Supporting Information

Discovery and Characterization of Novel Potent PARP-1 Inhibitors Endowed with Neuroprotective Properties: From TIQ-A to HYDAMTIQ.

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Chemistry.

General Methods. Melting points were determined with a electrothermal apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz, ^{13}C NMR spectra were recorded at 101 MHz using the solvents indicated below on Bruker AC spectrometer. The chemical shifts are reported in parts per million (ppm). The abbreviations used are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; m, multiplet. Flash column chromatography was performed using silica gel 60 (0.040-0.063 mm). TLC was carried out on precoated TLC plates with silica gel 60 F-254. Spots were visualized by UV lamp (254 nm). All reactions were carried out under a argon atmosphere. All tested compounds **1**-**3** showed an HPLC purity more than 95% in two different systems: i. analytical column: Grace Smart RP-18 250 x 4.6 mm, 5 μm, 100 Å; eluent: H_2O/CH_3CN: 85:15 (v/v) + 0.1% TFA; flow rate: 1 ml/min; detection at 230 and 254 nm; ii. column Luna C-18(2) 250 x 4.6 mm, 5 μm, 100 Å; eluent: $H_2O/CH_3CN/CH_3OH$: 75:20:5 (v/v/v) + 0.1% TFA; flow rate: 1 ml/min; detection (DAD) 190-400 nm. In particular analytical data for compound **1** were reported elsewhere,[1] derivative **2** displayed a RP-HPLC purity > 99% in both systems, while HYDAMTIQ (**3**) purity was > 97%. The HPLC analyses were carried out on a Shimadzu LC workstation with a EZ Start chromatography data software, a LC-10 ATVP pump, a SCL-10AVP system controller, a FCV-10ALVP low pressure gradient formation unit, a DGU-14A on-line degasser and a 7725i injector with a 20 μL stainless steel loop; DAD or SPD-10A variable-wavelength UV-Vis were used as detectors. Elemental analyses were carried out on a Carlo Erba 1106 elemental analyzer. Mass spectra were recorded on Agilent 1100 Series LC/MSD high performance ion trap mass spectrometers.

General method A. Commercially available 3-Bromo-2-thiophenecarboxylic acid (1 mmol) dissolved in ethylenglicoledimethylether (105 ml) was added of $Pd(Ph_3P)_4$ (936 mg, 0.81 mmol) and stirred at room temperature for 15 min. Successively, the mixture was treated with the suitable benzene boronic derivative (1.1 mmol) and an aqueous solution of 2M $NaHCO_3$ (4 mmol). The reaction mixture was then refluxed up to starting material disappearance (usually 10 h). After the mixture was cooled to room temperature, the solvent was partially removed under reduced pressure and the resulted mixture was extracted twice with diethyl ether. The water layer was acidified with 10% HCl and extracted with ethyl acetate (5 times). The collected organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was crystallized with a mixture of solvents, n-hexane/ethyl acetate (about 8:2) thus obtaining 3-aryl-2-thiophenecarboxylic acid derivatives as pure solid.

General method B. Thionyl chloride (1 ml) was added to a suspension of 3-aryl-2-thiophenecarboxylic acid derivatives (1 mmol) in 10 ml of dry benzene and the mixture was refluxed for 2 h. The solvent and the excess of thionyl chloride were removed under vacuum and the residue was taken up using 10 ml of dry THF, and NaN_3 (1.5 mmol) dissolved in the minimal amount of water was quickly added to this solution stirred at 0 °C. The mixture was stirred for 1 h at room temperature and poured into 100 ml of cracked ice and water and extracted with diethyl ether (4 x 100 ml), the collected organic layers were dried under Na_2SO_4. The filtrate was evaporated under vacuum and the residue was dissolved in 10 ml of *o*-dichlorobenzene and reflux for 5 h. The mixture was then cooled, and directly submitted to the flash chromatography, elution with dichloromethane/methanol (99/1) afforded to the cyclized derivatives as pure solid.

General method C. Derivatives from general procedure B (1 mmol) were dissolved in a mixture of dry dimethylformamide (1 ml) and dry acetonitrile (2 ml) and treated with N,N-dimethyl(methylene)ammonium chloride (2 mmol). The reaction mixture was refluxed overnight.

The formed solid was then filtered and washed with dry diethyl ether to give the desired 2-dimethylaminomethyl derivatives as hydrochloride salts.

3-Phenyl-2-thiophenecarboxylic acid[1] **(7).** Following the method A and starting from 3-bromo-2-thiophenecarboxylic acid (1.68 g, 8.2 mmol) and benzeneboronic acid (1.4 g, 8.2 mmol), **7** was prepared as pure solid (75% yield). mp: 166-168°C. ^{1}H-NMR (DMSO-d6) δ 7.25 (d, *J* = 5.1 Hz, 1H, H-Th), 7.35-7.41 (m, 3H, H-Ph), 7.44-7.47 (m, 2H, H-Ph), 7.94 (d, *J* = 5.1 Hz, 1H, H-Th), 12.85 (s, 1H, CO_2H). ^{13}C-NMR (DMSO-d6) δ 127.65, 127.76 (2C), 128.05, 129.29 (2C), 131.07, 135.51, 147.19, 162.84.

Thieno[2,3-c]isoquinolin-5(*4H*)-one[1] **(1).** Following the method B and starting from 3-phenyl-2-thiophenecarboxylic acid (0.5 g, 2.45 mmol), **1** was prepared as pure solid (44% yield). mp: 252-254°C. ^{1}H-NMR (DMSO-d6) δ 7.23 (d, *J* = 5.6 Hz, 1H, H-Ar), 7.51 (m, 1H, H-Ar), 7.70 (d, *J* = 5.3 Hz, 1H, H-Ar), 7.78 (m, 1H, H-Ar), 8.10 (dd, *J* = 7.4, 1.1 Hz, 1H, H-Ar), 8.25 (dd, *J* = 8.0, 0.9 Hz, 1H, H-Ar), 12.3 (s, 1H, CONH). ^{13}C-NMR ($CDCl_3$) δ 116.4, 119.2, 120.6, 122.6, 123.3, 126.2, 128.4, 133.1, 134.1, 139.9, 163.2. MS (ES^+) m/z 224.4 (M+Na; 100%); (ES^-) m/z 200.4 (M-H; 100%). Elemental Anal. Calc. (%) for $C_{11}H_7NOS$: C, 65.65; H, 3.51; N, 6.96. Found: C, 66.03; H, 3.44; N, 6.81.

