (m, 6H); GC/MS(EI) [M+] 518; Purity = 100 % (as determined by RP-HPLC, Method B, t_R = 11.99 min).

2-[2-(4-adamantan-1-yl-phenoxy)acetamido]-*N*-[2-(pyrrolidin-1-yl)ethyl]isonicotinamide (12f): Afforded as a pale yellow foam (75 mg, yield 60%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.02 (s, 1H), 8.57 (s, 1H), 8.40 (d, J = 5.1 Hz, 1H), 7.53 (dd, J = 1.5 Hz, 5.1 Hz, 1H), 7.33 (d, J = 9.6 Hz, 2H), 6.94 (d, J = 9.0 Hz, 2H), 4.62 (s, 2H), 3.63 (dd, J = 6.0 Hz, 2H), 2.83 (t, J = 6.0 Hz, 2H), 2.71 (b, 4H), 2.09 (b, 3H), 1.88-1.89 (m, 10H),

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1.71-1.82 (m, 6H); GC/MS(EI) [M+] 502; Purity = 100% (as determined by RP-HPLC, Method B, t_R = 12.40 min).

***tert*-Butyl 2-[2-(4-adamantan-1-yl-phenoxy)acetamido]isonicotinate (12h):** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.02 (s, 1H), 8.39 (s, 1H), 8.37 (d, *J* = 5.1 Hz, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 7.15 (dd, *J* = 1.5 Hz, 6.0 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 2H), 4.61 (s, 2H), 2.09 (b, 3H), 1.88-1.89 (m, 6H), 1.72-1.82 (m, 6H), 1.56 (s, 9H); GC/MS(EI) [M+] 462; Purity = 100 % (as determined by RP-HPLC, Method B, t_R = 28.29 min).

2-(Dimethylamino)ethyl 2-[2-(4-adamantan-1-yl-phenoxy) acetamido]isonicotinate (12i): Obtained as a white solid (26 mg, yield 44%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.04 (s, 1H), 8.81 (s, 1H), 8.44 (d, *J* = 5.1 Hz, 1H), 7.65 (dd, *J* = 1.5 Hz, 5.1 Hz, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 4.64 (s, 2H), 4.47 (t, *J* = 6.0 Hz, 2H), 2.74 (t, *J* = 6.0 Hz, 2H), 2.35 (s, 6H), 2.09 (b, 3H), 1.88-1.89 (m, 6H), 1.71-1.82 (m, 6H); GC/MS (EI) [M+] 477; Purity = > 96% (as determined by RP-HPLC, Method B, t_R = 24.95 min).

General procedure for coupling reaction (12j-m): The compound **5a** (100 mg, 1 equvi) was suspended in acetonitrile and Et_3N (4.0 equvi), 50% PPAA (1.2 equvi) were added at room temperature. The reaction solution was stirred at room temperature for 30min and amine (**13a-d**, 1.2 equvi) was added. The reaction solution was stirred overnight and evaporated under reduced pressure. The residue was purified by column chromatography to obtain compound (**12j-m**).

Ethyl 2-[2-(4-adamantan-1-yl-phenoxy)acetamido]isonicotinate (12j): Obtained as a white solid (65 mg, yield 43%) from corresponding ester **13a**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.04 (s, 1H), 8.80 (s, 1H), 8.45 (d, *J* = 4.8 Hz, 1H), 7.66 (dd, *J* = 1.2 Hz, 4.8 Hz, 1H), 7.33 (d, *J* = 9.0 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 4.64 (s, 2H), 4.43 (q, *J* = 7.2 Hz, 2H), 2.10 (b, 3H), 1.88-1.89 (m, 6H), 1.72-1.82 (m, 6H), 1.42 (t, *J* = 7.2 Hz, 3H); GC/MS(EI) [M+] 434; Purity = 100% (as determined by RP-HPLC, Method B, t_R = 26.27 min).

2-Methoxyethyl 2-[2-(4-adamantan-1-yl-phenoxy)acetamido]isonicotinate (12k): Obtained as a white solid (38 mg, yield 23%) from corresponding ester **13b**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.04 (s, 1H), 8.82 (s, 1H), 8.45 (d, *J* = 5.4 Hz, 1H), 7.67 (dd, *J* = 1.2 Hz, 5.1 Hz, 1H), 7.33 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 4.64 (s, 2H), 4.52 (t, *J* = 4.2 Hz, 2H), 3.75 (t, *J* = 4.5 Hz, 2H), 3.44 (s, 3H), 2.09 (b, 3H), 1.88-1.89 (m, 6H), 1.71-1.81 (m, 6H); GC/MS(EI) [M+] 464; Purity = 100% (as determined by RP-HPLC, Method B, t_R = 24.60 min).

2-(Pyrrolidin-1-yl)ethyl 2-[2-(4-adamantan-1-yl-phenoxy) acetamido]isonicotinate (12l): Obtained as a white solid (34 mg, yield 19%) from corresponding ester **13c**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.04 (s, 1H), 8.81 (s, 1H), 8.45 (d, *J* = 4.8 Hz, 1H), 7.66 (dd, *J* = 1.8 Hz, 5.4 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 4.64 (s, 2H), 4.51 (t, *J* = 6.0 Hz, 2H), 2.91 (t, *J* = 6.0 Hz, 2H), 2.65 (m, 4H), 2.09 (b, 3H), 1.88-1.89 (m, 6H), 1.81 (m, 4H), 1.71-1.81 (m, 6H); GC/MS(EI) [M+] 503; Purity = > 98% (as determined by RP-HPLC, Method B, t_R = 24.98 min).

2-Morpholinoethyl 2-[2-(4-adamantan-1-yl-phenoxy)acetamido]isonicotinate (12m): Obtained as a pale yellow foam (50 mg, yield 28%) from corresponding ester **13d**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.10 (s, 1H), 8.81 (s, 1H), 8.45 (d, *J* = 4.8 Hz, 1H), 7.64 (dd, *J* = 1.2 Hz, 5.1 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 4.63 (s, 2H), 4.54 (t, *J* = 6.3 Hz, 2H), 3.76 (t, *J* = 4.2 Hz, 4H), 2.88 (t, *J* = 5.7 Hz, 2H), 2.69 (t, *J* = 4.8 Hz, 4H), 2.08 (b, 3H), 1.87-1.89 (m, 6H), 1.72-1.82 (m, 6H); GC/MS(EI) [M+] 519; Purity = > 96% (as determined by RP-HPLC, Method B, t_R = 13.04 min).

2-[2-(4-Nitrophenoxy)acetamido]isonicotinamide (8): A solution of 4-nitrophenoxy acetic acid (100 mg, 0.5 mmol), 2-amino-4-pyridine carboxamide (104 mg, 0.76 mmol), PyBOP (528 mg, 1.01 mmol) and DMAP (124 mg, 1.01 mmol) in DMF was stirred at room temperature for 16 h. The reaction was monitored by TLC and quenched with water. The mixture was extracted with methanol and MC. The organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to obtain 40 mg compound **8** (yield 50%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.88 (s, 1H), 8.46 (d, *J* = 5.4 Hz, 1H), 8.40 (s, 1H), 8.23 (d, *J* = 9.0 Hz, 2H), 8.20 (s, 1H), 7.68 (s, 1H), 7.50 (d, *J* = 5.7 Hz, 1H), 7.19 (d, *J* = 9.0 Hz, 2H), 5.03 (s, 2H); GC/MS(EI) [M+] 316; Purity = 96% (as determined by RP-HPLC, Method A, t_R = 2.76 min).

