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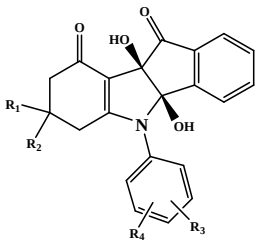

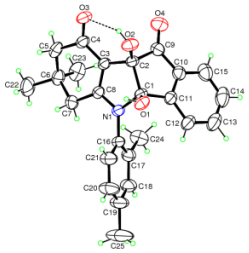

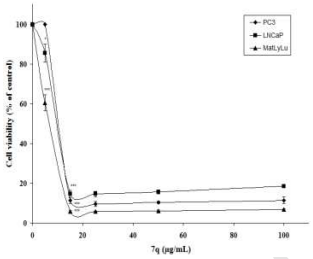

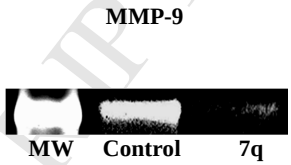

Synthesis, crystal structure and effect of indeno[1,2-b]indole derivatives on prostate cancer *in vitro*. Potential effect against MMP-9.

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1. Introduction

Prostate cancer (PCa) represents the second most frequently diagnosed malignancy in men and the sixth leading cause of cancer death worldwide [1]. Many antitumor compounds have been developed, but treatment options in patients with advanced stage of PCa have limited efficacy and are associated with significant side effects and reduced quality of life. Therefore, developing new alternatives for this malignancy is of great importance. The ability of indenoindoles as potential lipid peroxidation inhibitors [2], potassium channel openers [3], DNS intercalators and topoisomerase II inhibitors [4], estrogenic agents [5], or inhibitors of proteins kinase CK2 [6,7], have been reported. They are considered as a novel class of potent inhibitors of the human proteins kinase CK2, which is a second-messenger and phosphorylation independent constitutively active S/T protein kinase. Up to date the literature reports more than 400 potential physiological targets, showing their important roles in a variety of non cancer-related diseases (such as neurodegenerative disorders,

inflammatory processes, angiogenesis-related diseases and viral infections), as well as in different types of cancer (prostate, colon, breast and lung) [8-11].

However, there are no data available about the effect of the indenoindoles structures on the extracellular matrix (ECM). ECM proteins play important roles during proliferation, adhesion, migration and invasion of cancer cells as well as in angiogenesis in developing tumors [12-14]. Each of these events is regulated by the proteolysis of the ECM components and, thus, by matrix metalloproteinases (MMPs). MMP-9, which activity is correlated with the progression and the degree of malignancy, contributes to the invasion and metastatic spread of tumor cells, being recognized as a potential target for the development of new anti-cancer drugs [15,16]. On the other hand , special attention has been focused on the indole structure as responsible of the biological properties of some heterocyclic compounds. Thus, isatin derivatives which contain two carbonyl groups on the indole core have been tested as potential MMPs inhibitors; however, theses analogs did not show important effects on the metalloproteinase activities with no significant inhibitions reported [17].

Despite recent advances in molecular biology and the progress in combinatorial synthetic methodology, the rate of introduction of new pharmaceutical products has decreased markedly over the past two decades. Structural diversity in a focused collection of potential therapeutics is believed to increase the positive hit rate. Most pharmaceutical products in use are still small synthetic organic molecules that often contain a heterocyclic ring. However, the range of easily accessible and suitably functionalized heterocyclic building blocks for the synthesis of structurally diverse libraries is rather limited. The development of new, rapid, and clean synthetic routes toward focused libraries of such compounds is therefore of great importance to both medicinal and synthetic chemists. As part of our

general interest in the preparation of heterocyclic compounds with potential anticancer activities [18-23], herein we report the synthesis of indeno[1,2-b]indole derivatives, and their characterization by X-ray diffraction analysis. Docking simulation was performed using X-ray crystallographic structure of MMP-9 in complex with the most active inhibitor to explore the binding mode of the compound at the active site.

2. Results and discussion

2.1. Synthesis

Among the existing procedures for the preparation of indenoindoles, the Fischer indolization starting with an indanone, via the respective phenylhydrazones, serves as the most common method [24,25]. Recently, two new syntheses by transformation and reduction of 2-nitrobenzylidenephtalide, generated either by intramolecular cyclization of 2-(2-nitrophenylethyl)benzoic acid [26], or by reaction of a phthalidyl-phosphonium bromide with 2-nitrobenzaldehyde [27] and cyclization of the resulting amino compounds, have been published. The formation of *vic*-dihydroxy-indenoindolones by the reaction of ninhydrine **1** with aliphatic, and aromatic amines, or alicyclic, and cyclic enaminones has been reported elsewhere [28-33].

The intermediates **4** and **5** were achieved according to published procedures by refluxing cyclohexane-1,3-dione with aromatic amine and a catalytic amount of *p*-toluensulfonic acid in toluene and removal of water as an azeotrope with a Dean-Stark water trap [34]. To prepare the *vic*-dihydroxy-indenoindiole **6a-q** and **7a-q** derivatives, a solution of equimolar amounts of the corresponding enaminone **4** and **5** and ninhydrin **1** in chloroform, stirred at room temperature for 24 h. TLC (EtAc:Hx 1:1) showed, that only one compound was produced (*cf.* Scheme). Spectroscopic data (^{1}H and ^{13}C NMR) revealed that this was a

cyclization product with two ^{1}H resonances due to OH functionalities at 5 and 7 ppm and two ^{13}C resonances at 83 and 96 ppm approximately.

2.2. Biological

The effect of compounds **6p-q** and **7p-q**, on cell viability showed a dose-dependent response in all the cancer cell lines tested with inhibition in viability from 5µg/ml onwards. Compound **7q** showed the maximal cytotoxic effects. The structure-activity relationship studies on these compounds revealed that the presence of a dimethyl at position 7 and dichloro phenyl at position 5 are determinant for the cell viability since compounds which lack these substitutions were less toxic to the tumor cell lines. In addition, the anti-tumor activity is also affected by the presence of the H group at position 7 and methoxy and methyl phenyl groups at position 5. In this context, compound **7q** was selected for further evaluations. This derivative was also active in other human and non-human tumor prostate cell lines, such as LNCaP and MatLyLu (Table I, Figure 1).

Insert Table I here

Insert Figure 1 here

To examine the effect of this compound on the migration/motility properties of the tumor cells, the wound-healing assay was used [35]. Compound **7q**-free cultures of PC-3 cells (control vehicle) largely displayed wound recovery within 24 h and cells migrated to the wound (Figure 2a, 2c). The ability to close the scrape wound was significantly reduced by compound **7q** treatment at its cytotoxic IC_{50} (Figure 2b, 2d). On the other hand, the results of cell invasion assay using the Boyden chamber coated with Matrigel revealed that this compound also decreased the invasion in LNCaP cells after 18 h of incubation (Figure 3).

Insert Figure 2 here

Insert Figure 3 here

As shown in Figure 4, compound **7q** decreased MMP-9 activity in PC-3 cells at its cytotoxic IC_{50}. The inhibition on this metalloprotease would suggest a consequent inhibition on the ability of cells to migrate and invade surrounding areas. No MMP2 activity was reported for these cell lines, as previously shown [36].

Insert Figure 4 here

The anchorage-independent growth is considered an *in vitro* test, which correlates with tumorigenesis *in vivo* [37]. Thus, we examined the ability of PC-3, LNCaP and MatLyLu cells to grow in a semisoft agar medium after **7q** treatment. Cell colonies significantly grew in vehicle-treated agar on day 14 as previously reported [38]. On the other hand, colonies were either reduced in size and number or completely absent in cells treated with compound **7q** (Figure 5, Table II).

Insert Table II here

Insert Figure 5 here

New tissue formation, invasion and tumor cell metastasis all depend on cell motility and migration [39]. Cell migration constitutes an attractive target for the development of potential antitumor compounds and our results showed that the analog **7q** could be considered as an inhibitor of cell migration after 24 hour-incubation, which could lead to further decreases in cell invasion and metastasis prevention. Indeed, the ability of this structure to inhibit cell invasion was also confirmed.

The degree of tumor malignancy is related to the ability of neoplastic cells to invade other tissues and spreading to other organs. An important role is made by different metalloproteinases such as MMP-9 which degrades the extracellular matrix (ECM) that is

required for migration, invasion and metastasis [40]. This secreted gelatinase is highly expressed in cancer cells, making it valuable in finding patients who are at high risk of tumor development [41], thus, compounds that inhibit this protease could represent possible structures against cancer. Our results showed that compound **7q** decreased the activity of this gelatinase, suggesting that this enzyme could be a molecular target of this structure and proposing a possible mechanism for its antitumor action.

On the other hand, a specific feature of tumor cells is that they are able to grow in soft agar. This phenotypic transformation has been positively correlated with the *in vivo* tumor growth and metastasis [42]. Our results indicated that a suspension of PC-3, LNCaP or MatLyLu cells with vehicle successfully developed into relatively large colonies, whereas those cultured with the compound **7q** resulted in fewer and smaller-sized colonies or even a complete abolishment in the development of colonies, indicating a loss of the transformed phenotype and providing possible insights about the behavior of this compound on metastasis *in vivo*.

2.3. X-ray crystallography

To confirm the proposed molecular structures, X-ray single-crystal structure analyses of one compound of each series, *viz*. **6k** and **7k**, were carried out. The molecular structures are shown in Figure 6 and the relevant bond lengths and angles are given in Table III.

Insert Figure 6 here

Insert Table III here

The two stereocenters (C1 and C2) have the same configurations in **6k** and **7k**. Since both compounds crystallize in centrosymmetric space groups (*cf.* Table V), both crystals consist of equimolar mixtures of the RR and SS diastereomers (Figure 6 shows the RR configurations).

As expected, both compounds display very similar molecular geometries, which are also closely related the methoxy derivative previously reported by us [19]. Actually, 7k is isostructural with the OMe derivative. Within experimental error (*i.e.* three e.s.d.'s) bond distances are equal in both compounds; the only exception being C5-C6 and C6-C7, which are significantly longer in **7k** due to the C6 methyl substituents (*cf.* Table IV). The tetracyclic systems are V-shaped, with the two 5-membered rings making dihedral angles of 113.75(7)° (**6k**) and 114.91(7)° (**7k**). The N-bonded phenyl rings are approximately perpendicular to the heterocyclic rings [dihedral angles: 74.85(7)° (**6k**) and 78.52(7)° (**7k**)]. The phenyl rings are quite planar (r.m.s. deviations: 0.0037- 0.0089 Å) and the two 5-membered rings are approximately planar (r.m.s. deviations: 0.022-0.029 Å). The C3-C4-C5-C6-C7-C8 rings display a semi-sofa conformation (*i.e.* intermediate between boat and sofa), with distances from the C4, C5, C7, C8 mean plane of 0.166(2) Å and 0.142(2) Å for the C3 atoms, and of 0.614(2) Å and 0.599(2) Å for the C6 atoms. The puckering parameters [43] are: q_2 = 0.4522(16) Å, q_3 = -0.1927(16) Å, ϕ_2 = 3.3(2)°, Q = 0.4915(17) Å for **6k**, and q_2 = 0.4310(146) Å, q_3 = 0.-1911(14) Å, ϕ_2 = 6.15(18)°, Q = 0.4715(15) Å) for **7k**.

The molecules form O-H···O(keto) intramolecular hydrogen bonds (*cf* Figure 6). In addition, in the crystal structure there are intermolecular hydrogen bonds of the types O-H···O(keto) and (possible) weaker C-H···O(hydroxyl) and C-H···O(keto) (cf. Table IV), which link the molecules to form a three dimensional network.

Insert Table IV here

2.4. Molecular docking simulations

In order to seek the structure-activity relationships of these synthetic derivative, molecular docking of the most potent inhibitor **7q** and the inactive compound **6k** into the catalytic domain of the MMP-9 enzyme PDB ID: 1GKC [44] (Figure 7) was performed. The catalytic centre of the active-site includes a zinc ion coordinated by three histidine residues (401, 405 and 411) and a glutamic acid residue (402). The main differences between the catalytic domains of various MMPs occur in the S1'subsite or selectivity pocket (residues 425- 431 in MMP-9). It has been found that chain A is participating in the interactions.

Insert Figure 7 here

The highly active compound **7q** binds in the catalytic domain through three hydrogen bond. Carbonyl group of indene rings acts as receptor of the hydrogen bond formed with the NH group of His 401 (distance 1.88 Å). This interaction is the most important. Furthermore the dichlorophenyl group is placed into the hinge region (residues 420-431) (Figure 8). Also it has a very small overall interaction energy (-12.88 kcal/mol). These indicate that this compound binds to MMP-9 S1' subsite.

Insert Figure 8 here

The binding energy of compound **6k**, which has lower activity, is also smaller (-8.93 kcal/mol). This compound forms several hydrogen bonds but places the phenyl group outside S1' pocket.

Insert Figure 9 here

The docking results revealed that His 401 residue located in the catalytic centre of MMP-9 is important to your interaction with **7q**, which were stabilized by two hydrogen bonds two other residues and hydrophobic interactions to the Pro 430 residue within the S1' pocket. The compound **7q** that binds in the S1'subsite can be a specific inhibitor of MMP-9 mainly through an indirect mechanism by interacting far from the coordinating Zinc atom.

3. Conclusions

In conclusion, compounds were easily synthesized and with highly regiospecificity. The X-ray diffraction studies clearly confirmed the structure of the compounds. All tested compounds proved to be moderately active and, except **7q**, showed cytotoxic effects in different human and non-human prostate cancer cell lines. The mechanism of the antitumor activity of this structure seems to be related to the decrease of migration, invasion and clonogenicity possibly by inhibition of MMP-9. Of notice is the inhibition of the clonogenic potential which might lead to a possible *in vivo* anticancer effect of this compound. The structure-activity relationship studies on these compounds revealed that the presence of a dimethyl at 7 positions and dichloro phenyl at 5 positions are determinant for the cell viability since compounds which lack these substitutions were less toxic to the tumor cell lines. Docking studies shows a set of interactions in specific sites that are import for an inhibitory activity. Further studies will be needed to confirm this hypothesis.

4. Experimental

4.1. Chemistry

Melting points were determined on a Thomas micro hot stage apparatus and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu model 470 spectrophotometer. The ^{1}H NMR, ^{13}C NMR spectra were recorded using a Jeol Eclipse 270 (270 MHz/67.9 MHz) spectrometer using $CDCl_3$ or $DMSO_{d6}$, and are reported in ppm downfield from the residual $CHCl_3$ or DMSO. Elemental analyses were obtained using a Perkin Elmer 2400 CHN elemental analyzer, the results were within ± 0.4% of the predicted values. Chemical reagents were obtained from Aldrich Chemical Co, USA. All solvents were distilled and dried in the usual manner. The intermediates **4** and **5** were achieved according to published procedures [34].

4.1.2. General procedure to obtain 5-(substitutedphenyl)-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione derivatives, **6a-q, 7a-q**.

Enaminones (1.35 mmol) and **1** (1.12 mmol) were dissolved in chloroform 5 mL and stirred at rt. (24 h). The solvent was evaporated to dryness under reduced pressure, the solid thus obtained was collected by filtration, washed with diethyl ether and recrystallized from ethanol to afford the title compound.

4.1.2.1. 5-(2-methoxyphenyl)-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydro-indeno[1,2-b]-indole-9,10-dione, **6a**

Yield: 68% , mp: 234-236 ^{o}C, IR cm^{-1}: 3504, 3120 (OH), 1718 (C=O); NMR-^{1}H, ppm δ: 1.92 (m, 2H, H_7), 2.12 (m, 2H, H_6), 2.35 (m, 2H, H_8), 3.95 (s, 3H, OCH_3), 5.74 (s, 1H, OH), 6.64 (dd, 1H, H_4, J: 7.9, 2.3Hz), 6.81(dd, 1H, $H_{3'}$, J:7.8, 2.1Hz), 6.90(t, 1H, $H_{4'}$, J:7.6, 2.1Hz), 7.12(t, 1H, $H_{5'}$, J:7.8, 2.2Hz), 7.8 (m, 3H, OH, Ar), 7.51 (t, 1H, H_2, J:7.6, 1.8Hz), 7.83 (dd, 1H, H_1, J: 8.2, 2.1Hz). NMR-^{13}C, ppm δ: 21.8, 24.1, 37.3, 55.9, 82.7, 96.8, 106.3, 114.7, 115.1, 124.7, 125.2, 127.0, 127.7, 129.8, 131.3, 135.7, 135.4, 149.9, 150.0, 167.7, 193.1, 197.5. Anal. $C_{22}H_{19}NO_5$: C, 70.02; H, 5.07; N, 3.71. Found: C, 69.92; H, 5.10; N, 3.80 %.

4.1.2.2. 5-(3-methoxyphenyl)-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydro-indeno[1,2-b]-indole-9,10-dione, **6b**

Yield: 80%, mp: 128-130 ^{o}C, IR cm^{-1}: 3504, 3152 (OH), 1712 (C=O); NMR-^{1}H, ppm δ: 1.90 (m, 2H, H_7), 2.22 (m, 4H, $H_{6, 8}$), 3.83 (s, 3H, OCH_3), 5.52 (brs, 1H, OH), 6.79(d, 1H, $H_{4'}$, J: 7.9Hz), 6.90 (m, 2H, Ar), 7.03(d, 1H, $H_{6'}$, J:7.9Hz), 7.13(m, 1H, $H_{5',2'}$), 7.45(m, 2H, $H_{2',3}$), 7.81 (d, 1H, H_1, J: 7.6Hz). NMR-^{13}C, ppm δ: 21.8, 23.8, 36.7, 55.6, 82.9, 96.3, 106.3, 114.5, 114.6, 124.6, 125.2, 126.4, 127.9, 130.3, 131.0, 135.0, 135.3, 147.8, 159.8,

167.2, 193.1, 197.9. Anal. $C_{22}H_{19}NO_5$: C, 70.02; H, 5.07; N, 3.71. Found: C, 70.11; H, 5.18; N, 3.97 %.

4.1.2.3. 5-(4-methoxyphenyl)-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydro-indeno[1,2-b]-indole-9,10-dione, **6c**

Yield: 59% , mp: 204 ^{o}C, IR cm^{-1}: 3632, 3232 (OH),1708 (C=O); NMR-^{1}H, ppm δ: 1.92 (m, 2H, H_7), 2.17 (m, 4H, $H_{6,8}$), 3.86 (s, 3H, OCH_3), 5.60 (brs, 1H, OH), 6.90(dd, 1H, H_4, J:8.2, 2.1Hz), 6.94 (d, 2H, $H_{3',5'}$, J:8.2Hz), 7.16(d, 2H, $H_{2',6'}$, J:78.2Hz), 7.48(t, 1H, H_3, J:7.6Hz), 7.49(s, 1H, OH), 7.50 (t, 1H, H_2, J: 7.6Hz), 7.83(dd, 1H, H_1, J:8.4, 2.0Hz). NMR-^{13}C, ppm δ: 21.8, 23.9, 36.8, 55.6, 82.9, 96.4, 106.3, 114.5, 124.6, 125.3, 127.9, 130.0, 135.0, 135.3, 147.8, 159.8, 167.3, 193.1, 197.2. Anal. $C_{22}H_{19}NO_5$: C, 70.02; H, 5.07; N, 3.71. Found: C, 70.04; H, 5.08; N, 3.75 %.

4.1.2.4. 5-(2,4-dimethoxyphenyl)-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydro-indeno[1,2-b]-indole-9,10-dione, **6d**

Yield: 93%, mp: 188-190 ^{o}C, IR cm^{-1}: 3568, 2896 (OH), 1712 (C=O), NMR-^{1}H, ppm δ: 1.94 (m, 2H, H_7), 2.17 (m, 4H, $H_{6,8}$), 3.85 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 5.27 (brs, 1H, OH), 6.36(d, 1H, $H_{3'}$, J:1.9Hz), 6.61 (dd, 1H, $H_{5'}$, J:7.9, 1.9Hz), 6.74 (m, 2H, Ar), 7.42 (s, 2H, OH, Ar), 7.58 (m, 1H, Ar), 7.81(dd, 1H, H_1, J:8.0, 2.1Hz). NMR-^{13}C, ppm δ: 21.7, 23.5, 36.8, 55.2, 55.7, 81.5, 95.5, 99.3, 104.6, 105.9, 116.9, 124.4, 124.6, 129.9, 132.0, 134.8, 135.0, 148.2, 157.0, 161.4, 193.4, 198.0. Anal. $C_{23}H_{21}NO_6$: C, 67.80; H, 5.20; N, 3.44. Found: C, 67.83; H, 5.26; N, 3.63 %.

4.1.2.5. 5-(3,4-dimethoxyphenyl)-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydro-indeno[1,2-b]-indole-9,10-dione, **6e**

Yield: 87%, mp: 220-222 ^{o}C, IR cm^{-1}: 3536, 3105 (OH), 1715 (C=O); NMR-^{1}H, ppm δ: 1.96 (m, 2H, H_7), 2.28 (m, 4H, $H_{6,8}$), 3.86 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.03 (brs,

1H, OH), 6.74(dd, 1H, H_4, J:8.4, 2.1Hz), 6.90 (d, 1H, $H_{5'}$, J:8.6Hz), 7.01(s, 1H, $H_{2'}$), 7.03 (dd, 1H, H6´, J:8.4, 1.5Hz), 7.46 (t, 1H, H_4, J:7.5Hz), 7.48 (s, 1H, OH), 7.51 (t, 1H, H_2, J:7.5Hz), 7.83(dd, 1H, H_1, J:8.3, 1.8Hz). NMR-^{13}C, ppm δ: 21.8, 23.9, 36.7, 56.1, 56.3, 83.1, 96.8, 106.2, 110.8, 113.5, 122.2, 124.4, 125.5, 128.1, 130.3, 135.1, 147.9, 149.2, 149.3, 167.3, 193.0, 197.8. Anal. $C_{23}H_{21}NO_6$: C, 67.80; H, 5.20; N, 3.44. Found: C, 68.04; H, 5.33; N, 3.57 %.

4.1.2.6. 5-(2,5-dimethoxyphenyl)-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydro-indeno[1,2-b]-indole-9,10-dione, **6f**

Yield: 87%, mp: 142-144 ^{o}C, IR cm^{-1}: 3504, 3104 (OH), 1712 (C=O); NMR-^{1}H, ppm δ: 1.93 (m, 2H, H_7), 2.15 (t, 2H, H_6, J: 6.8Hz), 2.36 (t, 2H, H8, J: 6.7Hz), 3.10 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.32 (brs, 1H, OH), 6.72(d, 1H, H_4, J:7.9Hz), 6.75 (d, 1H, $H_{3'}$, J:8.9Hz), 6.96 (dd, 1H, $H_{4'}$, J: 8.9, 3.0Hz), 7.00(s, 1H, OH), 7.3 (d, 1H, $H_{6'}$, J: 3.0Hz), 7.45 (m, 2H, $H_{2,3}$), 7.83(d, 1H, H_1, J:7.9Hz). NMR-^{13}C, ppm δ: 21.7, 23.6, 36.3, 55.6, 56.0, 82.8, 96.3, 106.5, 112.4, 116.0, 116.7, 124.6, 125.0, 130.0, 134.9, 135.0, 148.1, 150.0, 154.0, 169.3, 192.9, 198.0. Anal. $C_{23}H_{21}NO_6$: C, 67.80; H, 5.20; N, 3.44. Found: C, 67.81; H, 5.35; N, 3.59 %.

4.1.2.7. 5-(3,4,5-trimethoxyphenyl)-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydro-indeno[1,2-b]-indole-9,10-dione, **6g**

Yield: 46%, mp: 140-142 ^{o}C, IR cm^{-1}: 3525, 2986 (OH), 1723 (C=O); NMR-^{1}H, ppm δ: 1.78 (m, 2H, H_7), 2.10 (m, 4H, $H_{6,8}$), 3.73 (s, 9H, OCH_3), 5.95 (s, 1H, OH), 6.67(s, 2H, $H_{2',6'}$), 6.93 (d, 1H, H_4, J:7.7Hz), 7.13(s, 1H, OH), 7.55 (t, 1H, H_3, J:7.7Hz), 7.66 (t, 1H, H_2, J:7.7Hz), 7.73(d, 1H, H_1, J:7.6Hz). NMR-^{13}C, ppm δ: 22.1, 24.0, 37.6, 56.6, 60.7, 84.0, 97.0, 106.6, 108.2, 123.6, 125.8, 130.7, 131.7, 135.3, 135.4, 137.7, 147.8, 153.1, 165.5,

190.2, 198.1. Anal. $C_{24}H_{23}NO_7$: C, 65.90; H, 5.30; N, 3.20. Found: C, 66.08; H, 5.37; N, 3.47 %.

4.1.2.8. 5-[(2-methylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **6h**

Yield: 55%, mp: 219-222 ^{o}C, IR cm^{-1}: 3560, 3184 (OH), 1718 (C=O); NMR-^{1}H, ppm δ: 1.15 (s, 3H, CH_3), 1.78 (m, 2H, H_7), 1.98 (m, 2H, H_6), 2.16 (m, 2H, H_8), 5.91 (s, 1H, OH), 6.62 (d, 1H, H_4, J: 5.4), 7.27 (m, 2H, OH, Ar), 7.40 (m, 2H, Ar), 7.56 (m, 2H, $H_{2,3}$), 7.65 (m, 2H, Ar), 7.76 (d, 1H, H_1, J: 8.9). NMR-^{13}C, ppm δ: 17.0, 21.6, 23.8, 36.0, 83.1, 97.2, 106.4, 124.9, 126.0, 126.2, 127.4, 129.5, 129.7, 130.4, 131.0, 132.0, 135.2, 135.6, 148.0, 168.1, 192.4, 198.0. Anal. $C_{22}H_{19}NO_4$: C, 73.12; H, 5.30; N, 3.88. Found: C, 73.17; H, 5.31; N, 4.07 %.

4.1.2.9. 5-[(3-methylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **6i**

Yield: 75%, mp: 211-212 °C, IR cm^{-1}: 3424, 2992 (OH), 1708 (C=O); NMR-^{1}H, ppm δ: 1.79 (m, 2H, H_7), 2.01 (m, 2H, H_6), 2.11 (m, 2H, H_8), 2.34 (s, 3H, CH_3), 5.95 (s, 1H, OH), 6.65 (d, 1H, H_4, J: 8.2), 7.08 (d, 1H, Ar, J: 7.7), 7.15 (s, 1H, Ar_2), 7.17 (s, 1H, OH), 7.27 (d, 1H, Ar, J: 7.7), 7.36 (t, 1H, Ar_5, J: 7.7), 7.55 (m, 2H, $H_{2,3}$), 7.71 (dd, 1H, H_1, J: 8.2, 1.7).). NMR-^{13}C, ppm δ: 18.5, 21.4, 24.0, 35.8, 83.5, 97.2, 107.5, 124.7, 125.3, 126.4, 129.1, 129.8, 130.0, 130.5, 134.9, 135.2, 135.4, 139.5, 147.7, 167.6, 191.7, 197.6. Anal. $C_{22}H_{19}NO_4$: C, 73.12; H, 5.30; N, 3.88. Found: C, 73.15; H, 5.38; N, 4.12 %.

