

NOVEL FIXED-RING ANALOGUES OF TAMOXIFEN: SYNTHESIS AND BIOLOGICAL EVALUATION

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Molecular Docking Studies

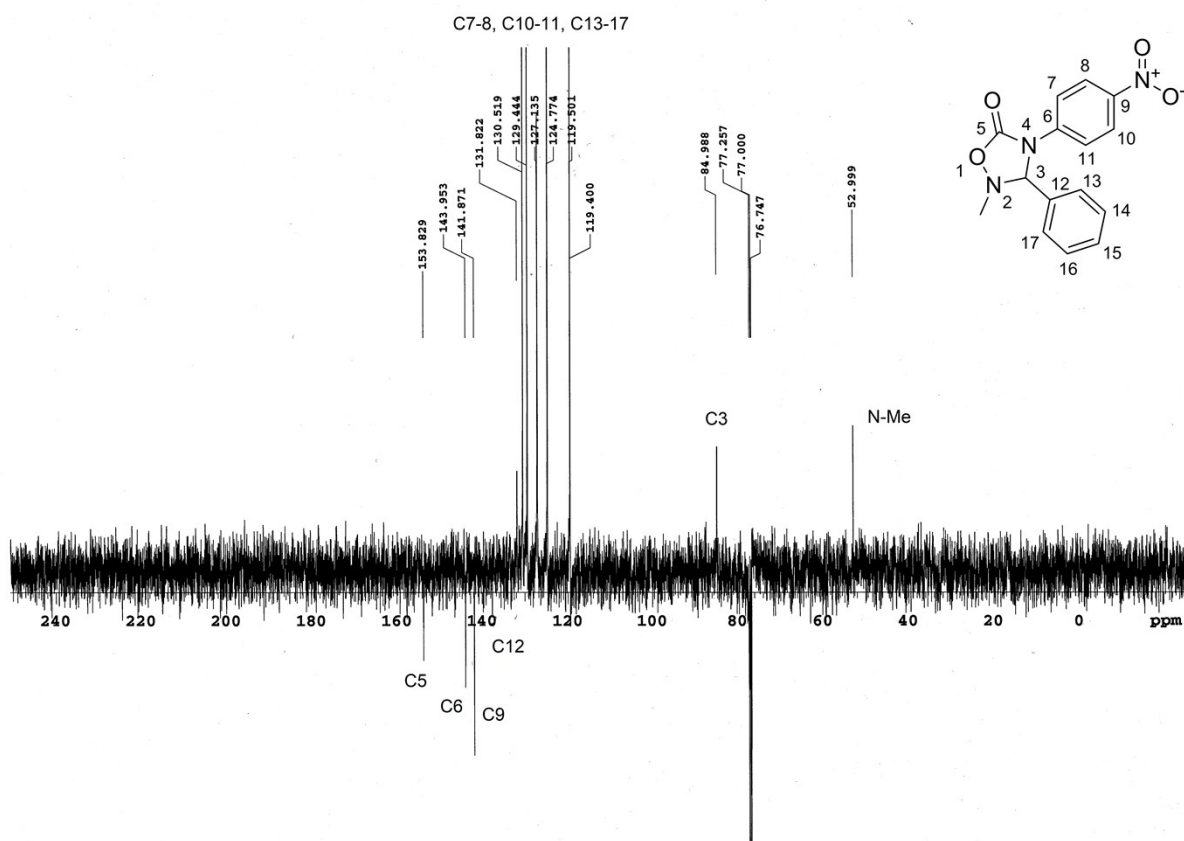
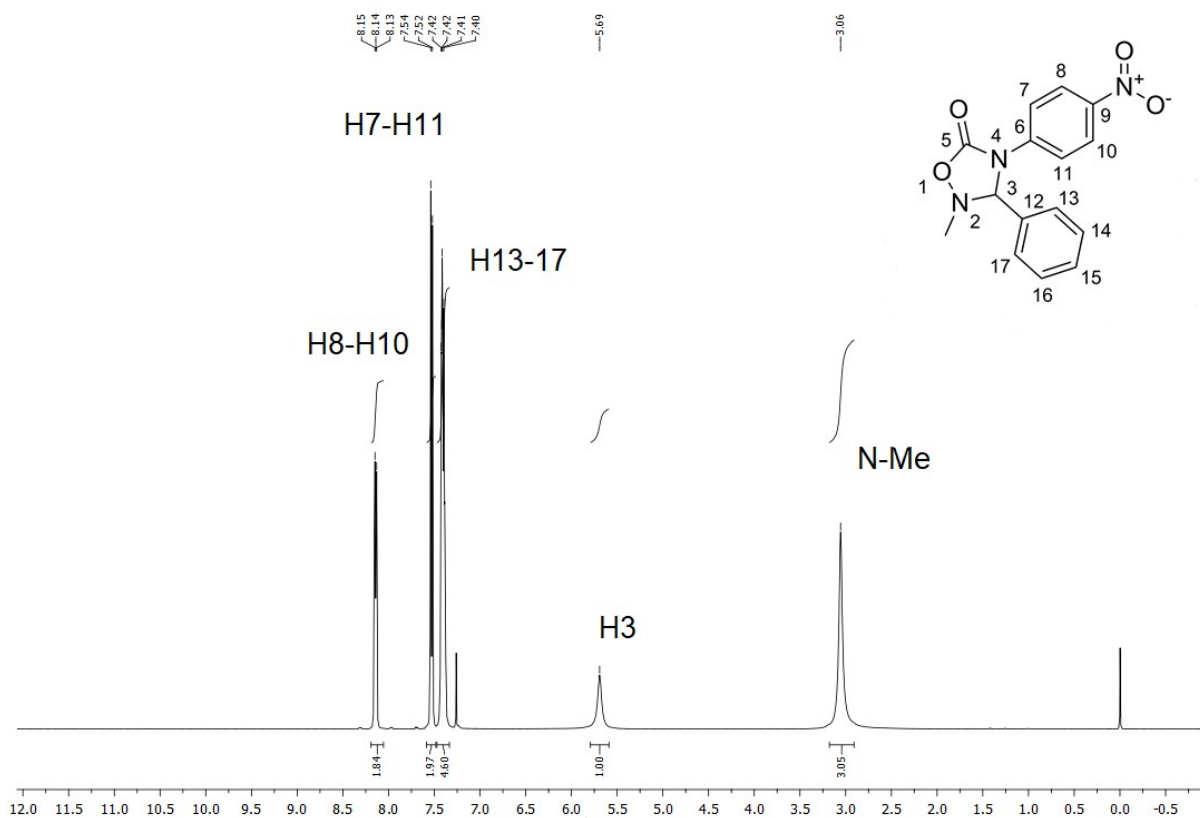
Receptor and Ligands Preparation

The X-ray crystal structure of ER α in complex with 4-OH-TAM (PDB code: 3ERT) was retrieved from the protein data bank database (<http://www.rcsb.org/pdb>). The AutoDock Tools software has been used to remove ligands and water molecules, adding all the hydrogen atoms, and to calculate Gasteiger charges for each atom of the macromolecules. The chemical structures of compounds were generated using ChemOffice v12.0 Ultra software package and optimized with ADT 4.2 for docking studies. The determination of ligand interactions for each pose within the binding pocket of receptor were analysed by BIOVIA Discovery Studio 2017.

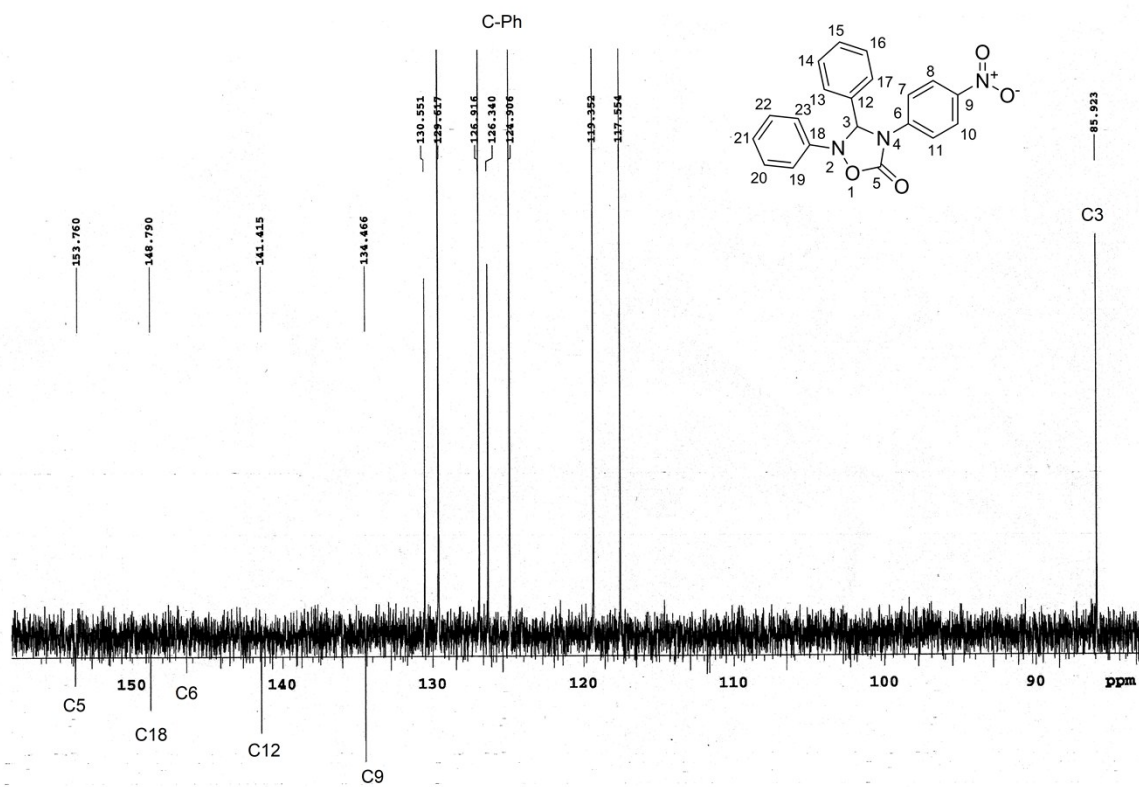
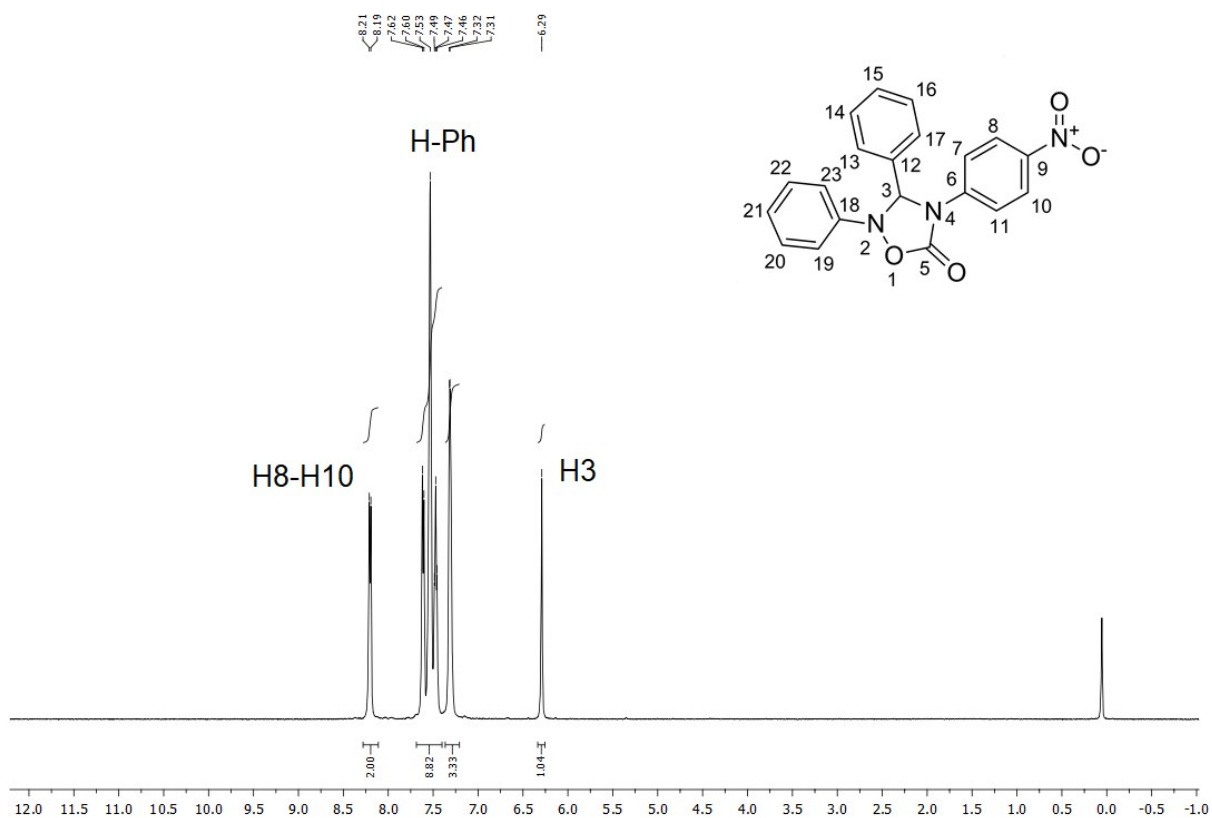
Docking Protocol