2-Dimethylaminomethylthieno[2,3-c]isoquinolin-5(*4H*)-one hydrochloride (2). Following the method C and starting from thieno[2,3-c]isoquinolin-5(*4H*)-one (**1**) (0.03 g, 0.15 mmol), **8** was prepared as pure solid (76% yield). mp: 244-245°C. ^{1}H-NMR (DMSO-d6) δ 2.78 (s, 6H, $N(CH_3)_2$), 4.55 (s, 2H, NCH_2), 7.58 (t, *J* = 7.6 Hz, 1H, H-Ph), 7.86 (t, *J* = 7.6 Hz, 1H, H-Ph), 7.95 (s, 1H, H-Th), 8.06 (d, *J* = 7.9 Hz, 1H, H-Ph), 8.29 (d, *J* = 8.0 Hz, 1H, H-Ph), 10.99 (bs, 1H, HCl), 12.57 (s, 1H, CONH). ^{13}C-NMR (DMSO-d6) δ 41.1, 53.8, 117.2, 121.5, 123.0, 123.6, 126.3, 126.5, 127.9,

133.2, 142.8, 161.1. MS (ES^+) m/z 259.5 (M-Cl; 80%), 214.4 (M-C_2H_7ClN; 100%). Elemental Anal. Calc. (%) for $C_{14}H_{15}ClN_2OS$: C, 57.04; H, 5.13; N, 9.50. Found: C, 57.11; H, 5.43; N, 9.31.

3-(2-Methoxyphenyl)-2-thiophenecarboxylic acid[1] **(8).** Following the method A and starting from 3-bromo-2-thiophenecarboxylic acid (5.0 g, 24.2 mmol) and 2-methoxybenzeneboronic acid (5 g, 28.9 mmol), **8** was prepared (70% yield). ^{1}H-NMR (DMSO-d6) δ 3.68 (s, 3H, CH_3), 6.95 (t, *J* = 7.4 Hz, 1H, H-Ph), 7.03-7.07 (m, 2H, H-Ph and H-Th), 7.19 (dd, *J* = 7.5, 1.6 Hz, 1H, H-Ph), 7.32 (t, *J* = 7.4 Hz, 1H, H-Ph), 7.78 (d, *J* = 5.1 Hz, 1H, H-Th), 12.64 (s, 1H, CO_2H). ^{13}C-NMR (DMSO-d6) δ 55.29, 111.15, 119.91, 124.98, 129.14, 129.61, 130.44, 131.90, 143.06, 156.41, 162.93.

9-Methoxythieno[2,3-c]isoquinolin-5(4*H*)-one[1] **(9).** Following the method B and starting from **8** (0.6 g, 2.75 mmol), **7** was prepared as pure solid (71% yield). mp: 198-201°C. ^{1}H-NMR (DMSO-d6) δ 4.00 (s, 3H, CH_3), 6.95 (d, *J* = 5.7 Hz, 1H, H-Ar), 7.20 (m, 1H, H-Ar), 7.44 (t, *J* = 8.0 Hz, 1H, H-Ar), 7.98 (d, *J* = 5.7 Hz, 1H, H-Ar), 8.13 (dd, *J* = 8.0, 1.1 Hz, 1H, H-Ar). ^{13}C-NMR (DMSO-d6) δ 56.8, 114.7, 115.7, 116.1, 120.4, 124.3, 125.9, 126.3, 127.5, 155.7, 161.7.

2-Dimethylaminomethyl-9-methoxythieno[2,3-c]isoquinolin-5(4*H*)-one hydrochloride (10). Following the general method C and starting from **7** (1.20 g, 5.50 mmol), **9** was prepared as pure solid (85% yield). mp: 264-265°C. ^{1}H-NMR (DMSO-d6) δ 2.72 (s, 6H, $N(CH_3)_2$), 4.01 (s, 3H, OCH_3), 4.51 (s, 2H, CH_2N), 7.42 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.49 (t, *J* = 7.9 Hz, 1H, H-Ar), 7.88 (d, J= 7.8 Hz, 1H, H-Ar), 8.15 (s, 1H, H-Ar), 10.53 (bs, 1H, HCl), 12.59 (s, 1H, NH). ^{13}C-NMR (DMSO-d6) δ 43.1, 56.0, 57.9, 116.0, 116.3, 121.4, 125.3, 127.1, 128.9, 132.4, 144.9, 156.6, 162.6.

2-Dimethylaminomethyl-9-hydroxythieno[2,3-c]isoquinolin-5(*4H*)-one hydrobromide (3). Boron tribromide 1 M dichloromethane solution (0.1 ml, 0.41 mmol) was added to a solution of 9-methoxy-4 H-thieno[2,3-c]isoquinolin-5(4*H*)-one (**9**) (0.03 g, 0.09 mmol) in dichloromethane (2 ml) and the reaction mixture was refluxed for 24 h. The mixture was cooled to r.t. and pored into ice

and the resulted mixture was and evaporated under reduced pressure. The mixture was purified by crystallization with 96% ethanol to obtain **3** (54% yield) as pure solid. mp: 214°C dec. ^{1}H-NMR (DMSO-d6) δ 2.77 (ps, 6H, $N(CH_3)_2$), 4.56 (ps, 2H, CH_2N), 7.27 (d, *J* = 7.9, 1H, H-Ph), 7.36 (t, *J* = 7.9, 1H, H-Ph), 7.75 (d, *J* = 7.9 Hz, 1H, H-Ph), 8.20 (s, 1H, H-Th), 9,71 (bs, 1H, HBr), 10.61 (s, 1H, OH), 12.44 (s, 1H, NH). ^{13}C-NMR (DMSO-d6) δ 43.2, 56.1, 117.0, 120.0, 120.2, 121.4, 124.0, 127.4, 128.9, 132.6, 144.0, 155.0, 162.8. MS (ES^+) m/z 275.6 (M-Br; 20%), 230.6 (M-C_2H_7BrN; 100%). Elemental Anal. Calc. (%) for $C_{14}H_{15}BrN_2O_2S$: C, 47.33; H, 4.26; N, 7.89. Found: C, 46.99; H, 4.47; N, 7.54.

Biology.

Measurement of PARP-1 activity. The compounds were evaluated as PARP-1 inhibitors in a fluorescence based multiwell assay,[2] using human recombinant PARP-1 enzyme.

Measurement of PARP-2 and Tankyrase-1 activity. The compounds were evaluated as PARP-2 and Tankyrase-1 inhibitors according to the methodology reported in reference 28 of the manuscript.