4.1.2.10. 5-[(4-methylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **6j**

Yield: 73%, mp: 228-231 °C, IR cm^{-1}: 3513, 3184 (OH), 1721 (C=O); NMR-^{1}H, ppm δ: 1.78 (m, 2H, H_7), 1.93 (m, 2H, H_6), 2.0 (m, 2H, H_8), 2.38 (s, 3H, CH_3), 5.92 (s, 1H, OH),

6.66 (d, 1H, H_4, J: 6.9), 7.14 (s, 1H, OH), 7.18 (d, 2H, $Ar_{3,5}$, J: 8.2), 7.29 (d, 2H, $Ar_{2,6}$, J: 8.2), 7.55 (m, 2H, $H_{2,3}$), 7.71 (dd, 1H, H_1, J: 7.6, 1.2); NMR-^{13}C, ppm δ: 21.3, 22.3, 24.2, 37.7, 84.1, 96.9, 107.0, 123.8, 125.5, 129.8, 130.0, 130.7, 133.8, 135.3, 135.4, 138.0, 147.9, 165.4, 190.2, 198.2. Anal. $C_{22}H_{19}NO_4$: C, 73.12; H, 5.30; N, 3.88. Found: C, 73.19; H, 5.32; N, 4.17 %.

4.1.2.11. 5-[(2,4-dimethylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno-[1,2-b]indole- 9,10-dione, **6k**

Yield: 88%, mp: 230-233 °C, IR cm^{-1}: 3567, 3184 (OH), 1715 (C=O); NMR-^{1}H, ppm δ: 1.11 (s, 3H, CH_3), 1.76 (m, 2H, H_7), 1.98 (m, 2H, H_6), 2.13 (m, 2H, H_8), 2.34 (s, 3H, CH_3), 5.88 (s, 1H, OH), 6.67 (d, 1H, H_4, J: 8.4), 7.05 (s, 1H, Ar_3), 7.21 (m, 2H, OH, Ar), 7.52 (d, 1H, Ar, J: 7.9), 7.58 (m, 2H, $H_{2,3}$), 7.75 (dd, 1H, H_1, J: 7.2). NMR-^{13}C, ppm δ: 17.0, 21.2, 21.6, 23.8, 36.0, 83.0, 97.1, 106.4, 124.9, 125.0, 128.1, 130.4, 130.7, 131.0, 135.1, 135.6, 136.5, 139.6, 148.0, 165.3, 191.8, 198.0. Anal. $C_{23}H_{21}NO_4$: C, 73.58; H, 5.64; N, 3.73. Found: C, 73.63; H, 5.71; N, 3.97 %.

4.1.2.12. 5-[(2,5-dimethylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno-[1,2-b]indole- 9,10-dione, **6l**

Yield: 79%, mp: 227-229 °C, IR cm^{-1}: 3497, 3168 (OH), 1715 (C=O); NMR-^{1}H, ppm δ: 1.09 (s, 3H, CH_3), 1.76 (m, 2H, H_7), 1.97 (m, 2H, H_6), 2.13 (m, 2H, H_8), 2.36 (s, 3H, CH_3), 5.89 (s, 1H, OH), 6.66 (m, 1H, H_4), 7.12 (d, 1H, Ar_3, J: 7.9), 7.21 (m, 2H, OH, Ar), 7.47 (s, 1H, Ar_6), 7.55 (m, 2H, $H_{2,3}$), 7.75 (dd, 1H, H_1, J: 8.6, 2.7). NMR-^{13}C, ppm δ: 16.5, 21.0, 21.6, 23.8, 35.6, 83.1, 97.2, 100.2, 124.9, 125.0, 130.3, 130.4, 130.7, 131.3, 133.3, 135.0, 135.6, 137.2, 147.9, 165.4, 191.7, 198.0. Anal. $C_{23}H_{21}NO_4$: C, 73.58; H, 5.64; N, 3.73. Found: C, 73.59; H, 5.67; N, 3.87 %.

4.1.2.13. 5-[(3,4-dimethylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno-[1,2-b]indole-9,10-dione, **6m**

Yield: 79%, mp: 233-236 ^{o}C, IR cm^{-1}: 3562, 3184 (OH),1718 (C=O); NMR-^{1}H, ppm δ: 1.77 (m, 2H, H_7), 2.01 (m, 2H, H_6), 2.10 (m, 2H, H_8), 2.23 (s, 3H, CH_3), 2.28 (s, 3H CH_3), 5.92 (s, 1H, OH), 6.69 (d, 1H, H_4, J: 6.7), 7.01 (m, 1H, Ar_6), 7.10 (s, 2H, OH, Ar), 7.23 (d, 1H, Ar_5, J: 8.2), 7.55 (m, 2H, $H_{2,3}$), 7.71 (dd, 1H, H_1, J: 7.4, 1.2); NMR-^{13}C, ppm δ: 19.6, 19.9, 22.2, 24.1, 37.7, 84.0, 96.8, 106.8, 123.7, 125.5, 127.2, 130.3, 130.6, 130.7, 134.0, 135.2, 135.3, 136.7, 137.3, 147.9, 165.4, 190.1, 198.2. Anal. $C_{23}H_{21}NO_4$: C, 73.58; H, 5.64; N, 3.73. Found: C, 73.72; H, 5.69; N, 4.01 %.

4.1.2.14. 5-[(3,5-dimethylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno-[1,2-b] indole- 9,10-dione, **6n**

Yield: 60%, mp: 215-217 °C, IR cm^{-1}: 3543, 3200 (OH), 1718 (C=O); NMR-^{1}H, ppm δ: 1.78 (m, 2H, H_7), 1.95 (m, 2H, H_6), 2.01 (m, 2H, H_8), 2.29 (s, 6H, CH_3), 5.91 (s, 1H, OH), 6.69 (d, 1H, H_4, J: 6.9), 6.92 (s, 2H, Ar_4), 7.09 (s, 1H, $Ar_{2,6}$), 7.10 (s, 1H, OH), 7.55 (m, 2H, $H_{2,3}$), 7.71 (d, 1H, H_1, J: 6.4); NMR-^{13}C, ppm δ: 21.4, 22.3, 24.3, 37.7, 84.1, 96.9, 107.1, 123.7, 125.6, 127.4, 129.9, 130.7, 135.3, 136.4, 138.5, 147.9, 165.3, 190.3, 198.2. Anal. $C_{23}H_{21}NO_4$: C, 73.58; H, 5.64; N, 3.73. Found: C, 73.71; H, 5.67; N, 3.91 %.

4.1.2.15. 5-[(4-bromophenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **6o**

Yield: 73%, mp: 200 °C, IR cm^{-1}: 3549, 3184 (OH),1721 (C=O); NMR-^{1}H, ppm δ: 1.75 (m, 2H, H_7), 1.93 (m, 2H, H_6), 2.11 (m, 2H, H_8), 6.00 (s, 1H, OH), 6.67 (d, 1H, H_4, J: 7.2Hz), 7.27 (m, 3H, $H_{3',5'}$, OH), 7.53 (t, 1H, $H_{3,}$, J: 7.2Hz), 7.68 (d, 2H, $H_{2',6'}$, J: 8.7Hz), 7.72 (d, 1H, $H_{1,}$ J: 7.2Hz); NMR-^{13}C, ppm δ: 22.2, 24.0, 37.7, 84.0, 97.0, 107.6, 121.6, 123.8, 125.3,

131.8, 132.5, 135.2, 135.6, 136.0, 147.6, 164.9, 190.4, 198.0. Anal. $C_{21}H_{16}BrNO_4$: C, 59.17; H, 3.78; N, 3.29. Found: C, 59.23; H, 3.83; N, 3.47 %.

4.1.2.16. 5-[(4-chlorophenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **6p**

Yield: 73%, mp: 170-172 °C, IR cm^{-1}: 3485, 3184 (OH), 1721 (C=O); NMR-^{1}H, ppm δ: 1.87 (m, 2H, H_7), 1.96 (m, 2H, H_6), 2.23 (m, 2H, H_8), 4.05 (brs, 1H, OH), 6.91 (d, 1H, H_4, J: 7.6Hz), 7.28 (d, 2H, $H_{3',5'}$, J: 8.9Hz), 7.44 (d, 1H, $H_{2',6'}$, J: 8.9Hz), 7.49 (t, 1H, H_3, J: 7.6Hz), 7.51 (s, 1H, OH), 7.54 (t, 1H, H_2, J: 7.5Hz), 7.82 (d, 1H, H_1, J: 7.6Hz) ; NMR-^{13}C, ppm δ: 21.7, 23.9, 35.3, 82.8, 97.6, 107.7, 119.5, 124.9, 125.1, 129.7, 130.7, 133.8, 134.7, 135.2, 135.7, 147.6, 164.9, 192.7, 197.4. $C_{21}H_{16}ClNO_4$: C, 66.06; H, 4.22; N, 3.67. Found: C, 66.14; H, 4.29; N, 3.83 %.

4.1.2.17. 5-[(3,4-dichlorophenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno-[1,2-b]-indole-9,10-dione, **6q**

Yield: 53%, mp: 160-162 °C, IR cm^{-1}: 3561, 3173 (OH), 1727 (C=O); NMR-^{1}H, ppm δ: 1.8-2.02 (m, 2H, H_7), 2.25-2.33 (m, 2H, H_6), 2.38-2.46 (m, 2H, H_8), 4.52 (brs, 1H, OH), 6.98 (d, 1H, H_4, J: 7.2Hz), 7.27 (dd, 1H, $H_{6'}$, J: 8.9, 2.2Hz), 7.47 (d, 1H, $H_{2'}$, J: 2.2Hz), 7.51-7.60 (m, 4H, $H_{2,3,5'}$, OH), 7.84 (d, 1H, H_1, J: 7.6Hz) ; NMR-^{13}C, ppm δ: 21.6, 23.9, 35.9, 82.9, 97.4, 107.8, 124.9, 125.0, 129.0, 130.7, 131.0, 131.3, 133.3, 133.5, 134.9, 135.0, 135.7, 147.4, 167.0, 192.6, 197.1. $C_{21}H_{15}Cl_2NO_4$: C, 60.59; H, 3.63; N, 3.36. Found: C, 60.62; H, 3.67; N, 3.61 %.

4.1.2.18. 7,7-dimethyl-5-[(2-methoxyphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **7a**

Yield: 81%, mp: 170-172 °C, IR cm^{-1}: 3600, 3104 (OH), 1712 (C=O); NMR-^{1}H, ppm δ: 0.8 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.95 (d, 2H, H_6, J: 17Hz), 2.00 (d, 2H, H_8, J: 17 Hz), 3.16

(s, 3H, OCH_3), 5.81 (s, 1H, OH), 6.52 (dd, 1H, H_4, J: 8.2, 2.1Hz), 7.05 (t, 1H, $H_{4'}$, J: 7.9Hz), 7.07 (m, 3H, OH, Ar), 7.12 (t, 1H, H_3, J: 7.6Hz),7.48 (m, 3H, H_2, Ar), 7.62 (dd, 1H, $H_{6'}$, J: 8.8, 1.8Hz), 7.69(d, 1H, H_1, J: 8.1Hz); NMR-^{13}C, ppm δ: 28.5, 28.6, 33.7, 36.9, 52.0, 55.5, 83.8, 96.6, 105.0, 112.5, 121.1, 123.6, 124.7, 125.0, 130.3, 130.8, 131.8, 134.9, 135.1, 148.4, 156.5, 165.6, 189.5, 198.5. Anal. $C_{24}H_{23}NO_5$: C, 71.10; H, 5.72; N, 3.45. Found: C, 71.19; H, 5.75; N, 3.68 %.

4.1.2.19. 7,7-dimethyl-5-[(3-methoxyphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, ***7b***

Yield: 95%, mp: 202-204 °C, IR cm^{-1}: 3552, 2992 (OH), 1715 (C=O); NMR-^{1}H, ppm δ: 0.8 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.92 (d, 2H, H_6, J: 17Hz), 2.01 (d, 2H, H_8, J: 17 Hz), 3.16 (s, 3H, OCH_3), 5.82 (s, 1H, OH), 6.51 (dd, 1H, H_4, J: 8.3, 2.2Hz), 7.03 (dd, 1H, $H_{4'}$, J: 8.4, 2.1Hz), 7.08 (s, 1H, OH), 7.12 (t, 1H, H_3, J: 7.6Hz),7.48 (m, 3H, Ar), 7.63 (dd, 1H, $H_{6'}$, J: 8.0, 1.5Hz), 7.69(d, 1H, H_1, J: 8.2Hz); NMR-^{13}C, ppm δ: 28.5, 28.6, 33.7, 36.7, 51.9, 55.5, 83.8, 96.5, 104.7, 112.5, 121.1, 123.5, 124.7, 125.0, 130.2, 130.7, 131.8, 134.9, 148.3, 156.4, 165.4, 189.3, 198.4. Anal. $C_{24}H_{23}NO_5$: C, 71.10; H, 5.72; N, 3.45. Found: C, 71.16; H, 5.81; N, 3.59 %.

4.1.2.20. 7,7-dimethyl-5-[(4-methoxyphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, ***7c***

Yield: 85%, mp: 120-122 °C, IR cm^{-1}: 3440, 3100 (OH), 1715 (C=O); NMR-^{1}H, ppm δ: 0.91 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 2.05 (d, 2H, H_6, J: 17Hz), 2.22 (dd, 2H, H_8, J: 16.1, 18Hz), 3.91 (s, 3H, OCH_3), 4.09 (brs, 1H, OH), 6.86 (dd, 1H, H_4, J: 8.3, 2.2Hz), 6.96 (d, 2H, $H_{3',5'}$, J: 8.7Hz), 7.12 (d, 2H, H2´,6´, J: 8.7Hz), 7.48 (m, 3H, OH, Ar), 7.87(d, 1H, H_1, J: 8.0Hz); NMR-^{13}C, ppm δ: 27.7, 29.5, 34.3, 37.6, 50.5, 55.6, 82.7, 96.8, 105.3, 114.6,

124.7, 125.2, 128.0, 130.3, 130.8, 135.0, 135.3, 147.7, 159.9, 166.1, 191.5, 197.5. Anal. $C_{24}H_{23}NO_5$: C, 71.10; H, 5.72; N, 3.45. Found: C, 71.27; H, 5.77; N, 3.70 %.

4.1.2.21. 7,7-dimethyl-5-[(2,4-dimethoxyphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione **7d [**19]

4.1.2.22. 7,7-dimethyl-5-[(3,4-dimethoxyphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione **7e**

Yield: 72%, mp: 156-158 °C, IR cm^{-1}: 3456, 3088 (OH), 1715 (C=O); NMR-^{1}H, ppm δ: 0.87 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.77 (d, 1H, H_6, J: 17.6Hz), 1.88 (d, 1H, H_8, J: 15.6Hz), 2.10 (d, 1H, H_8, J: 15.6Hz), 2.34 (d, 1H, H_6, J: 17.6Hz), 3.71 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 5.95 (s, 1H, OH), 6.73 (dd, 1H, $H_{6'}$, J: 8.3, 2.1Hz), 6.80 (d, 1H, H_4, J: 7.2Hz), 6.96 (d, 1H, $H_{2'}$, J: 2.1Hz), 7.03 (d, 1H, $H_{6'}$, J:8.4Hz), 7.15 (s, 1H, OH), 7.53 (t, 1H, H_3, J: 7.2Hz), 7.61 (t, 1H, H_2, J: 7.2Hz), 7.71 (d, 1H, H_1, J: 7.7Hz); NMR-^{13}C, ppm δ: 28.4, 33.5, 36.7, 51.9, 55.4, 55.9, 83.7, 96.3, 99.4, 104.6, 105.3, 117.2, 123.4, 125.0, 130.1, 132.3, 134.8, 135.0, 148.4, 157.3, 161.1, 165.7, 189.2, 198.4. Anal. $C_{25}H_{25}NO_6$: C, 68.95; H, 5.79; N, 3.22. Found: C, 68.97; H, 5.81; N, 3.41 %.

4.1.2.23. 7,7-dimethyl-5-[(2,5-dimethoxyphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **7f**

Yield: 80%, mp: 210-212 °C, IR cm^{-1}: 3488, 3056 (OH), 1712 (C=O); NMR-^{1}H, ppm δ: 0.80 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.92 (d, 2H, H_6, J: 17.5Hz), 2.00 (d, 2H, H_8, J: 17,5Hz), 3.12 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 5.83 (s, 1H, OH), 6.60 (dd, 1H, H_4, J: 8.2, 1.5Hz), 6.97 (d, 1H, $H_{3'}$, J: 9.2Hz), 7.04 (dd, 1H, $H_{4'}$, J: 9.2, 2.9Hz), 7.09 (s, 1H, OH), 7.29 (d, 1H, $H_{6'}$, J:2.9Hz), 7.49 (t, 1H, H_3, J:7.2Hz), 7.54 (t, 1H, H_2, J: 7.2Hz), 7.69 (d, 1H, H_1, J: 8.1Hz); NMR-^{13}C, ppm δ: 28.4, 28.6, 36.9, 52.0, 55.7, 56.1, 56.6, 83.4, 96.7, 105.0,

113.0, 114.9, 118.1, 123.5, 125.2, 130.3, 134.9, 135.1, 148.3, 150.6, 153.4, 165.4, 189.5, 198.3. Anal. $C_{25}H_{25}NO_6$: C, 68.95; H, 5.79; N, 3.22. Found: C, 69.12; H, 5.86; N, 3.30 %.

4.1.2.24. 7,7-dimethyl-5-[(3,4,5-trimethoxyphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **7g**

Yield: 90%, mp: 198 °C, IR cm^{-1}: 3600, 3136 (OH), 1715 (C=O); NMR-^{1}H, ppm δ: 0.89 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 1.84 (d, 2H, H_6, J: 17Hz), 1.89 (d, 1H, H_8, J: 15Hz), 2.12(d, 1H, H8, J: 15Hz), 3.73 (s, 6H, OCH_3), 3.74 (s, 3H, OCH_3), 6.10 (brs, 1H, OH), 6.90 (d, 1H, H_4, J: 7.7Hz), 6.63 (s, 2H, $H_{2',6'}$), 7.17 (s, 1H, OH), 7.54 (t, 1H, H_3, J:6.7Hz), 7.65 (t, 1H, H_2, J:6.7Hz), 7.71 (d, 1H, H_1, J: 7.4Hz); NMR-^{13}C, ppm δ: 27.0, 30.0, 33.9, 37.4, 51.8, 56.7, 60.8, 84.0, 97.3, 105.6, 108.2, 123.6, 126.9, 130.8, 131.8, 135.3, 135.4, 137.8, 147.7, 153.2, 164.4, 189.7, 198.2. Anal. $C_{26}H_{27}NO_7$: C, 67.09; H, 5.85; N, 3.01. Found: C, 67.12; H, 5.91; N, 3.17 %.

4.1.2.25. 7,7-dimethyl-5-[(2-methylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno [1,2-b]indole-9,10-dione, **7h**

Yield: 82%, mp: 231-234 °C, IR cm^{-1}: 3424, 2996 (OH), 1712 (C=O); NMR-^{1}H, ppm δ: 0.89 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.86 (s, 2H, H_6), 2.04 (s, 2H, H_8), 5.88 (s, 1H, OH), 6.67 (d, 1H, H_4, J: 6.2), 7.27 (m, 2H, OH, Ar), 7.37 (m, 2H, $Ar_{4,5}$), 7.57 (m, 2H, $H_{2,3}$), 7.62 (m, 1H, Ar), 7.76 (dd, 1H, H_1, J: 7.4); NMR-^{13}C, ppm δ: 17.3, 28.4, 28.6, 33.8, 36.9, 52.0, 84.1, 97.3, 104.7, 124.2, 125.2, 127.2, 129.4, 130.7, 131.3, 132.0, 134.6, 135.4, 135.7, 137.9, 148.7, 164.3, 189.5, 198.7. $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.11; H, 6.01; N, 3.92 %.

4.1.2.26. 7,7-dimethyl-5-[(3-methylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno [1,2-b]indole-9,10-dione, **7i**

Yield: 56%, mp: 118-120 °C, IR cm^{-1}: 3524, 3092 (OH), 1718 (C=O); NMR-^{1}H, ppm δ: 0.88 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.92 (s, 2H, H_6), 2.10 (s, 2H, H_8), 2.35 (s, 3H, CH_3), 5.96 (s, 1H, OH), 6.65 (d, 1H, H_4, J: 7.9), 7.02 (d, 1H, Ar_6, J: 7.7), 7.15 (s, 1H, Ar_2), 7.20 (s, 1H, OH), 7.27 (d, 1H, Ar_4, J: 7.7), 7.36 (t, 1H, Ar_5, J: 7.7), 7.54 (m, 2H, $H_{2,3}$), 7.71 (dd, 1H, H_1, J: 8.1, 1.9); NMR-^{13}C, ppm δ: 21.6, 27.1, 30.0, 34.1, 37.7, 51.8, 84.0, 97.3, 106.0, 123.8, 125.6, 127.0, 129.2, 129.3, 130.4, 130.8, 135.3, 135.4, 136.6, 140.0, 147.8, 164.1, 189.8, 198.2. $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.07; H, 5.98; N, 3.73 %.

4.1.2.27. 7,7-dimethyl-5-[(4-methylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno [1,2-b]indole-9,10-dione, **7j**

Yield: 99%, mp: 206-207 °C, IR cm^{-1}: 3602, 3214 (OH), 1721 (C=O); NMR-^{1}H, ppm δ: 0.95 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.98 (d, 1H, H_6 J: 17Hz), 2.15 (d, 1H, H_6, J: 17Hz), 2.20 (d, 1H, H_8, J: 16Hz), 2.28 (d, 1H, H8, J: 16Hz), 2.44 (s, 3H, CH_3), 5.97 (s, 1H, OH), 6.86 (d, 1H, H_4, J: 8.7), 7.10 (d, 2H, $AH_{3',5'}$, J: 8.2Hz), 7.26 (d, 2H, $H_{2',6'}$, J: 8.2), 7.48 (m, 3H, $H_{2,3}$, OH), 7.86 (d, 1H, H_1, J: 8.6Hz); NMR-^{13}C, ppm δ: 21.3, 27.6, 30.5, 34.4, 37.6, 51.8, 82.7, 96.8, 105.6, 124.7, 125.2, 129.2, 130.0, 132.9, 134.9, 135.3, 138.8, 147.7, 165.7, 191.7, 197.5. $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 63.91; H, 6.13; N, 3.85 %.

4.1.2.28. 7,7-dimethyl-5-[(2,4-dimethylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **7k**

Yield: 59%), mp: 215-218 °C, IR cm^{-1}: 3583, 3184 (OH), 1718 (C=O); NMR-^{1}H, ppm δ: 0.82 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.13 (s, 3H, CH_3), 1.83 (s, 2H, H_6), 2.03 (s, 2H, H_8), 2.33 (s, 3H, CH_3), 5.86 (s, 1H, OH), 6.71 (d, 1H, H_4, J: 8.2), 7.05 (d, 1H, Ar_3, J: 1), 7.17 (dd, 1H, Ar_5, J: 8.2, 1.2), 7.22 (s, 1H, OH), 7.47 (d, 1H, Ar_6, J: 7.9), 7.57 (m, 2H, $H_{2,3}$), 7.75 (dd, 1H, H_1, J: 8.9, 1.2). NMR-^{13}C, ppm δ: 17.1, 21.2, 28.7, 34.1, 37.1, 50.9, 82.9,

97.0, 104.7, 124.9, 128.0, 130.3, 131.0, 131.6, 135.1, 135.2, 135.5, 139.5, 148.2, 166.5, 191.7, 197.8. $C_{25}H_{25}NO_4$: C, 74.42; H, 6.25; N, 3.47. Found: C, 74.56; H, 6.30; N, 3.75 %.

4.1.2.29. 7,7-dimethyl-5-[(2,5-dimethylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **7l**

Yield: 98%, mp: 173-175 °C, IR cm^{-1}: 3595, 3296 (OH), 1721 (C=O); NMR-^{1}H, ppm δ: 0.82 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.94 (m, 4H, $H_{6,8}$), 2.35 (s, 3H, CH_3), 5.86 (s, 1H, OH), 6.69 (m, 1H, H_4,), 7.12 (d, 1H, Ar_3, J: 7.9), 7.20 (m, 3H, OH, 2Ar), 7.42 (s, 1H, Ar_6), 7.56 (m, 2H, $H_{2,3}$), 7.74 (m, 1H, H_1). NMR-^{13}C, ppm δ: 16.7, 21.0, 28.7, 34.1, 37.2, 50.2, 82.8, 97.1, 105.0, 124.9, 130.4, 130.7, 131.6, 133.4, 133.7, 135.1, 135.5, 137.3, 148.1, 167.0, 191.6, 198.0. $C_{25}H_{25}NO_4$: C, 74.42; H, 6.25; N, 3.47. Found: C, 74.61; H, 6.39; N, 3.79 %.

4.1.2.30. 7,7-dimethyl-5-[(3,4-dimethylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **7m**

Yield: 58%, mp: 185-188 °C, IR cm^{-1}: 3520, 3168 (OH), 1718 (C=O); NMR-^{1}H, ppm δ: 0.82 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 1.93 (s, 2H, H_6), 2.06 (s, 2H, H_8), 2.21 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 5.95 (s, 1H, OH), 6.69 (d, 1H, H_4, J: 6.7), 6.86 (d, 1H, Ar_6, J: 8.2), 7.04 (s, 1H, Ar_2), 7.17 (s, 1H, OH), 7.21 (d, 1H, Ar_5, J: 8.2), 7.53 (m, 2H, $H_{2,3}$), 7.7 (d, 1H, H_1, J: 7.2); NMR-^{13}C, ppm δ: 19.57, 19.95, 27.16, 29.55, 33.86, 37.54, 51.37, 83.75, 97.15, 105.31, 123.81, 125.53, 127.24, 130.43, 130.64, 130.93, 133.65, 134.99, 135.54, 137.11, 137.64, 147.73, 165.08, 190.59, 198.36. $C_{25}H_{25}NO_4$: C, 74.42; H, 6.25; N, 3.47. Found: C, 74.43; H, 6.27; N, 3.59 %.

4.1.2.31. 7,7-dimethyl-5-[(3,5-dimethylphenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione, **7n**

Yield: 64%), mp: 143-145 °C, IR cm^{-1}: 3561, 3168 (OH), 1705 (C=O); NMR-^{1}H, ppm δ: 0.84 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 1.94 (s, 2H, H_6), 2.07 (s, 2H, H_8), 2.26 (s, 6H, CH_3), 5.95 (s, 1H, OH), 6.67 (d, 1H, H_4, J: 6.7), 6.81 (s, 1H, Ar_4), 7.08 (s, 2H, $Ar_{2,6}$), 7.19 (s, 1H, OH), 7.54 (m, 2H, $H_{2,3}$), 7.70 (dd, 1H, H_1, J: 7.4, 2.2); NMR-^{13}C, ppm δ: 21.3, 27.5, 29.6, 34.5, 37.7, 50.7, 82.7, 96.7, 105.6, 124.7, 125.3, 126.9, 130.4, 130.5, 134.9, 135.2, 135.4, 139.1, 147.7, 165.6, 191.7, 197.6. $C_{25}H_{25}NO_4$: C, 74.42; H, 6.25; N, 3.47. Found: C, 74.47; H, 6.28; N, 3.62 %.

4.1.2.32. 7,7-dimethyl-5-[(4-bromophenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno-[1,2-b]indole-9,10-dione, **7o**

Yield: 75%, mp: 140-142 °C, IR cm^{-1}: 3644, 3008 (OH), 1718 (C=O); NMR-^{1}H, ppm δ: 0.93 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.96 (d, 1H, H_6, J:17 Hz), 2.05 (d, 1H, H_6, J:17 Hz), 2.20 (d, 1H, H_8, J:15 Hz), 2.29 (d, 1H, H_8, J: 15Hz), 3.60 (brs, 1H, OH), 6.86 (d, 1H, H_4, J:6.7 Hz), 7.16 (d, 2H, $H_{3',5'}$, J:8.2 Hz), 7.49 (m, 3H, $H_{2,3}$, OH), 7.60 (d, 2H, $H_{2',6'}$, J: 8.2 Hz), 7.83 (d, 1H, H_1, J: 7.6 Hz); NMR-^{13}C, ppm δ: 27.7, 29.4, 34.4, 37.7, 50.0, 82.8, 123.0, 124.9, 125.1, 128.8, 130.6, 131.0, 132.7, 134.5, 134.6, 134.8, 135.7, 147.5, 191.4, 197.3. $C_{23}H_{20}BrNO_4$: C, 60.81; H, 4.44; N, 3.08. Found: C, 60.93; H, 4.52; N, 3.27 %.