Molecular Docking was carried out using AutoDock 4.2. Grid Maps were centered on the ER α active site with 44 points per dimension and a step size of 0.375. The Lamarckian genetic algorithm implemented in AutoDock has been employed to dock compounds **14a-k**. We have carried out comparative docking experiments of compounds **14a-k**. Each docking experiment consisted of 100 docking runs with 150 individuals and 2.5×10^6 energy evaluations. Other parameters were left to their default values. 100 highest-scoring docking poses were saved and binding affinity of the best mode was selected. The cluster with the lowest free energy of binding was visually analyzed using Python Molecular Viewer 1.4.3 (PMV). The Autodock docking parameters were validated to ensure that the ligand orientation and the position obtained from the docking studies represent valid reasonable binding modes of inhibitors. The ligand linezolid was extracted and docked back into the corresponding binding pocket. The results of docking simulation predicted the binding conformation of 4-OH-TAM with a root mean square deviation (RMSD) of 1.9 Å, compared with conformation of co-crystallized structure, thus indicating a valid docking protocol.

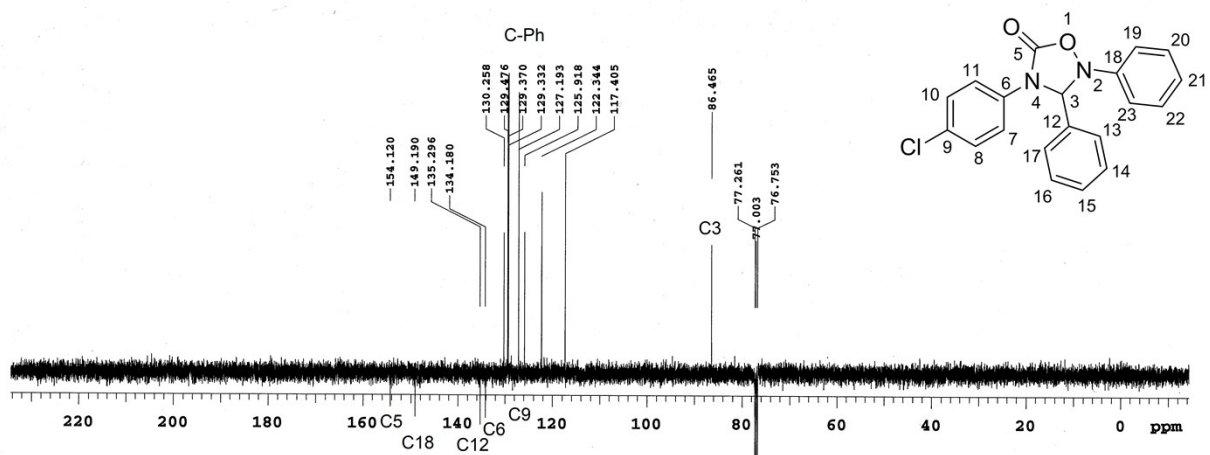
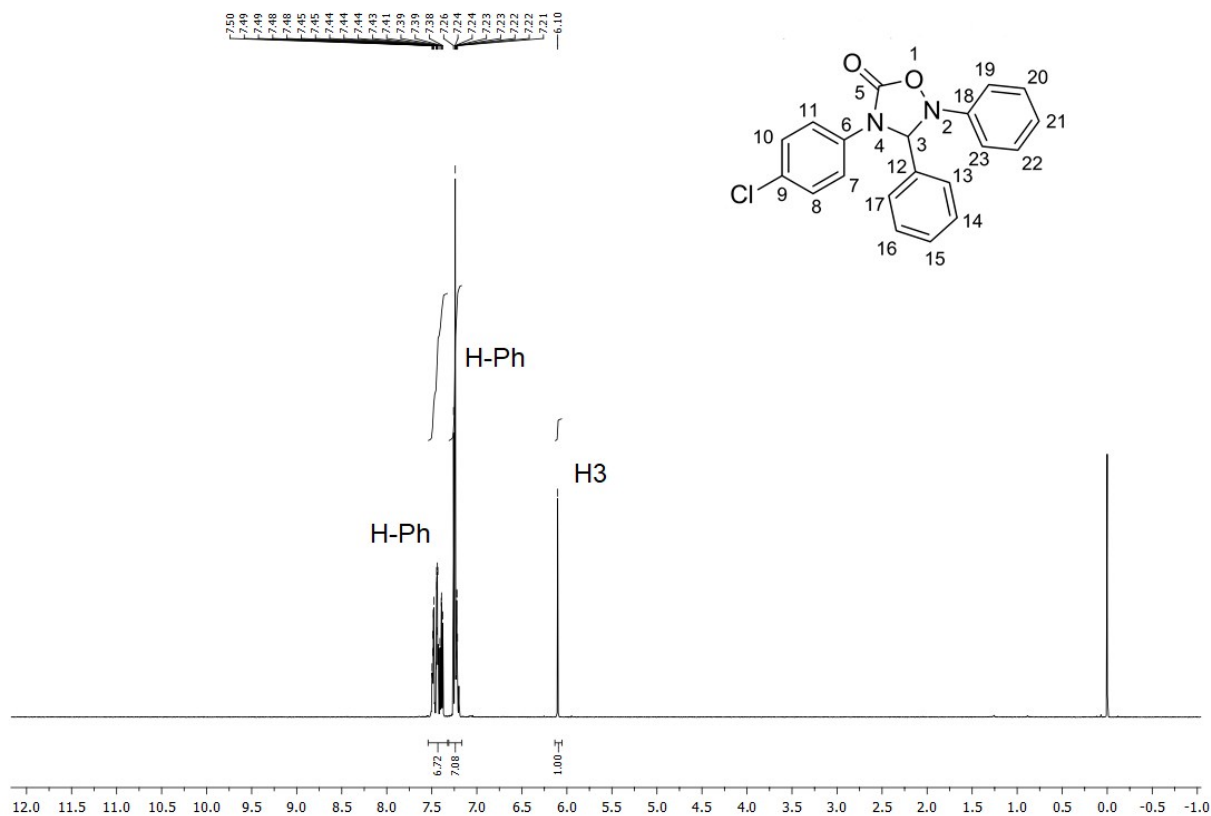
^1H and ^{13}C Attached-Proton-Test (APT) NMR of compound **14a**