2-[2-(4-Fluorophenoxy)acetamido]isonicotinamide (9): A solution of 4-fluorophenoxy acetic acid (80 mg, 0.47 mmol), 2-amino-4-pyridine carboxamide (128 mg, 0.94 mmol), PyBOP (489 mg, 0.94 mmol) and DMAP (114 mg, 0.94 mmol) in DMF was stirred at room temperature for 16 h. The reaction was monitored by TLC and quenched with water. The mixture was extracted with methanol and MC. The organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to obtain 80 mg compound **9** (yield 59%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.67 (s, 1H), 8.44 (d, *J* = 5.1 Hz, 1H), 8.20 (s, 1H), 7.68 (b, 1H), 7.49 (dd, *J* = 1.8 Hz, 5.4 Hz, 1H), 7.11-7.17 (m, 2H), 6.97-7.02 (m, 2H), 4.80 (s, 2H); GC/MS(EI) [M+] 289; Purity = 100% (as determined by RP-HPLC, Method A, t_R = 2.88 min).

[2-(2-Phenoxy)acetamido]isonicotinamide (10): A solution of phenoxy acetic acid (100 mg, 0.65 mmol), 2-amino-4-pyridine carboxamide (180 mg, 1.31 mmol), PyBOP (684 mg, 1.31 mmol) and DMAP (160 mg, 1.31 mmol) in DMF was stirred at room temperature for 16 h. The reaction was monitored by TLC and quenched with water. The mixture was extracted with methanol and MC. The organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to obtain 105 mg compound **10** (yield 59%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.66 (s, 1H), 8.44 (d, *J* = 5.1 Hz, 1H), 8.42 (s, 1H), 8.20 (s, 1H), 7.67 (s, 1H), 7.49 (dd, *J* = 1.8 Hz, 5.4 Hz, 1H), 7.28-7.34 (m, 2H), 6.94-6.99 (m, 3H), 4.81 (s, 2H); GC/MS(EI) [M+] 271; Purity = 100% (as determined by RP-HPLC, Method C, t_R = 2.33 min).

General procedure for esterification of isonicotinic acid (13a-d): A suspension of 2-aminopyridine-4-carboxylic acid (1 equiv), appropriate halides (1.2 equvi) and K_2CO_3 (4.0 equvi) in DMF was heated at 80 ^oC overnight. The reaction mixture was cooled to room temperature and evaporated. The residue was dissolved in EA, filtered and concentrated to obtain crude product.

Ethyl-2-aminoisonicotinate (13a): ^{1}H NMR (CD_3OD, 300 MHz) δ 8.0 (d, *J* = 5.1 Hz, 1H), 7.11 (s, 1H), 7.04 (dd, *J* = 1.5 Hz, 5.4 Hz, 1H), 4.35 (q, *J* = 7.2 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H).

2-Methoxyethyl-2-aminoisonicotinate (13b): ^{1}H NMR (CD_3OD, 300 MHz) δ 8.01 (d, *J* = 5.4 Hz, 1H), 7.12 (s, 1H), 7.06 (dd, *J* = 1.2 Hz, 5.7 Hz, 1H), 4.43 (t, *J* = 4.5 Hz, 2H), 3.71 (t, *J* = 4.5 Hz, 2H), 3.40 (s, 3H).

2-(Pyrrolidin-1-yl)ethyl-2-aminoisonicotinate (13c): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.18 (d, *J* = 5.7 Hz, 1H), 7.17 (dd, *J* = 1.5 Hz, 5.1 Hz, 1H), 7.07 (s, 1H), 4.59 (b, 2H), 4.45 (t, *J* = 6.6 Hz, 2H), 2.86 (t, *J* = 5.7 Hz, 2H), 2.61 (m, 4H), 1.80 (m, 4H).

2-Morpholinoethyl-2-aminoisonicotinate (13d): ^{1}H NMR (CD_3OD, 300 MHz) δ 8.01 (d, *J* = 5.4 Hz, 1H), 7.12 (s, 1H), 7.06 (dd, *J* = 1.8 Hz, 5.7 Hz, 1H), 4.46 (t, *J* = 5.4 Hz, 2H), 3.69 (t, *J* = 4.2 Hz, 4H), 2.28 (t, *J* = 5.7 Hz, 2H), 2.58 (t, *J* = 4.2 Hz, 4H).

The spectral data of compounds **2**, **7**, **11** and **12g** have been published previously in supplementary materials.[1]

Cell culture. Human colorectal carcinoma HCT116 cells were cultured in RPMI 1640 with 10% fetal bovine serum (FBS; Lonza, Inc.). The media contained 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco). The cells were cultured in an atmosphere of 5% CO_2 at 37 ^oC, and hypoxia was induced by culturing cells in a hypoxia chamber flushed with a mixed gas of 1% O_2, 5% CO_2, and 94% N_2.

Reporter assay. The ability of the compounds to inhibit HIF-1 was determined by a reporter assay, as previously described.[2] At 75 - 90% confluence, HCT116 cells were transiently co-transfected with pGL3-HRE-Luciferase plasmid containing six copies of HREs from human *VEGF* genes and pRL-SV40 plasmid-encoding renilla luciferase (Promega, Madison, WI, USA). After 12 h of incubation, the cells were treated with various concentrations of the tested compound and incubated for 16 h in hypoxic condition. The luciferase assay was performed using a dual-luciferase reporter assay system (Promega). Luciferase activity was determined in a Microlumat Plus luminometer (EG&G Berthold, Bad Wildbad, Germany). The results were normalized to the activity of renilla luciferase expressed by the co-transfected *Rluc* gene, under the control of a constitutive promoter. The degree of inhibition by compound (%) was calculated using following equation: I = 100 - {(Hc-N/Ho-N)} × 100. Where "Ho", HRE-Luc activity at hypoxia without compound; "N", HRE-Luc activity at normoxia; "Hc", HRE-Luc activity in the presence of compound.

Western blot analysis. Cells were lysed by adding sodium dodecyl sulfate (SDS) sample buffer and 0.03% (wt/vol) bromophenol blue. Total cell lysates were denaturated by boiling for 5 min, resolved on SDS-polyacrylamide gels, and transferred onto nitrocellulose membranes. The membranes were blocked in Tris-buffer saline containing 5% (wt/vol) skim milk and 0.1% Tween 20 for 2 h; they were then incubated with a primary antibody overnight, at 4 °C. The blot was developed using a horseradish peroxidase-conjugated secondary antibody (phototope-horseradish peroxidase Western blot detection kit; Millipore).