4.1.2.33. 7,7-dimethyl-5-[(4-chlorophenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno-[1,2-b]indole-9,10-dione **7p**

Yield: 67%, mp: 147-148 °C, IR cm^{-1}: 3637, 3018 (OH), 1714 (C=O); NMR-^{1}H, ppm δ: 0.90 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.98 (d, 1H, H_6, J:15 Hz), 2.05 (d, 1H, H_6, J:15 Hz), 2.23 (d, 1H, H_8, J:17 Hz), 2.31 (d, 1H, H_8, J: 17Hz), 3.67 (brs, 1H, OH), 6.81 (d, 1H, H_4, J:6.8 Hz), 7.14 (d, 2H, $H_{3',5'}$, J:8.0 Hz), 7.47 (m, 3H, $H_{2,3}$, OH), 7.62 (d, 2H, $H_{2',6'}$, J: 8.0 Hz), 7.83 (d, 1H, H_1, J: 7.6 Hz); NMR-^{13}C, ppm δ: 28.0, 30.1, 34.7, 36.9, 51.0, 81.7, 122.8,

125.3, 125.9, 128.3, 131.1, 131.7, 132.0, 133.9, 134.7, 135.2, 135.9, 147.7, 191.3, 197.2. $C_{23}H_{20}ClNO_4$: C, 67.40; H, 4.92; N, 3.42. Found: C, 67.44; H, 4.99; N, 3.63 %.

4.1.2.34. 7,7-dimethyl-5-[(3,4-dichlorophenyl)]-(4bRS,9bRS)-dihydroxy-4b,5,6,7,8,9b-hexahydroindeno[1,2-b]indole-9,10-dione **7q**

Yield: 47%, mp: 180-182 °C, IR cm^{-1}: 3568, 2647 (OH), 1724 (C=O); NMR-^{1}H, ppm δ: 0.96 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 2.00 (d, 1H, H_6, J: 17Hz), 2.17 (d, 1H, H_6, J: 17Hz), 2.20 (d, 1H, H_8, J: 15Hz), 2.26 (d, 1H, H_8, J: 15Hz), 3.7 (brs, 1H, OH), 6.93 (d, 1H, H_4, J: 7.2Hz), 7.19 (dd, 1H, $H_{6'}$, J: 8.9, 2.2Hz), 7.48-7.56 (m, 5H, $H_{2',5',3,2,}$OH), 7.84 (d, 1H, H_1, J: 7.6Hz) ; NMR-^{13}C, ppm δ: 27.5, 29.5, 37.7, 50.1, 82.6, 97.4, 106.7, 124.9, 128.8, 130.7, 131.1, 131.2, 133.4, 134.8, 135.1, 135.7, 147.4, 165.6, 168.9, 191.9, 197.1. $C_{23}H_{19}Cl_2NO_4$: C, 62.17; H, 4.31; N, 3.15. Found: C, 62.23; H, 4.35; N, 3.41 %.

4.2. Anticancer assays

4.2.1. Cell Culture

Human and non-human prostate tumor cell lines PC-3, LNCaP and MatLyLu were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Cells were grown in culture RPMI medium supplemented with fetal bovine serum (FBS, 10%), penicillin (50 units/ml) and streptomycin (50 µg/ml), in a humidified atmosphere (95% air, 5% CO_2, 37°C). Culture medium was purchased from Gibco-In Vitrogen, Karlsruhe, Germany and PAA Laboratories, Pasching, Austria.

4.2.2. Cell Viability Test

5×10^3 PC-3, 1.2×10^4 LNCaP or 1×10^4 MatLyLu cells were seeded in a 96-well microtiter plate containing 100 µl of culture growth RPMI medium/well. After 24 h of culture, cells were exposed to the compounds previously dissolved in DMSO (72h, 5-100 µg/ml). The final concentration of this solvent in the culture media was always lower than 0.2%, a

concentration that has neither a cytotoxic effect nor causes any interference with the colorimetric detection method. The dose-dependent effects of each compound on were assessed using the XTT test (Roche Applied Science, Mannheim, Germany). After 72 h of exposure with the compounds, cells were incubated with XTT (4h, 37°C) and the formazan was recorded at 492 nm (Microplate reader HT II, Anthos, Salzburg, Austria). The IC_{50} value was defined as the concentration of tested compound resulting in a 50% reduction in cell viability compared to vehicle-treated cells. Further evaluations of the best compound were performed using this IC_{50} value [36,45].

4.2.3. Cell Migration and Motility Evaluation

Cell migration and motility was tested using the scrape wound repair assay. 8×104 PC-3 cells were grown to confluence on 24-well plates in RPMI medium (48 h, 37°C). A sterilized micropipette tip was used to introduce a wound across the entire cell monolayer, and the medium was removed. After washing gently with PBS, the most cytotoxic compound on the XTT assay was added (IC_{50}) in fresh medium and incubated for 24 h in the presence of endothelial growth factor (1 pg/ml). Cover slips were mounted onto a light microscope and images of the wounds were captured on a computer system using a digital camera immediately following wounding (0 h) and after 24 h of compound or vehicle incubation. The wound area in each image was measured using the ImageJ program for Windows (http://rsb.info.nih.gov/ij/) and quantified by following the change in wound area over time compared with the original wound area. The results were expressed as the percentage of wound closure [35].

4.2.4. Invasion Assay.

Human tumor LNCaP cells (1×10^5 cells/ml) were pretreated with the compound (cytotoxic IC_{50}, 24h). Cells then were seeded into the upper part of a Boyden chamber membrane

coated with Matrigel (Becton Dickinson Biosciences, Heidelberg, Germany) in 50 µL of serum-free medium and incubated (18 h, 37°C). The bottom of the chamber contained 0.5 mL of standard medium (20% FBS). Invaded cells at the lower surface of the chamber were reacted with calcein (4 µg/ml) in Hank´s Buffered Salt Solution (1 h, 37°C). The fluorescence of invaded cells was read at 485/530 nm (Fluoroskan Ascent, Thermo Labsystems Oy, Helsinki, Finland) [22,36].

4.2.5. Measurement of Clonogenic Potential

The ability of cells to grow in an anchorage-independent manner was tested in cells grown in agar (0.6%). In short, 1 mL of a mixture of 1.2% Noble agar (Gibco-In Vitrogen, Karlsruhe, Germany) and RPMI medium (1:1) was added into each well of a six-well plate. PC3, LNCaP and MatLyLu cells (1×105) suspended in completed RPMI medium (FBS 20%) containing Noble agar (0.3%) were overlaid on the semisolid bottom layer. The plates were kept at room temperature for 15 min and incubated for 24 h (37°C, 95% O_2, 5% CO_2). The following day, 1 mL of medium with the compound at its IC_{50} concentrations was added to each well. After 2 weeks of incubation cells were stained with crystal violet (0.01%) for 18 h at 37°C. Pictures were taken under light microscopy, and the total number of colonies and the relative colony size were determined [22,46].

4.2.6. MMP Zymography

To measure MMP-2 and MMP-9 activities, PC-3 and LNCaP cells (80% confluent in six-well plates) were washed twice with PBS and treated with the compound at its respective IC_{50} concentration in serum-free medium (2.5 mL, 24 h, 37°C) in a humidified atmosphere (95% O_2, 5% CO_2). 22 µl of a mixture composed of the conditioned medium and sample buffer (without mercaptoethanol, 0.75:0.25) were subjected to electrophoresis on 10% SDS-polyacrylamide gels gelatin-copolymerized (1 mg/mL). MMP-2 and MMP-9

gelatinolytic activity in the conditioned culture medium was assayed as previously described [22,47]. Bands quantification was performed by using ImageJ Software for Windows. Pure human MMP-9 and MMP-2 protein were used as a positive control.

4.3. X-ray crystallography

Crystals of **6k** and **7k** suitable for X-ray diffraction were obtained by slow evaporation of a solution in ethanol. Crystal data, intensity data collection parameters and final refinement results are summarised in Table V. Diffraction data were measured on a Bruker Smart-Apex diffractometer using graphite-monochromated Mo-Kα radiation (λ = 0.71070 Å). The structure was solved by direct methods and refined on F^2 by full-matrix least-squares, using all reflections and weights $w = [\sigma^2(F_o^2) + (a\ P)^2 + b\ P]^{-1}$, with $P = (F_o^2 + 2\ F_c^2)/3$. The C-bonded H atoms were placed in calculated positions and refined using a riding atom model with fixed C-H distances (0.93 Å for CH, 0.97 Å for CH_2, 0.96 Å for CH_3), and with U_{iso} = p U_{eq}(parent atom) (p = 1.2 for CH and CH_2, 1.5 for CH_3). The O-bonded H atoms were placed at idealized tetrahedral geometries, with fixed O-H distances (0.82 Å) and U_{iso} = 1.5 U_{eq}(parent atom).

The following computer programs were used: data collection, SMART [48]; cell refinement and data reduction, SAINT [49]; absorption correction, SADABS [50]; structure solution and refinement, SHELXL-97 [51]; molecular graphics, ORTEP-3 [52]; geometrical calculations, PLATON [53]; The structure solution, the refinement and the drawings were carried out with the aid of the WinGX [54] suite of programs. Comprehensive crystallographic data (excluding structure factors) for the structural analysis of **6k** and **7k** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data (CIF files) can be obtained, free of charge, on application to CCDC, 12 Union Road,

Cambridge CB2 1EZ, UK, fax: +44-(0)1223-336033, or from www.ccdc.cam.ac.uk/data_request/cif, quoting deposition No. CCDC 1022641 (**6k**) and CCDC 1022819 (**7k**).

4.4. Molecular docking methodology

The three dimensional structure of MMP-9 was downloaded from the Protein Data Bank (http://www.rscb.org/pdb) server with PDB code: 1GKC. Internal ligands and the crystallographic water molecules were removed from the protein and missing Hydrogens were added. Ligands were built with the molecular editor of CAChe 6.0 [55] using the crystallographic data structures **7k** and **6k** as reference. They were geometrically optimized by using MM3 [56] force field by CAChe 6.0 and PM3 of the Arguslab 4.0.1 [57] with RMSD 0.0001. The active site of the enzyme was defined and the ligand was placed in a box with dimensions of X = 20 Å, Y = 20 Å, Z = 20 Å and a resolution of the grid of 0.4000 Å. Docking was performed using Arguslab 4.0.1employing genetic algorithm to generate the poses of the ligands at the binding site, allowing flexibility of the ligand. The results of docking were quantifies in terms of the score and minimized energy.

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Table 1. Selected bond lengths (Å) and angles (°) for **6k** and **7k**.

	6k	**7k**
C1-C2	1.5796(16)	1.5765(15)
C1-C11	1.5032(17)	1.5060(17)
C1-N1	1.5011(16)	1.4994(14)
C2-C3	1.5009(17)	1.4978(16)
C2-C9	1.5462(17)	1.5440(18)
C3-C4	1.4108(17)	1.4117(16)
C3-C8	1.3710(17)	1.3657(16)
C4-C5	1.5082(19)	1.5099(19)
C5-C6	1.516(2)	1.535(2)
C6-C7	1.532(2)	1.5440(18)
C7-C8	1.4861(18)	1.4870(16)
C9-C10	1.4765(18)	1.4753(19)
N1-C8	1.3427(16)	1.3443(14)
N1-C16	1.4341(15)	1.4310(14)
O1-C1	1.3788(14)	1.3803(14)
O2-C2	1.4126(15)	1.4118(14)
O3-C4	1.2524(16)	1.2500(16)
O4-C9	1.2081(15)	1.2043(17)
C2-C1-C11	105.29(9)	105.19(10)
C2-C1-N1	102.20(9)	102.21(8)
C2-C1-O1	117.03(10)	117.00(9)
C11-C1-N1	109.24(10)	110.02(9)
C11-C1-O11	110.52(10)	110.24(9)
N1-C1-O1	111.97(10)	111.70(10)
C1-C2-C3	103.62(9)	103.66(9)
C1-C2-C9	103.84(9)	104.10(9)
C1-C2-O2	112.31(9)	112.04(9)
C3-C2-C9	111.91(10)	112.27(10)
C3-C2-O2	114.39(10)	114.27(10)
C9-C2-O2	110.12(10)	109.92(10)
C2-C3-C4	126.45(11)	126.84(11)
C2-C3-C8	109.72(10)	109.91(10)
C4-C3-C8	121.58(12)	121.38(11)
C3-C4-C5	116.97(12)	116.93(11)
C3-C4-O3	122.59(12)	122.61(12)
C5-C4-O3	120.31(11)	120.31(11)
C4-C5-C6	113.58(11)	116.39(10)
C5-C6-C7	111.84(12)	109.31(11)
C6-C7-C8	108.88(12)	111.12(11)

C3-C8-C7	123.23(11)	123.57(11)
C3-C8-N1	112.47(11)	112.39(10)
C7-C8-N1	124.18(12)	123.85(10)
C2-C9-C10	108.37(10)	108.15(10)
C2-C9-O4	124.33(12)	124.40(13)
C10-C9-O4	127.24(12)	127.42(13)
C9-C10-C11	110.23(11)	110.57(11)
C1-C11-C10	111.82(10)	111.72(11)
C1-N1-C8	111.55(10)	111.48(9)

Table 2. Crystal data, intensity data collection parameters and final refinement results for **6k** and **7k**

Compound	**6k**	**7k**
CCDC deposit No.	CCDC 1022641	CCDC 1022819
Crystal Data		
Formula	$C_{23}H_{21}NO_4$	$C_{25}H_{25}NO_4$
MW	375.31	403.46
Color	pale yellow	pale yellow
Morphology	prism	prism
Specimen size (mm)	0.55x0.52x0.16	0.50x0.48x0.43
T (K)	296(2)	296(2)
a (Å)	10.5702(6)	9.6597(4)
b (Å)	14.0438(7)	18.0974(8)
c (Å)	13.4084(7)	12.2889(5)
β (°)	110.1540(10)	93.7970(10)
V (Å^3)	1868.55(17)	2143.57(16)
Crystal system	monoclinic	monoclinic
Space group (No.)	$P2_1/c$ (No. 14)	$P2_1/n$ (No. 14)
Z	4	4
D_c (g cm^{-3})	1.334	1.250
F(000)	792	856
μ(Mo-Kα) (mm^{-1})	0.091	0.084
θ range (°) for cell	2.57-25.69	2.25-28.67
No. refls. for cell	5024	7826
Data Collection		
θ range (°)	2.05-28.77	2.01-28.82
h range	-14, 13	-13, 13
k range	-18, 18	-23, 24
l range	-17, 15	-15, 16
Mean ΔI for checks (%)	-0.3	-0.1
No. refls. measured	9865	17049
No. refls. unique	4447	5250
No. refls. I>2σ(I)	3582	4287
Abs. correction	multi-scan	multi-scan
Trans. coeff. (T_{min}, T_{max})	0.937-0.969	0.951-0.973
R_{int}	0.0140	0.0161

Refinement (last cycle)

Weighting scheme (a,b)	0.0638, 0.3320	0.0822, 0.3249
No. params. refined	257	277
R1 [I>2σ(I)]	0.0437	0.0471
R1 (all data)	0.0.538	0.0572
wR2 [I>2σ(I)]	0.1156	0.1299
wR2 (all data)	0.1231	0.1388
S (g.o.f.) (all data)	1.032	1.027
Δ/σ max.	0.001	0.001
Δ/σ mean	<0.0005	<0.0005
$\Delta\rho_r$ (min., max.) (e Å^{-3})	-0.18, 0.30	-0.18, 0.31

Table 3. Possible hydrogen bonds for **6k** and **7k** (Å and °).

		6k		
D-H···A	D-H	H···A	D···A	DHA
O1-H1···O3[i]	0.82	1.96	2.7715(13)	173.3
O2-H2···O3	0.82	2.18	2.8528(14)	139.4
C12-H12···O1[ii]	0.93	2.52	3.4039(16)	158.5
C15-H15···O4[iii]	0.93	2.54	3.3893(17)	151.2
		7k		
D-H···A	D-H	H···A	D···A	DHA
O1-H1···O3[iv]	0.82	1.97	2.7839(14)	175.2
O2-H2···O3	0.82	2.22	2.8811(14)	138.5
C5-H5B···O2[iv]	0.97	2.57	3.2341(17)	125.8

Symmetry codes: i) 1-x, 2-y, 1-z; ii) -x,2-y,1-z; iii) 1-x, 2-y, 2-z; iv) -x, -y, 1-z

Table 4. Cytotoxic effects of compounds on human and non-human tumor cells.

Compound	R_1, R_2	R_3	PC-3	LNCaP	MatLyLu
6p	**H**	**4Cl**	67.91 ± 4.37	79.17 ± 2.73	>100
7p	**H**	**3,4-Cl**	76.59 ± 5.41	66.25 ± 2.16	>100
6q	**CH_3**	**4Cl**	47.77 ± 3.11	53.92 ± 3.88	75.17 ± 2.35
7q	**CH_3**	**3,4-Cl**	10.70 ± 0.07**	9.57 ± 0.55**	5.96 ± 0.28
DQ			24.60 ± 2.22	4.94±0.66	10.33±2.17

6a-o, **7a-o**: > 100 µg/mL

Results are expressed as the mean ± SEM of the half inhibitory concentration or IC_{50} (µg/mL). Each experiment was performed three times in five different wells. DQ: control dequalinium. ***p*<0.01 compared to DQ.

Table 5. Inhibition of clonogenic potential by compound **7q**.

	Colony formation (% of control)		Relative colony size	
Cell line	**Control**	**7q**	**Control**	**7q**
PC-3	100±17.6	0	1±0.3	0
LNCaP	100±18.3	0	1±0.3	0
MatLyLu	100±17.6	6.35±1.98**	1±0.05	0.16±0.04***

Colony growth relative to vehicle-treated controls PC-3, LNCaP and MatLyLu cells were quantified by light microscopy. The results are given as means ± SEM. of three independent experiments. ***p*< 0.01 and ***p<0.001compared to control vehicle.

Figure Captions

Scheme. The general reaction protocols leading to compounds **6a-q** and **7a-q.**

Figure 1. Molecular structures of compounds (a) **6k** and (b) **7k** showing the atomic numbering. The displacement ellipsoids are drawn at 50% probability. Dashed lines indicate intramolecular hydrogen bonds.

Figure 2. Effect of compound **7q** on PC-3, LNCaP and MatLyLu cell viability. Results are expressed as the mean ± SEM of three different experiments. *p<0.05, ** p<0.01 and ***p<0.001 compared to the previous concentration at the same cell line.

Figure 3. Effect of compound **7q** on PC-3 wound closure. **(a)** Representative images of PC-3 cells were captured at the time of wounding (a) and (b), and 24 h following the wound (c) and (d), to illustrate recovery from scrape wound. **(e)** The percentage of wound area closed at 24 hours post-treatment is plotted for cell monolayer with or without **7q**. Data shown in (a), (b), (c) and (d) are from a representative experiment carried out independently three times. Data in (e) represents the mean ± SEM. **p<0.01 compared to control vehicle.

Figure 4. Effect of compound **7q** in the invasion of LNCaP cells. Cells treated with the compound or vehicle were seeded onto a Matrigel coated 0.8 µm porous membrane for 18 h, and the inhibition of invasion relative to the control vehicle treated cells was determined. The results represent the mean ± SEM of three independent experiments. **p<0.01 compared to control vehicle.

Figure 5. Activity of MMP-9 metalloproteinase by gelatin zymography in PC-3 cells when exposed to compound **7q** for 24h. (a) Conditioned medium prepared from subconfluent cultures were collected, resolved in non-reducing gels containing gelatin (1 mg/ml) and processed for zones of gel degradation activity. (b) Results were quantified in relation to control vehicle and are presented as the mean ± SEM of percentage of activity in three different experiments. ***p<0.001 compared to control vehicle.

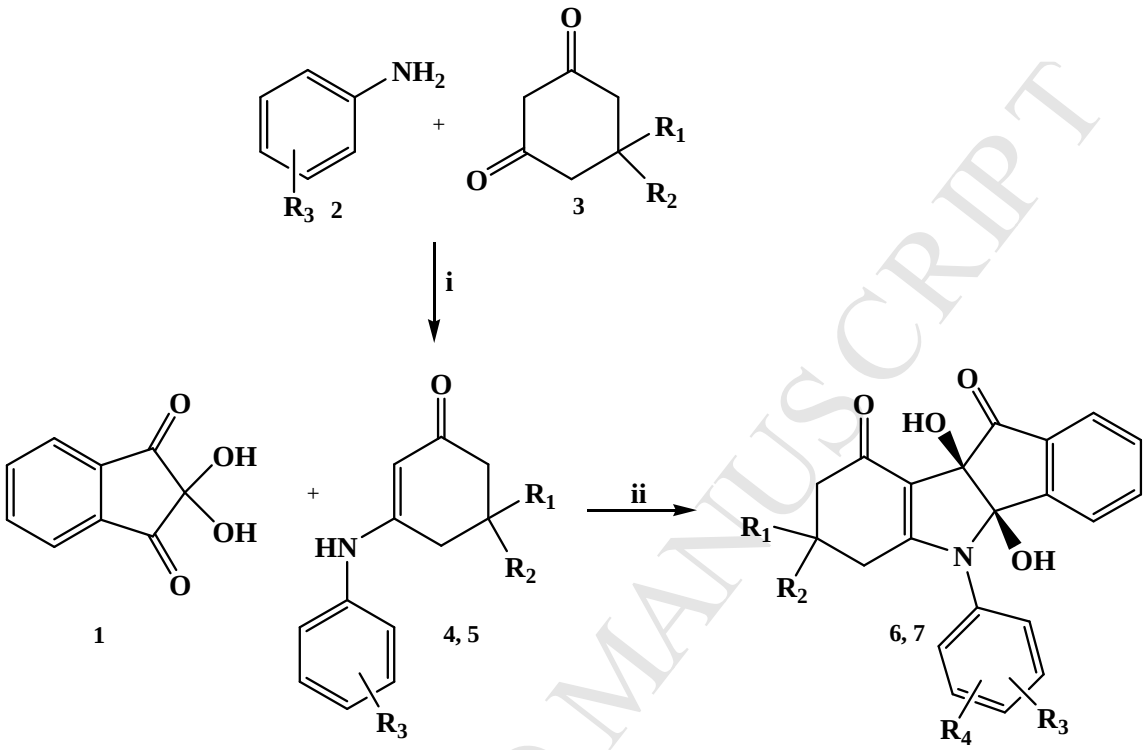
Figure 6. Effect of compound **7q** on the formation of tumor colonies in soft agar. PC-3 (a), LNCaP cells (b) and MatLyLu cells (c) were plated over a semi-solid layer of soft agar and treated with the compound **7q** or vehicle (control) and incubated for 14 days. The results represent standard images of three different experiments.

Figure 7. Catalytic centre and S1'subsite of the enzyme MMP-9 human (PDB ID: 1GKC).

Figure 8. Binding mode of highly active compound **7q** at the catalytic centre and S1'subsite of the enzyme MMP-9 human.

Figure 9. Binding mode of **6k** at the catalytic centre and S1'subsite of the enzyme MMP-9 human.

Scheme.

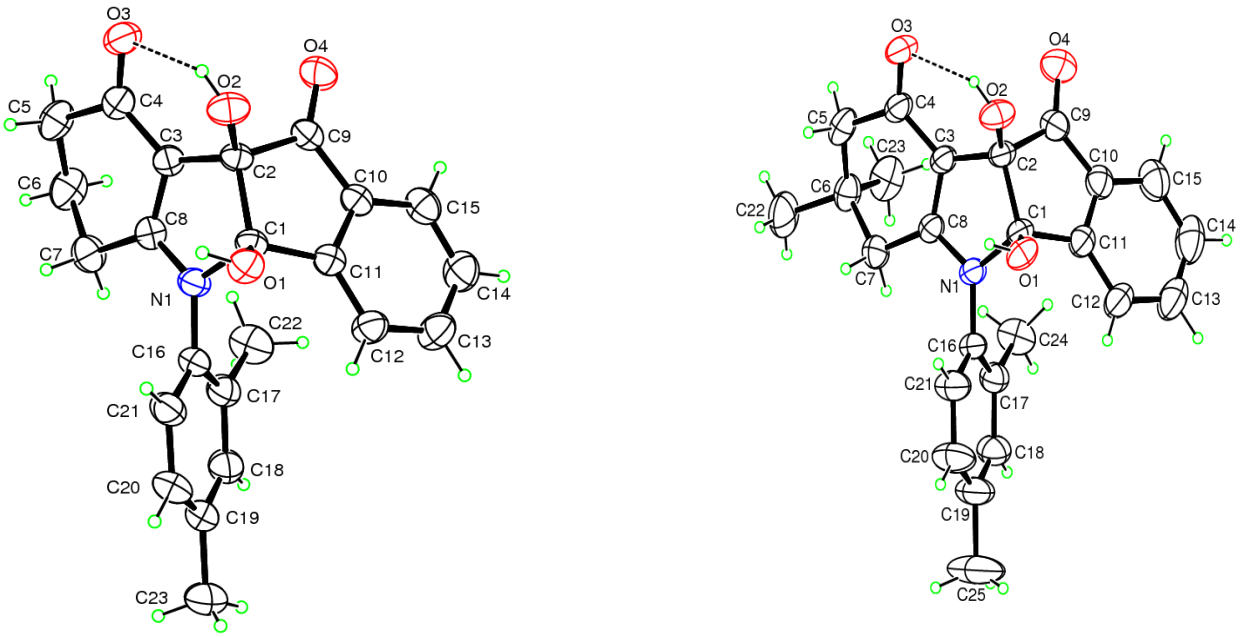


i: cat. p-TsOH, PhMe, Δ; **ii**: $CHCl_3$, rt.

R_1, R_2: H; R_1, R_2: CH_3; R_3, R_4: CH_3, OCH_3, Br, Cl.

6a-q R_1, R_2: H. 7a-q R_1, R_2: CH_3

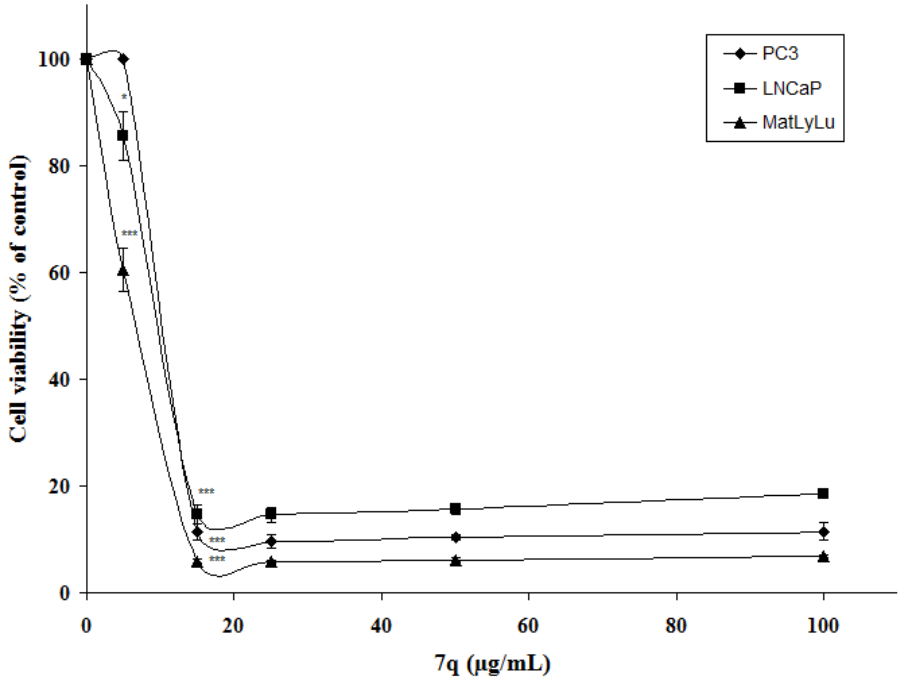
Figure 1.