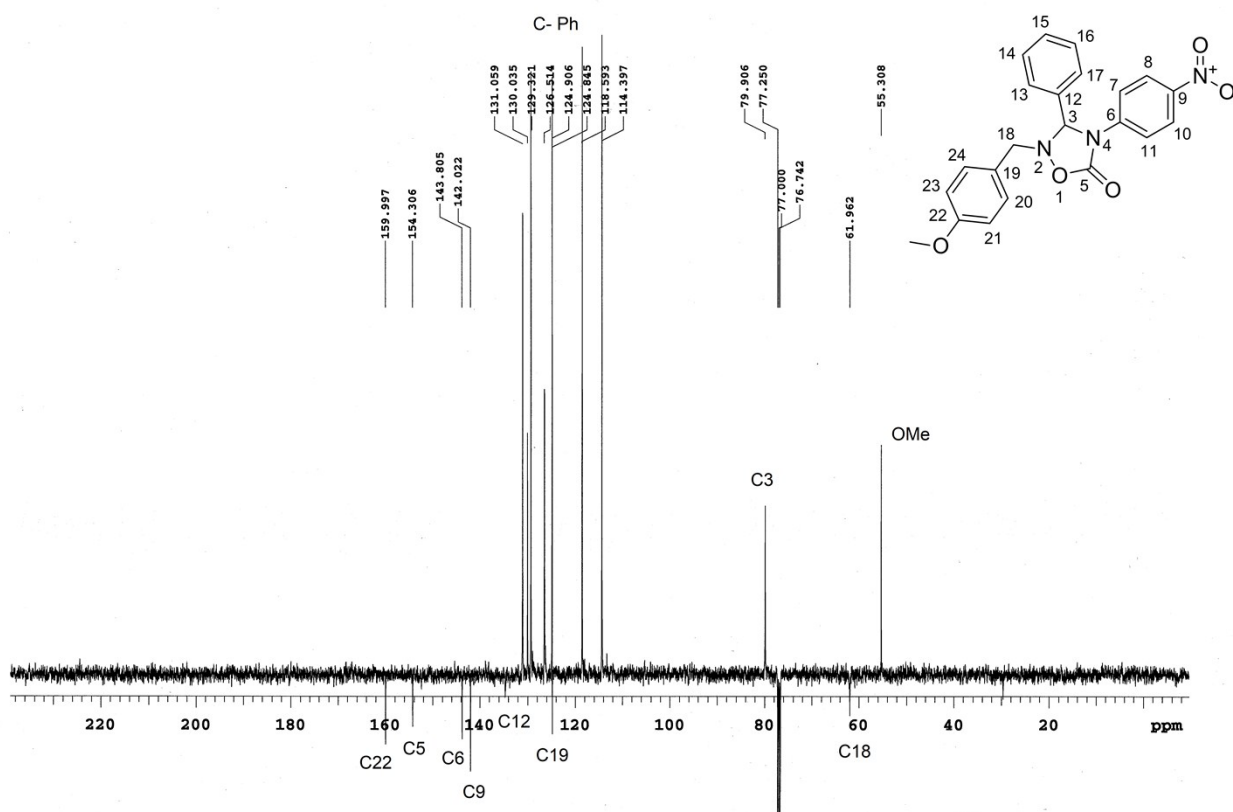
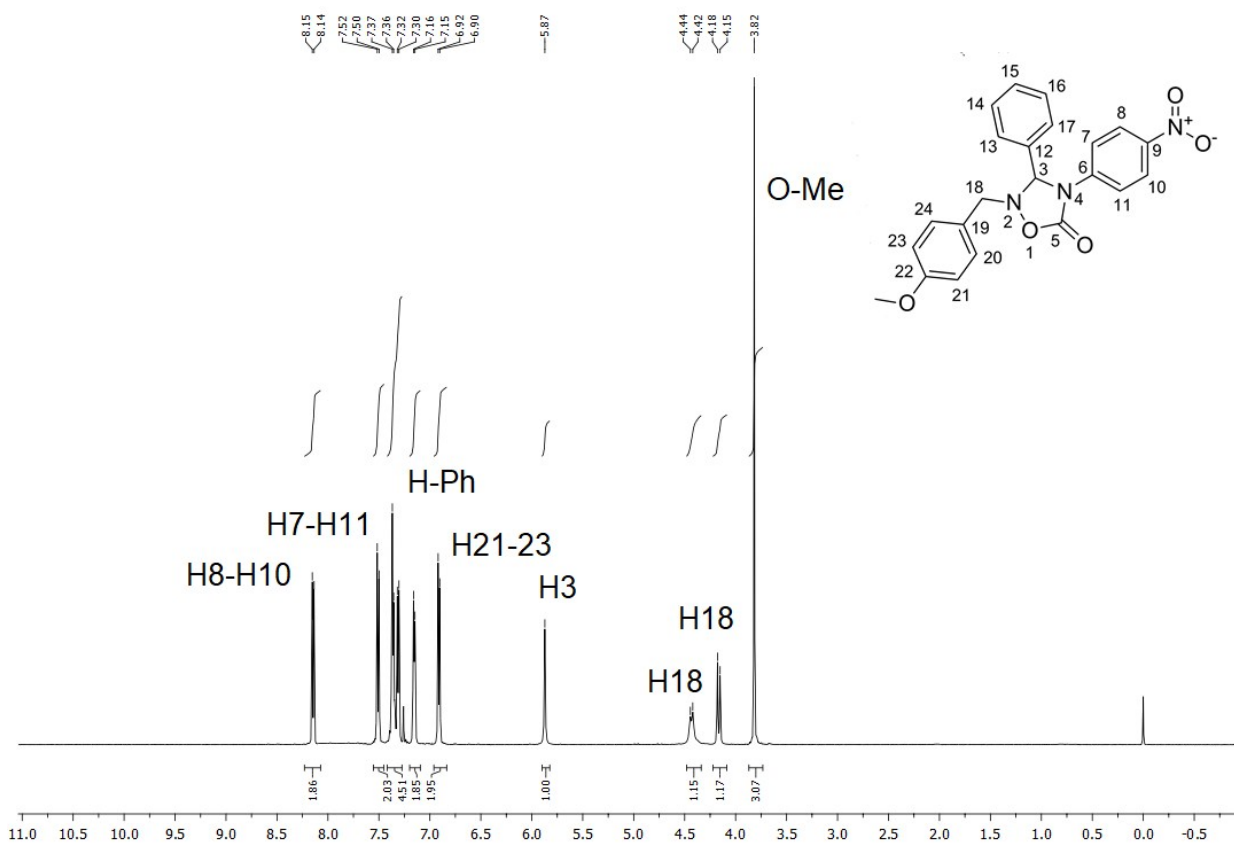
^1H and ^{13}C Attached-Proton-Test (APT) NMR of compound **14b**



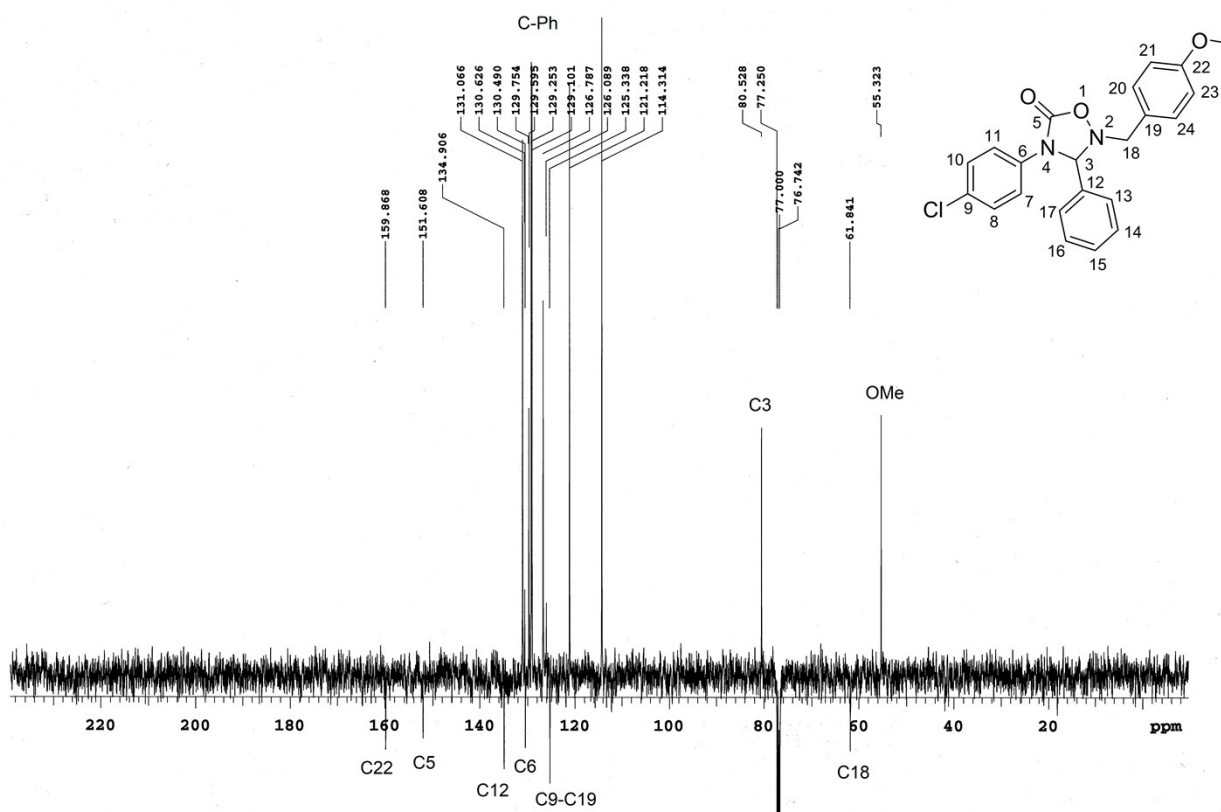
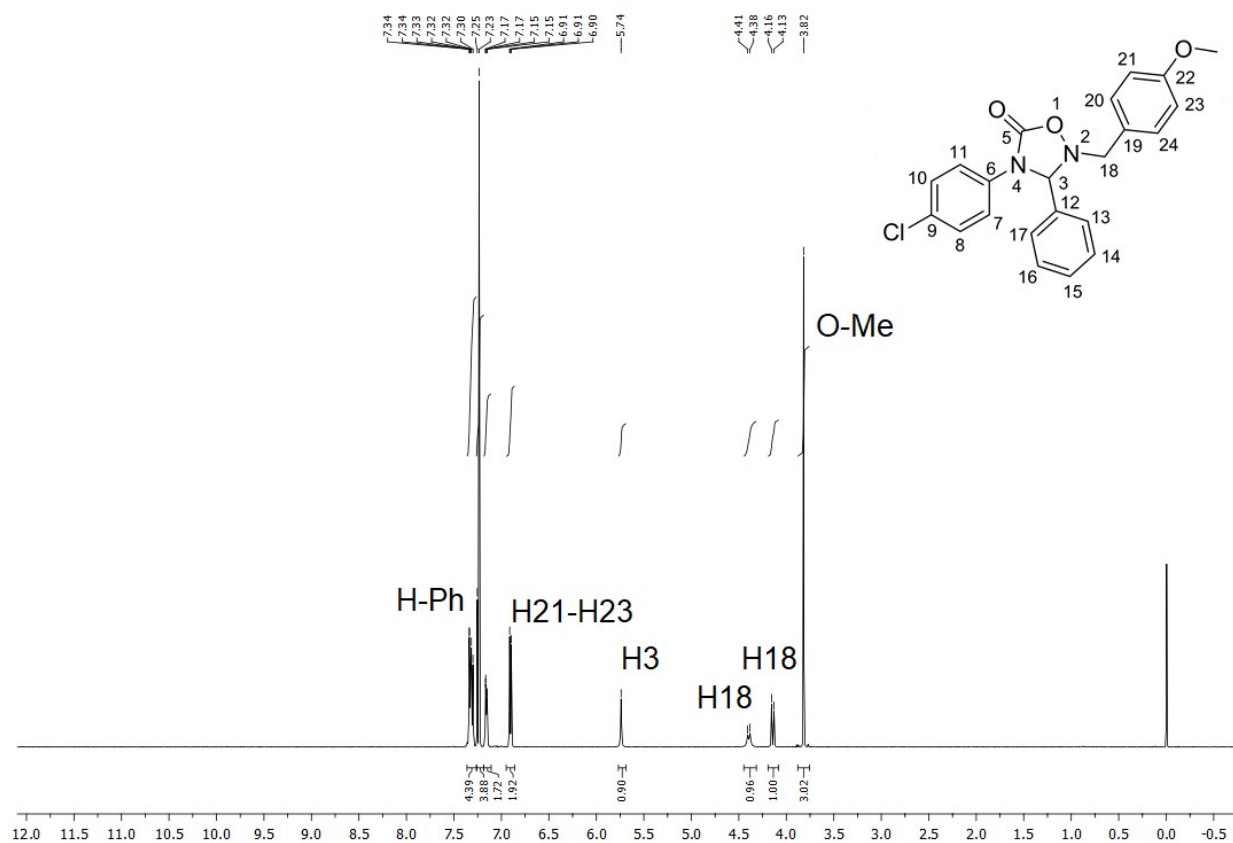
^1H and ^{13}C Attached-Proton-Test (APT) NMR of compound **14c**