RNA extraction and RT-PCR. Total RNA was extracted from cells by using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA samples (1 μg) were subjected to reverse transcription using the Maxime RT PreMix for cDNA synthesis, which was used as a template PCR premix (Bioneer). The primer sequences used were as follows: *VEGF* forward, 5′-GGTGG ACATCTTCCAGAGTA-3′; *VEGF* reverse, 5′-GGCTTGTC ACATCTGCAAGTA-3′; *EPO* forward, 5′TATGCCTGGAA GATGGAGGTC-3′; *EPO* reverse, 5′TGTCAGCAGTGATT GTTCGGAAG3′; HIF1α forward, 5′CTATATCCCAATGG ATGATGATGA-3′; HIF1α reverse, 5′ATCATGTTCCATT TTTCGCTT3′; GAPDH forward, 5′ATGGGGAAGGTGAA GGTCGG3′; and GAPDH reverse, 5′-CAGGAGGCATTGC TGATGAT-3′.

Intestinal permeability experiments *in vitro*. Caco-2 cells were cultured in supplemented Dulbecco's modified eagle medium with 10% fetal bovine serum and seeded onto polycarbonate membranes for test compound transport experiments. Caco-2 cell membranes were grown by seeding on Snapwell supports incubated at 37 °C with 5% CO_2/95% O_2 and approximately 95% humidity for 15 to 21 days. Drug permeability experiments were performed at 37 °C at a final drug concentration of 5 mM in HBSS buffer supplemented with 10 mM HEPES (pH 7.4) in the apical chamber (donor side in the apical to basolateral (a-b) permeability study). These concentrations did not show any cytotoxic effect on Caco-2 cells. The sample was obtained from the apical side and from the basolateral chamber at regular time intervals and snap frozen on dry ice/methanol. Drug concentrations were determined by API2000 LC/MS/MS system. Metoprolol and ranitidine were evaluated as controls.

***In vivo* animal model.** The *in vivo* antitumor activity of **12g** was evaluated in mice using HCT116 cells (six-week-old female athymic nude mice, Crj:BALB/c nu/nu; Charles River). When the tumor volume reached approximately 100 mm^3, the mice received the following treatment *via* intravenous (i.v.) injection: group 1 (control group; six mice), vehicle solution; group 2 (six mice), **12g** administered on day 0, 1, 2, 3 and 8 at a dose of 30 mg/kg per animal. The treatments were continued for 9 days. Tumor volume (V) was determined using the following equation:

$$V = (L \times W^2) \times 0.5$$

Where L = long side and s = short side. Tumor growth inhibition was analyzed for statistical significance, using a Student's *t*-test.

Statistical analysis. Each experiment was performed at least three times, and representative data are shown. Data in the table are given as mean values ± standard deviation from separate experiments. Means were checked for statistical differences by using the Student's *t*-test with error probabilities of $p < 0.05$.