(a) **(b)**

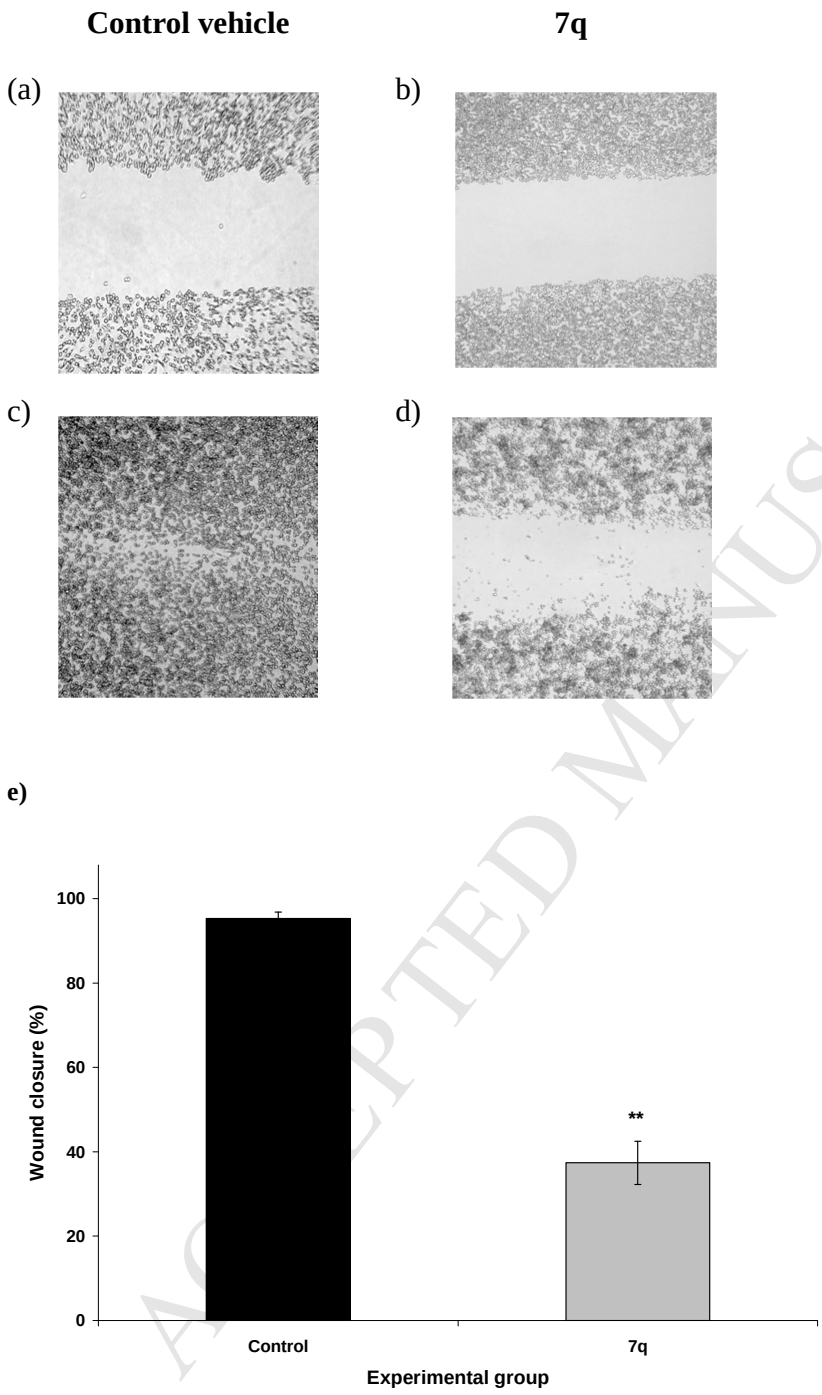
The displacement ellipsoids are drawn at 50% probability. Dashed lines indicate intramolecular hydrogen bonds.

Figure 2.



Results are expressed as the mean ± SEM of three different experiments. *p<0.05, ** p<0.01 and ***p<0.001 compared to the previous concentration at the same cell line.

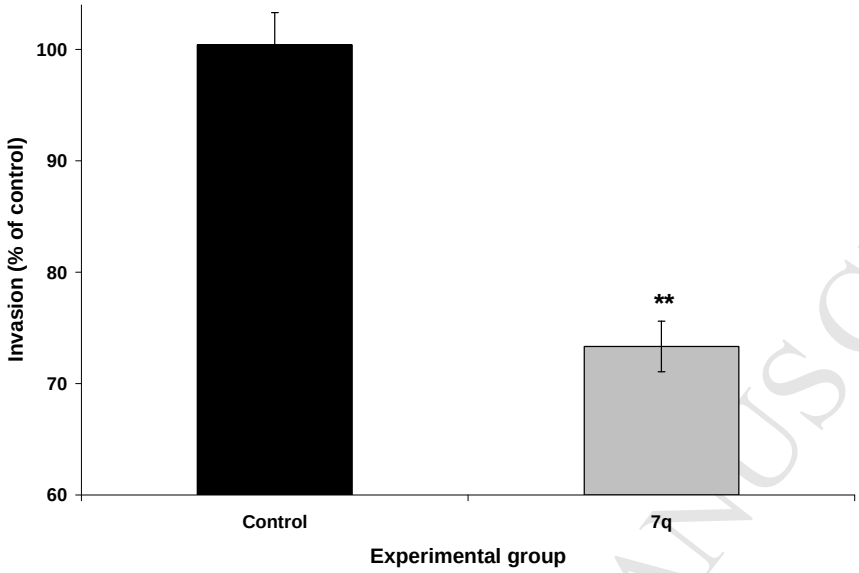
Figure 3.



(a) Representative images of PC-3 cells were captured at the time of wounding (a) and (b), and 24 h following the wound (c) and (d), to illustrate recovery from scrape wound. **(e)** The percentage of wound area closed at 24 hours post-treatment is plotted for cell monolayer with or without **7q**. Data shown in (a), (b), (c) and (d) are from a representative experiment

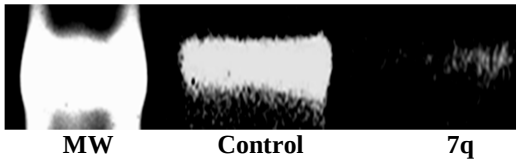
carried out independently three times. Data in (e) represents the mean ± SEM. $^{**}p<0.01$ compared to control vehicle.

Figure 4.

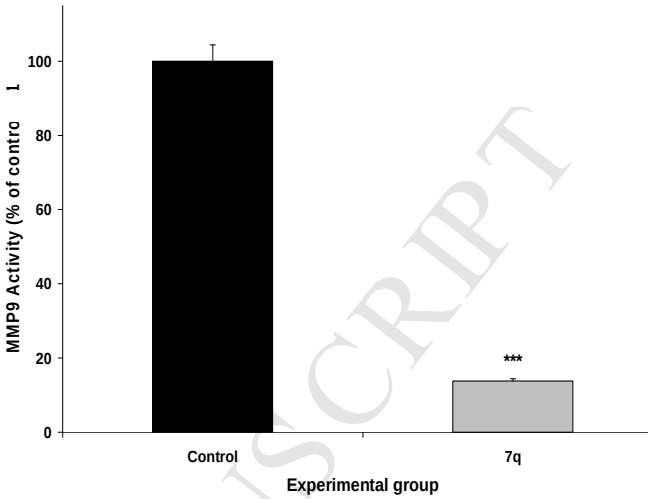


Cells treated with the compound or vehicle were seeded onto a Matrigel coated 0.8 µm porous membrane for 18 h, and the inhibition of invasion relative to the control vehicle treated cells was determined. The results represent the mean ± SEM of three independent experiments. ** $p<0.01$ compared to control vehicle.

Figure 5.



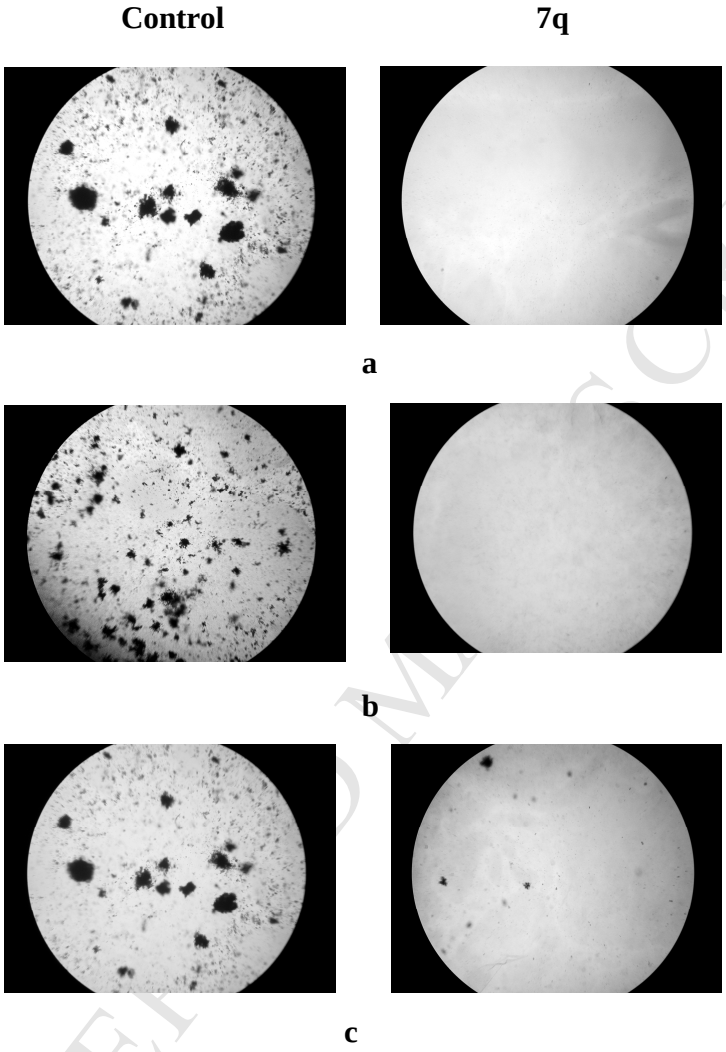
a)



b)

(a) Conditioned medium prepared from subconfluent cultures were collected, resolved in non-reducing gels containing gelatin (1 mg/ml) and processed for zones of gel degradation activity. (b) Results were quantified in relation to control vehicle and are presented as the mean ± SEM of percentage of activity in three different experiments. ***p<0.001 compared to control vehicle.

Figure 6.



(a) PC-3, (b) LNCaP and (c) MatLyLu cells were plated over a semi-solid layer of soft agar and treated with the compound **7q** or vehicle (control) and incubated for 14 days. The results represent standard images of three different experiments.

Figure 7.

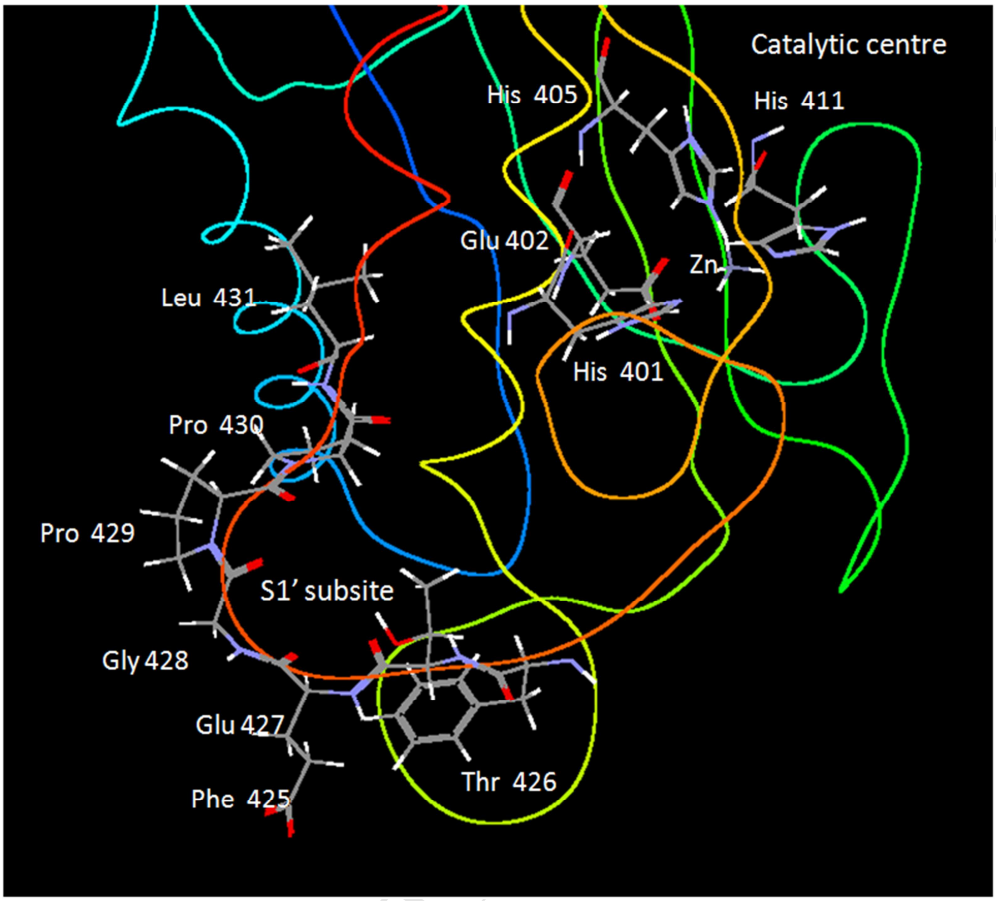


Figure 8.

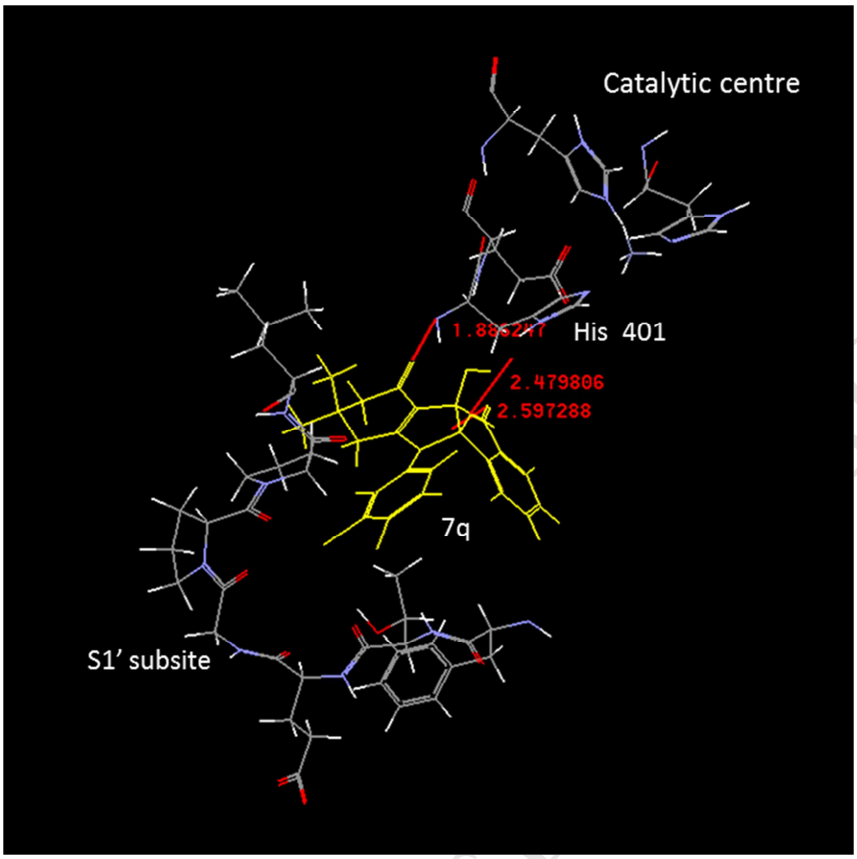
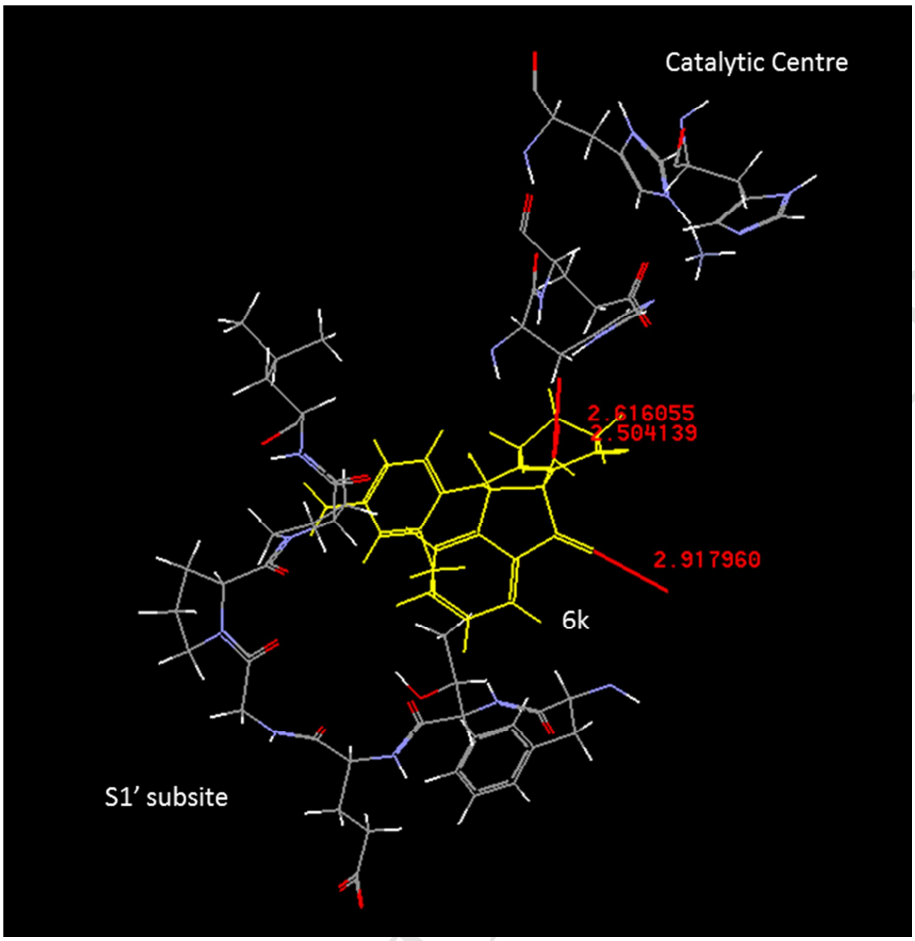
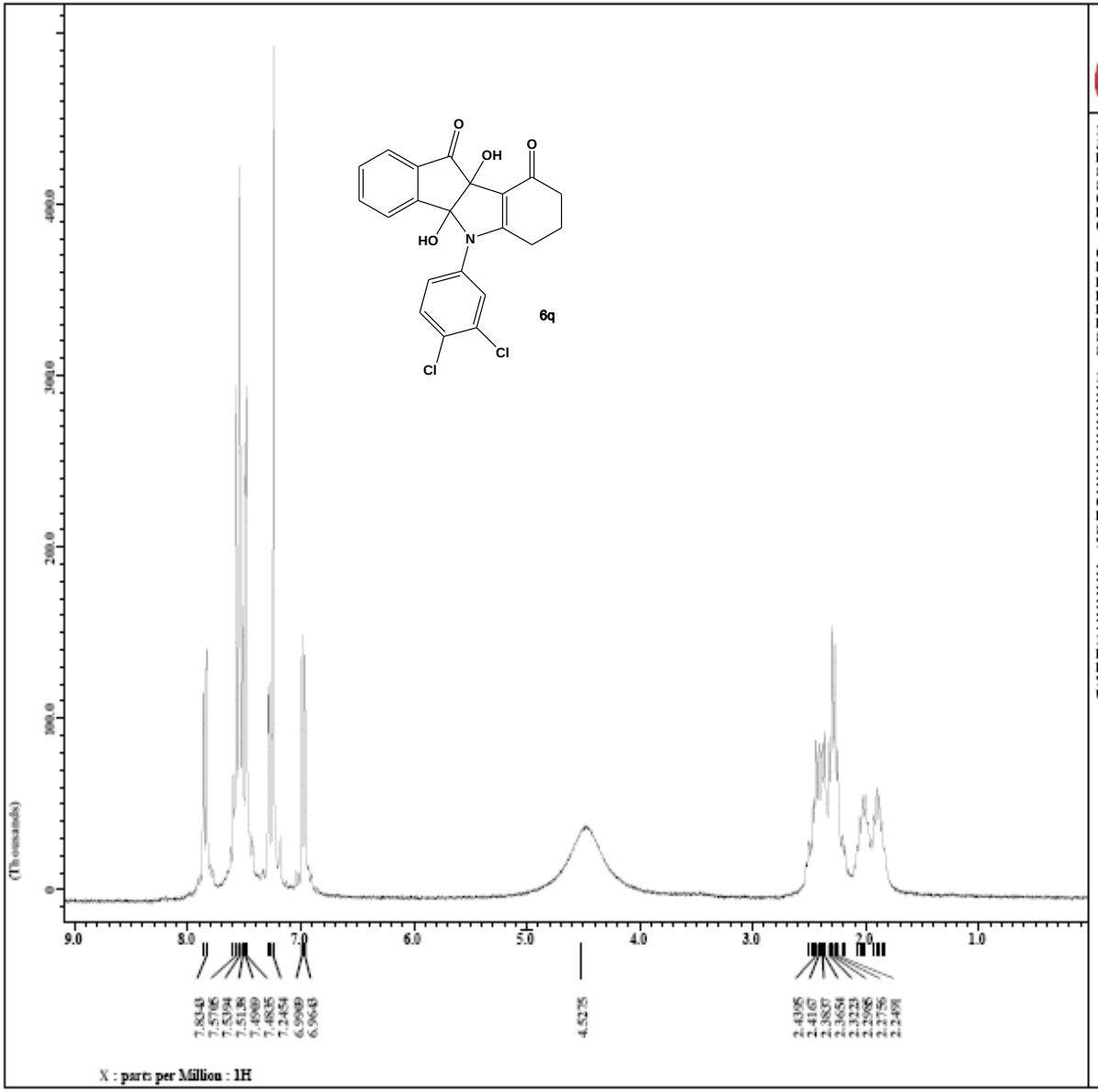


Figure 9.

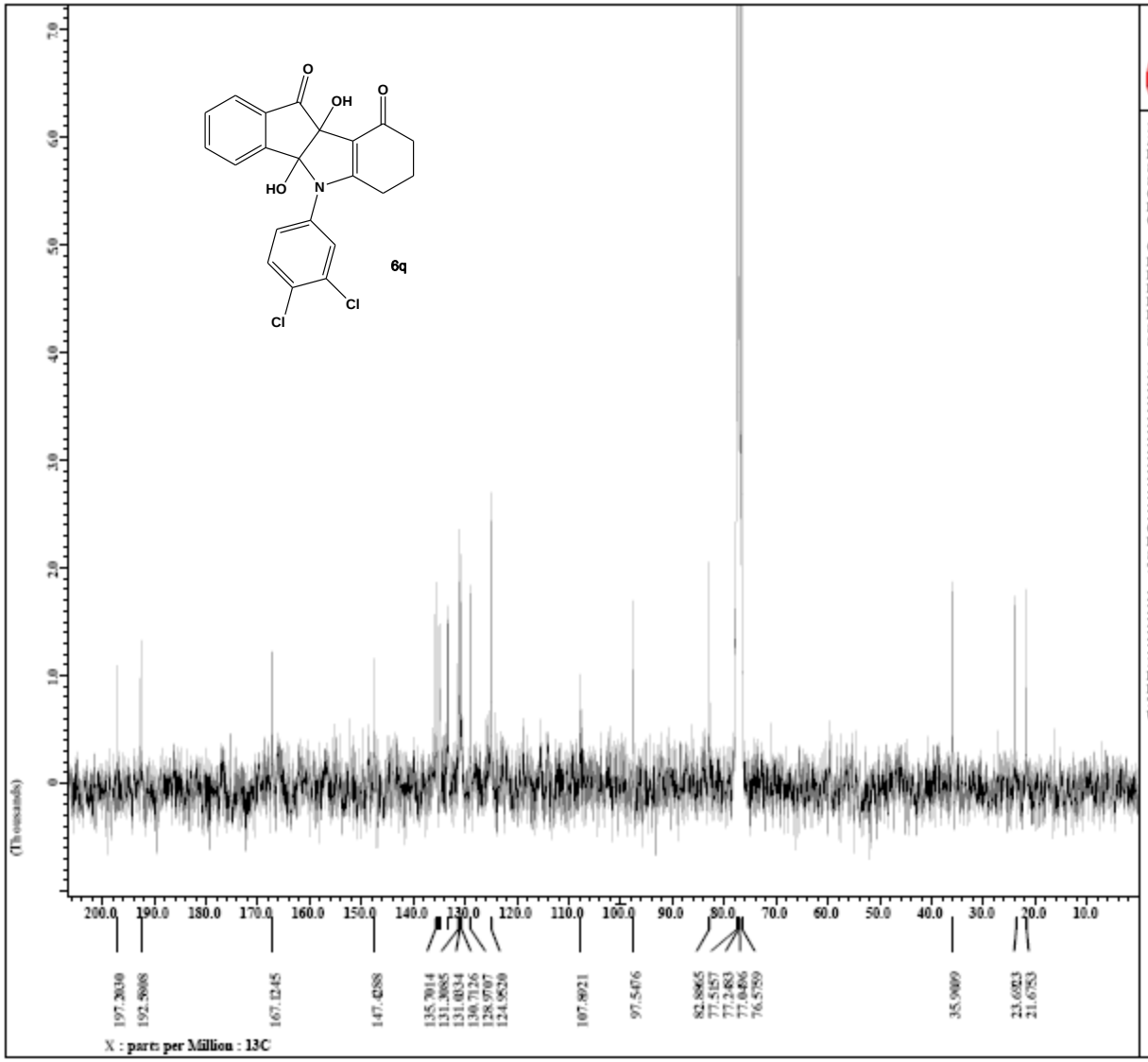


- Compounds were easily synthesized and with highly regiospecificity.
- Crystals consist of equimolar mixtures of the RR and SS diastereomers.
- All tested compounds proved to be moderately active, except one.
- The antitumor activity seems to be related with inhibition of MMP-9.
- Docking studies shows a set of interactions in specific sites.