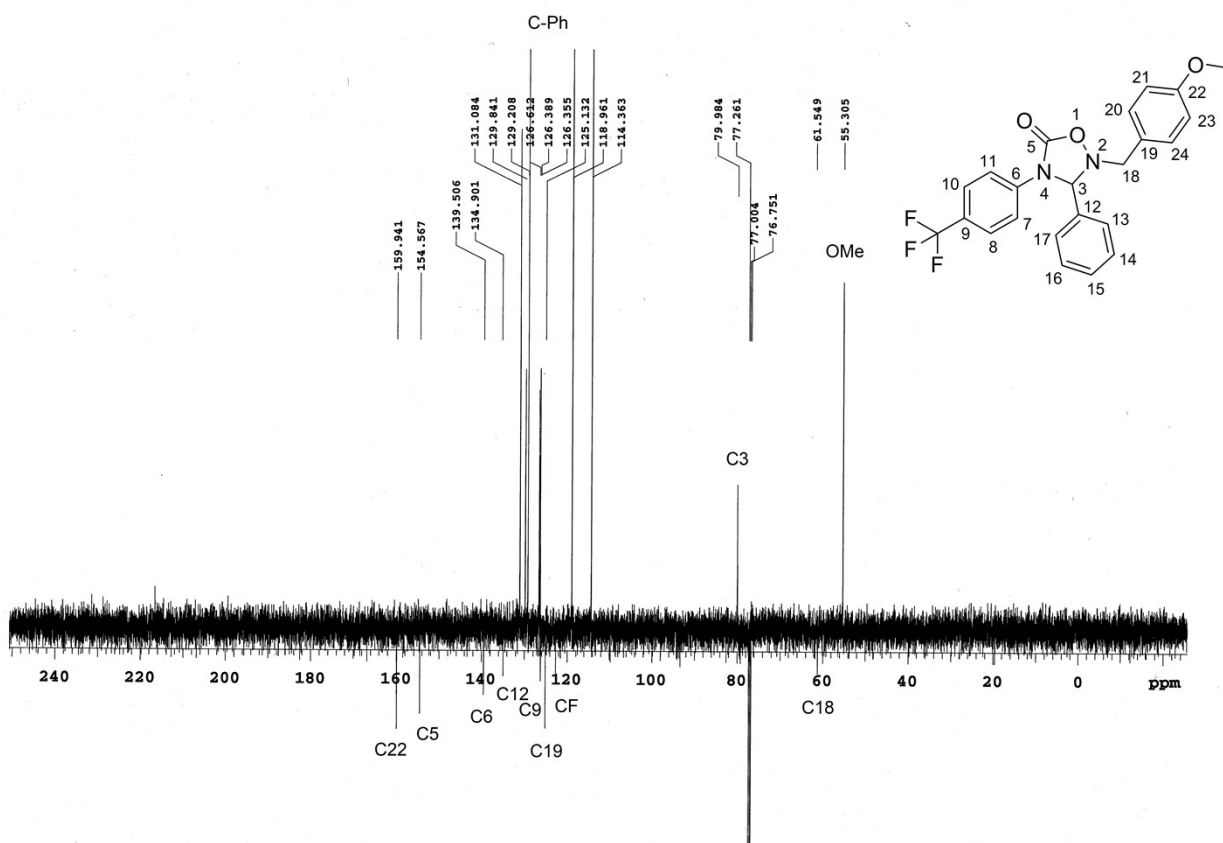
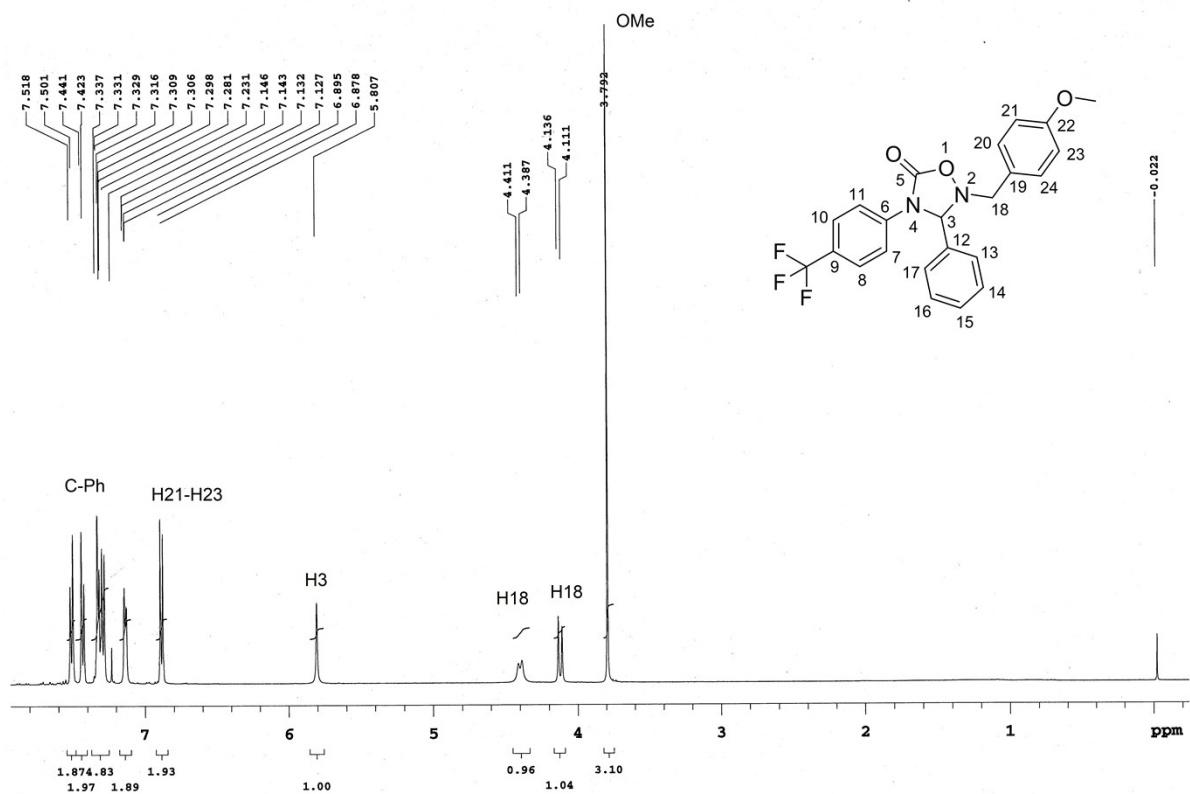
^1H and ^{13}C Attached-Proton-Test (APT) NMR of compound **14d**