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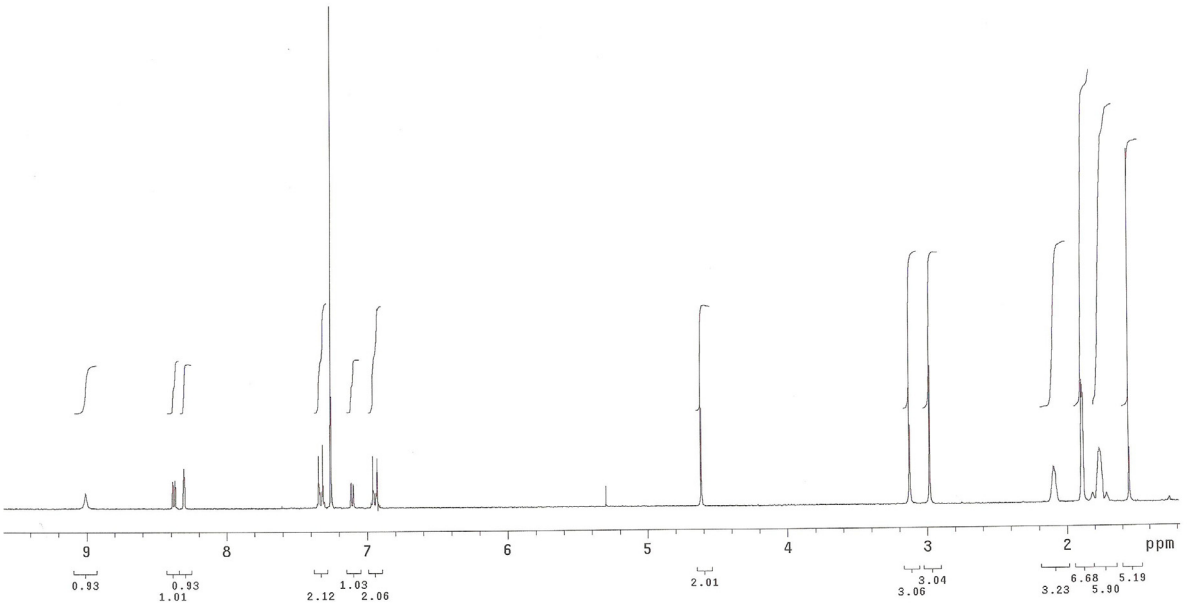
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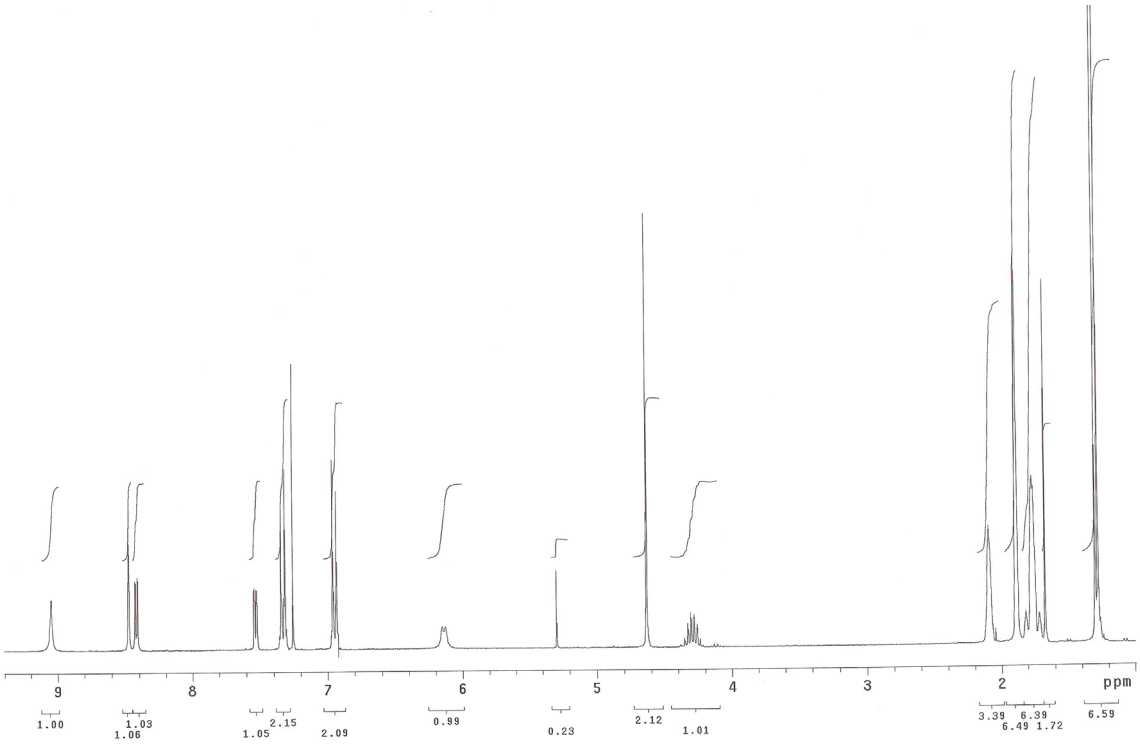
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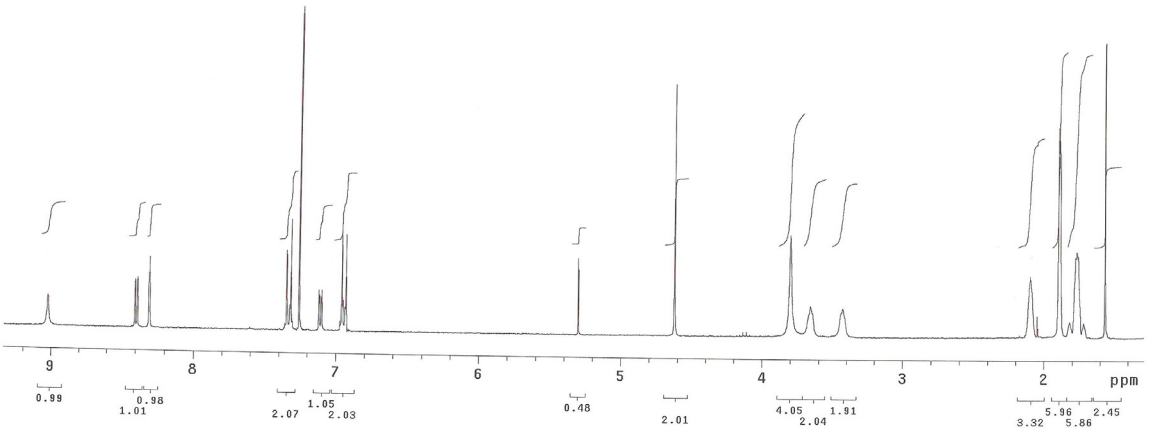
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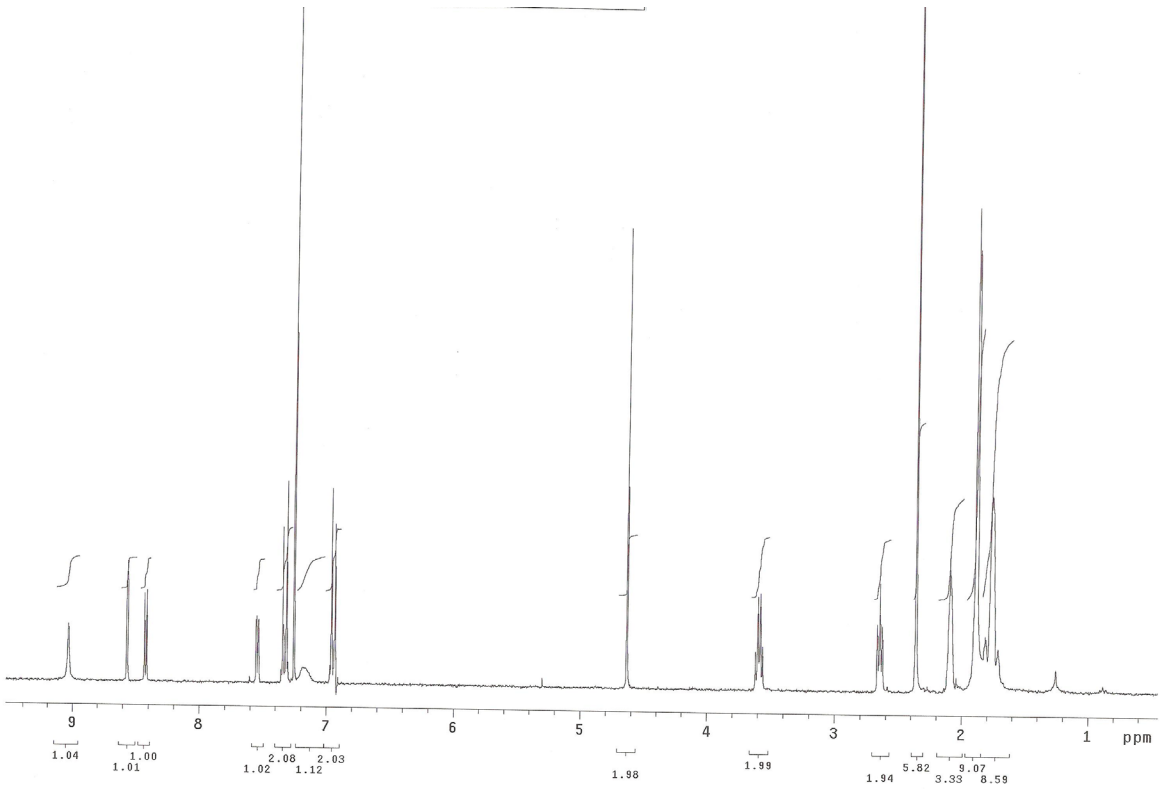
12c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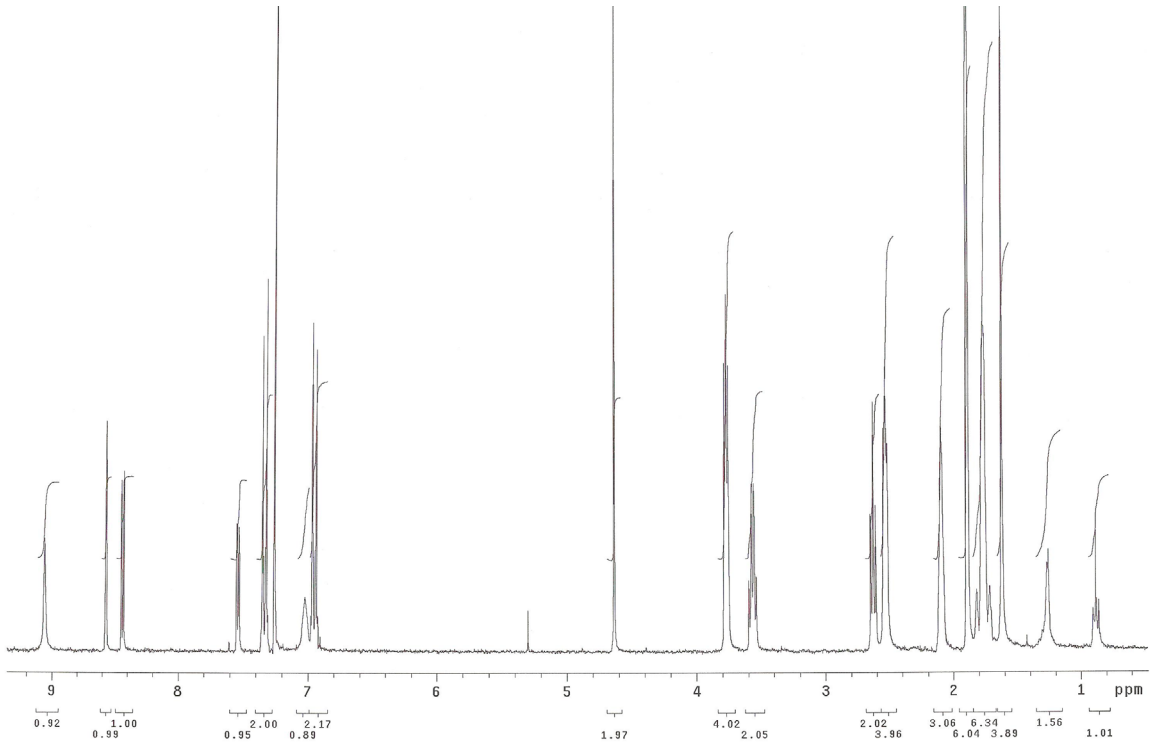
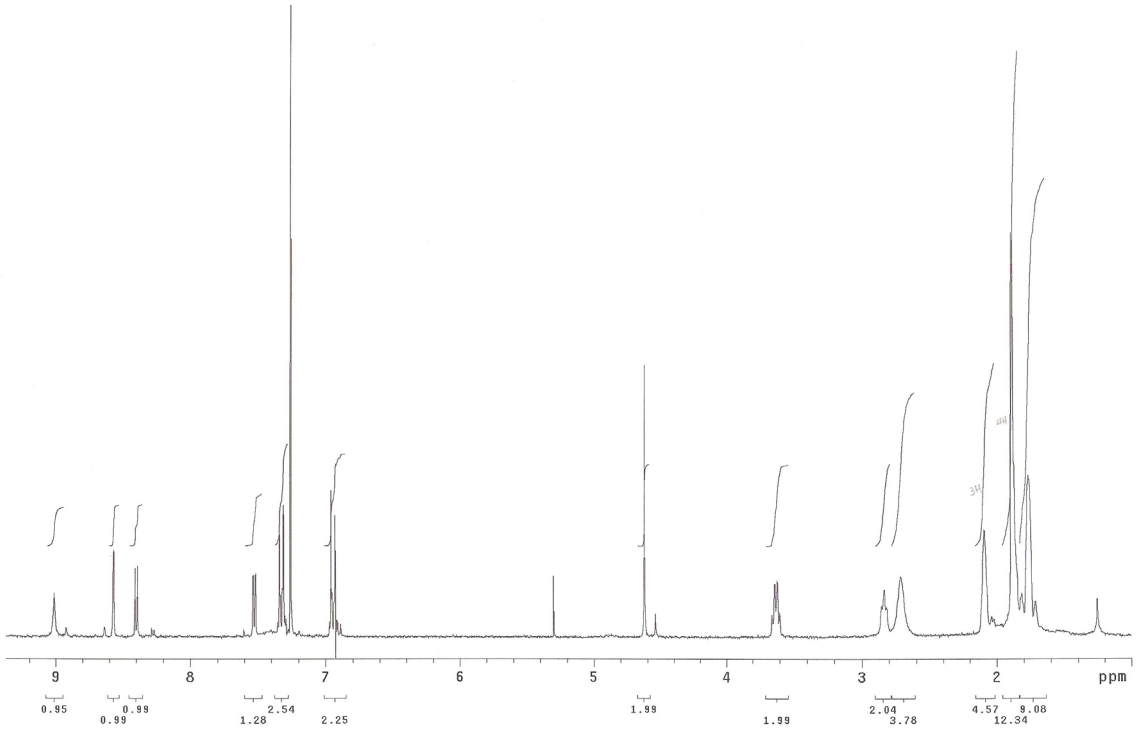
2.03
1.02
1.12
1.98
1.99
1.94
5.82
9.07
3.33
8.59