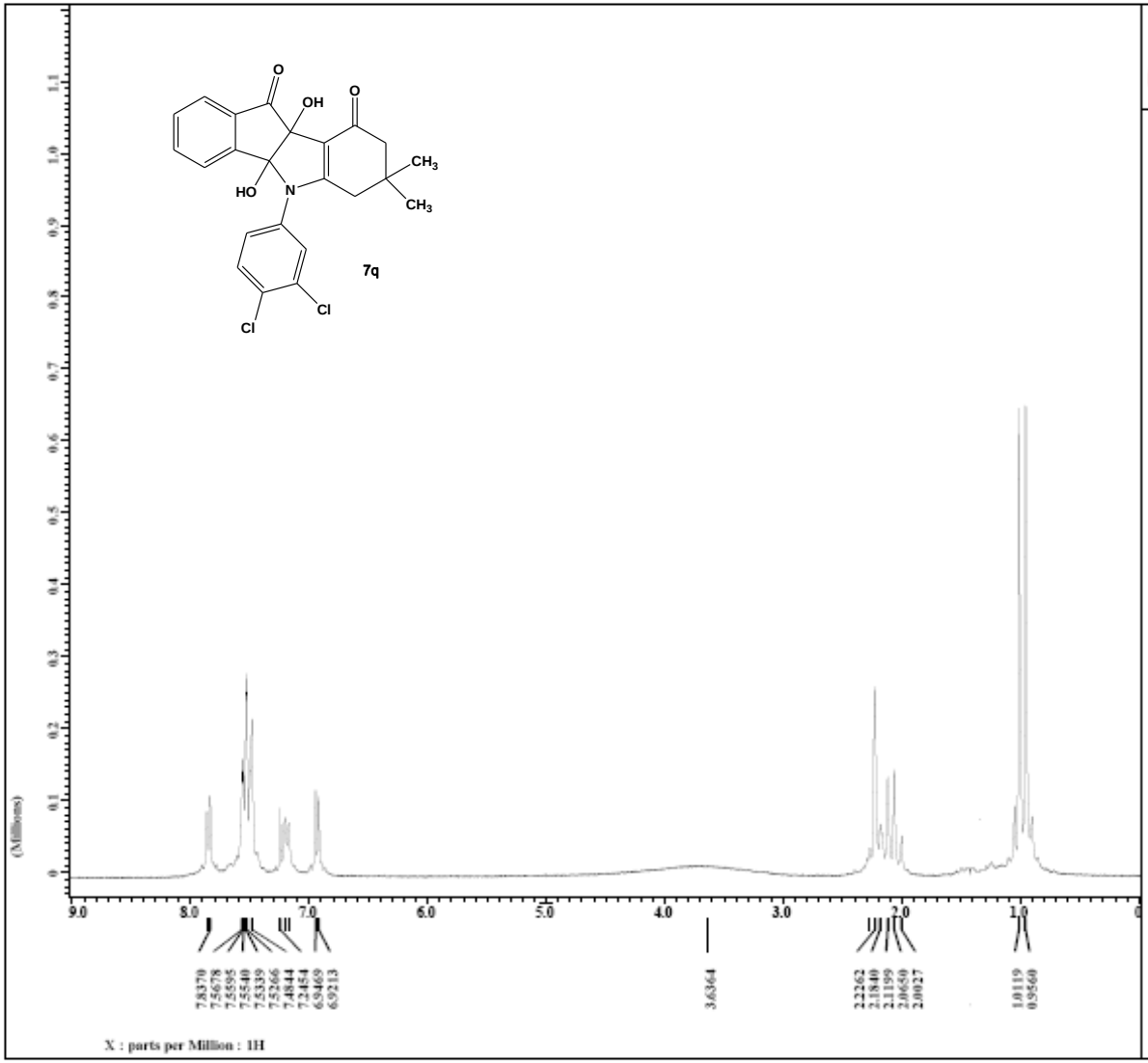
JEOL

```
Filename          = JcharrisZL205-1H-4.jd
Author            = runlab
Experiment        = single_pulse.exp
Sample_id         = JcharrisZL205
Solvent           = CHLOROFORM-D
Creation_time     = 30-DEC-2013 20:16:49
Revision_time     =  2-MAR-2015 17:04:54
Current_time      =  2-MAR-2015 17:05:06

Content           = Single Pulse Experime
Data_format       = 1D COMPLEX
Dim_size          = 16384
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T] (270[MHz]
X_acq_duration    = 4.0419328[s]
X_domain          = 1H
X_freq            = 270.16608844[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.24740639[Hz]
X_sweep           = 4.05350628[kHz]
Clipped           = FALSE
Mod_return        = 1
Scans             = 8
Total_scans       = 8

X_90_width        = 11.7[us]
X_acq_time        = 4.0419328[s]
X_angle           = 45[deg]
X_pulse           = 5.85[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 4[s]
Temp_get          = 20.2[dC]
Unblank_time      = 2[us]
```



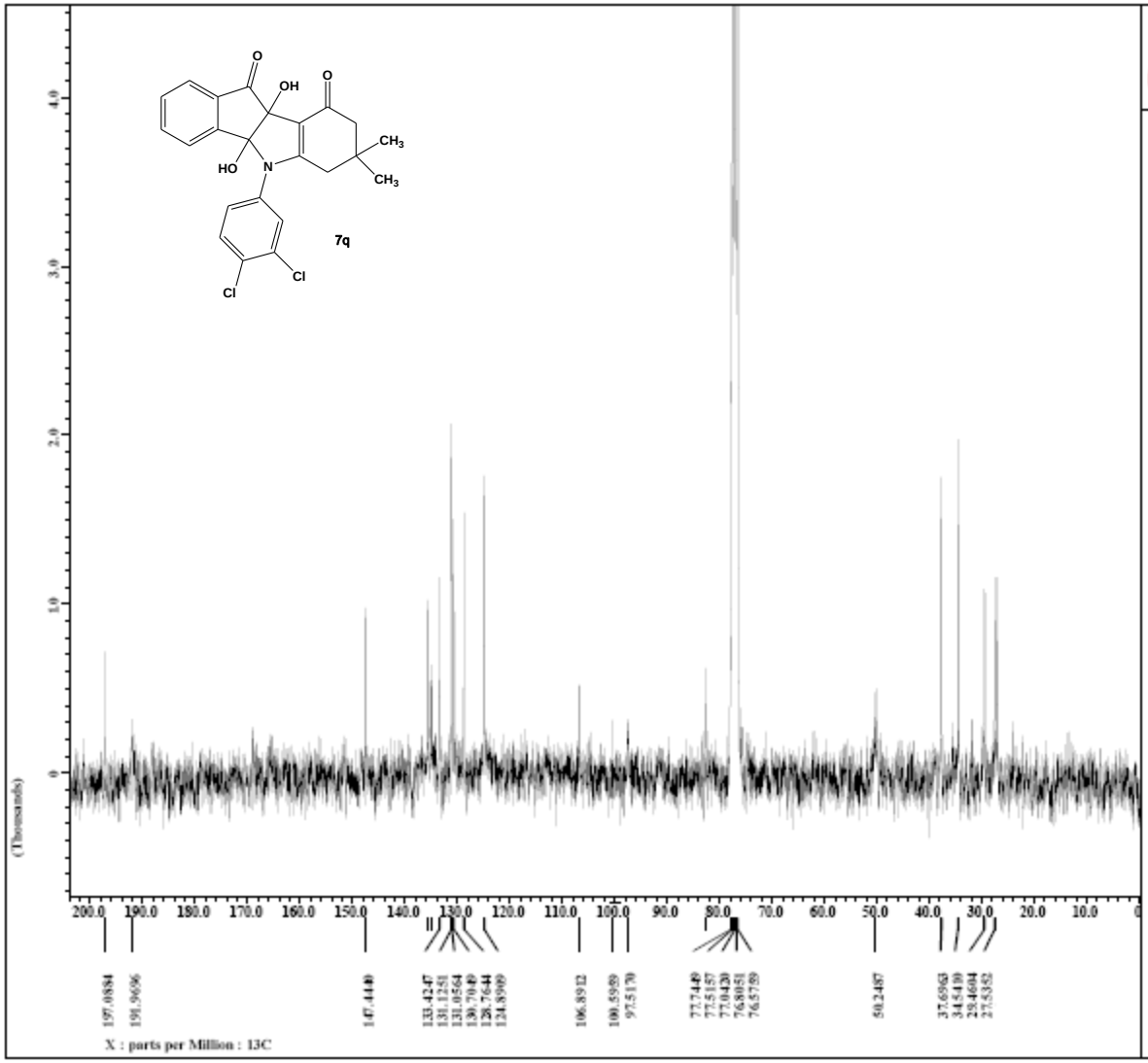
JEOL

```
Filename          = Jcharris@L205-13C-3.j
Author            = rmnlab
Experiment        = single_pulse_dec
Sample_id         = Jcharris@L205
Solvent           = CHLOROFORM-D
Creation_time     = 31-DEC-2013 00:58:23
Revision_time     =  2-MAR-2015 17:06:58
Current_time      =  2-MAR-2015 17:07:06

Content           = Single Pulse with Bro
Data_format       = 1D COMPLEX
Dim_size          = 32768
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T] (270[MHz])
X_acq_duration    = 1.9267584[s]
X_domain          = 13C
X_freq            = 67.93330993[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.51900643[Hz]
X_sweep           = 17.00680272[kHz]
Irr_domain        = 1H
Irr_freq          = 270.16608844[MHz]
Irr_offset        = 5[ppm]
Clipped           = FALSE
Mod_return        = 1
Scans             = 5762
Total_scans       = 5762

X_90_width        = 7.8[us]
X_acq_time        = 1.9267584[s]
X_angle           = 30[deg]
X_pulse           = 2.6[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 1[s]
Temp_get          = 27.1[dC]
Unblank_time      = 2[us]
```



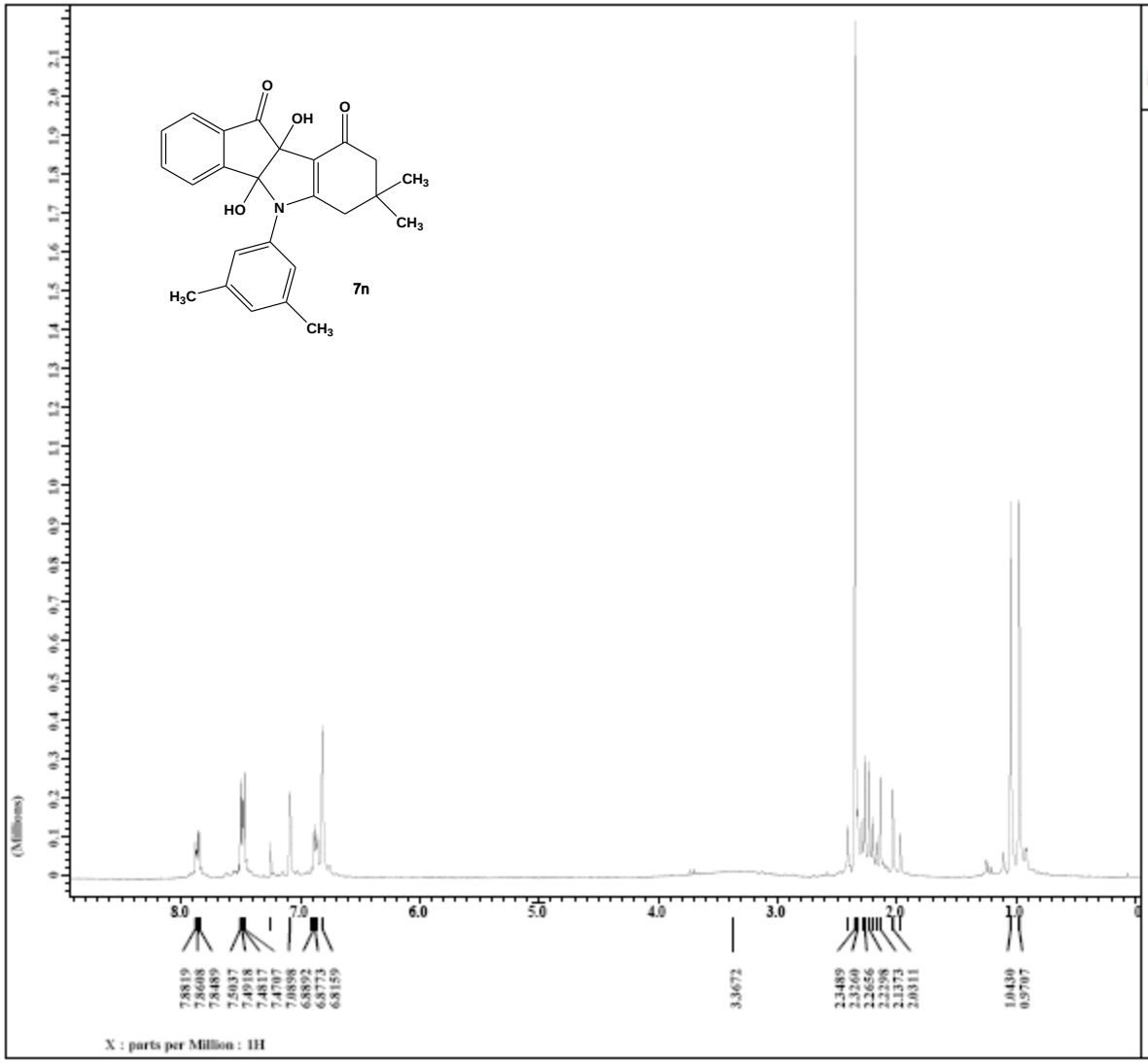
JEOL

```
filename          = jcharrisGL208-1H-3.jd
Author            = rmnlab
experiment        = single_pulse.exp
sample_id         = jcharrisGL208
solvent           = CHLOROFORM-D
creation_time     = 31-DEC-2013 01:05:33
Revision_time     =  2-MAR-2015 17:08:31
current_time      =  2-MAR-2015 17:08:40

content           = single pulse experime
data_format       = 1D COMPLEX
dim_size          = 16384
dim_title         = 1H
dim_units         = [ppm]
dimensions        = X
site              = eclipse+ 400
spectrometer      = DELTA_NMR

field_strength    = 6.345446[T]  (270[MHz]
x_acq_duration    = 4.0419328[s]
x_domain          = 1H
x_freq            = 270.16608844[MHz]
x_offset          = 5[ppm]
x_points          = 16384
x_prescans        = 0
x_resolution      = 0.24740639[Hz]
x_sweep           = 4.05350628[kHz]
clipped           = FALSE
mod_return        = 1
scans             = 8
total_scans       = 8

x_90_width        = 11.7[us]
x_acq_time        = 4.0419328[s]
x_angle           = 45[deg]
x_pulse           = 5.85[us]
initial_wait      = 1[s]
phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 4[s]
temp_get          = 20.6[dc]
unblank_time      = 2[us]
```



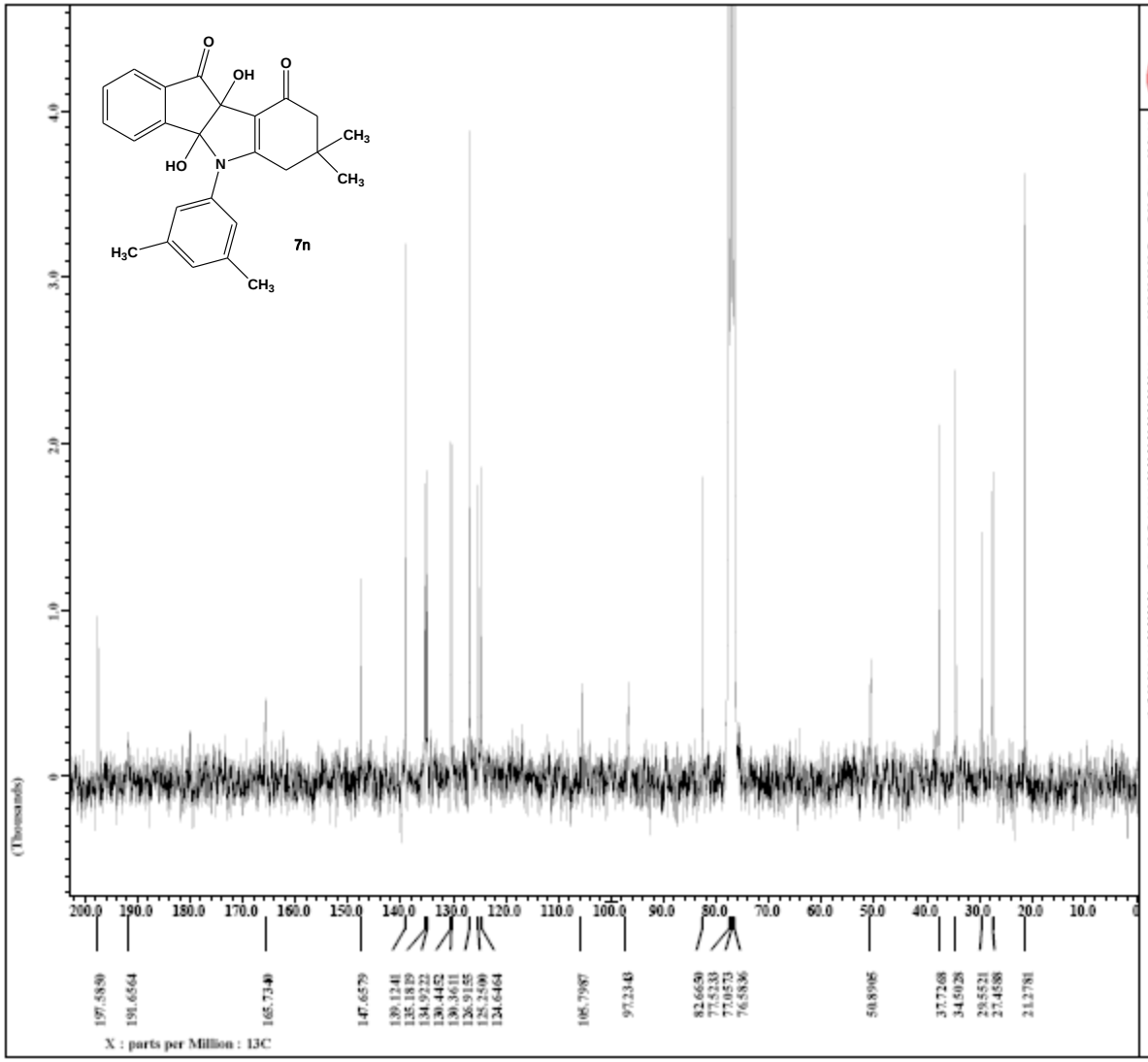
JEOL

```
filename          = jcharrisGL208-13c-3.j
Author            = rmnlab
Experiment        = single_pulse_dec
sample_id         = jcharrisGL208
solvent           = CHLOROFORM-D
creation_time     = 31-DEC-2013 10:51:25
Revision_time     =  2-MAR-2015 17:09:58
current_time      =  2-MAR-2015 17:10:12

content           = single pulse with ero
Data_format       = 1D COMPLEX
Dim_size          = 32768
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 1.9267584[s]
X_domain          = 13C
X_freq            = 67.93330993[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.51900643[Hz]
X_sweep           = 17.00680272[kHz]
Xfr_domain        = 1H
Xfr_freq          = 270.16608844[MHz]
Xfr_offset        = 5[ppm]
clipped           = FALSE
Mod_return        = 1
scans             = 12000
Total_scans       = 12000

X_90_width        = 7.8[us]
X_acq_time        = 1.9267584[s]
X_angle           = 30[deg]
X_pulse           = 2.6[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 1[s]
Temp_get          = 26.2[dc]
Unblank_time      = 2[us]
```



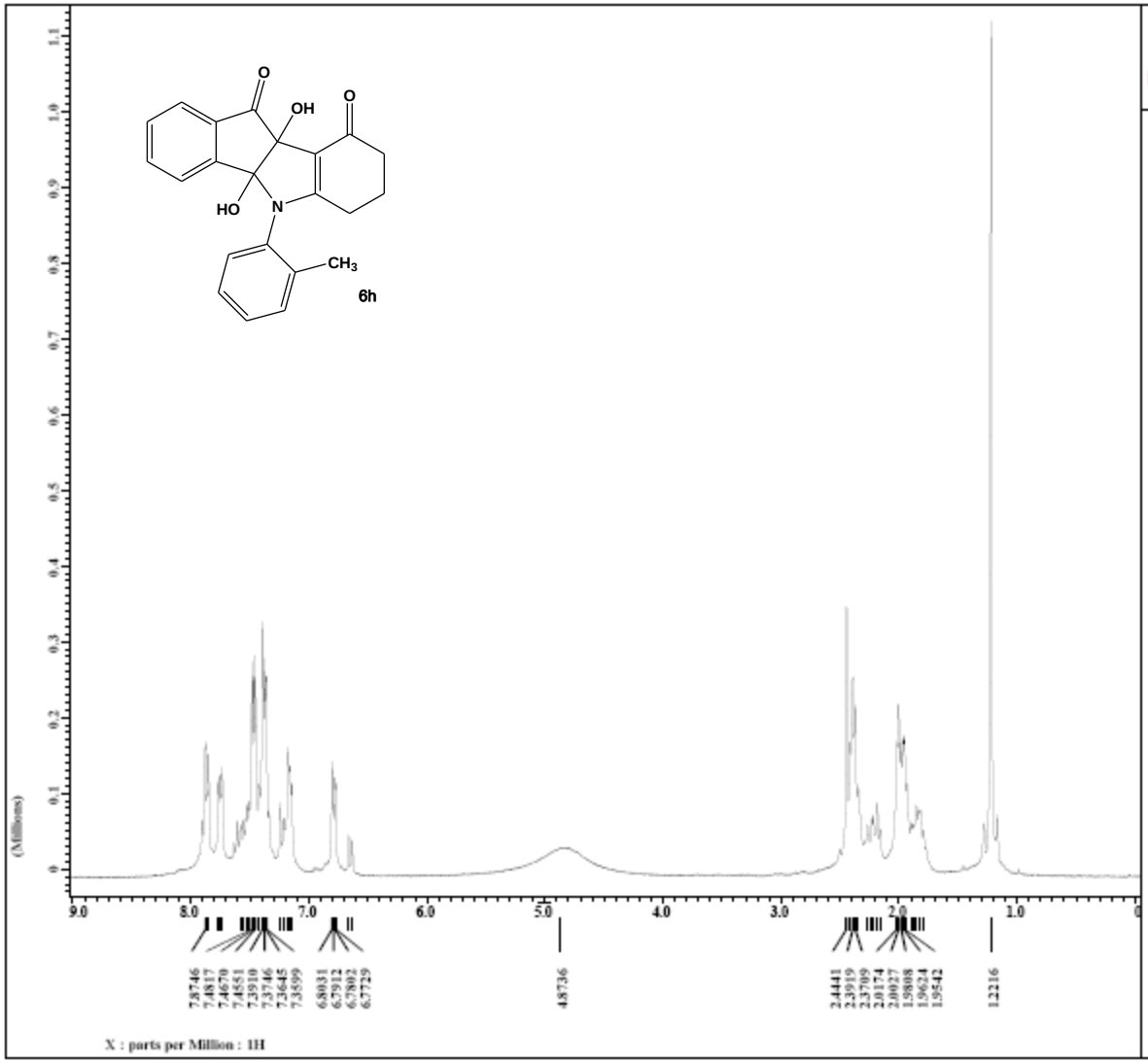
JEOL

```
filename          = jcharrismz5729-1h-3.j
Author            = rmnlab
experiment        = single_pulse.exp
sample_id         = jcharrismz5729
solvent           = CHLOROFORM-D
creation_time     = 24-DEC-2013 20:24:25
Revision_time     =  2-MAR-2015 17:37:41
current_time      =  2-MAR-2015 17:37:57

content           = single pulse experime
Data_format       = 1D COMPLEX
Dim_size          = 16384
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 4.0419328[s]
X_domain          = 1H
X_freq            = 270.16608844[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.24740639[Hz]
X_sweep           = 4.05350628[kHz]
clipped           = FALSE
Mod_return        = 1
scans             = 16
Total_scans       = 16

X_90_width        = 11.7[us]
X_acq_time        = 4.0419328[s]
X_angle           = 45[deg]
X_pulse           = 5.85[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 4[s]
Temp_get          = 25.2[dc]
Unblank_time      = 2[us]
```