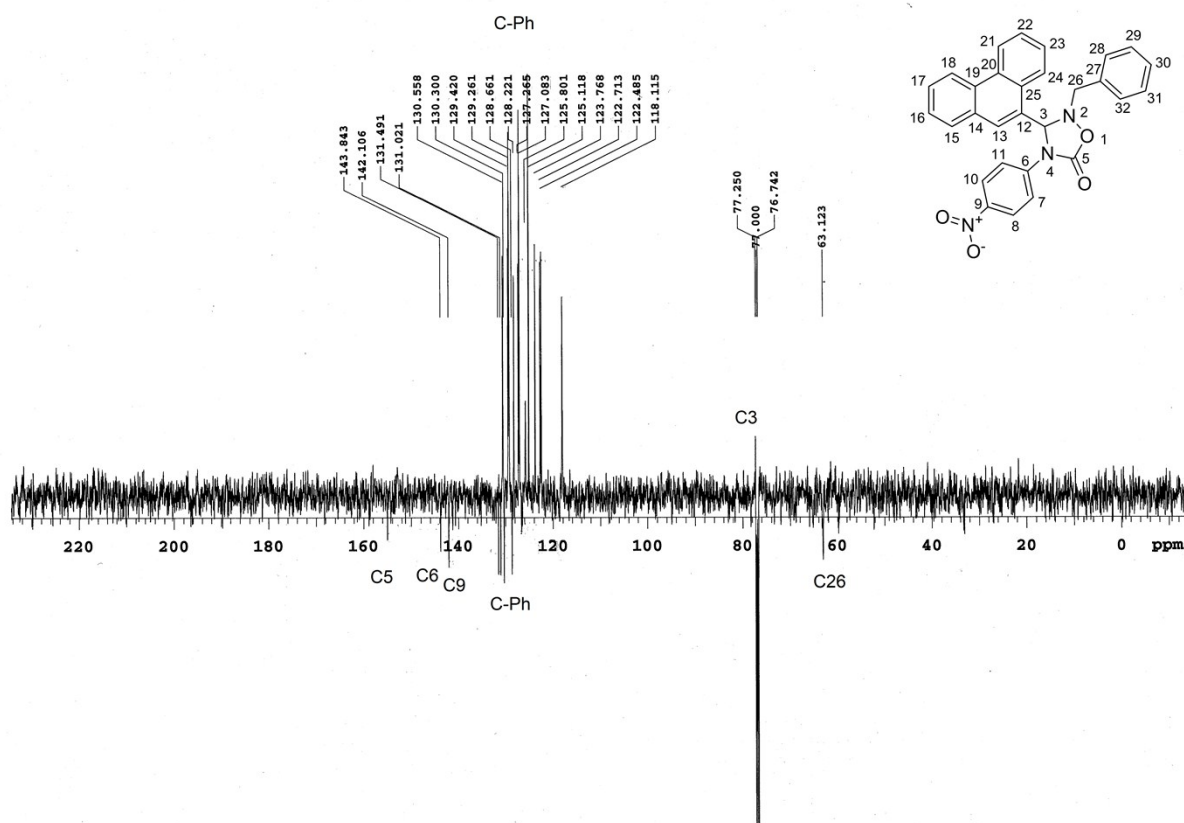
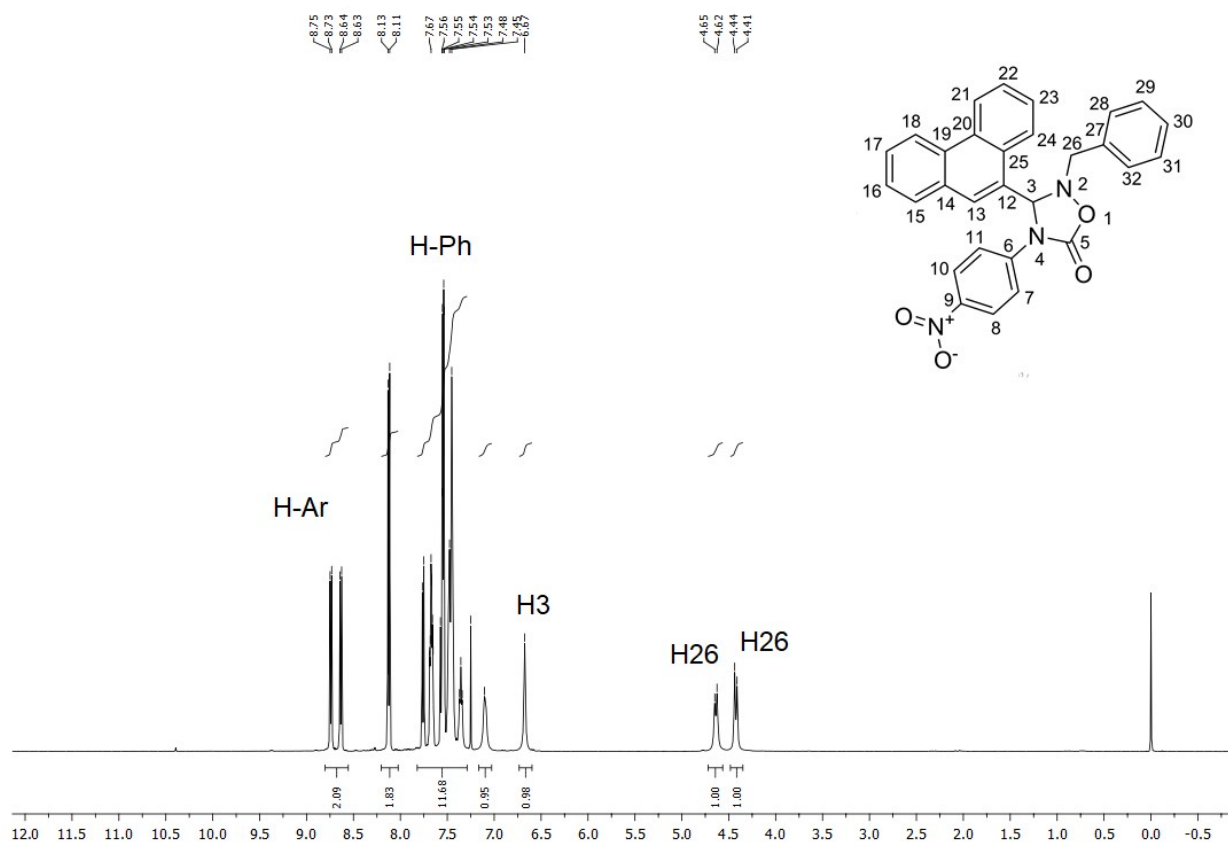
^1H and ^{13}C Attached-Proton-Test (APT) NMR of compound **14e**



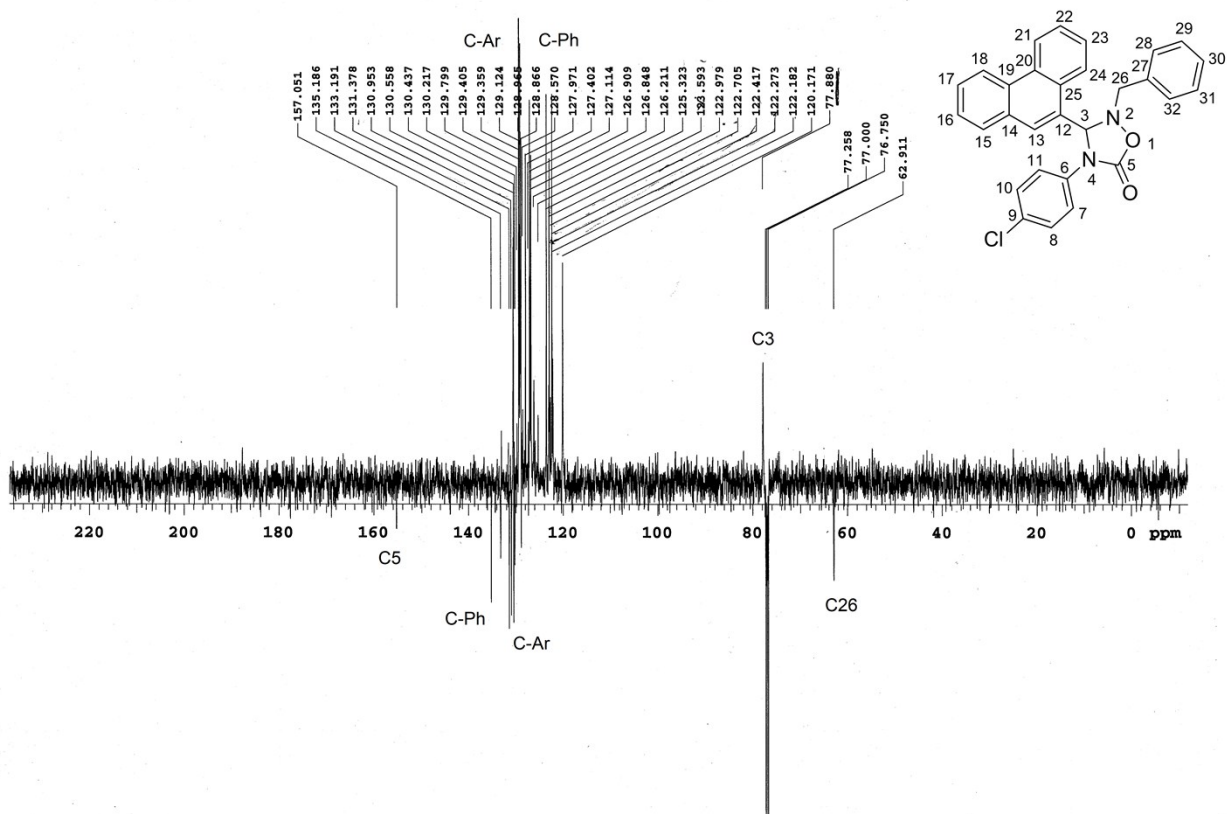
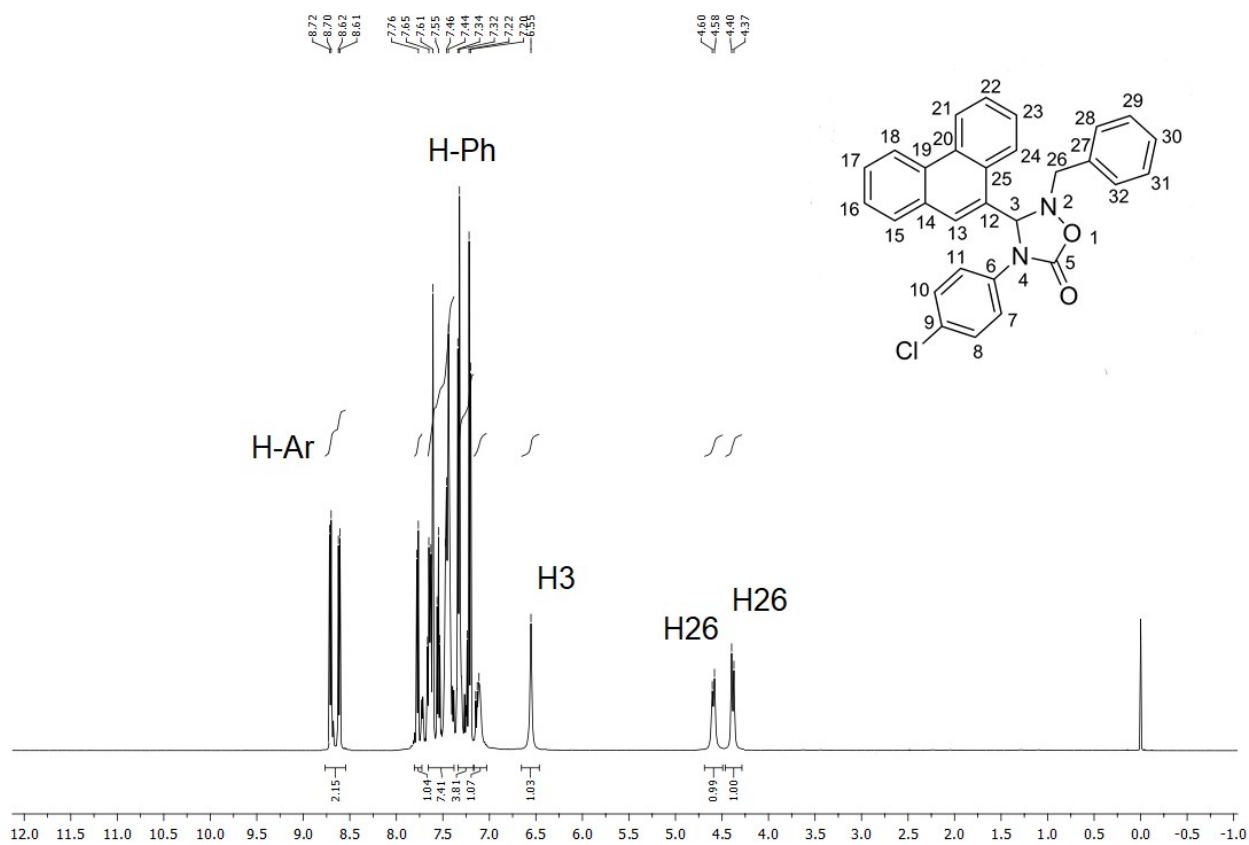
^1H and ^{13}C Attached-Proton-Test (APT) NMR of compound **14f**