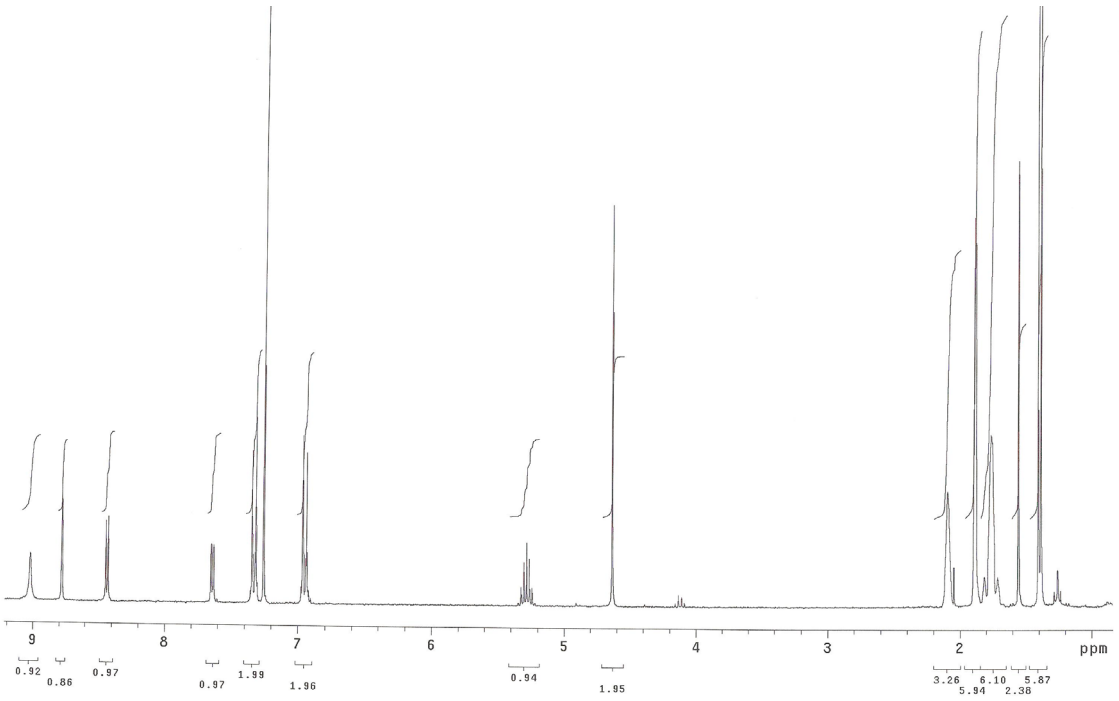
12d


9
8
7
6
5
4
3
2
1
ppm
0.92
1.00
0.99
0.95
2.00
2.17
0.89
1.97
4.02
2.05
2.02
3.96
3.06
6.34
6.04
3.89
1.56
1.01