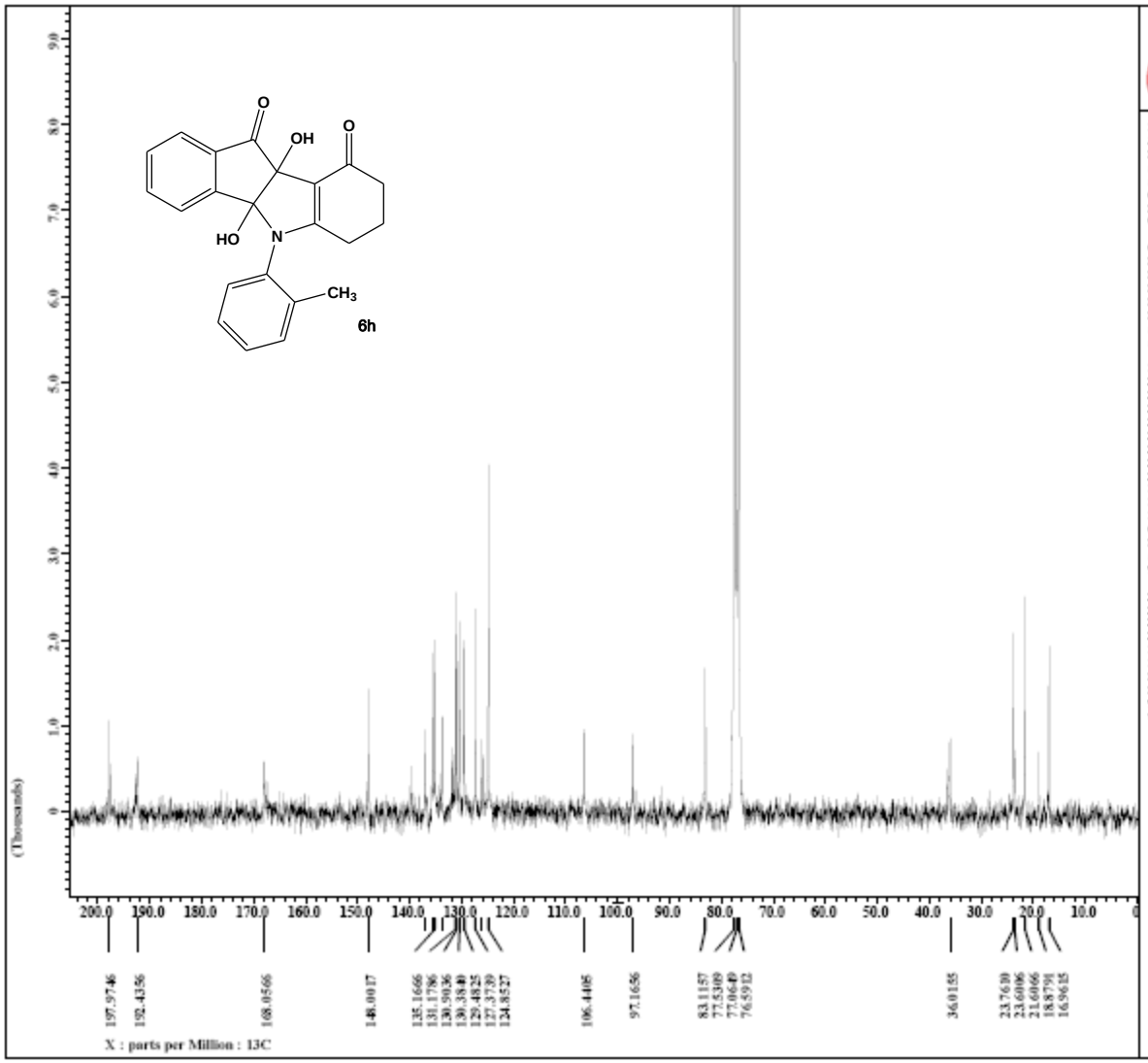


JEOL

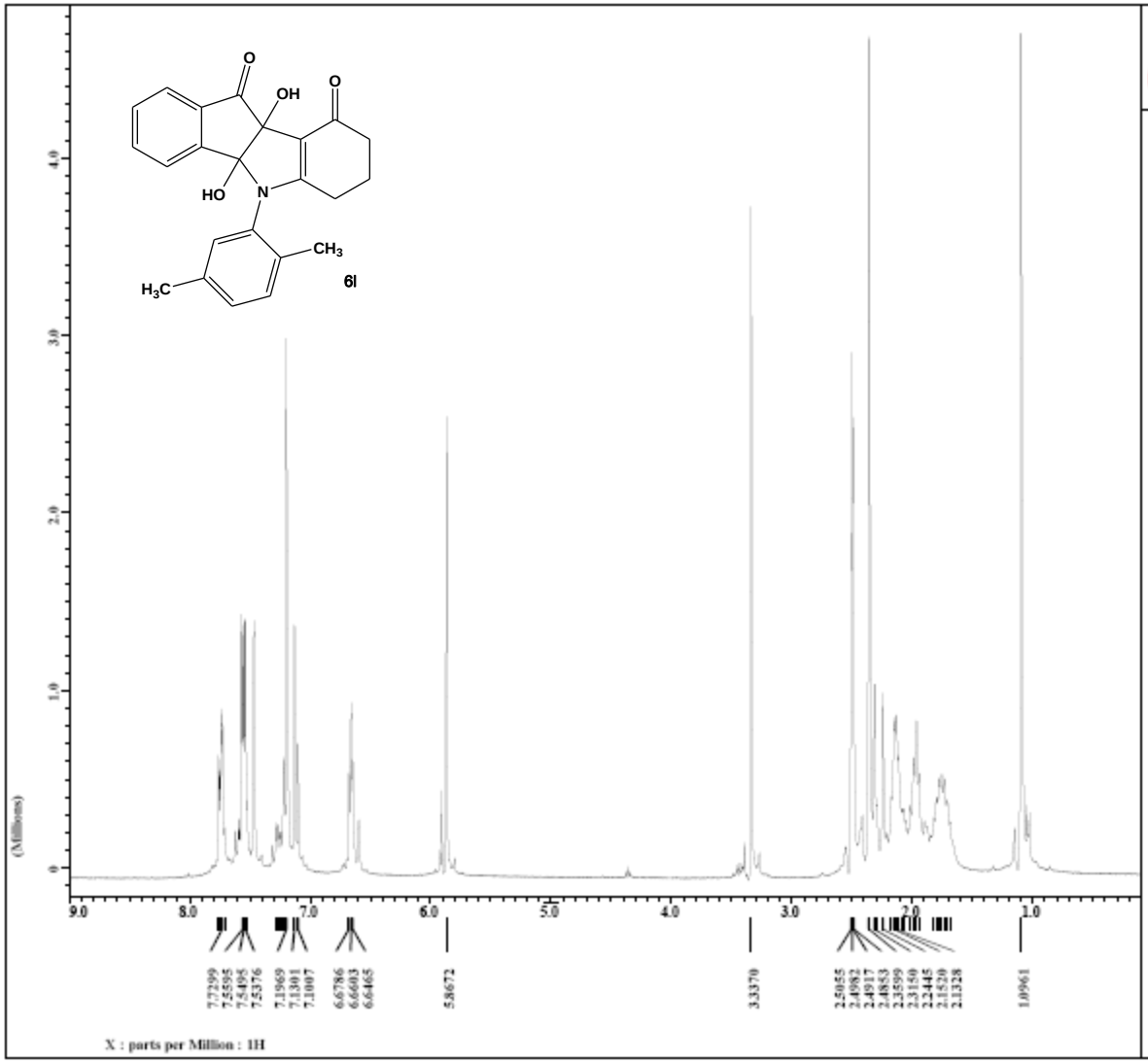
```
filename          = jcharrisnz5729-13c-3.
Author            = rmnlab
experiment        = single_pulse_dec
sample_id         = jcharrisnz5729
solvent           = CHLOROFORM-D
creation_time     = 24-DEC-2013 20:21:56
Revision_time     =  2-MAR-2015 17:39:22
current_time      =  2-MAR-2015 17:39:39

content           = single pulse with nro
Data_format       = 1D COMPLEX
Dim_size          = 32768
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 1.9267584[s]
X_domain          = 13C
X_freq            = 67.93330993[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.51900643[Hz]
X_sweep           = 17.00680272[kHz]
Xfr_domain        = 1H
Xfr_freq          = 270.16608844[MHz]
Xfr_offset        = 5[ppm]
clipped           = FALSE
mod_return        = 1
scans             = 8395
total_scans       = 8395

X_90_width        = 7.8[us]
X_acq_time        = 1.9267584[s]
X_angle           = 30[deg]
X_pulse           = 2.6[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 1[s]
Temp_get          = 27[dC]
Unblank_time      = 2[us]
```



```
filename          = jcharrissm2915-1h-4.j
Author            = rmnlab
experiment        = single_pulse.exp
sample_id         = jcharrissm2915
solvent           = CHLOROFORM-D
creation_time     = 16-DEC-2013 13:38:03
Revision_time     =  2-MAR-2015 17:50:33
current_time      =  2-MAR-2015 17:50:45

content           = single pulse experime
Data_format       = 1D COMPLEX
Dim_size          = 16384
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 4.0419328[s]
X_domain          = 1H
X_freq            = 270.16608844[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.24740639[Hz]
X_sweep           = 4.05350628[kHz]
clipped           = FALSE
Mod_return        = 1
scans             = 16
Total_scans       = 16

X_90_width        = 11.7[us]
X_acq_time        = 4.0419328[s]
X_angle           = 45[deg]
X_pulse           = 5.85[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 4[s]
Temp_get          = 17.3[dc]
Unblank_time      = 2[us]
```




```
filename          : jcharrissm2915-13c-3.
Author            : rmnlab
Experiment        : single_pulse_dec
sample_id         : jcharrissm2915
solvent           : CHLOROFORM-D
creation_time     : 16-DEC-2013 20:21:17
Revision_time     :  2-MAR-2015 17:51:39
current_time      :  2-MAR-2015 17:51:52

content           : single pulse with ero
Data_format       : 1D COMPLEX
Dim_size          : 32768
Dim_title         : 13C
Dim_units         : [ppm]
Dimensions        : X
Site              : Eclipse+ 400
Spectrometer      : DELTA_NMR

Field_strength    : 6.345446[T]  (270[MHz]
X_acq_duration    : 1.9267584[s]
X_domain          : 13C
X_freq            : 67.93330993[MHz]
X_offset          : 100[ppm]
X_points          : 32768
X_prescans        : 4
X_resolution      : 0.51900643[Hz]
X_sweep           : 17.00680272[kHz]
Irr_domain        : 1H
Irr_freq          : 270.16608844[MHz]
Irr_offset        : 5[ppm]
Clipped           : FALSE
Mod_return        : 1
Scans             : 8242
Total_scans       : 8242

X_90_width        : 7.8[us]
X_acq_time        : 1.9267584[s]
X_angle           : 30[deg]
X_pulse           : 2.6[us]
Initial_wait      : 1[s]
Phase_preset      : 3[us]
Recvr_gain        : 15
Relaxation_delay  : 1[s]
Temp_get          : 25.7[dc]
Unblank_time      : 2[us]
```

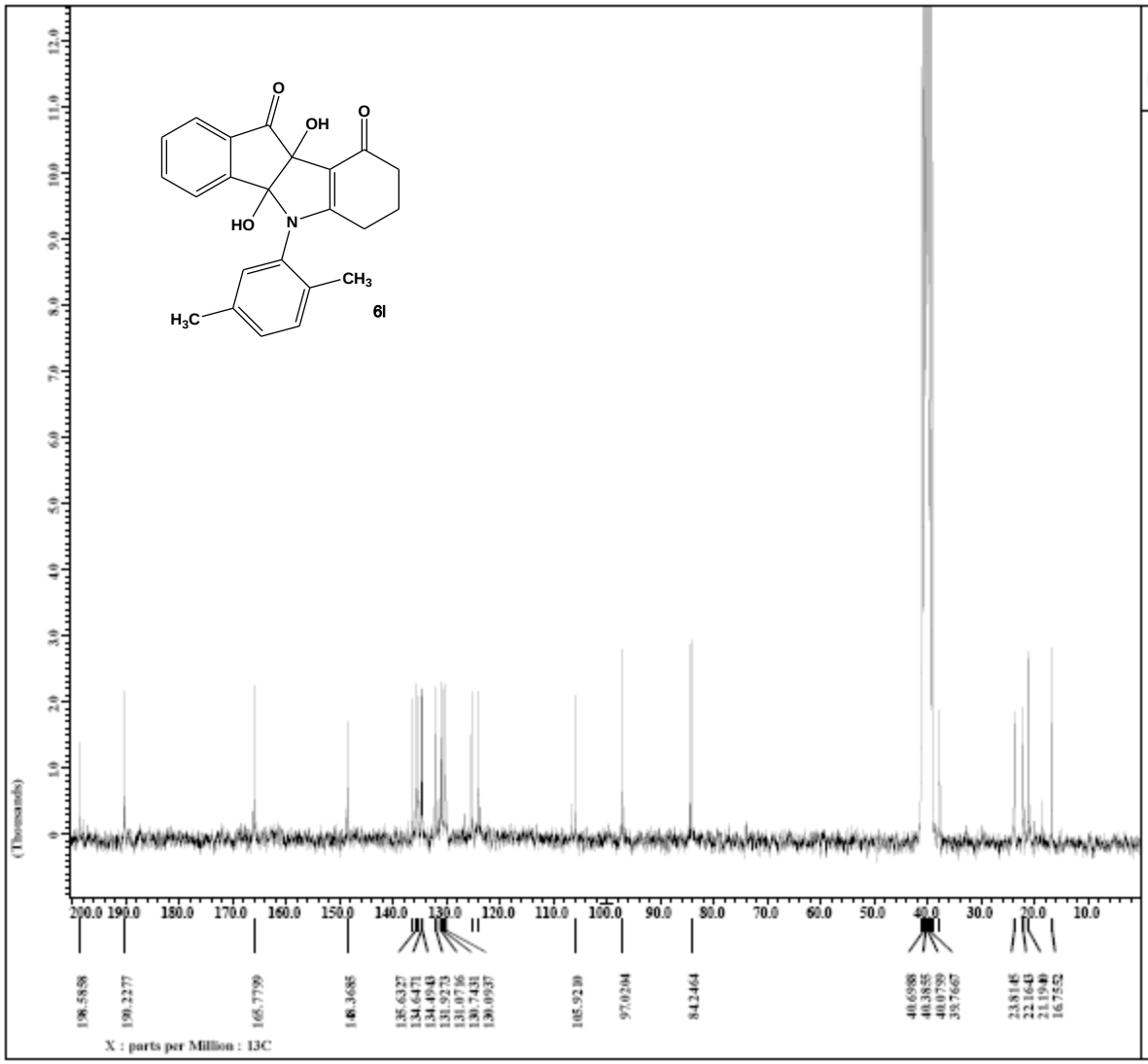


filename = jcharrison-2111-1h-4.
Author = alirica
experiment = single_pulse.exp
sample_id = jcharrison-2111
solvent = DMSO-D6
creation_time = 25-FEB-2009 15:38:25
Revision_time = 2-MAR-2015 17:59:08
current_time = 2-MAR-2015 17:59:23

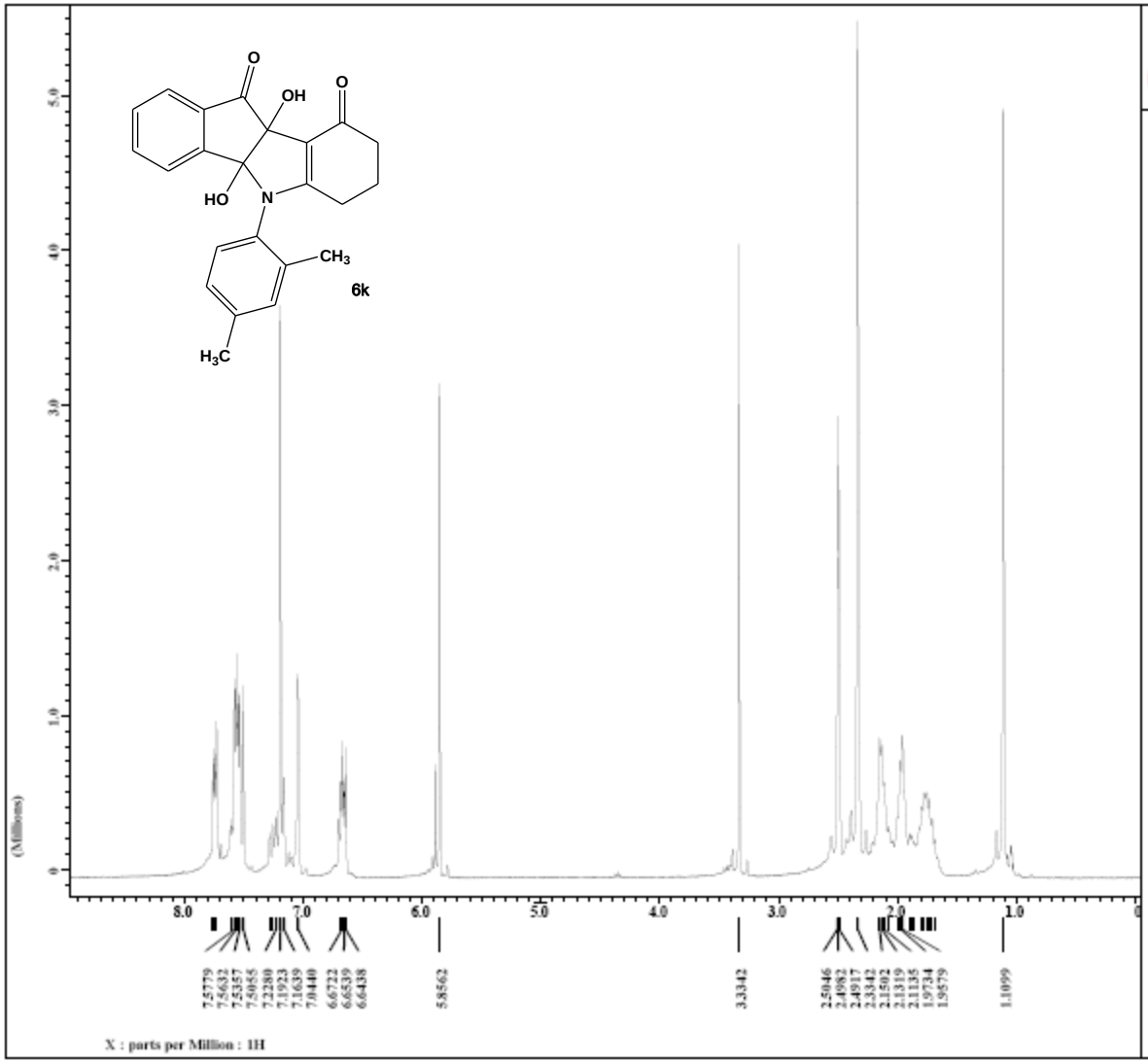
content = single pulse experime
Data_format = 1D COMPLEX
Dim_size = 16384
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Site = Eclipse+ 400
Spectrometer = DELTA_NMR

Field_strength = 6.345446[T] (270[MHz]
X_acq_duration = 4.0419328[s]
X_domain = 1H
X_freq = 270.16608844[MHz]
X_offset = 5[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.24740639[Hz]
X_sweep = 4.05350628[kHz]
clipped = FALSE
Mod_return = 1
scans = 16
Total_scans = 16

X_90_width = 11.7[us]
X_acq_time = 4.0419328[s]
X_angle = 45[deg]
X_pulse = 5.85[us]
Initial_wait = 1[s]
Phase_preset = 3[us]
Recvr_gain = 19
Relaxation_delay = 4[s]
Temp_get = 20.3[dc]
Unblank_time = 2[us]

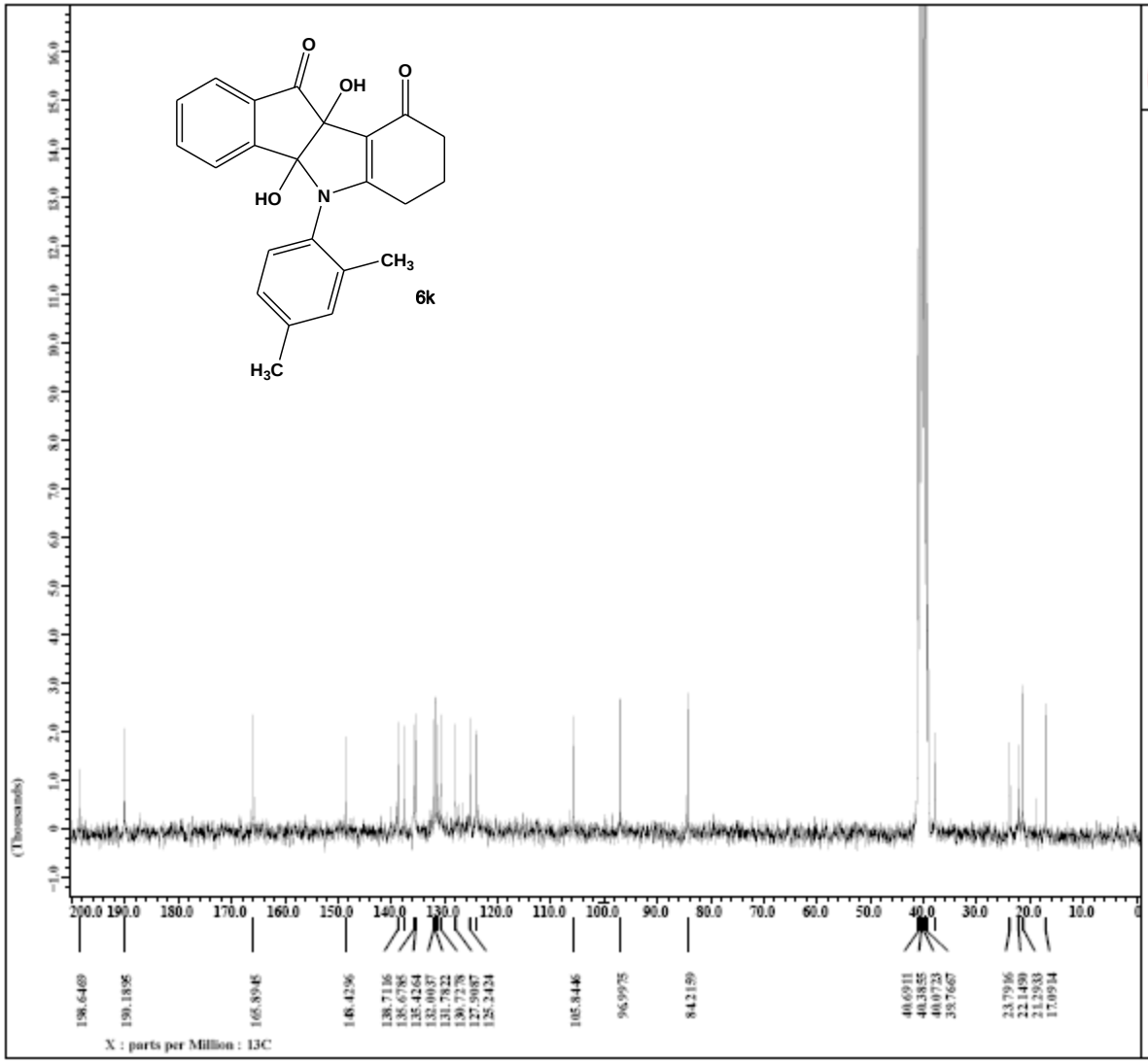


filename	: jcharriss-2111-13c-2
Author	: alirica
Experiment	: single_pulse_dec
sample_id	: jcharriss-2111
solvent	: DMSO-D6
creation_time	: 26-FEB-2009 02:08:29
Revision_time	: 2-MAR-2015 18:00:06
current_time	: 2-MAR-2015 18:00:26
content	: single pulse with nro
Data_format	: 1D COMPLEX
Dim_size	: 32768
Dim_title	: 13C
Dim_units	: [ppm]
Dimensions	: X
Site	: eclipse+ 400
Spectrometer	: DELTA_NMR
field_strength	: 6.345446[T] (270[MHz]
x_acq_duration	: 1.9267584[s]
x_domain	: 13C
x_freq	: 67.93330993[MHz]
x_offset	: 100[ppm]
x_points	: 32768
x_prescans	: 4
x_resolution	: 0.51900643[Hz]
x_sweep	: 17.00680272[kHz]
xfr_domain	: 1H
xfr_freq	: 270.16608844[MHz]
xfr_offset	: 5[ppm]
clipped	: FALSE
mod_return	: 1
scans	: 6000
total_scans	: 6000
x_90_width	: 7.8[us]
x_acq_time	: 1.9267584[s]
x_angle	: 30[deg]
x_pulse	: 2.6[us]
xnitial_wait	: 1[s]
phase_preset	: 3[us]
Recvr_gain	: 15
Relaxation_delay	: 1[s]
Temp_get	: 21.2[dc]
Unblank_time	: 2[us]



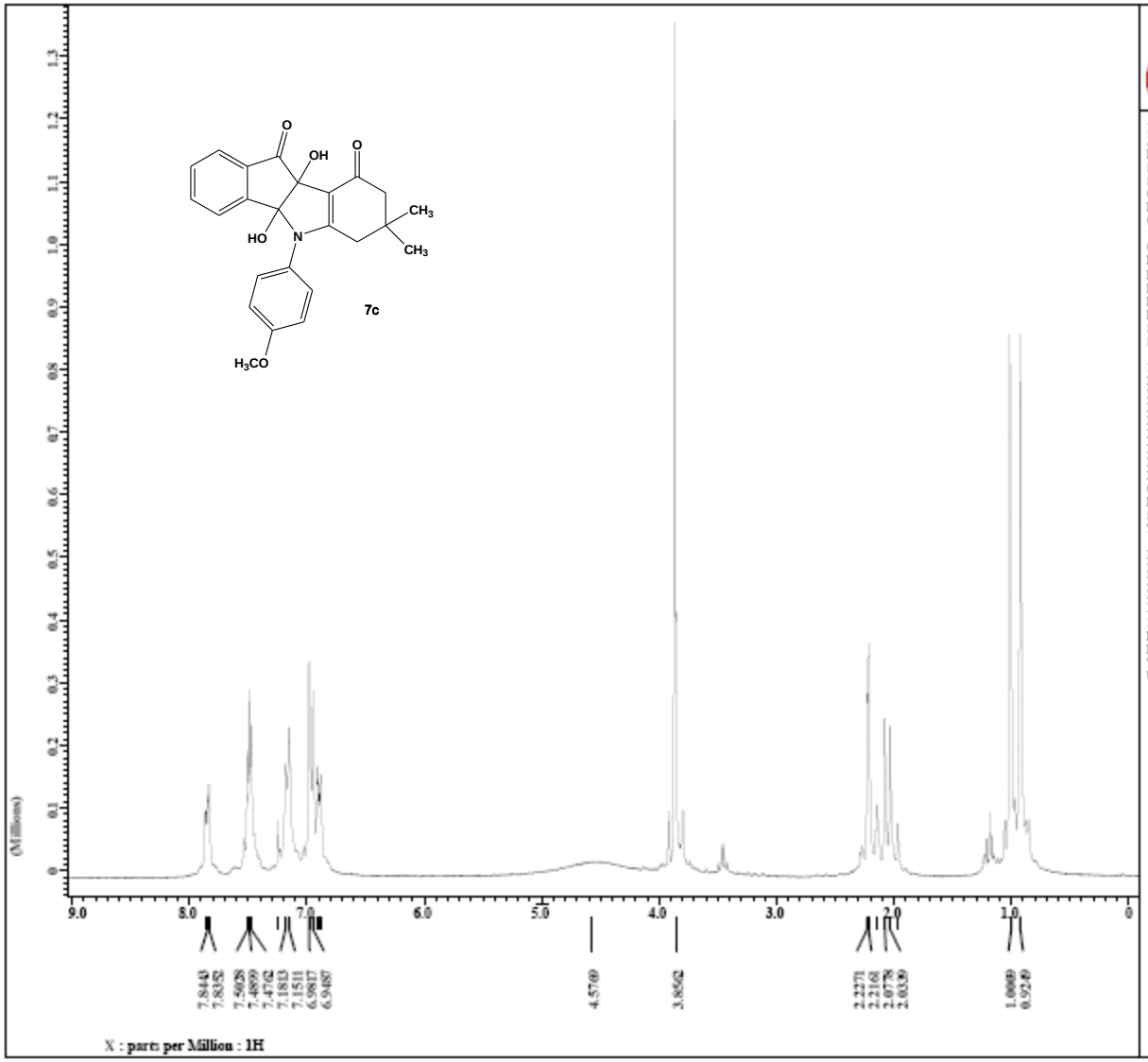
JEOL

```
filename          = jcharrissm-2513-1m-3.
Author            = alirica
experiment        = single_pulse.exp
sample_id         = jcharrissm-2513
solvent           = DMSO-D6
creation_time     = 25-FEB-2009 15:44:57
Revision_time     =  2-MAR-2015 18:02:08
current_time      =  2-MAR-2015 18:02:15

content           = single pulse experime
Data_format       = 1D COMPLEX
Dim_size          = 16384
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 4.0419328[s]
X_domain          = 1H
X_freq            = 270.16608844[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.24740639[Hz]
X_sweep           = 4.05350628[kHz]
clipped           = FALSE
Mod_return        = 1
scans             = 16
Total_scans       = 16

X_90_width        = 11.7[us]
X_acq_time        = 4.0419328[s]
X_angle           = 45[deg]
X_pulse           = 5.85[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 19
Relaxation_delay  = 4[s]
Temp_get          = 20.7[dc]
Unblank_time      = 2[us]
```