Human embryonic kidney 293 cells and ATP measurements. HEK293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine, 5% fetal bovine serum, and antibiotics. Cultures were brought to 50–70% confluence and exposed to MNNG and the PARP-1 inhibitors. Cellular ATP was measured using a kit (Perkin-Elmer, Life Siences) as described in previous work.[3]

Oxygen-Glucose Deprivation in Cortical Cell Cultures. Cultures of mixed cortical cells containing both neuronal and glial elements were prepared, used at 14 days in vitro, and exposed to

OGD as previously described in detail.[4] Briefly, culture medium was replaced by a glucose-free balanced salt solution saturated with 95% N_2 / 5% CO_2 and heated to 37°C. Multiwells were then sealed into an airtight incubation chamber equipped with inlet and outlet valves, and 95% N_2 / 5%

CO_2 was blown through the chamber for 10 min to ensure maximal removal of oxygen. The chamber was then sealed and placed into the incubator at 37°C for 60 min. OGD was terminated by removing the cultures from the chamber, replacing the exposure solution with oxygenated medium, and returning the multiwells to the incubator under normoxic conditions. The extent of neuronal death was assessed 24 h later. OGD-induced cell injury was quantitatively evaluated by measuring the amount of LDH released from injured cells into culture media 24 h following exposure to OGD, as previously described.[5, 6]

Middle cerebral artery occlusion (MCAO). Sprague Dawley 275-300 g male rats were anesthetized with isofluorane, 5% for induction, and 2% for maintenance in air. Body temperature was measured with a rectal probe and kept at 37°C with a heating pad.

The external and internal right carotid arteries were dissected under an operating microscope and a silk suture was tied loosely around the external carotid stump. A silicone-coated nylon filament (diameter: 0.28 mm) was then inserted through the external into the internal carotid artery up to the Willis circle to occlude the right middle cerebral artery. The silk suture was tightened around the intraluminal filament to prevent bleeding. 2 hours later the filament was removed and the rats were sacrificed after 2 days. A laser Periflux 5000 doppler system connected to a 418-1 probe, attached to the skull 4 mm lateral and 2 mm posterior of bregma, was used to monitor regional cerebral blood flow (rCBF) before ischemia, during MCAO, and after reperfusion. The MCAO was considered adequate if rCBF showed a sharp decrease to 25% of baseline (pre-ischemia) level. Cerebral blood flow was constantly monitored during the experiments and rats were utilized only if

the average cerebral blood flow in the region, during the 2 hour ischemic period, decreased by at least 70 % and recovered to approximately the basal levels (± 10 %) within a few minutes after the withdrawing of the filament. The brains were rapidly removed and frozen in dry ice for cryostat

sectioning. Toluidine blue stained coronal sections were imaged by using the Image 3.0 ProPlus analysis software as previously described.[7] Infarct areas were calculated subtracting the area of intact tissue in the ipsilateral hemisphere from the area of the contra lateral hemisphere to minimize the error that is introduced by oedema. Infarct volumes were calculated multiplying the infarct area by the distance among sections.[8] A small group of animals was again anaesthetized 24 h after surgery in pMCAO experiments and 48 h in tMCAO, the chest was open and 10 ml of a solution containing 2% 2,3,5-triphenyltetrazolieum chloride (TTC) in saline was slowly injected into the left cardiac ventricle. Twenty min later brains were removed and placed in 4% buffered formalin. Within two days, 2 mm thick coronal slices were prepared and the infarct areas were measured using the above- mentioned computer assisted image analysis system. The brain infarct volumes measured with toluidine blue staining were not different than those measured with TTC staining.

Crystallography.

PARP-1 catalytic domain (residues 654-1014) at 11 mg/ml in 25 mM Tris pH 8.5, 3.4 mM beta-mercaptoethanol, 0.011 mM beta-octylglucopyranoside was co-crystallized by hanging drop vapor diffusion in drops mixed 1:1 with 100 mM sodium cacodylate, pH 7.4, 20-23% PEG 8000, 200 mM magnesium acetate. Crystals appeared within one week. Crystals were frozen without additional cryo-protection and X-ray data to a resolution of 2.25 Å was collected on a Rigaku FR-E rotating anode x-ray source (see also Table 2 in the text). Data was processed using xia2 in space group P 21 21 2.[9] The structure was solved by molecular replacement using Phaser[10] with pdb code 2RD6[11] as

the search model. The structure was refined to an R-work/R-free of 0.202/0.281 using Phenix[12] and AutoBUSTER,[13] the latter to try to better resolve the orientation of the tertiary amine. Overall, the electron density for the ligand was very clear.

Table 1. Crystallographic data.

Data Collection	**High resolution shell shown in parentheses**
Resolution	32.35 – 2.25 (2.31 – 2.25)
R-merge overall	0.109 (0.69)
Total No. Observations	117850 (1923)
Total Number Unique	17676 (513)
Mean ((I)/sd(I))	14.1 (2.8)
Completeness %	90.1 (36.5)
Multiplicity	6.7 (3.7)
Refinement	
R-work/R-free	0.202/0.281
Space Group	P21212
Cell length (a,b,c)	67.3, 92.3, 64.7
Cell angle	90, 90, 90
Number of reflections	16122

Molecular Modelling.

All the calculation were performed with the Schrodinger Suite 2009. The CαRMSD calculation have been performed using the *Protein Structure Alignment* tool with the HYDAMTIQ (**3**) complex used as reference, compared with the other PARP-1 x-rays availavable to date on the Protein DataBank.

DAMTIQ (**2**) was prepared for the docking study with the default options of the LigPrep module that assign all the possible protonation states at pH 7.0 ± 2. The crystal structure of PARP-1 in complex with HYDAMTIQ (**3**) was used for docking experiments after being prepared with the standard options of the Protein Preparation Wizard protocol. The docking grid was calculated placing the coordinates of the center of mass of the co-crystallized ligand as center of a cubic box, having a side length of 10 Å. Two H-bond constraints were added on the carbonylic oxygen and on the amidic hydrogen of the backbone atoms of the Gly293 and used as constraints during the pose search. All docking calculations were performed using the extra precision (XP) mode of Glide 5.5. The best poses were stored and used for visual inspection.

WaterMap simulations were performed on the crystal structure of HYDAMTQ (**3**) bound to PARP-1 structure after removing the ligand.

The system was prepared using the Protein preparation wizard of Maestro 9.0. Hydrogen additions and the adjustment of protonation and tautomerization states of binding site residues were carried out at pH 7.4. The crystal structure water molecules were retained during preparation of the system. Bond orders and assigned charged states of co-crystallized ligand were visually inspected for any structural discrepancies. Finally, the all-hydrogen protein-ligand complexes were subjected to restrained molecular mechanics refinement to the RMSD of 0.3 Å using the OPLS-2005 force field incorporated in the IMPREF minimization panel of protein preparation. The final refined structures were used for Watermap calculations.

Watermap is an innovative tool which predicts the location of hydration sites and calculates thermodynamics of the water molecules in the protein-binding site using clustering technique applied to an explicit solvent molecular dynamics (MD) simulation.[14, 15] In this analysis, a molecular dynamics simulation of the active site is firstly performed. Then, the resulting trajectory is analyzed for regions highly occupied by water, and an assessment of the thermodynamic nature of each resulting water was calculated.

For Watermap simulation, residues within the 5 Å distance of any atom of the corresponding ligand were considered as binding site whereas residues within 15 Å from co-crystallized ligand were defined as simulation boundary. Existing crystalline waters were treated as solvent. Watermap simulations were performed for 2 ns of the simulation time. The analysis of Watermap simulation was done using Watermap analysis module in Maestro 9.0.