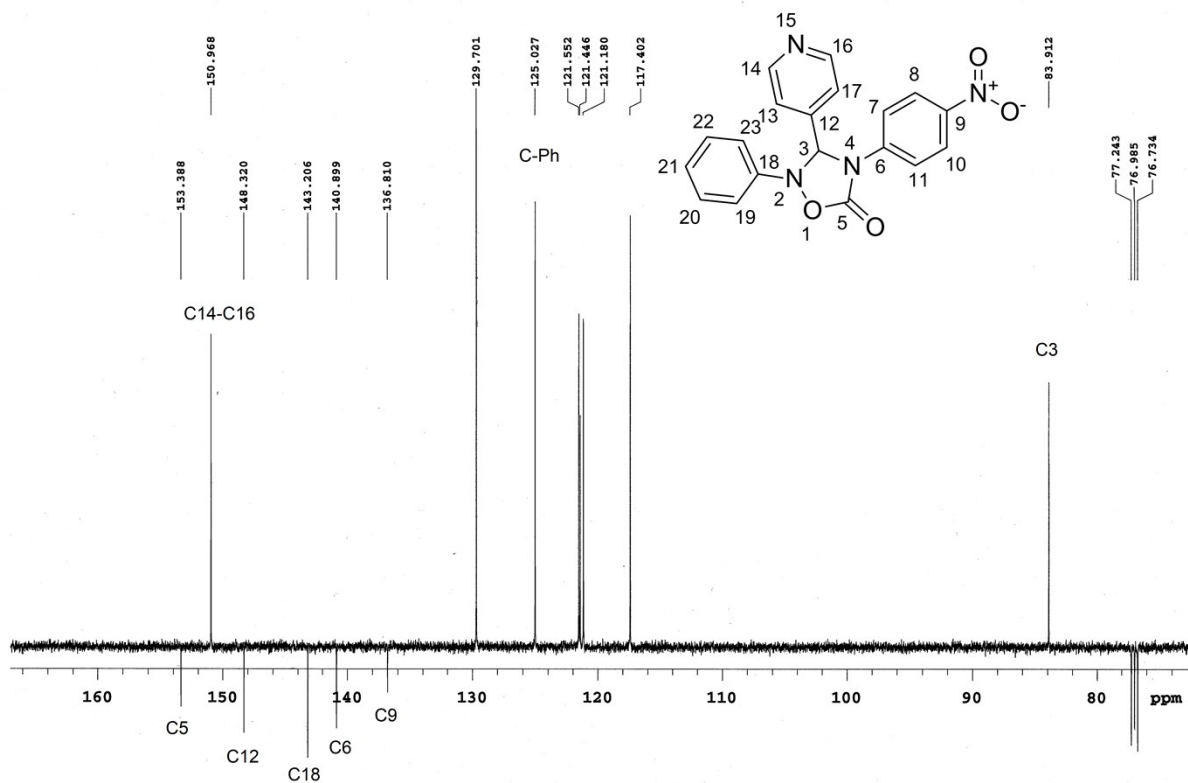
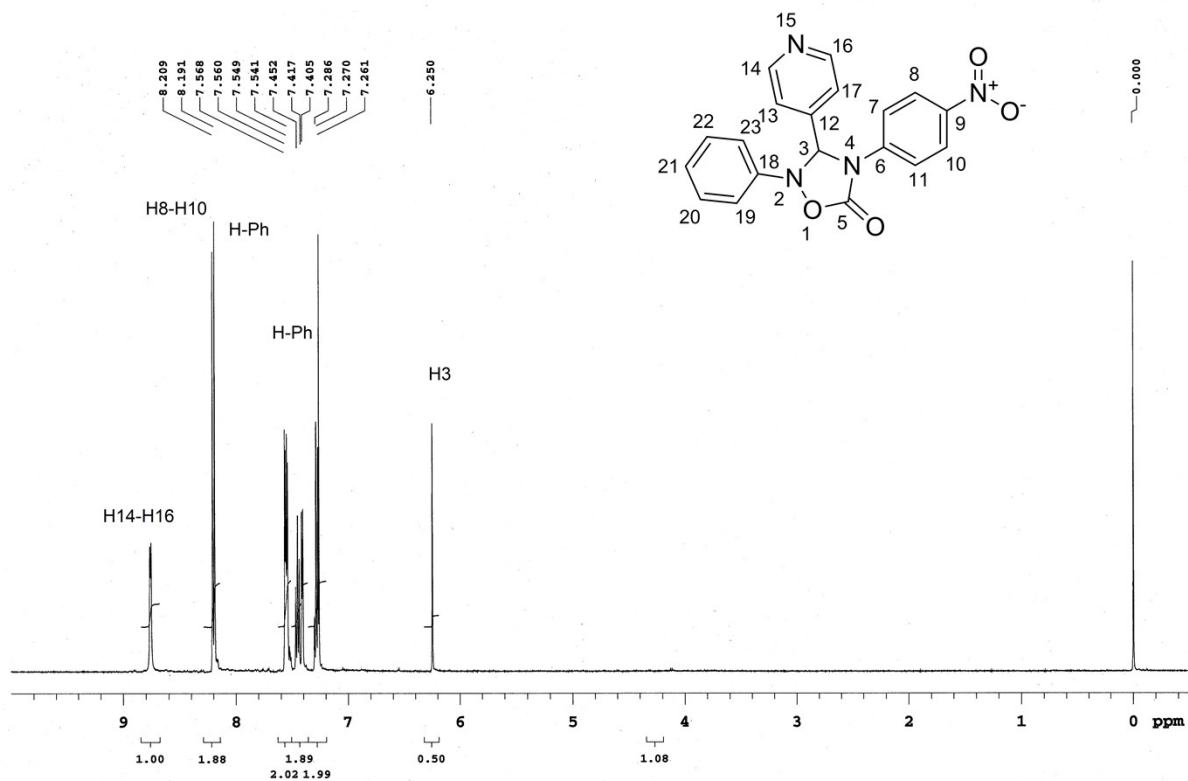
^1H and ^{13}C Attached-Proton-Test (APT) NMR of compound **14g**



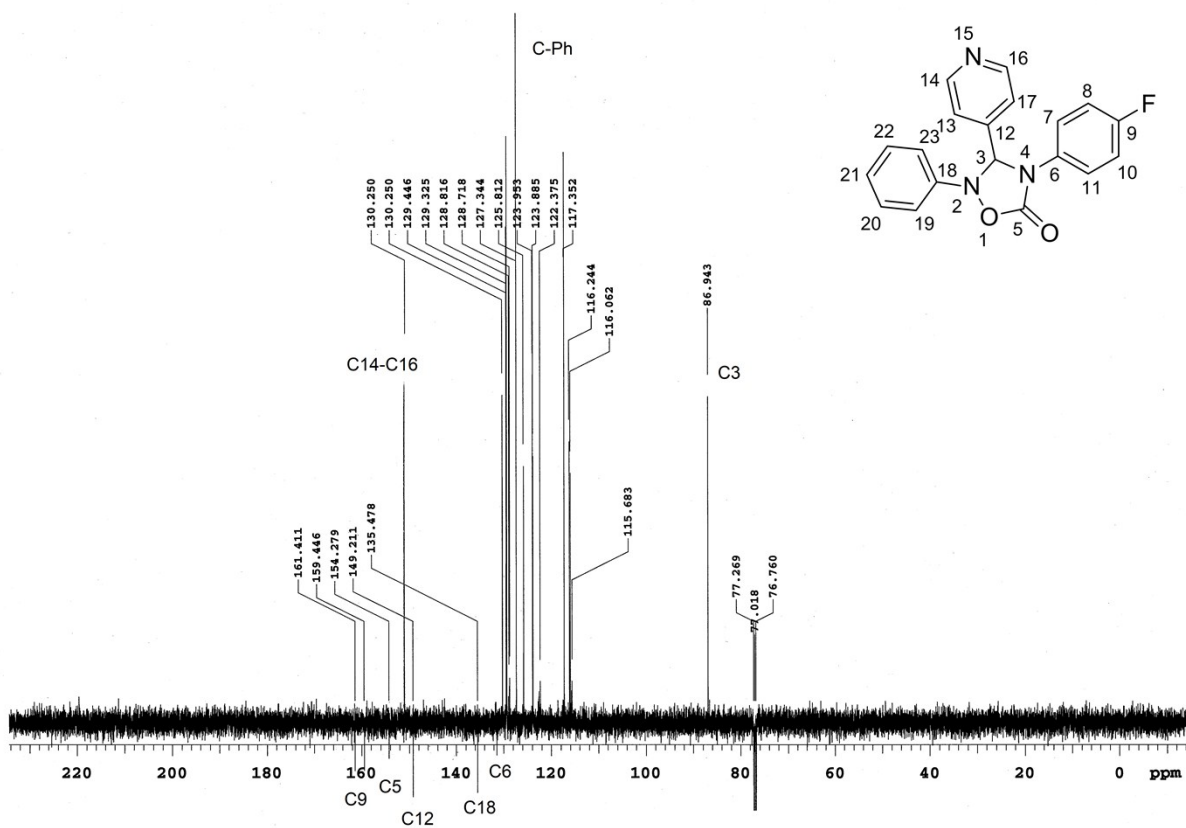
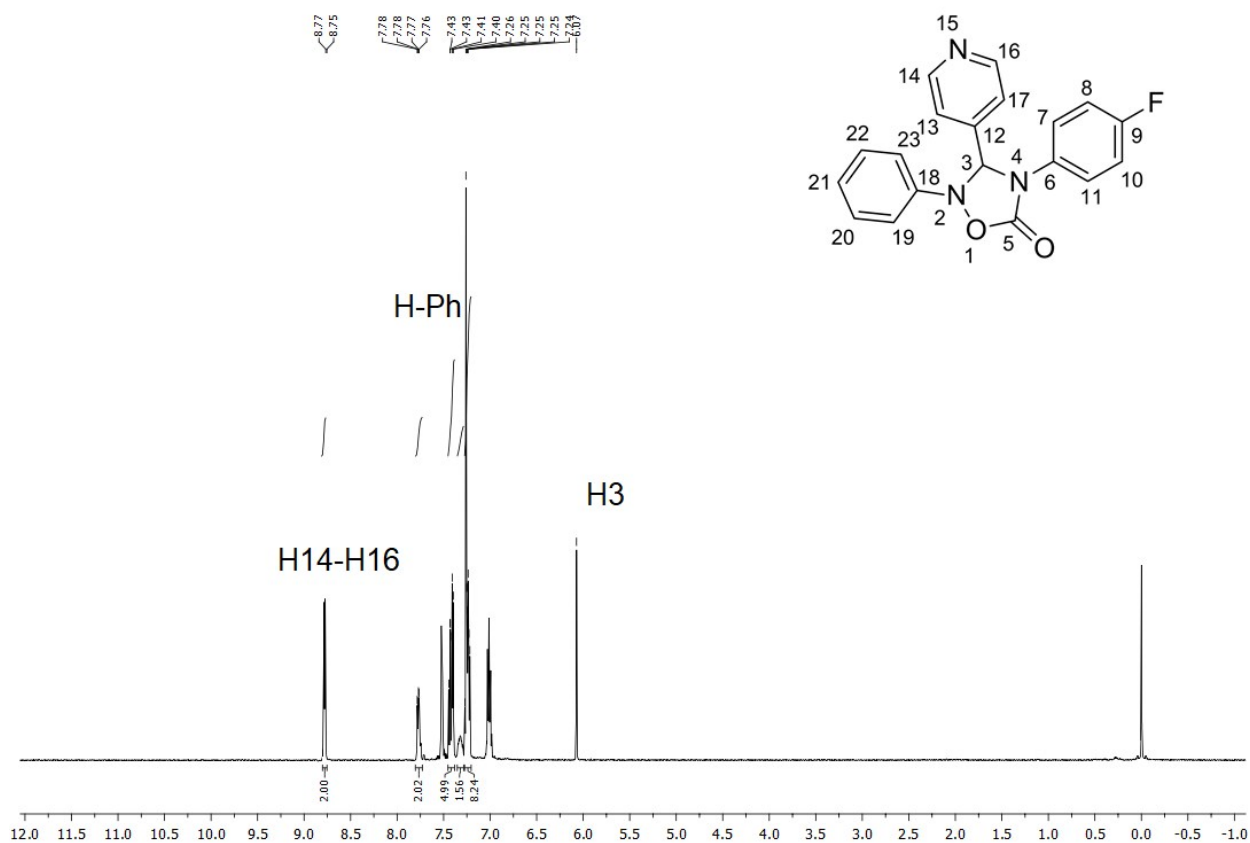
^1H and ^{13}C Attached-Proton-Test (APT) NMR of compound **14h**