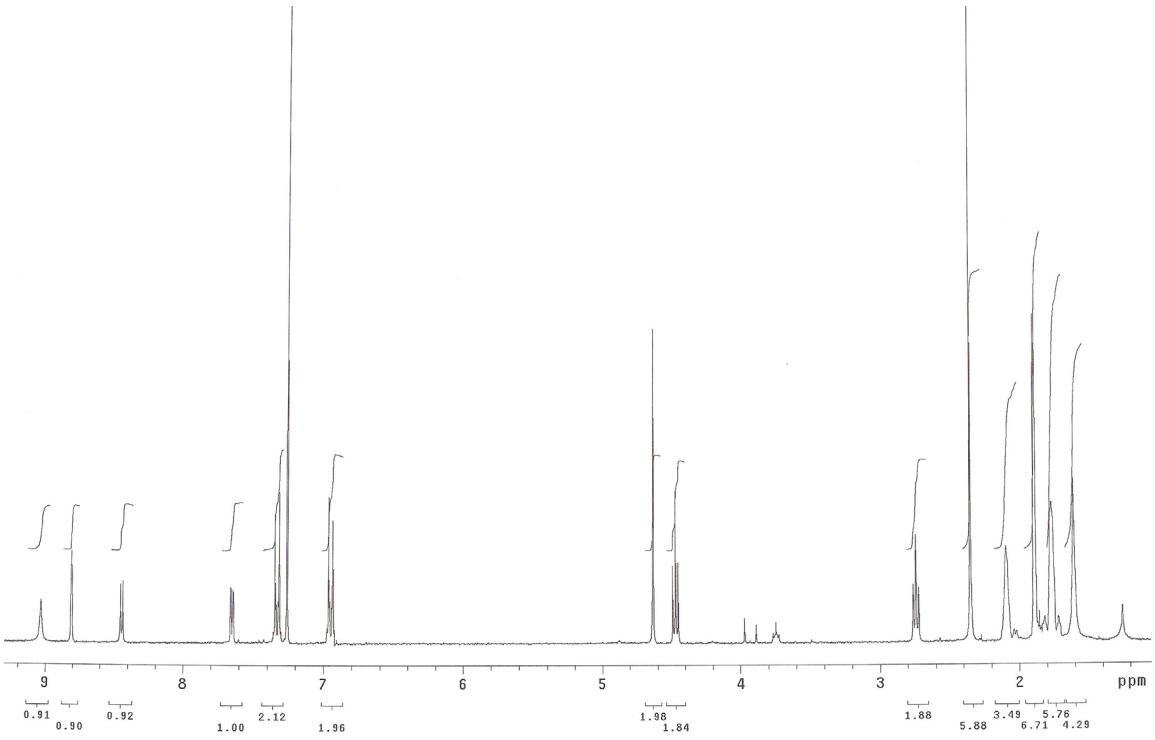
12e


9
8
7
6
5
4
3
2
ppm
0.95
0.99
0.99
0.99
1.28
2.54
2.25
1.99
1.99
2.04
3.78
4.57
9.08
12.34

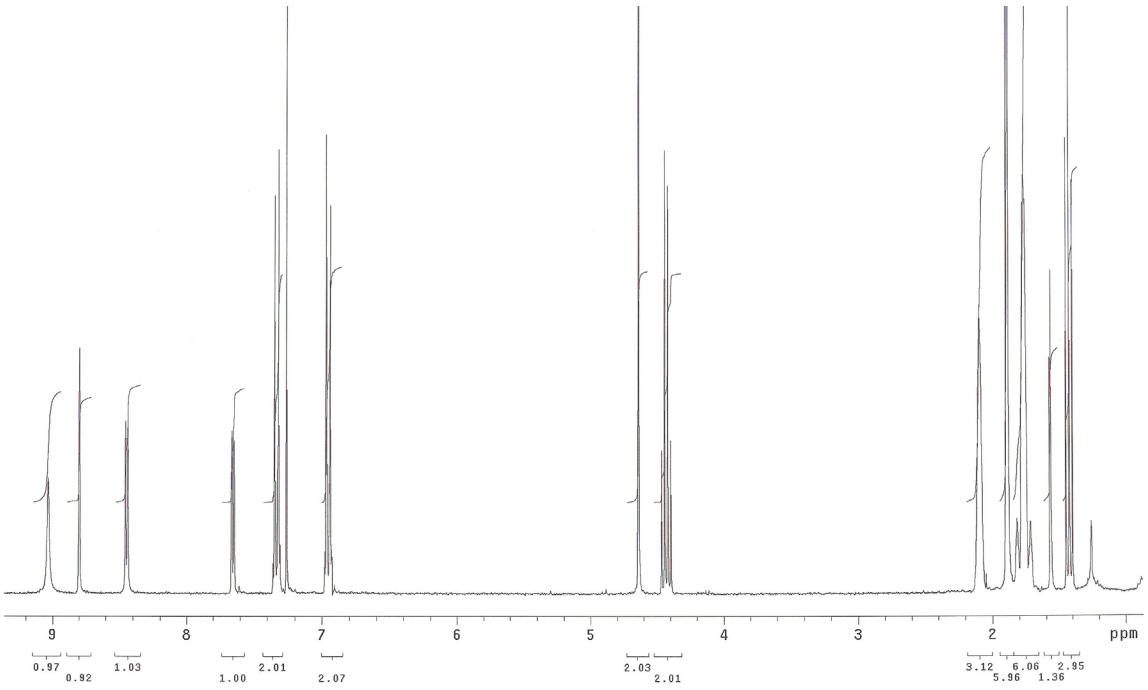
12f


9
8
7
6
5
4
3
2
ppm
0.92
0.86
0.97
0.97
1.99
1.96
0.94
1.95
3.26
6.10
5.87
5.94
2.38

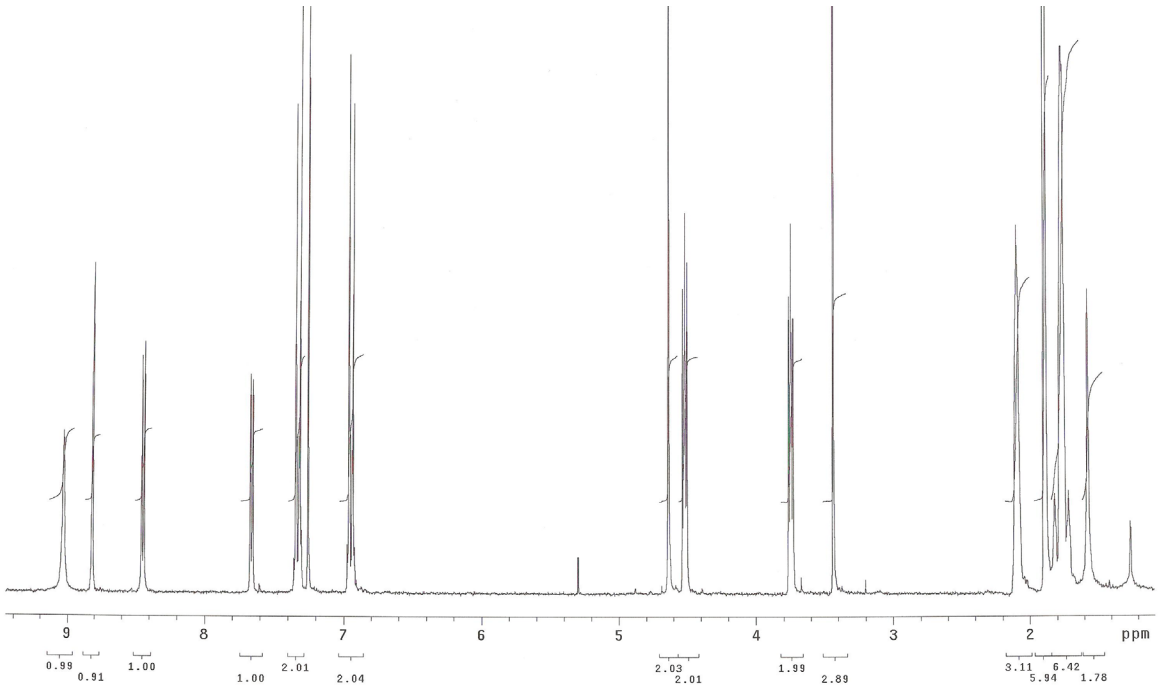
12h


9
8
7
6
5
4
3
2
ppm
0.91
0.90
0.92
1.00
2.12
1.96
1.98
1.84
1.88
5.88
3.49
6.71
5.76
4.29