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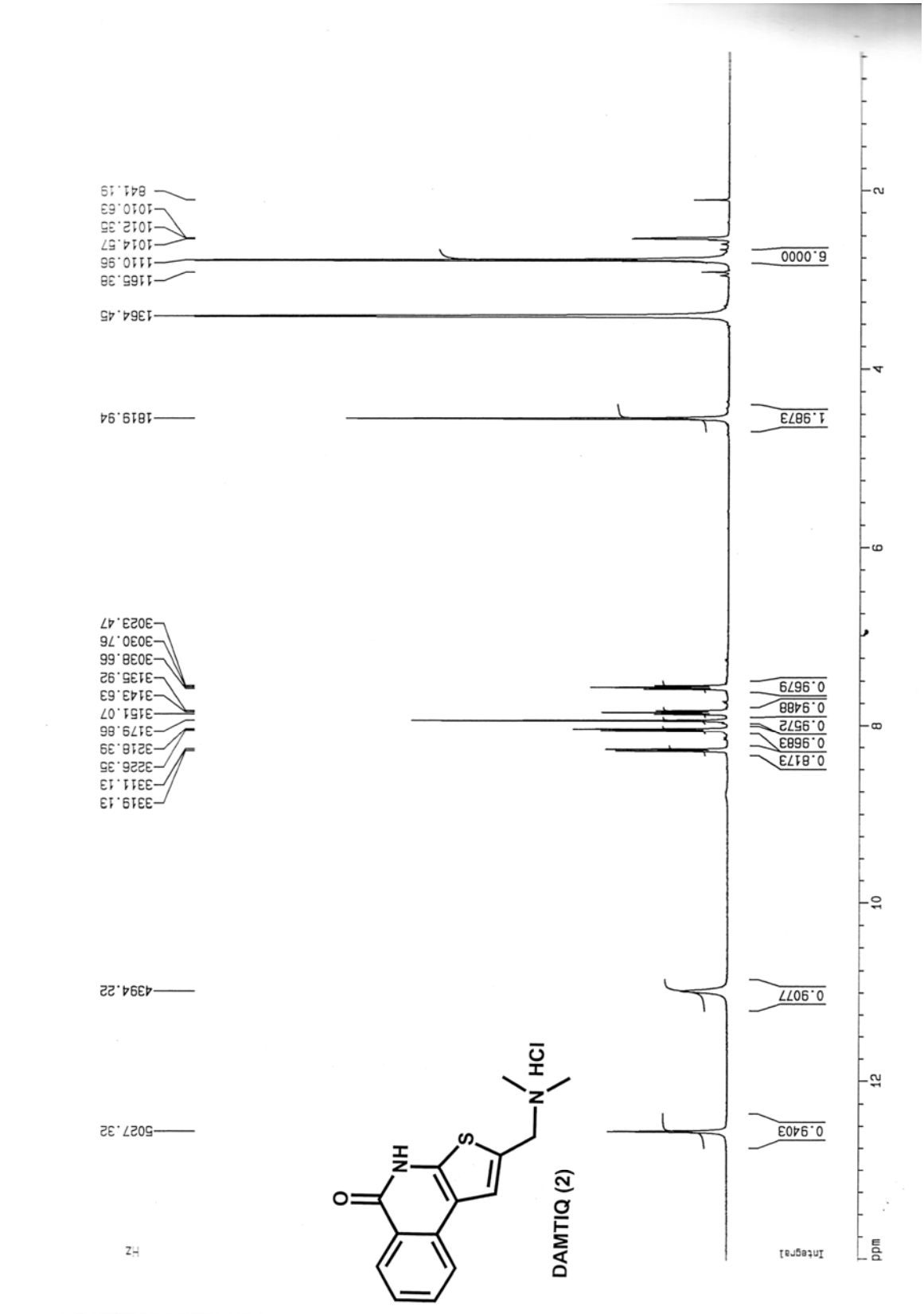
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