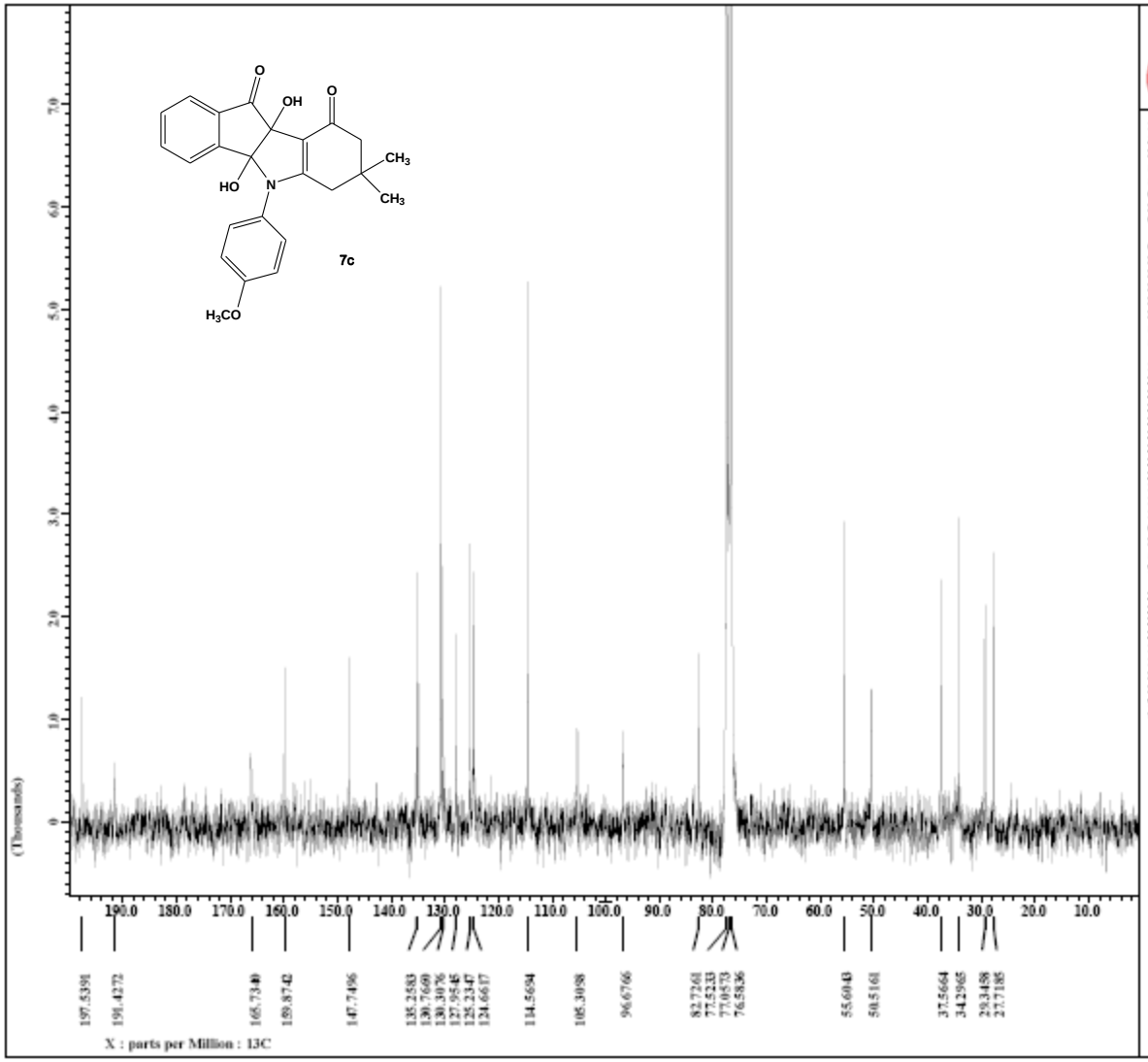
JEOL

```
filename          = jcharrison-2513-13c-2
Author            = alirica
Experiment        = single_pulse_dec
sample_id         = jcharrison-2513
solvent           = DMSO-D6
creation_time     = 25-FEB-2009 18:12:18
Revision_time     =  2-MAR-2015 18:02:48
current_time      =  2-MAR-2015 18:03:02

content           = single pulse with nro
Data_format       = 1D COMPLEX
Dim_size          = 32768
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 1.9267584[s]
X_domain          = 13C
X_freq            = 67.93330993[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.51900643[Hz]
X_sweep           = 17.00680272[kHz]
Xfr_domain        = 1H
Xfr_freq          = 270.16608844[MHz]
Xfr_offset        = 5[ppm]
clipped           = FALSE
mod_return        = 1
scans             = 3000
total_scans       = 3000

X_90_width        = 7.8[us]
X_acq_time        = 1.9267584[s]
X_angle           = 30[deg]
X_pulse           = 2.6[us]
Initial_wait      = 1[s]
phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 1[s]
Temp_get          = 21.3[dc]
Unblank_time      = 2[us]
```



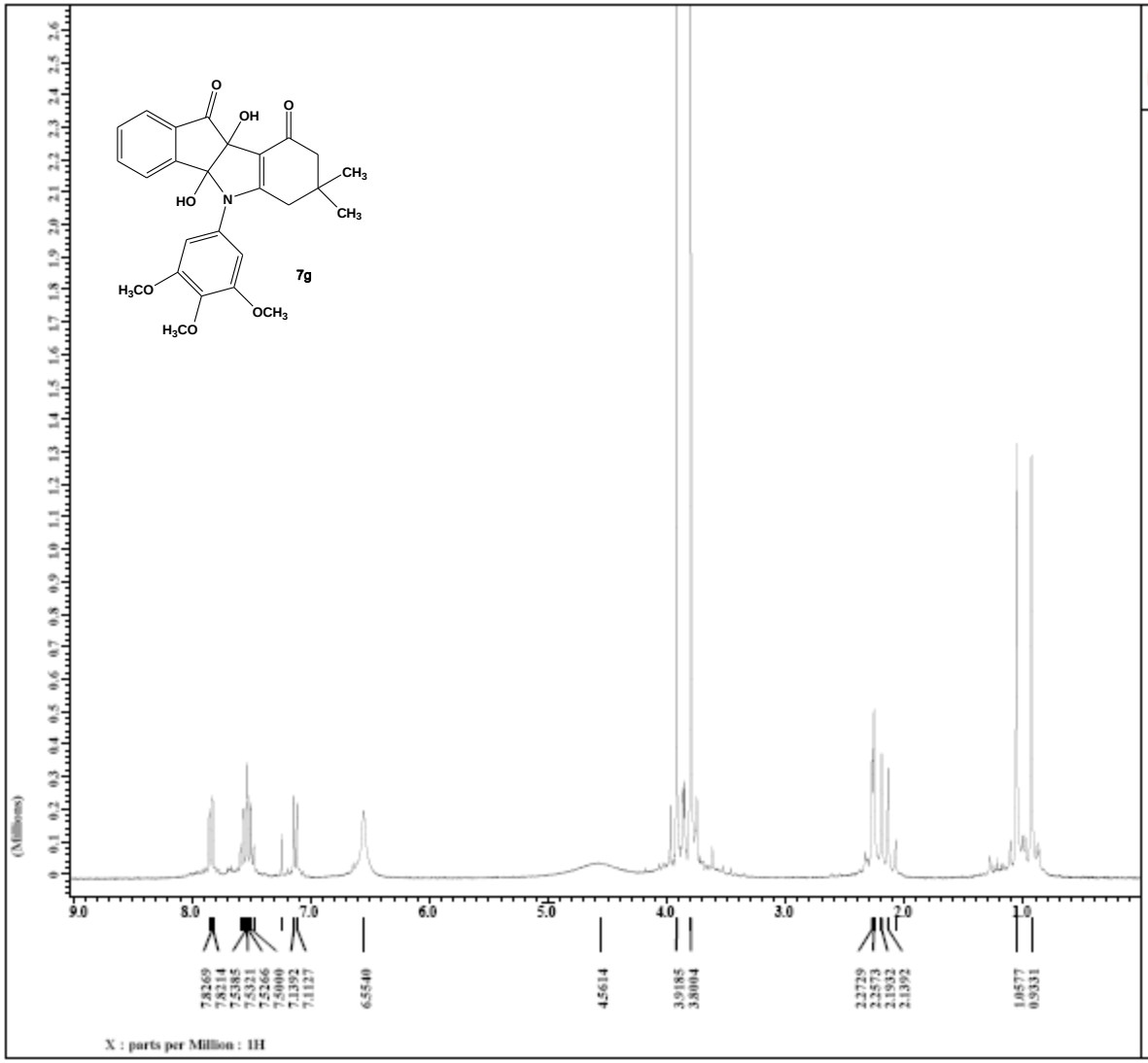
JEOL

```
Filename          = JcharrisGL202-1H-3.jd
Author            = rmnlab
Experiment        = single_pulse.exp
Sample_id         = JcharrisGL202
Solvent           = CHLOROFORM-D
Creation_time     = 30-DEC-2013 13:38:48
Revision_time     =  2-MAR-2015 17:03:24
Current_time      =  2-MAR-2015 17:03:35

Content           = Single Pulse Experime
Data_format       = 1D COMPLEX
Dim_size          = 16384
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T] (270[MHz]
X_acq_duration    = 4.0419328[s]
X_domain          = 1H
X_freq            = 270.16608844[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.24740639[Hz]
X_sweep           = 4.05350628[kHz]
Clipped           = FALSE
Mod_return        = 1
Scans             = 8
Total_scans       = 8

X_90_width        = 11.7[us]
X_acq_time        = 4.0419328[s]
X_angle           = 45[deg]
X_pulse           = 5.85[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 4[s]
Temp_get          = 17.6[dC]
Unblank_time      = 2[us]
```

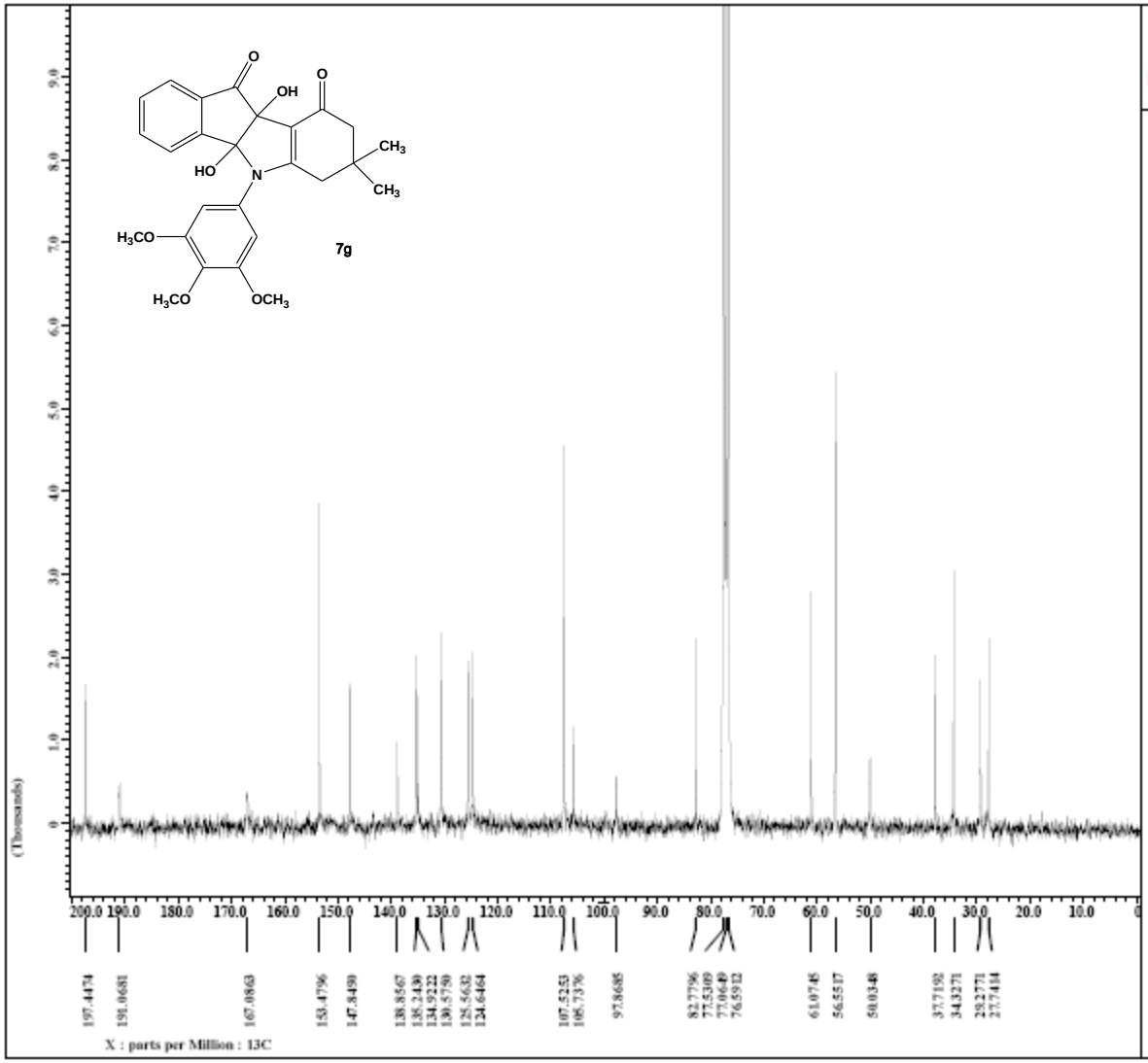


JEOL

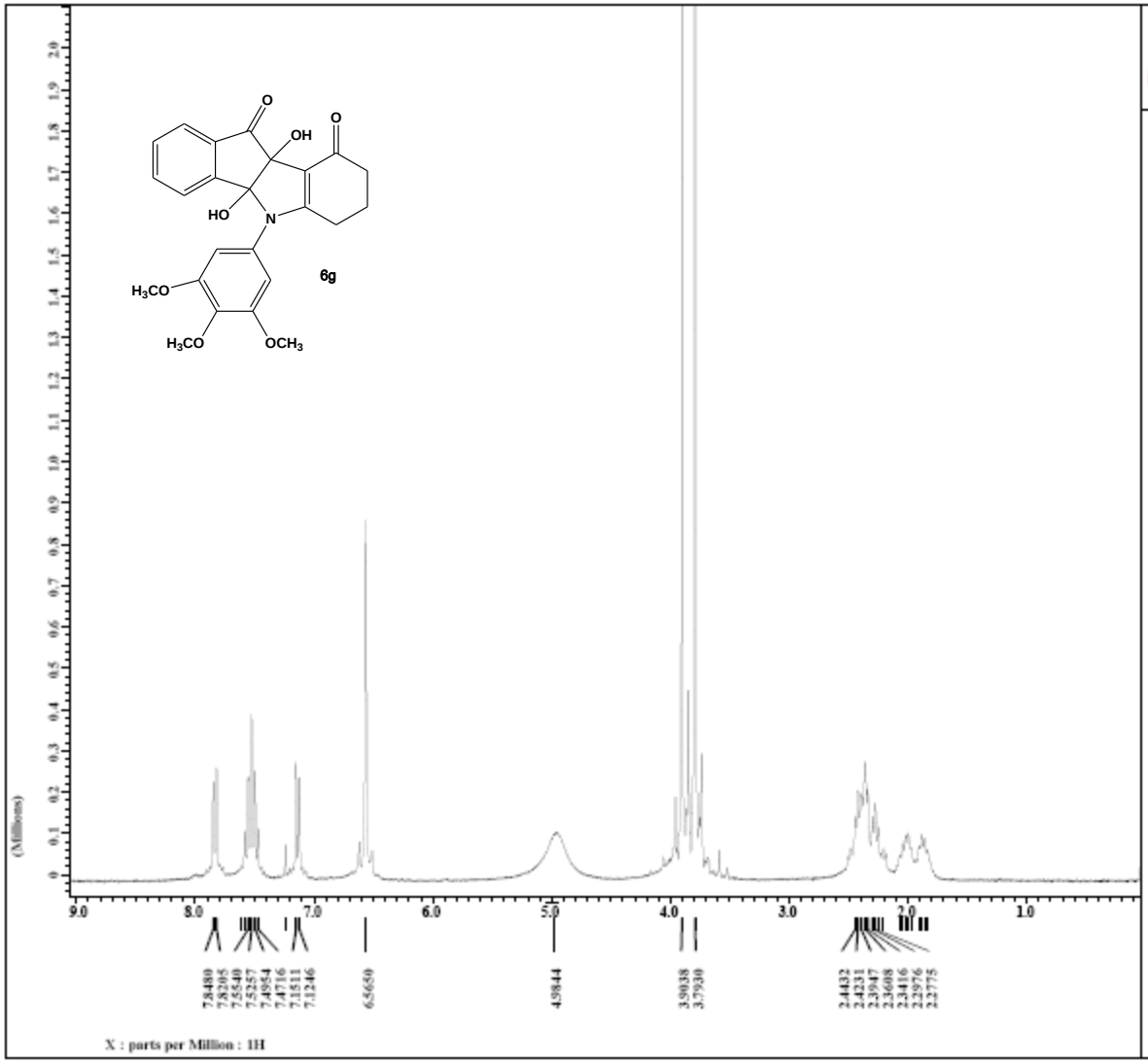
filename	: jcharrisGL202-13c-3.j
Author	: rmnlab
Experiment	: single_pulse_dec
sample_id	: jcharrisGL202
solvent	: CHLOROFORM-D
creation_time	: 30-DEC-2013 20:08:57
Revision_time	: 2-MAR-2015 17:01:22
current_time	: 2-MAR-2015 17:01:38
content	: single pulse with nro
Data_format	: 1D COMPLEX
Dim_size	: 32768
Dim_title	: 13c
Dim_units	: [ppm]
Dimensions	: X
Site	: Eclipse+ 400
Spectrometer	: DELTA_NMR
Field_strength	: 6.345446[T] (270[MHz]
x_acq_duration	: 1.9267584[s]
x_domain	: 13c
x_freq	: 67.93330993[MHz]
x_offset	: 100[ppm]
x_points	: 32768
x_prescans	: 4
x_resolution	: 0.51900643[Hz]
x_sweep	: 17.00680272[kHz]
xfr_domain	: 1H
xfr_freq	: 270.16608844[MHz]
xfr_offset	: 5[ppm]
clipped	: FALSE
mod_return	: 1
scans	: 7988
total_scans	: 7988
x_90_width	: 7.8[us]
x_acq_time	: 1.9267584[s]
x_angle	: 30[deg]
x_pulse	: 2.6[us]
Initial_wait	: 1[s]
phase_preset	: 3[us]
Recvr_gain	: 15
Relaxation_delay	: 1[s]
Temp_get	: 26.7[dc]
Unblank_time	: 2[us]



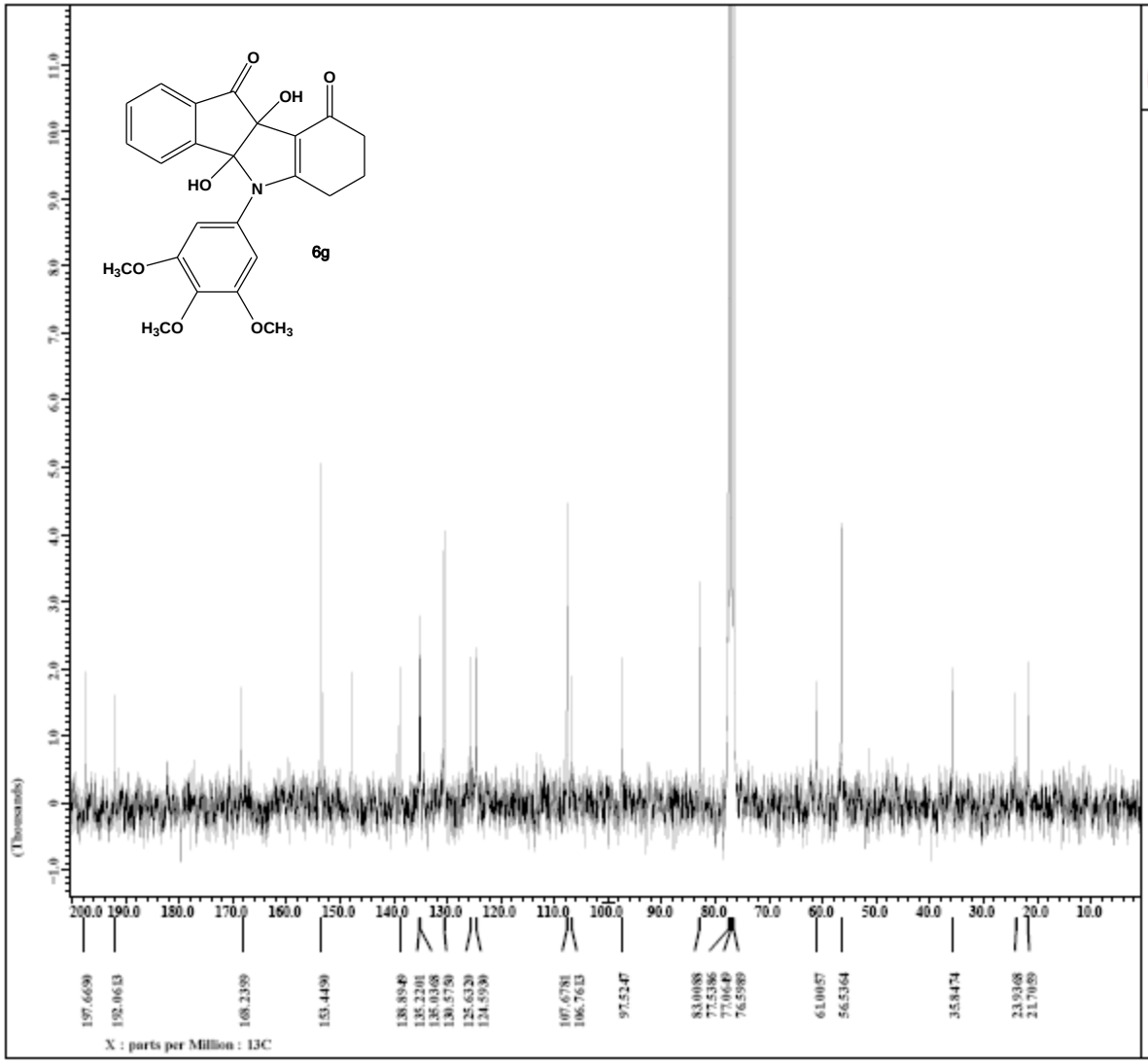

```
filename          = jcharrisGL374-1H-5.jd
Author            = rmnlab
Experiment        = single_pulse.exp
sample_id         = jcharrisGL374
solvent           = CHLOROFORM-D
creation_time     =  3-FEB-2015 00:32:38
Revision_time     =  3-MAR-2015 18:25:25
current_time      =  3-MAR-2015 18:25:26

content           = single pulse experime
Data_format       = 1D COMPLEX
Dim_size          = 16384
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    =  6.345446[T]  (270[MHz]
X_acq_duration    = 4.0419328[s]
X_domain          = 1H
X_freq            = 270.16608844[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.24740639[Hz]
X_sweep           = 4.05350628[kHz]
clipped           = FALSE
Mod_return        = 1
scans             = 8
Total_scans       = 8

X_90_width        = 11.7[us]
X_acq_time        = 4.0419328[s]
X_angle           = 45[deg]
X_pulse           = 5.85[us]
Initial_wait      = 1[s]
phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 4[s]
Temp_get          = 17.6[dc]
Unblank_time      = 2[us]
```



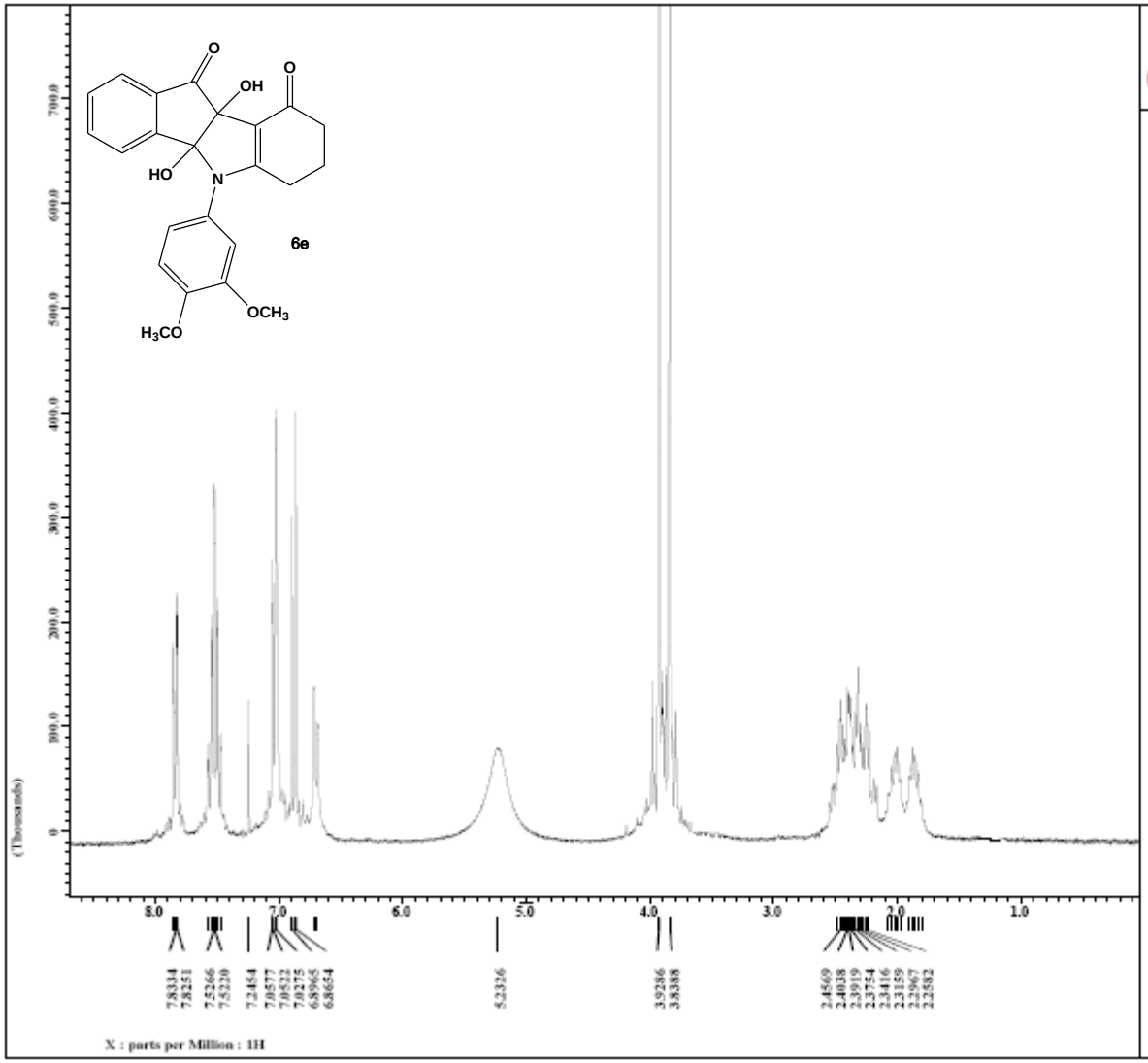

```
Filename          = jcharrisGL374-13c-5.j
Author            = rmnlab
Experiment        = single_pulse_dec
Sample_id         = jcharrisGL374
Solvent           = CHLOROFORM-D
Creation_time     =  3-FEB-2015 13:05:28
Revision_time     =  4-MAR-2015 06:54:31
Current_time      =  4-MAR-2015 06:54:55

Content           = single pulse with ero
Data_format       = 1D COMPLEX
Dim_size          = 32768
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 1.9267584[s]
X_domain          = 13C
X_freq            = 67.93330993[MHz]
X_offset          = 100[ppm]
X_points          = 32769
X_prescans        = 4
X_resolution      = 0.51900643[Hz]
X_sweep           = 17.00680272[kHz]
Irr_domain        = 1H
Irr_freq          = 270.16608844[MHz]
Irr_offset        = 5[ppm]
Clipped           = FALSE
Mod_return        = 1
Scans             = 15416
Total_scans       = 15416

X_90_width        = 7.8[us]
X_acq_time        = 1.9267584[s]
X_angle           = 30[deg]
X_pulse           = 2.6[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 1[s]
Temp_get          = 26[dC]
Unblank_time      = 2[us]
```



```
filename          = jcharrisGL366-1H-4.jd
Author            = rmnlab
experiment        = single_pulse.exp
sample_id         = jcharrisGL366
solvent           = CHLOROFORM-D
creation_time     =  3-FEB-2015 20:14:18
Revision_time     =  4-MAR-2015 14:04:04
current_time      =  4-MAR-2015 14:04:24

content           = single pulse experime
Data_format       = 1D COMPLEX
Dim_size          = 16384
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 4.0419328[s]
X_domain          = 1H
X_freq            = 270.16608844[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.24740639[Hz]
X_sweep           = 4.05350628[kHz]
clipped           = FALSE
Mod_return        = 1
scans             = 8
Total_scans       = 8

X_90_width        = 11.7[us]
X_acq_time        = 4.0419328[s]
X_angle           = 45[deg]
X_pulse           = 5.85[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 4[s]
Temp_get          = 25[dC]
Unblank_time      = 2[us]
```




```
Filename          = jcharrisGL366-13C-3.j
Author            = rmnlab
Experiment        = single_pulse_dec
Sample_id         = jcharrisGL366
Solvent           = CHLOROFORM-D
Creation_time     =  3-FEB-2015 20:12:52
Revision_time     =  4-MAR-2015 14:02:03
Current_time      =  4-MAR-2015 14:02:23

Content           = single pulse with ero
Data_format       = 1D COMPLEX
Dim_size          = 32768
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 1.9267584[s]
X_domain          = 13C
X_freq            = 67.93330993[MHz]
X_offset          = 100[ppm]
X_points          = 32769
X_prescans        = 4
X_resolution      = 0.51900643[Hz]
X_sweep           = 17.00680272[kHz]
Irr_domain        = 1H
Irr_freq          = 270.16608844[MHz]
Irr_offset        = 5[ppm]
Clipped           = FALSE
Mod_return        = 1
Scans             = 8262
Total_scans       = 8262

X_90_width        = 7.8[us]
X_acq_time        = 1.9267584[s]
X_angle           = 30[deg]
X_pulse           = 2.6[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 1[s]
Temp_get          = 25.7[dc]
Unblank_time      = 2[us]
```