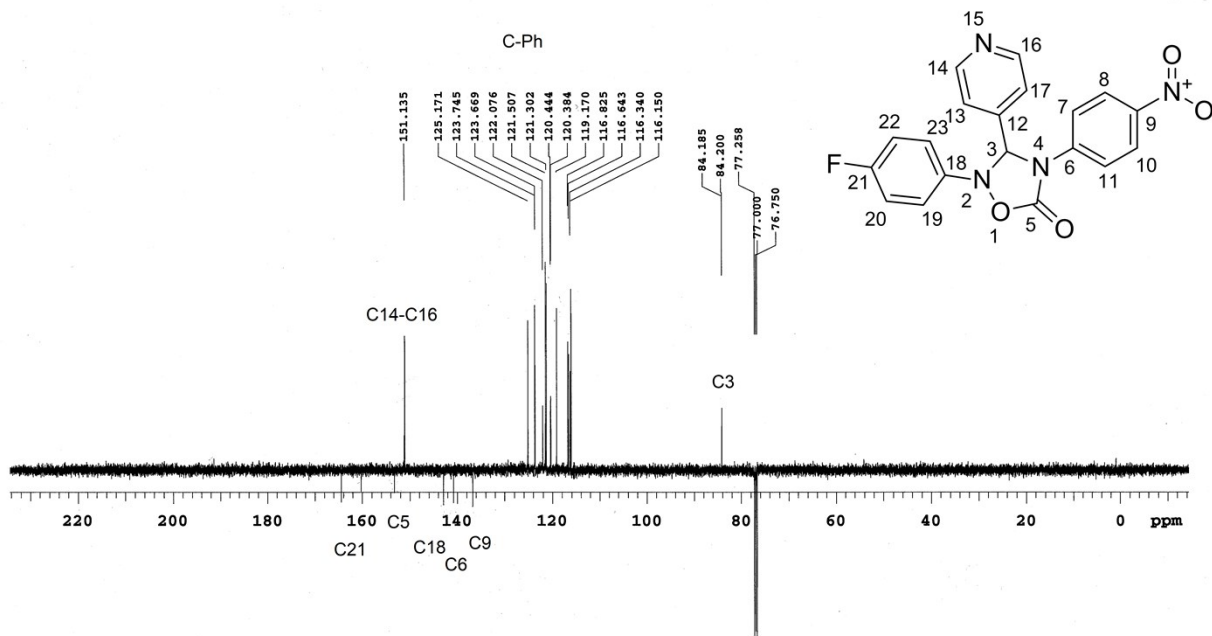
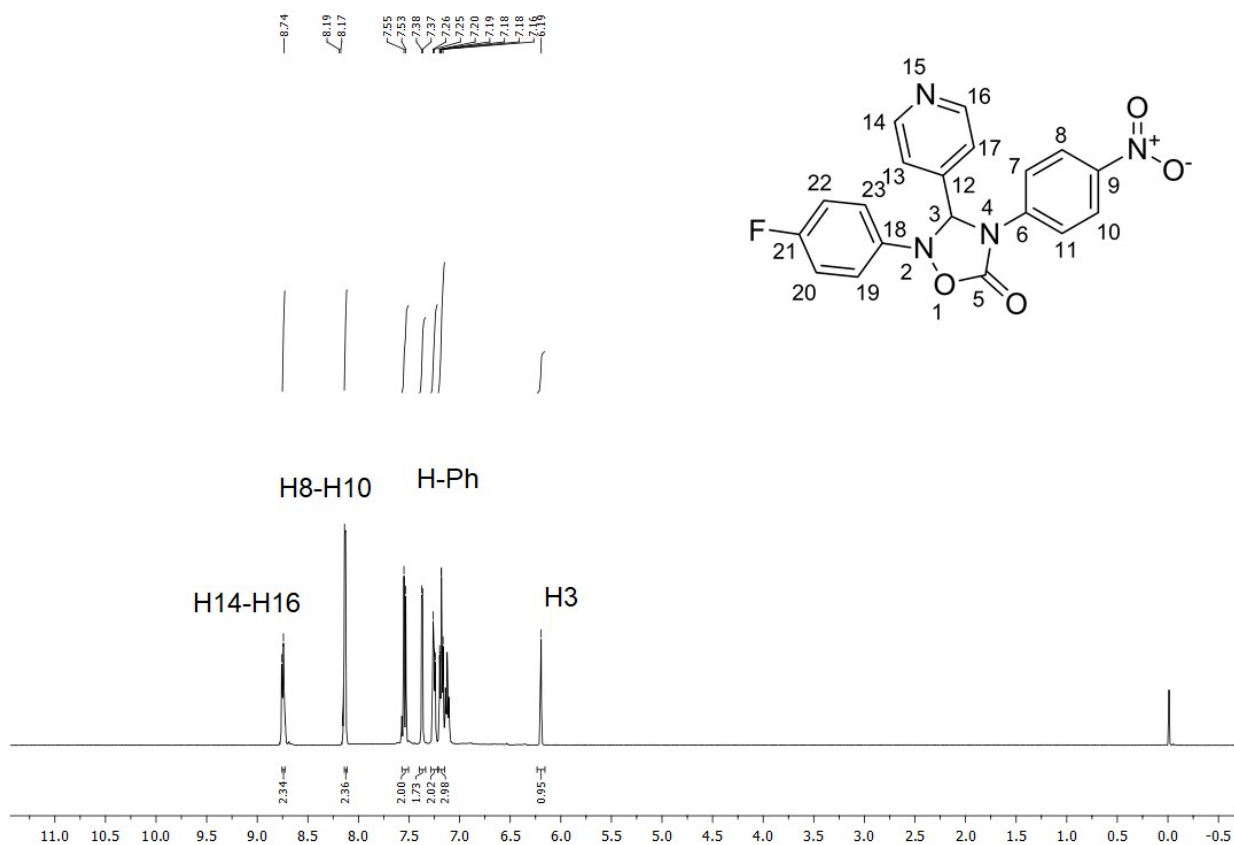
^1H and ^{13}C Attached-Proton-Test (APT) NMR of compound **14i**



¹H and ¹³C Attached-Proton-Test (APT) NMR of compound **14j**



¹H and ¹³C Attached-Proton-Test (APT) NMR of compound **14k**



M062X/cc-pVTZ Energies and Cartesian Coordinates

12b

E = -631.850927

G = -631.680305

Imaginary Frequencies: 0

N	0.47066900	-0.49433400	-0.11430700
O	0.23690200	-1.71929400	-0.34224900
C	-0.43824400	0.41765600	0.09694600
H	-0.06115600	1.39711000	0.34475700
C	1.87638700	-0.12485500	-0.05249900
C	2.32451600	1.10287600	-0.51424100
C	2.75405900	-1.07035700	0.45254600
C	3.67751400	1.39930400	-0.43297400
H	1.63574200	1.80647100	-0.96068600
C	4.10117200	-0.75875200	0.53573400
H	2.36284800	-2.02736300	0.76474800
C	4.56571400	0.47489200	0.09757200
H	4.03747700	2.35035500	-0.80142900
H	4.79279100	-1.48549200	0.94004600
H	5.61982600	0.70939000	0.15643700
C	-1.87323500	0.22258600	0.05909500
C	-2.66104600	1.34848700	0.34356300
C	-2.51182600	-0.98901300	-0.23726700
C	-4.04018400	1.27360300	0.33463800
H	-2.17501400	2.28924300	0.57300200
C	-3.89786000	-1.05215200	-0.24529800
H	-1.91619200	-1.85960300	-0.45439900
C	-4.66534000	0.06744900	0.03882500
H	-4.62899800	2.15297500	0.55875400
H	-4.38171000	-1.99229200	-0.47455600
H	-5.74526300	0.00355300	0.03162100