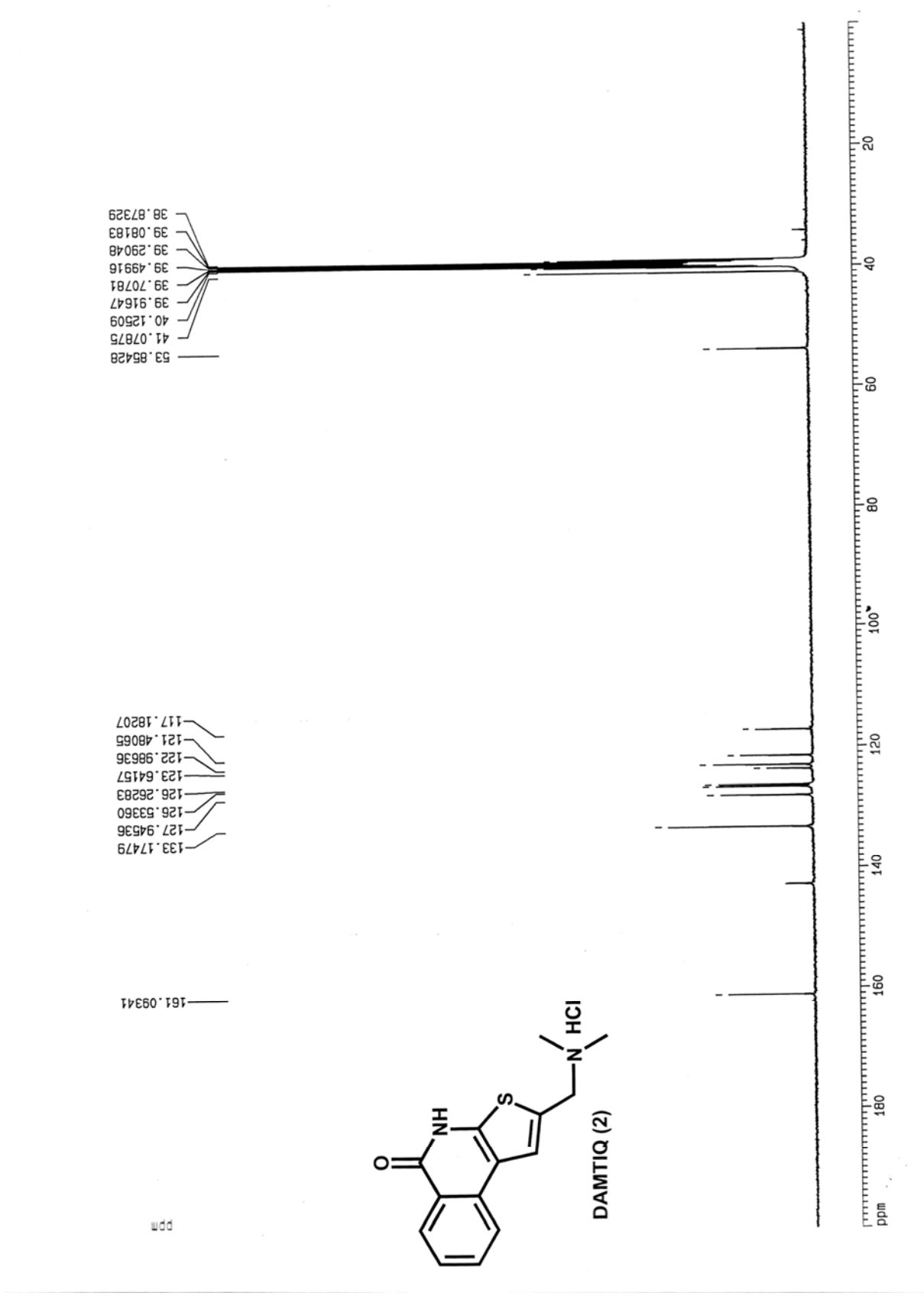
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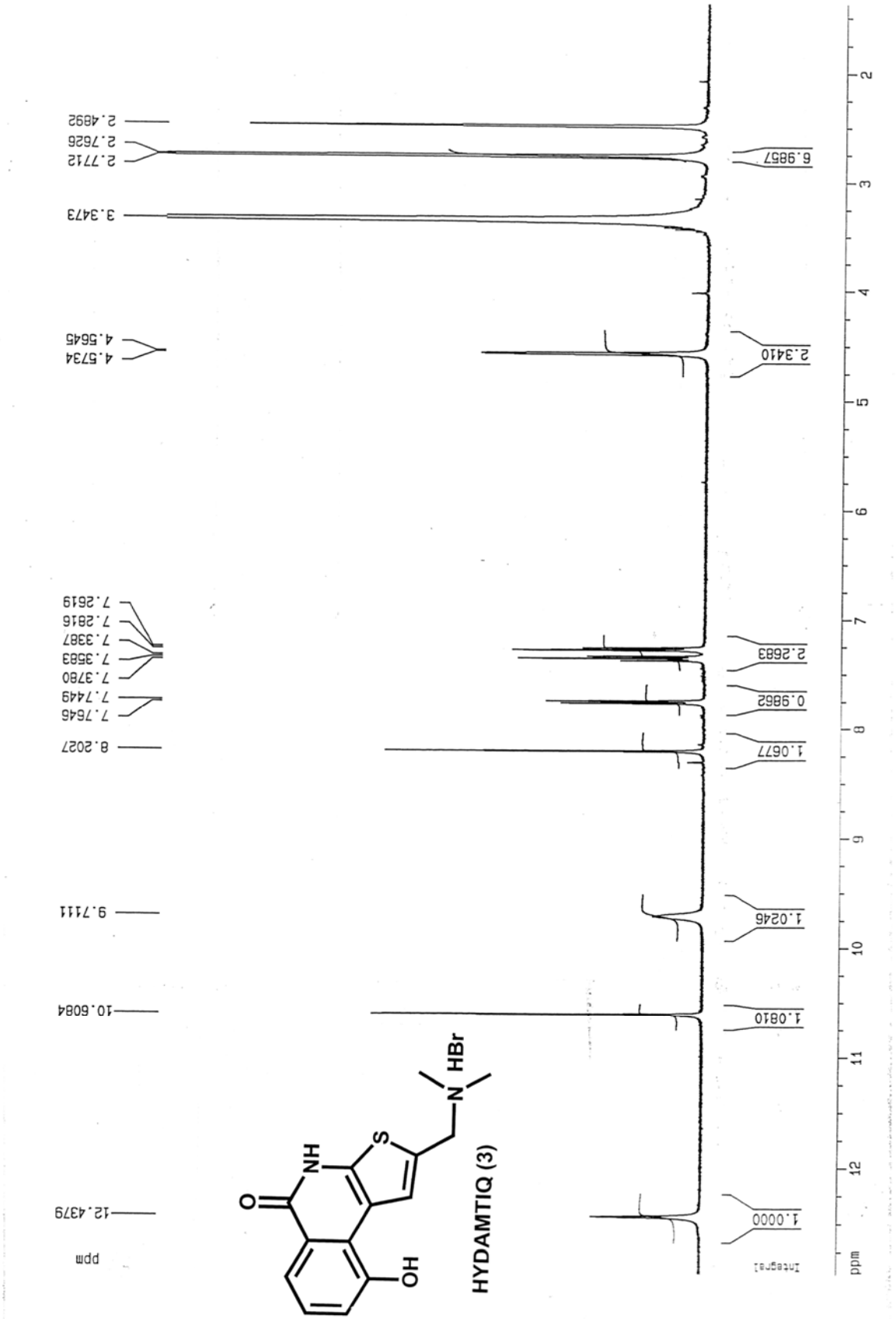
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