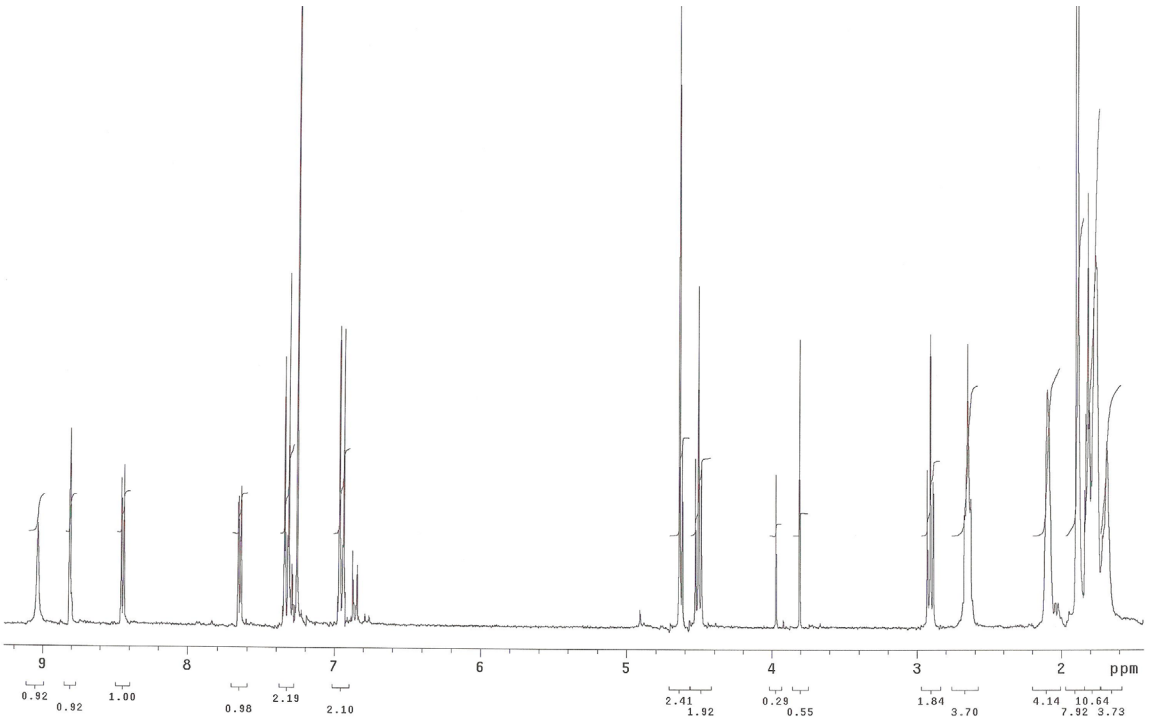
12i


9
8
7
6
5
4
3
2
ppm
0.97
0.92
1.03
1.00
2.01
2.07
2.03
2.01
3.12
6.06
2.95
5.96
1.36

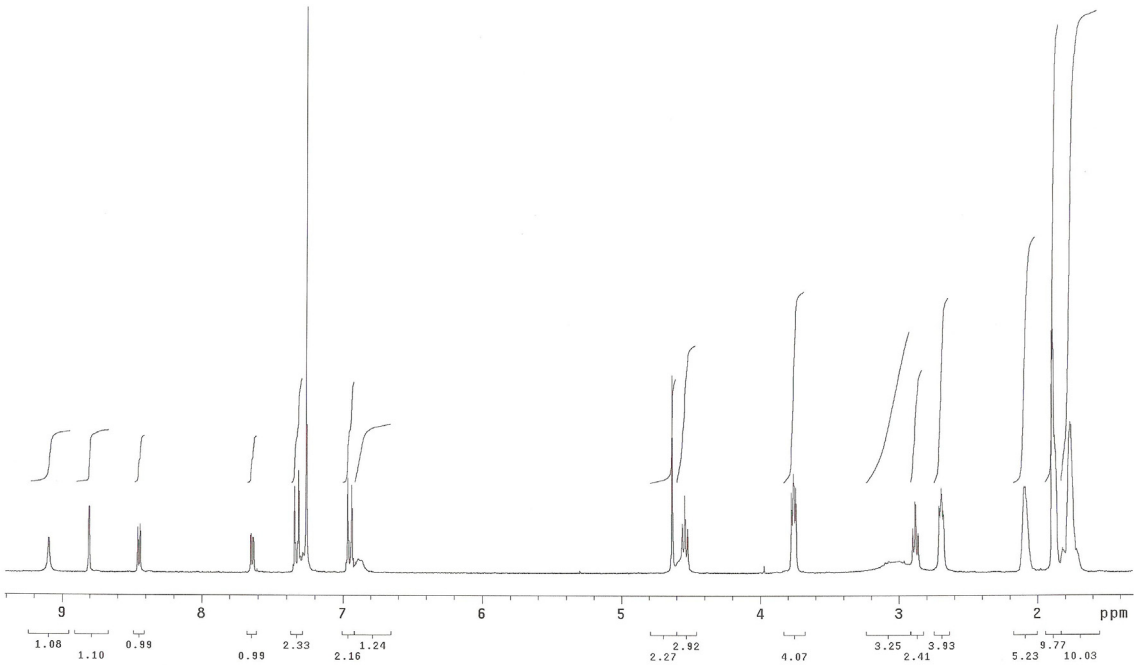
12j


9
8
7
6
5
4
3
2
ppm
0.99
0.91
1.00
1.00
2.01
2.04
2.03
2.01
1.99
2.89
3.11
6.42
5.94
1.78