13a

E = -604.210549

G = -604.139547

Imaginary Frequencies: 0

N	0.47066900	-0.49433400	-0.11430700
O	0.23690200	-1.71929400	-0.34224900
C	-0.43824400	0.41765600	0.09694600
H	-0.06115600	1.39711000	0.34475700
C	1.87638700	-0.12485500	-0.05249900
C	2.32451600	1.10287600	-0.51424100
C	2.75405900	-1.07035700	0.45254600
C	3.67751400	1.39930400	-0.43297400
H	1.63574200	1.80647100	-0.96068600
C	4.10117200	-0.75875200	0.53573400
H	2.36284800	-2.02736300	0.76474800
C	4.56571400	0.47489200	0.09757200
H	4.03747700	2.35035500	-0.80142900
H	4.79279100	-1.48549200	0.94004600
H	5.61982600	0.70939000	0.15643700
C	-1.87323500	0.22258600	0.05909500
C	-2.66104600	1.34848700	0.34356300

C	-2.51182600	-0.98901300	-0.23726700
C	-4.04018400	1.27360300	0.33463800
H	-2.17501400	2.28924300	0.57300200
C	-3.89786000	-1.05215200	-0.24529800
H	-1.91619200	-1.85960300	-0.45439900
C	-4.66534000	0.06744900	0.03882500
H	-4.62899800	2.15297500	0.55875400
H	-4.38171000	-1.99229200	-0.47455600
H	-5.74526300	0.00355300	0.03162100

TS3

E = -1236.08668

G = -1235.819529

Imaginary Frequencies: -263.03

N	-0.41485800	-0.61609800	0.74707500
N	-2.44315900	0.64583500	0.10059800
O	-2.12697400	0.81882200	1.40257600
C	-0.85604700	0.07225900	1.76848200
O	-0.55467900	0.28786300	2.90564200
C	-1.45931500	0.79836500	-0.75219400
H	-1.61722400	0.29573600	-1.69941900
C	-0.34351200	1.72166400	-0.67929700
C	0.63883800	1.56431000	-1.66119500
C	-0.18863300	2.70490500	0.30529800
C	1.76894600	2.36305600	-1.65661700
H	0.52014200	0.79816600	-2.41708500
C	0.93266000	3.51370200	0.28637300
H	-0.93592900	2.82971300	1.07496300
C	1.91391600	3.34129300	-0.68440200
H	2.53231300	2.22181300	-2.40848000
H	1.04929300	4.27774100	1.04207100
H	2.79401400	3.96949800	-0.67848300
C	0.93200100	-0.86294100	0.59188400
C	1.31327400	-1.77098100	-0.40967100
C	1.93678200	-0.17988900	1.29891500
C	2.64103200	-1.98437200	-0.71024400
H	0.53384700	-2.29663500	-0.94454700
C	3.26775900	-0.38656800	0.99684700
H	1.66050500	0.50842800	2.08354700
C	3.60675600	-1.28102900	-0.00545100
H	2.94501600	-2.67824100	-1.47928900
H	4.05034200	0.13782300	1.52429600
C	-3.57383100	-0.17763800	-0.16859100
C	-4.01535700	-1.07705100	0.79107300
C	-4.21450000	-0.05382100	-1.39407700
C	-5.10207700	-1.88383000	0.49473100
H	-3.51234900	-1.13902400	1.74423300
C	-5.29221000	-0.87618500	-1.67888200
H	-3.89119100	0.69680500	-2.10295300
C	-5.73666800	-1.79284300	-0.73672800
H	-5.45158900	-2.59243600	1.23253100
H	-5.79584200	-0.78439300	-2.63093300
H	-6.58426900	-2.42628600	-0.95744500
N	5.01768300	-1.48520500	-0.33422500
O	5.84311200	-0.85772500	0.29621000
O	5.28433700	-2.26706900	-1.22376300

TS4**E = -1236.038093****G= -1235.772465****Imaginary Frequencies: -256.50**

N	-2.02645700	0.40428000	-0.51412100
O	-1.05009600	0.43172200	-1.26152200
C	-1.93273600	-0.62150000	0.46978400
H	-2.66318700	-0.46832400	1.25722100
C	-1.94124600	-2.04548200	-0.02589300
C	-2.02676200	-3.04962400	0.93561200
C	-1.88065300	-2.38744100	-1.37190600
C	-2.05280600	-4.38084600	0.55383200
H	-2.05470900	-2.78012300	1.98269200
C	-1.91681300	-3.72277200	-1.74844200
H	-1.79778700	-1.62359100	-2.13088900
C	-1.99997400	-4.72132300	-0.79048000
H	-2.11750000	-5.15246600	1.30860200
H	-1.87361000	-3.97958100	-2.79803200
H	-2.02230000	-5.76021500	-1.08993000
C	-2.74681100	1.61087500	-0.25947300
C	-2.16577300	2.82087500	-0.61509100
C	-4.01036300	1.54646400	0.31440300
C	-2.86469800	3.98958200	-0.36942700
H	-1.18013300	2.82349600	-1.05538500
C	-4.69782400	2.72548500	0.54784300
H	-4.45906500	0.59107500	0.54867700
C	-4.12616800	3.94508000	0.21102700
H	-2.41979400	4.94069300	-0.62597500
H	-5.68430000	2.68934000	0.98779600
H	-4.66558500	4.86281600	0.39917200
N	0.24877200	0.51463000	0.40629500
C	-0.45784600	-0.31832200	1.13091100
O	-0.27802700	-0.87786000	2.19588400
C	1.61804500	0.45636700	0.28586600
C	2.37585300	-0.63678200	0.73590500
C	2.26884400	1.53983000	-0.32197200
C	3.74872500	-0.63212500	0.59225400
H	1.87883100	-1.46952300	1.21099700
C	3.63830000	1.54585500	-0.47080000
H	1.66891600	2.36829200	-0.67119300
C	4.36025400	0.45628000	-0.00821400
H	4.35355300	-1.45844800	0.93340100
H	4.15708700	2.37000700	-0.93573400
N	5.81972700	0.45475300	-0.16674800
O	6.32854400	1.41790000	-0.69977300
O	6.43057900	-0.50825500	0.24368200

TS5**E = -1236.07495****G= -1235.810121****Imaginary Frequencies: -377.59**

C	-0.58851700	-0.14653100	1.41324500
O	-0.55263200	0.19164700	-0.09161200
C	-2.73051900	-0.22882500	-0.00485600
H	-3.69129400	0.22870700	0.20140600

N	-1.79502400	0.60257400	-0.42353800
O	-1.74719600	-0.20364600	1.83994800
N	0.55430400	-0.28023200	1.94095100
C	1.74134900	-0.24148800	1.22333400
C	2.74190600	0.65389700	1.61808300
C	2.01150800	-1.13599800	0.17843800
C	3.96210200	0.68241500	0.97216700
H	2.53845300	1.31907100	2.44539200
C	3.22844800	-1.11812800	-0.47028100
H	1.25052600	-1.85061700	-0.10430600
C	4.18830900	-0.20282400	-0.06859400
H	4.74074800	1.37204700	1.26104700
H	3.45143700	-1.79891800	-1.27772600
C	-2.68707900	-1.67607500	-0.17863200
C	-3.65030400	-2.43582100	0.48321600
C	-1.75538100	-2.29937200	-1.00931600
C	-3.67096800	-3.81090000	0.33474200
H	-4.36432300	-1.94428800	1.13143700
C	-1.79135100	-3.67411600	-1.16498400
H	-1.01774700	-1.70708500	-1.53198300
C	-2.74087900	-4.43028000	-0.48944500
H	-4.41036300	-4.39903900	0.85958400
H	-1.07532500	-4.15790400	-1.81453300
H	-2.75800400	-5.50483100	-0.60958700
N	5.48032000	-0.17917000	-0.75832400
O	5.65131900	-0.96669400	-1.66562600
O	6.30606600	0.62757500	-0.38615900
C	-1.99783900	2.01574100	-0.45990000
C	-1.05596600	2.86478300	0.10167800
C	-3.14041800	2.50680500	-1.07580100
C	-1.28968500	4.23054100	0.06783800
H	-0.16147600	2.45989800	0.55211900
C	-3.36391700	3.87393300	-1.09160000
H	-3.83112400	1.82890000	-1.55975800
C	-2.44071300	4.73729500	-0.51874200
H	-0.56511900	4.90216800	0.50654200
H	-4.25210000	4.26417100	-1.56832700
H	-2.61245400	5.80415700	-0.54088500

TS6**E = -1236.022598****G= -1235.756068****Imaginary Frequencies: -318.54**

O	1.93616300	-0.24937900	-1.70766800
C	1.91458600	0.40314900	0.37638900
H	2.06360400	0.03710800	1.38806300
N	2.52686400	-0.49026800	-0.58949000
C	2.32349900	1.85432200	0.24385900
C	1.67477000	2.79760800	1.03520200
C	3.33648900	2.25542400	-0.61633200
C	2.03917300	4.13239800	0.96342500
H	0.87869100	2.48008800	1.69617300
C	3.69811100	3.59373100	-0.68446900
H	3.84180400	1.52773400	-1.23546900
C	3.05199300	4.53379500	0.10311800
H	1.53013800	4.86015300	1.58023600
H	4.48726900	3.89969800	-1.35752700
H	3.33516500	5.57593400	0.04682000

C	2.66160000	-1.87444300	-0.22361200
C	2.03916700	-2.86421500	-0.96785400
C	3.45379200	-2.18471700	0.87299800
C	2.21074400	-4.18831900	-0.59235200
H	1.42851000	-2.59507400	-1.81677600
C	3.61372100	-3.51126500	1.23710000
H	3.95355400	-1.39586900	1.41993100
C	2.99222100	-4.51497600	0.50636000
H	1.72324900	-4.96798800	-1.16097100
H	4.23110300	-3.76119600	2.08858400
H	3.11987500	-5.54970600	0.79161300
C	0.42393100	0.19582100	-0.02705900
O	0.32418200	-0.20056500	-1.26843400
N	-0.49127800	0.38185800	0.83576300
C	-1.84851300	0.24338100	0.50321300
C	-2.38815200	0.56602400	-0.74551700
C	-2.69920200	-0.18769100	1.52465500
C	-3.74674700	0.44273700	-0.97060600
H	-1.74287600	0.91974800	-1.53545000
C	-4.05409300	-0.32988200	1.30393500
H	-2.26814200	-0.40889700	2.49065100
C	-4.55773800	-0.01107300	0.05404600
H	-4.18471200	0.68939900	-1.92575000
H	-4.72399500	-0.67316200	2.07726500
N	-6.00028600	-0.15274500	-0.18926800
O	-6.68714100	-0.55860600	0.72310900
O	-6.41775900	0.14345800	-1.28768400