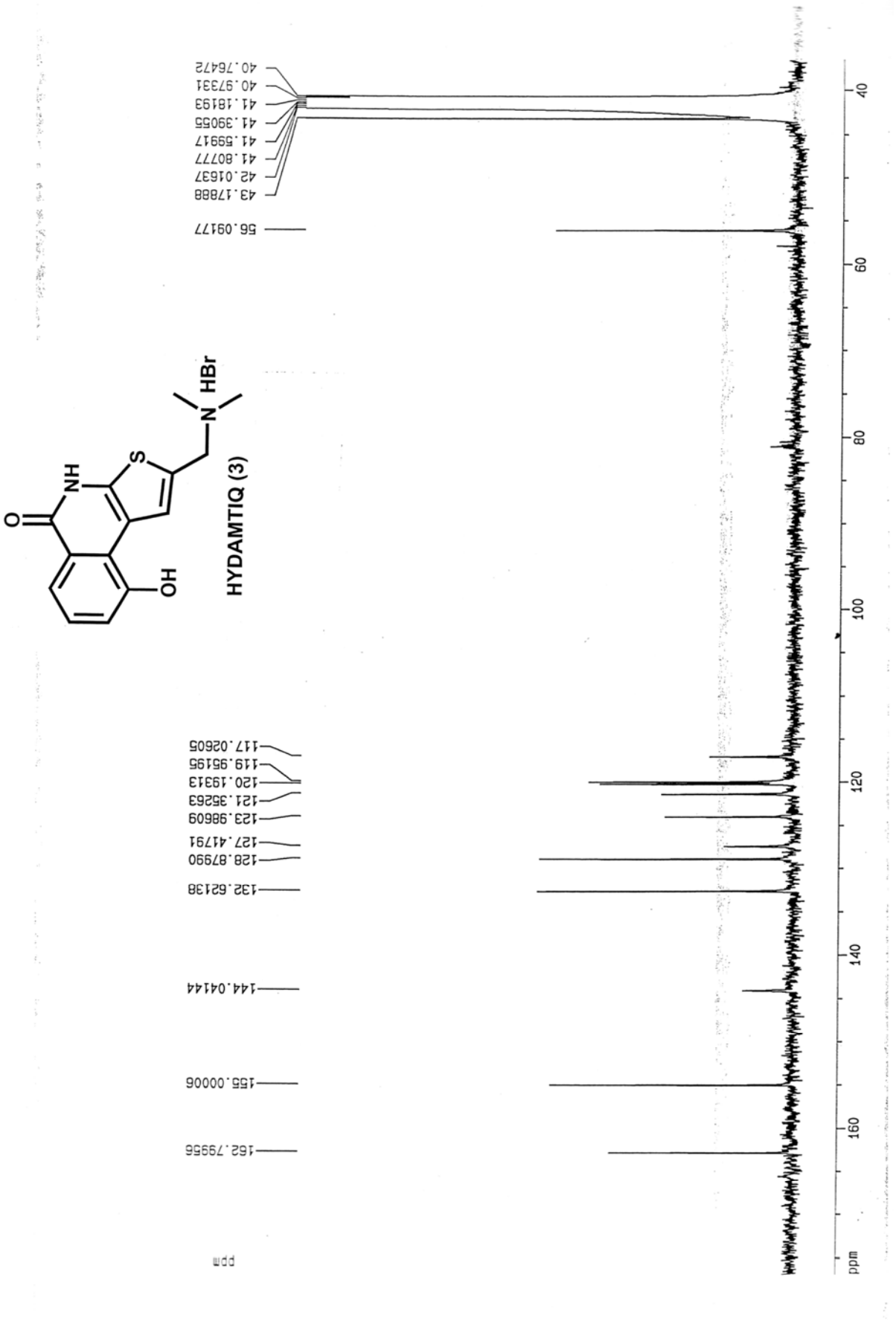
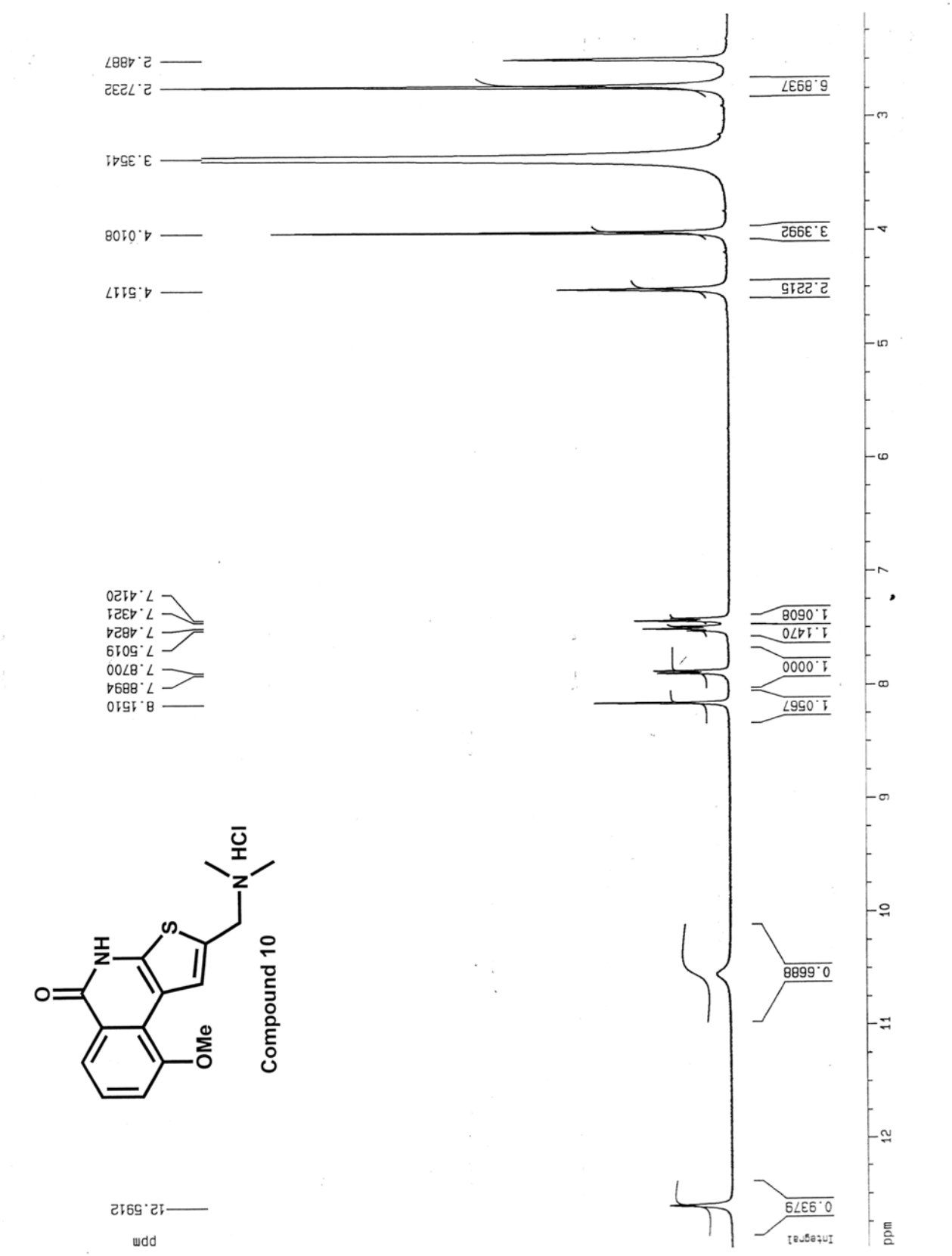
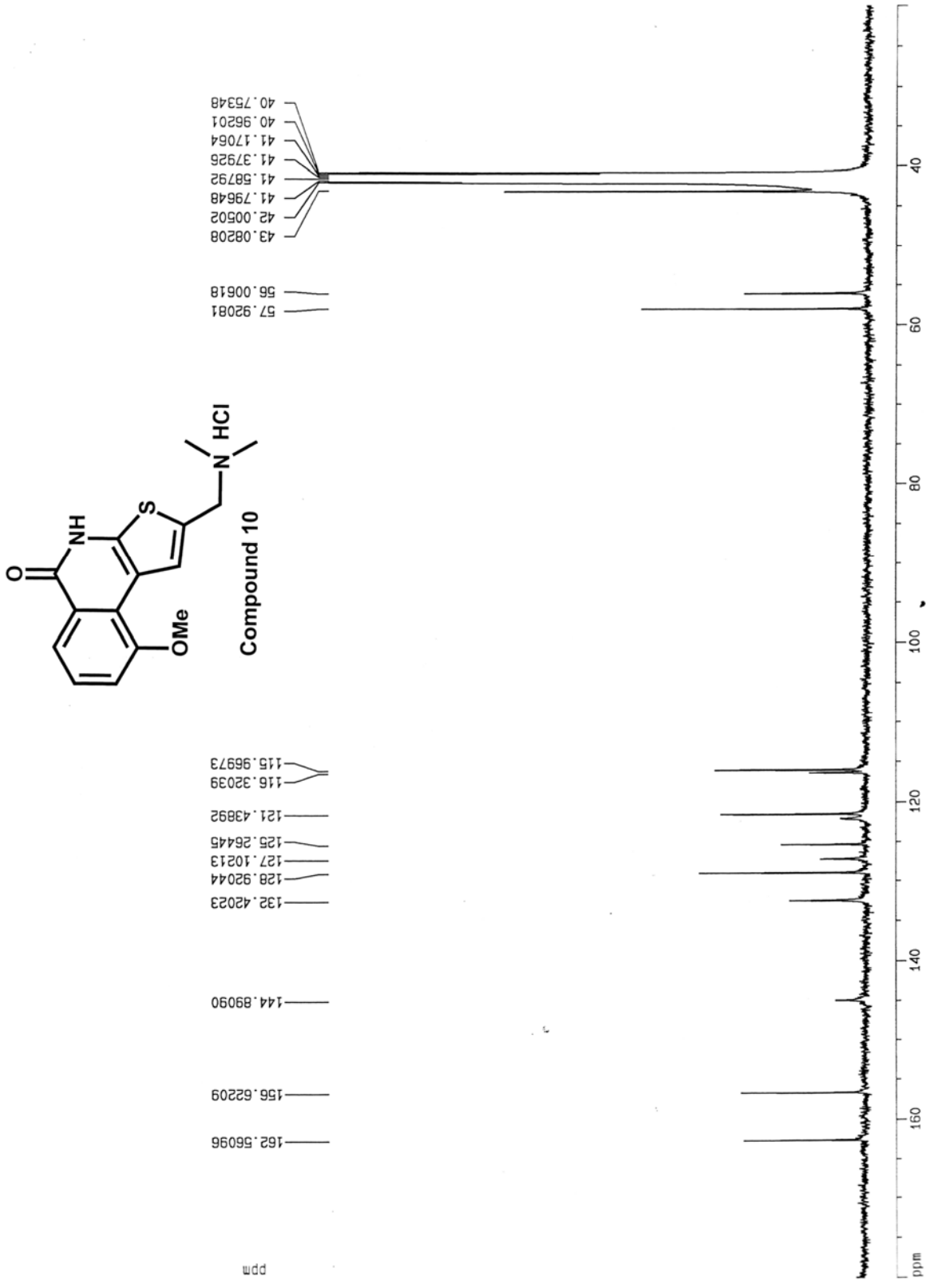
S18