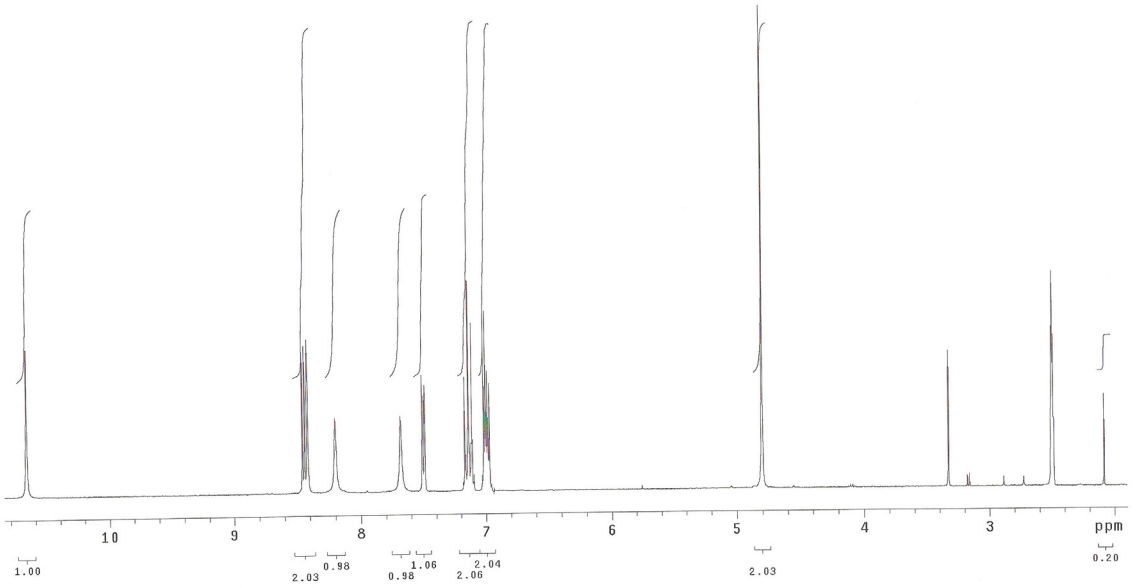
12k


9
8
7
6
5
4
3
2
ppm
0.92
0.92
1.00
0.98
2.19
2.10
2.41
1.92
0.29
0.55
1.84
3.70
4.14
10.64
7.92
3.73

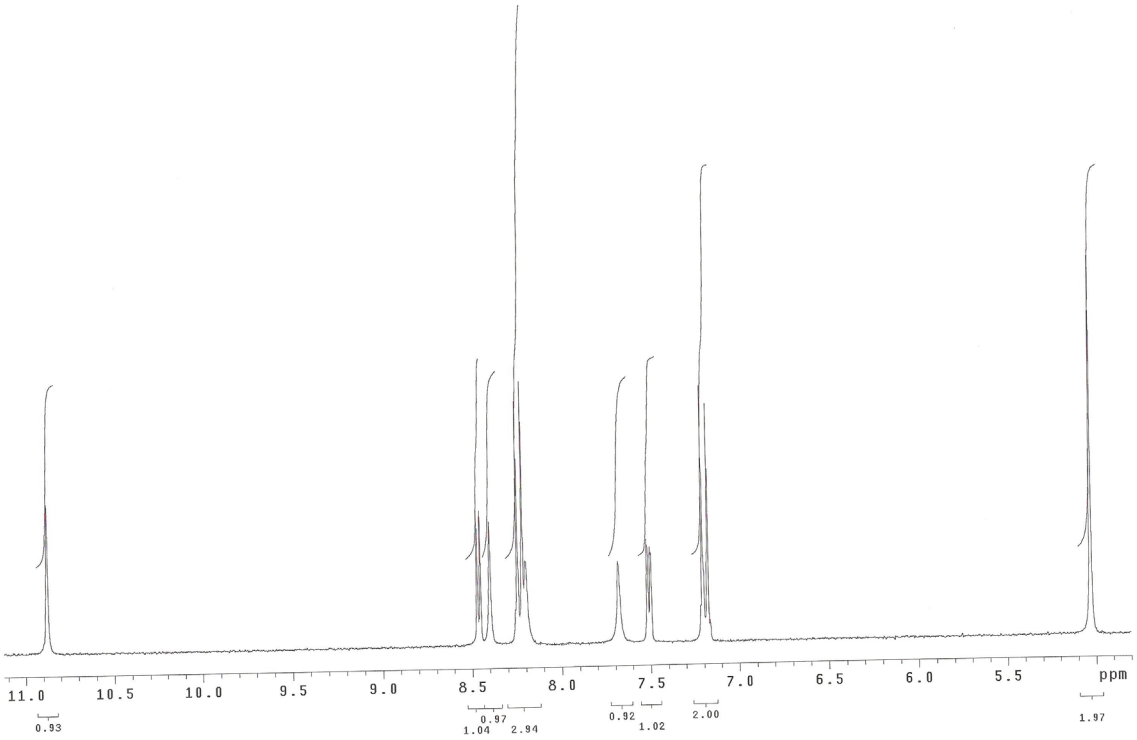
12l


9
8
7
6
5
4
3
2
ppm
1.08
1.10
0.99
0.99
2.33
1.24
2.16
2.92
2.27
4.07
3.25
3.93
2.41
5.23
9.77
10.03

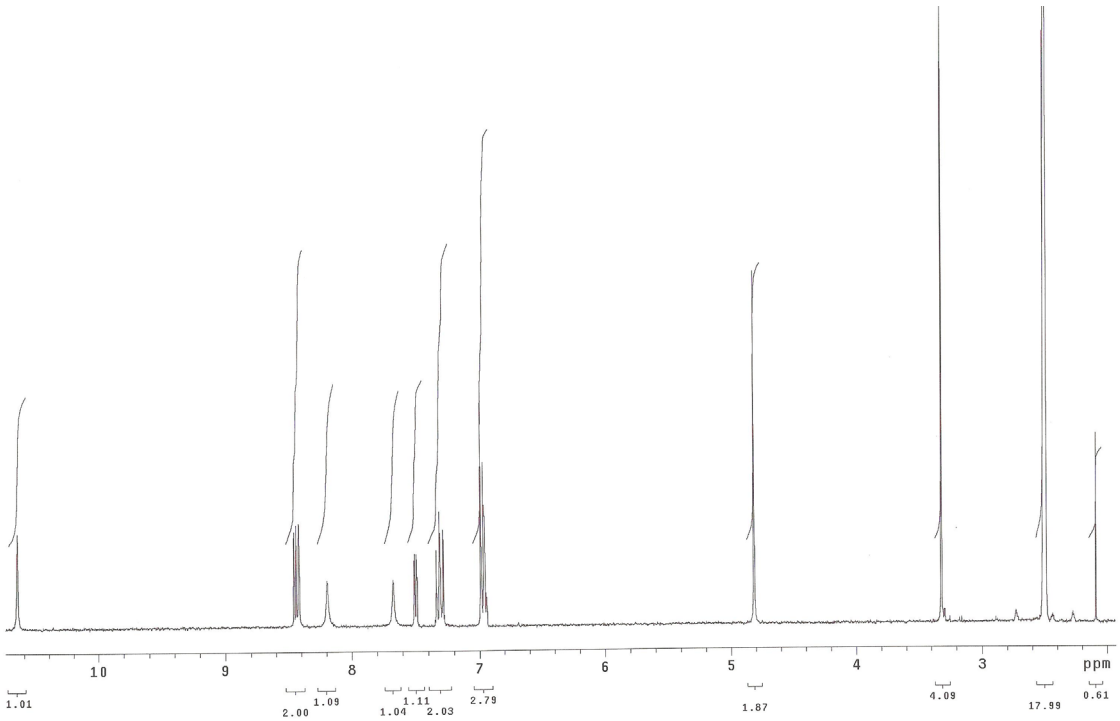
12m


10
9
8
7
6
5
4
3
ppm
1.00
2.03
0.98
0.98
1.06
2.06
2.04
2.03
0.20

8


11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
ppm
0.93
1.04
0.97
2.94
0.92
1.02
2.00
1.97

9



10