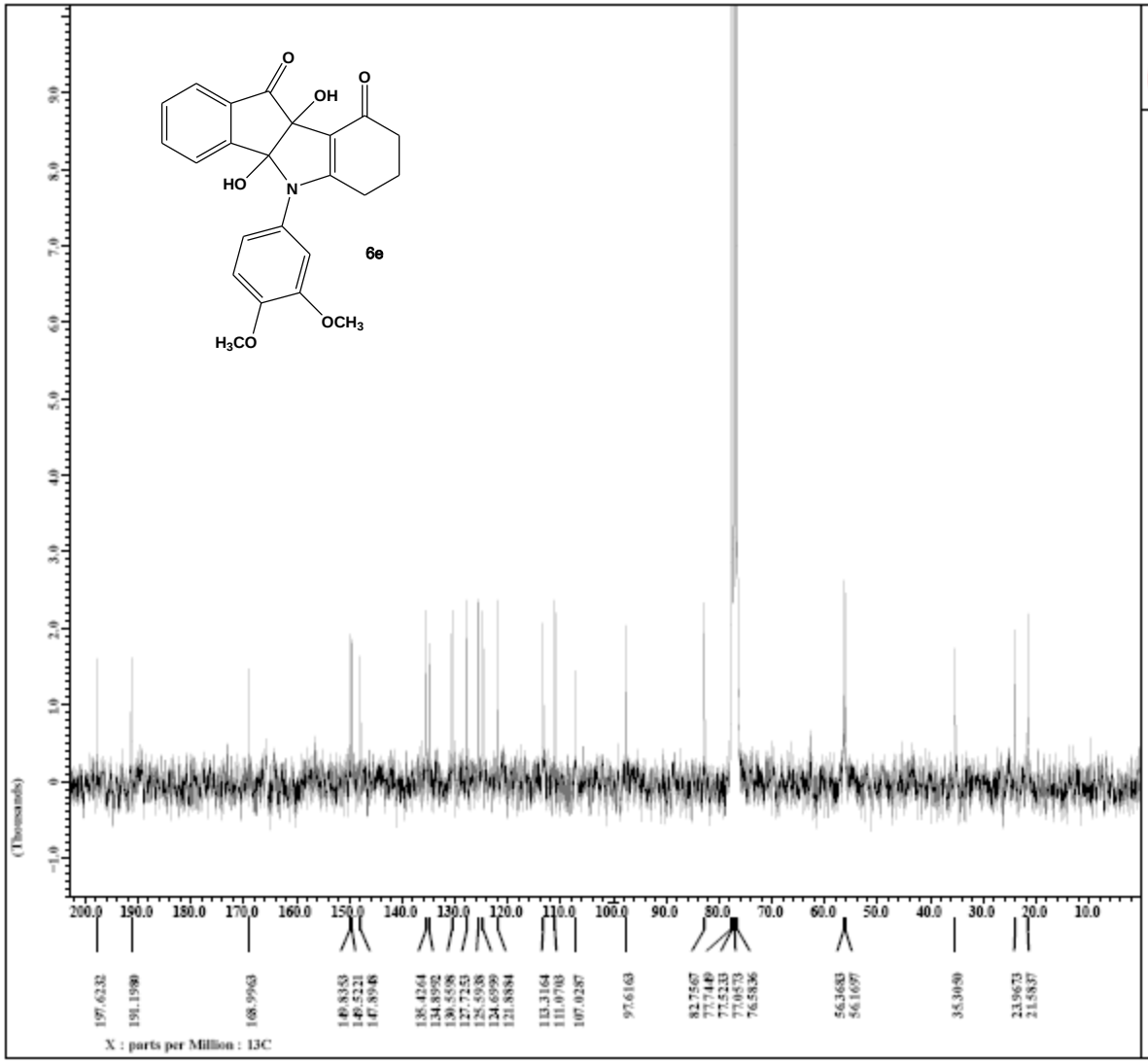


filename = jcharrisGL367-1H-4.jd
Author = rmnlab
experiment = single_pulse.exp
sample_id = jcharrisGL367
solvent = CHLOROFORM-D
creation_time = 4-FEB-2015 22:18:41
revision_time = 5-MAR-2015 16:08:22
current_time = 5-MAR-2015 16:08:37

content = single pulse experime
data_format = 1D COMPLEX
dim_size = 16384
dim_title = 1H
dim_units = [ppm]
dimensions = X
site = eclipse+ 400
spectrometer = DELTA_NMR

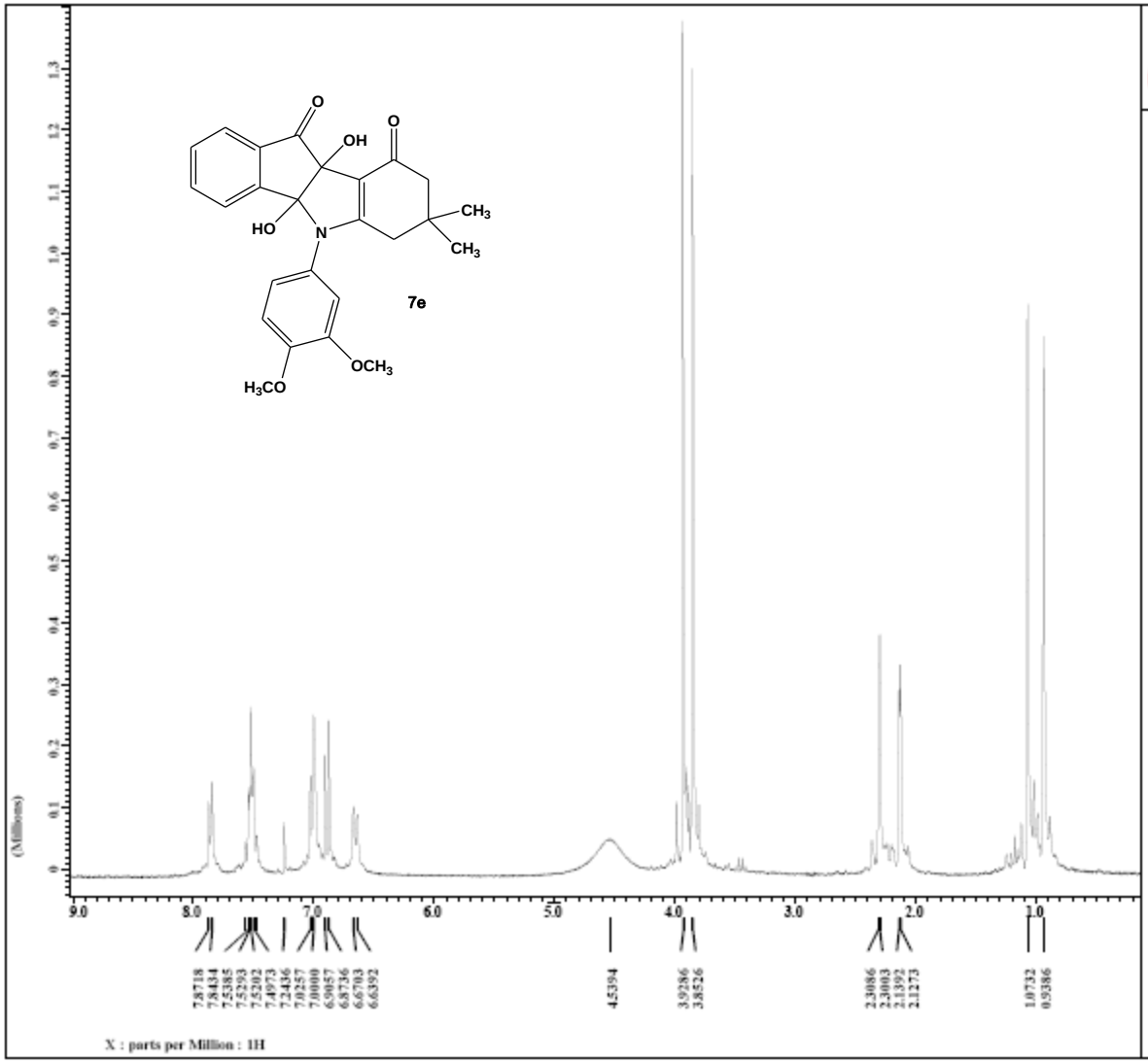
field_strength = 6.345446[T] (270[MHz]
x_acq_duration = 4.0419328[s]
x_domain = 1H
x_freq = 270.16608844[MHz]
x_offset = 5[ppm]
x_points = 16384
x_prescans = 0
x_resolution = 0.24740639[Hz]
x_sweep = 4.05350628[kHz]
clipped = FALSE
mod_return = 1
scans = 16
total_scans = 16

x_90_width = 11.7[us]
x_acq_time = 4.0419328[s]
x_angle = 45[deg]
x_pulse = 5.85[us]
initial_wait = 1[s]
phase_preset = 3[us]
recvr_gain = 15
relaxation_delay = 4[s]
temp_get = 20[dc]
unblank_time = 2[us]



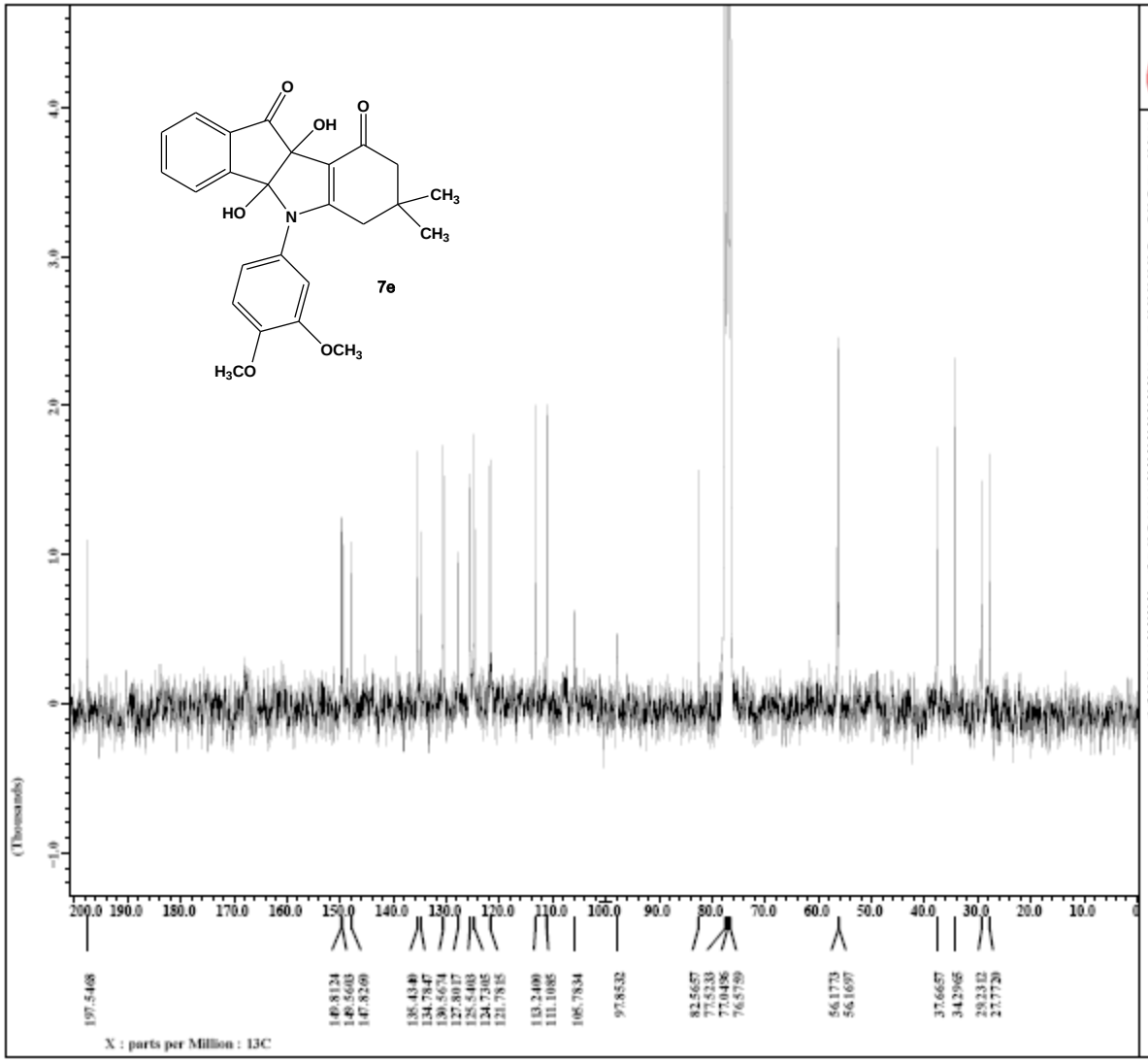
JEOL

```
filename          = jcharrisGL367-13c-3.j
Author            = rmnlab
Experiment        = single_pulse_dec
sample_id         = jcharrisGL367
solvent           = CHLOROFORM-D
creation_time     =  4-FEB-2015 22:07:59
Revision_time     =  5-MAR-2015 16:05:14
current_time      =  5-MAR-2015 16:05:39

content           = single pulse with dro
Data_format       = 1D COMPLEX
Dim_size          = 32768
Dim_title         = 13c
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 1.9267584[s]
X_domain          = 13c
X_freq            = 67.93330993[MHz]
X_offset          = 100[ppm]
X_points          = 32769
X_prescans        = 4
X_resolution      = 0.51900643[Hz]
X_sweep           = 17.00680272[kHz]
Xfr_domain        = 1H
Xfr_freq          = 270.16608844[MHz]
Xfr_offset        = 5[ppm]
clipped           = FALSE
mod_return        = 1
scans             = 10000
total_scans       = 10000

X_90_width        = 7.8[us]
X_acq_time        = 1.9267584[s]
X_angle           = 30[deg]
X_pulse           = 2.6[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 1[s]
Temp_get          = 26.4[dC]
Unblank_time      = 2[us]
```



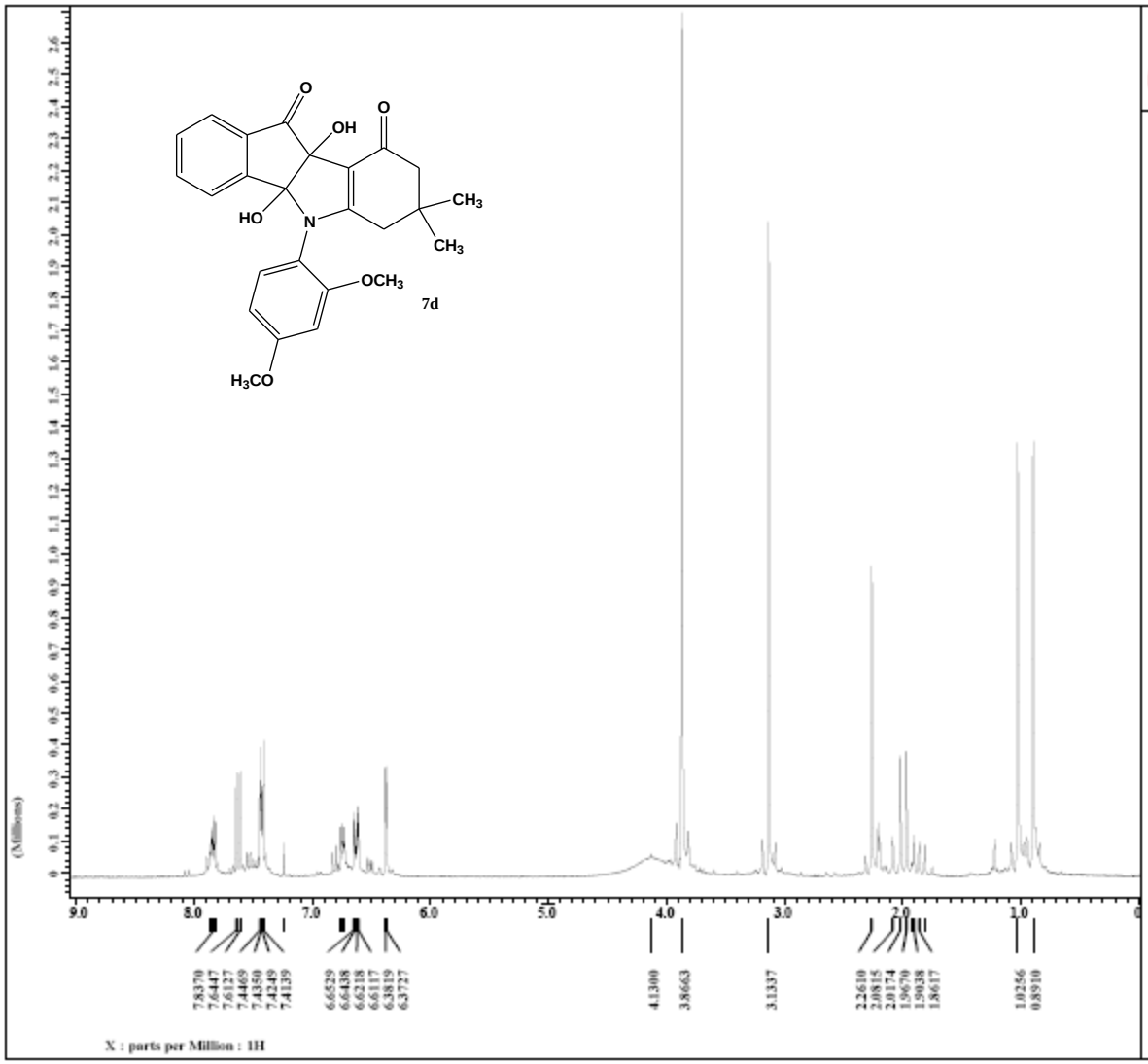
JEOL

```
filename          = jcharrisGL377-1H-5.jd
Author            = rmnlab
experiment        = single_pulse.exp
sample_id         = jcharrisGL377
solvent           = CHLOROFORM-D
creation_time     =  5-FEB-2015 12:51:07
Revision_time     =  6-MAR-2015 07:07:16
current_time      =  6-MAR-2015 07:07:25

content           = single pulse experime
Data_format       = 1D COMPLEX
Dim_size          = 16384
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 4.0419328[s]
X_domain          = 1H
X_freq            = 270.16608844[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.24740639[Hz]
X_sweep           = 4.05350628[kHz]
clipped           = FALSE
Mod_return        = 1
scans             = 16
Total_scans       = 16

X_90_width        = 11.7[us]
X_acq_time        = 4.0419328[s]
X_angle           = 45[deg]
X_pulse           = 5.85[us]
Initial_wait      = 1[s]
phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 4[s]
Temp_get          = 24.9[dc]
Unblank_time      = 2[us]
```



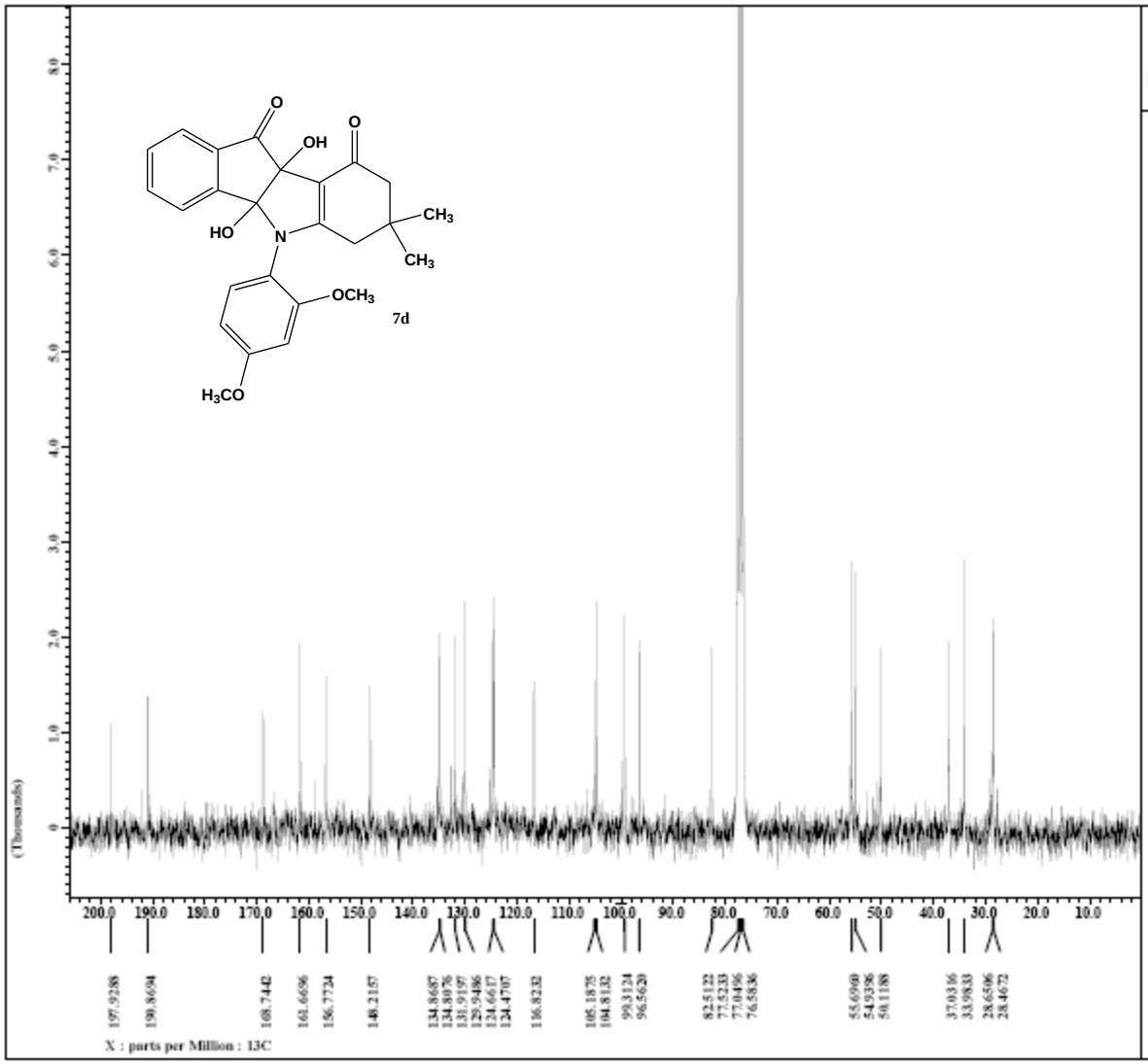
JEOL

```
filename          = jcharrisGL377-13c-3.j
Author            = rmnlab
Experiment        = single_pulse_dec
sample_id         = jcharrisGL377
solvent           = CHLOROFORM-D
creation_time     =  5-FEB-2015 12:48:40
Revision_time     =  6-MAR-2015 07:05:20
current_time      =  6-MAR-2015 07:05:28

content           = single pulse with dro
Data_format       = 1D COMPLEX
Dim_size          = 32768
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 1.9267584[s]
X_domain          = 13C
X_freq            = 67.93330993[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.51900643[Hz]
X_sweep           = 17.00680272[kHz]
Xfr_domain        = 1H
Xfr_freq          = 270.16608844[MHz]
Xfr_offset        = 5[ppm]
clipped           = FALSE
Mod_return        = 1
scans             = 16000
Total_scans       = 16000

X_90_width        = 7.8[us]
X_acq_time        = 1.9267584[s]
X_angle           = 30[deg]
X_pulse           = 2.6[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 1[s]
Temp_get          = 26.7[dc]
Unblank_time      = 2[us]
```



JEOL

```
filename          = jcharrisGL377-1H-6.jd
Author            = rmnlab
experiment        = single_pulse.exp
sample_id         = jcharrisGL377
solvent           = CHLOROFORM-D
creation_time     =  5-FEB-2015 17:24:56
Revision_time     =  6-MAR-2015 11:15:36
current_time      =  6-MAR-2015 11:15:39

content           = single pulse experime
Data_format       = 1D COMPLEX
Dim_size          = 16384
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = Eclipse+ 400
Spectrometer      = DELTA_NMR

Field_strength    = 6.345446[T]  (270[MHz]
X_acq_duration    = 4.0419328[s]
X_domain          = 1H
X_freq            = 270.16608844[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 0
X_resolution      = 0.24740639[Hz]
X_sweep           = 4.05350628[kHz]
clipped           = FALSE
Mod_return        = 1
scans             = 16
Total_scans       = 16

X_90_width        = 11.7[us]
X_acq_time        = 4.0419328[s]
X_angle           = 45[deg]
X_pulse           = 5.85[us]
Initial_wait      = 1[s]
Phase_preset      = 3[us]
Recvr_gain        = 15
Relaxation_delay  = 4[s]
Temp_get          = 24.8[dc]
Unblank_time      = 2[us]
```



JEOL

filename	= jcharrisGL367-13c-3.j
Author	= rmnlab
Experiment	= single_pulse_dec
sample_id	= jcharrisGL367
solvent	= CHLOROFORM-D
creation_time	= 5-FEB-2015 17:22:23
Revision_time	= 6-MAR-2015 11:10:37
current_time	= 6-MAR-2015 11:16:09
content	= single pulse with nro
Data_format	= 1D COMPLEX
Dim_size	= 32768
Dim_title	= 13C
Dim_units	= [ppm]
Dimensions	= X
Site	= Eclipse+ 400
Spectrometer	= DELTA_NMR
Field_strength	= 6.345446[T] (270[MHz]
x_acq_duration	= 1.9267584[s]
x_domain	= 13C
x_freq	= 67.93330993[MHz]
x_offset	= 100[ppm]
x_points	= 32768
x_prescans	= 4
x_resolution	= 0.51900643[Hz]
x_sweep	= 17.00680272[kHz]
xfr_domain	= 1H
xfr_freq	= 270.16608844[MHz]
xfr_offset	= 5[ppm]
clipped	= FALSE
mod_return	= 1
scans	= 4950
total_scans	= 4950
x_90_width	= 7.8[us]
x_acq_time	= 1.9267584[s]
x_angle	= 30[deg]
x_pulse	= 2.6[us]
Initial_wait	= 1[s]
phase_preset	= 3[us]
Recvr_gain	= 15
Relaxation_delay	= 1[s]
Temp_get	= 26.7[dc]
Unblank_time	= 2[us]