HYDAMTIQ (3)
HBr
N
S
NH
O
OH
40.76472
40.97331
41.18193
41.39055
41.59917
41.80777
42.01637
43.17888
56.09177
117.02605
119.95195
120.19313
121.35263
123.98609
127.41791
128.87990
132.62138
144.04144
155.00006
162.79556
ppm
40
60
80
100
120
140
160

S19


2.4887
2.7232
3.3541
4.0108
4.5117
7.4120
7.4321
7.4824
7.5019
7.8700
7.8894
8.1510
12.5912
ppm
6.8937
3.3992
2.2215
1.0608
1.1470
1.0000
1.0567
0.6688
0.9379
Integral
ppm
Compound 10
HCl
N
S
NH
O
OMe

S20


40.75348
40.96201
41.17064
41.37925
41.58792
41.79648
42.00502
43.08208
56.00618
57.92081
115.96973
116.32039
121.43892
125.26445
127.10213
128.92044
132.42023
144.89090
156.62209
162.56096
ppm
Compound 10
HCl
N
S
NH
O
OMe

S21

HPLC Purity.

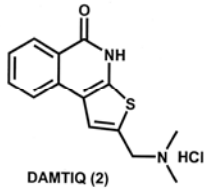
Two different systems were used

Method A: analytical column: Grace Smart RP-18 250 x 4.6 mm, 5 µm, 100 Å; eluent: H_2O/CH_3CN: 85:15 (v/v) + 0.1% TFA; flow rate: 1 ml/min; detection at 230 and 254 nm;

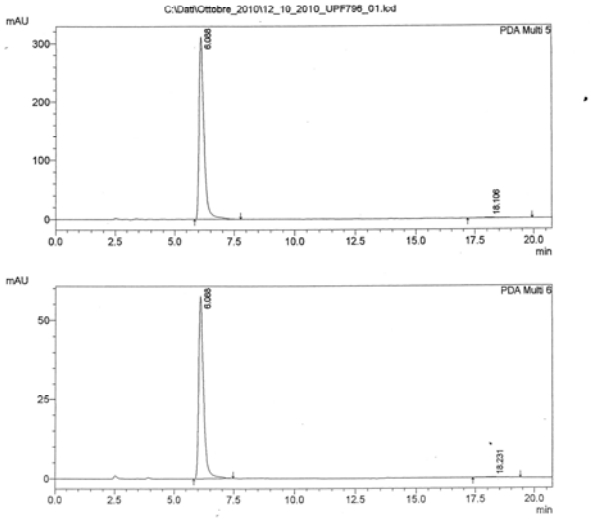
Method B: analytical. column Luna C-18(2) 250 x 4.6 mm, 5 µm, 100 Å; eluent: $H_2O/CH_3CN/CH_3OH$: 75:20:5 (v/v/v) + 0.1% TFA; flow rate: 1 ml/min; detection (DAD) 190-400 nm.

Method A

Acquired by : Admin
Sample Name : 12_10_2010_UPF796
Sample ID : 12_10_2010_UPF796
Vail # :
Injection Volume : 20 uL
Data File Name : 12_10_2010_UPF796_01.lcd
Method File Name : Giovanni1.lcm
Batch File Name :
Report File Name : Default.lcr
Data Acquired : 12/10/2010 11.19.55
Data Processed : 12/10/2010 11.40.42
Colonna: Luna phenomenex
Eluente: H2O/MeCN/meOH -75/25/5 (v,v) +TFA 0.1%
Flow rate: 1.00 mL/min
Detector: DAD (190-400 nm)
Temperatura colonna: non controllata
Strumento: Maserati
Operatore: Antonella Lisanti



DAMTIQ (2)



PeakTable

PDA Ch5 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.088	4347536	311043	98.255	99.670
2	18.106	77202	1028	1.745	0.330
Total		4424738	312071	100.000	100.000

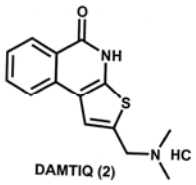
PeakTable

PDA Ch6 254nm 4nm

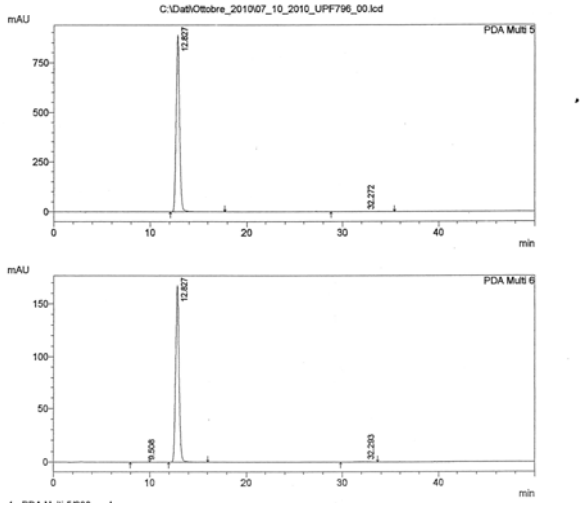
Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.088	797932	57485	98.653	99.689
2	18.231	10898	180	1.347	0.311
Total		808831	57665	100.000	100.000

Method B

Acquired by : Admin
Sample Name : 07_10_2010_UPF796
Sample ID : 07_10_2010_UPF796
Vail # :
Injection Volume : 20 uL
Data File Name : 07_10_2010_UPF796_00.lcd
Method File Name : Giovanni1.lcm
Batch File Name :
Report File Name : Default.lcr
Data Acquired : 07/10/2010 12.33.45
Data Processed : 07/10/2010 17.22.18
Colonna: Grace Smart RP-18
Eluente: H2O/MeCN -85/15 (v,v) + TFA 0.1%
Flow rate: 1.00 mL/min
Detector: DAD (190-400 nm)
Temperatura colonna: non controllata
Strumento: Maserati
Operatore: Valentina Meacci



DAMTIQ (2)



PeakTable

PDA Ch6 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.508	8187	112	0.202	0.067
2	12.827	4029923	167277	99.608	99.881
3	32.293	7689	87	0.190	0.052
Total		4045799	167476	100.000	100.000

PeakTable

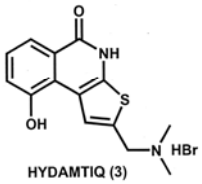
PDA Ch5 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	12.827	21420056	887182	99.706	99.947
2	32.272	63217	471	0.294	0.053
Total		21483273	887653	100.000	100.000

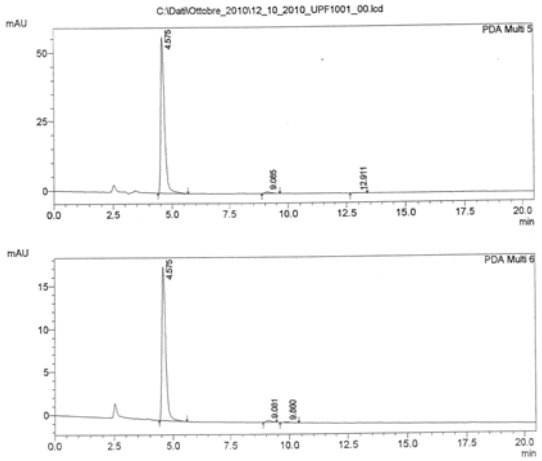
S22

Method A

Acquired by : Admin
Sample Name : 12_10_2010_UPF1001
Sample ID : 12_10_2010_UPF1001
Vail # :
Injection Volume : 20 uL
Data File Name : 12_10_2010_UPF1001_00.lcd
Method File Name : Giovanni1.lcm
Batch File Name :
Report File Name : Default.lcr
Data Acquired : 12/10/2010 9.41.23
Data Processed : 12/10/2010 10.01.56
Colonna: Luna phenomenex
Eluente: H2O/MeCN/meOH -75/25/5 (v,v) +TFA 0.1%
Flow rate: 1.00 mL/min
Detector: DAD (190-400 nm)
Temperatura colonna: non controllata
Strumento: Maserati
Operatore: Antonella Lisanti



HYDAMTIQ (3)



Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.575	639609	56575	98.092	98.807
2	9.085	10663	585	1.635	1.022
3	12.911	1778	98	0.273	0.170
Total		652049	57258	100.000	100.000

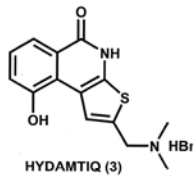
PeakTable

PDA Ch6 254nm 4nm

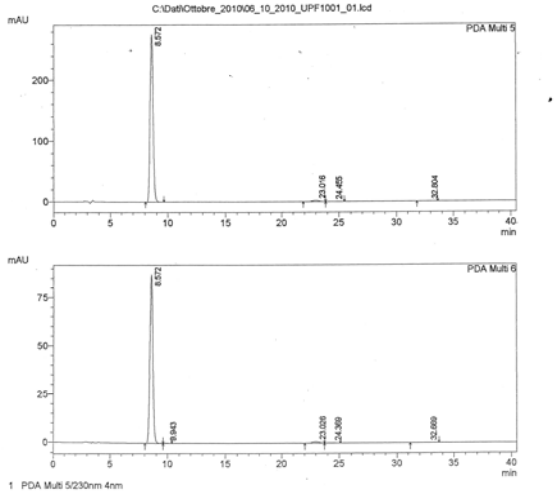
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.575	200543	17863	97.857	98.517
2	9.081	3344	210	1.632	1.159
3	9.860	1048	59	0.511	0.324
Total		204934	18132	100.000	100.000

Method B

C:\Dati\Ottobre_2010\06_10_2010_UPF1001_01.lcd
Acquired by : Admin
Sample Name : 06_10_2010_UPF1001
Sample ID : 06_10_2010_UPF1001
Vail # :
Injection Volume : 20 uL
Data File Name : 06_10_2010_UPF1001_01.lcd
Method File Name : Giovanni1.lcm
Batch File Name :
Report File Name : Default.lcr
Data Acquired : 06/10/2010 16.15.16
Data Processed : 06/10/2010 16.55.47
Colonna: Grace Smart RP-18
Eluente: H2O/MeCN -85/15 (v,v) + TFA 0.1%
Flow rate: 1.00 mL/min
Detector: DAD (190-400 nm)
Temperatura colonna: non controllata
Strumento: Maserati
Operatore: Valentina Meaoci



HYDAMTIQ (3)



PDA Ch5 230nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.572	4972468	275952	97.674	99.102
2	23.016	93974	2026	1.846	0.727
3	24.455	12327	265	0.242	0.095
4	32.804	12095	209	0.238	0.075
Total		5090864	278452	100.000	100.000

PeakTable

PDA Ch6 254nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.572	1578745	87519	96.617	98.713
2	9.943	2239	85	0.137	0.096
3	23.026	34792	747	2.129	0.842
4	24.369	11842	220	0.725	0.248
5	32.669	6403	89	0.392	0.101
Total		1634022	88660	100.000	100.000