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Graphical Abstract

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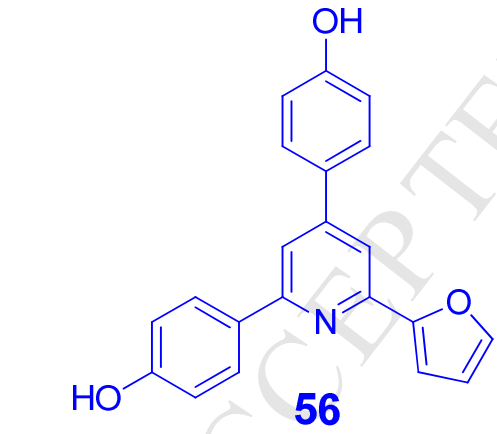
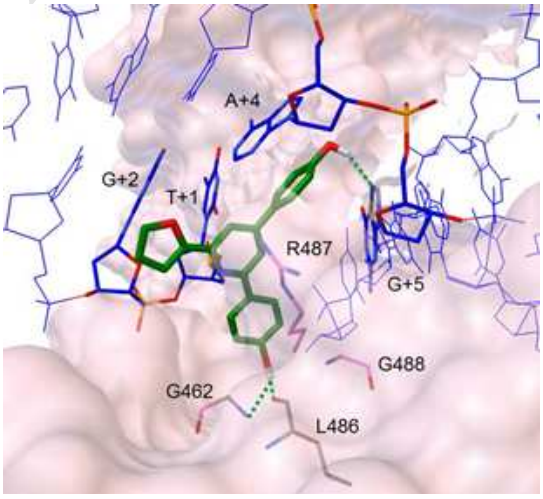
Radha Karki,[#[a]] Chanmi Park, [#[b]] Kyu-Yeon Jun,[[b]] Tata Man Kadayatt,[[a]] Eung-Seok Lee,*[[a]] and Youngjoo Kwon*[[b]]

[a]*College of Pharmacy, Yeungnam University, Gyeongsan 712-749 (Republic of Korea)*

[b]*College of Pharmacy, Graduate School of Pharmaceutical Sciences, Ewha Global Top 5 program Ewha Womans University, Seoul 120-750 (Republic of Korea)*



Potent Topoisomerase II Poison



Synthesis and Biological Activity of 2,4-Di-*p*-Phenolyl-6-*2*-Furanyl-Pyridine As a Potent Topoisomerase II Poison

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[a]*College of Pharmacy, Yeungnam University, Gyeongsan 712-749 (Republic of Korea)*

[b]*College of Pharmacy, Graduate School of Pharmaceutical Sciences, Ewha Global Top 5 program Ewha Womans University, Seoul 120-750 (Republic of Korea)*

[#]Authors are equal contributors

*Corresponding Authors:

Tel: +82 53 810-2827, fax: +82 53 810-4654, email: eslee@ynu.ac.kr (E.-S.L.)

Tel: +82 2 3277-4653, fax: +82 2 3277-2851, email: ykwon@ewha.ac.kr (Y.K.)

Abstract

Dihydroxylated 2,4-diphenyl-6-aryl pyridine derivatives were simply achieved using *Claisen-Schmidt* condensation reaction and modified Kröhnke pyridine synthetic method. Total forty-five compounds were designed and synthesized which contain hydroxyl groups at *ortho*, *meta* or *para* position of 2- and 4-phenyl rings attached to the central pyridine. They were evaluated for topoisomerase I and II inhibitory activity, and cytotoxicity against several human cancer cell lines for the development of novel antitumor agents. Most of the prepared compounds exhibited significant antiproliferative activity on human cancer cell lines, HCT15 and K562, as well as potent topo II inhibitory activity comparable to or stronger than etoposide. The structure-activity relationship demonstrated that compounds with hydroxyl group at *meta* or *para* position of 2-phenyl ring in combination with hydroxyl at *ortho*, *meta* or *para* position of 4-phenyl ring displayed the most potent topoisomerase II inhibitory activity and cytotoxicity. Positive correlation between topoisomerase II inhibition and cytotoxicity was obtained for several compounds (**30**, **35**, **36**, **40**-**45**, **49**, **54**, **56**). Compound **56** showed the most potent topoisomerase II inhibitory activity at low concentration and functioned as a topoisomerase poison like the mode of action of etoposide.

Keywords: Terpyridine bioisosteres; Topoisomerase poison; Antitumor agents; Dihydroxylated 2,4-diphenyl-6-aryl pyridine; Cytotoxicity

1. Introduction

DNA topoisomerases, the molecular targets of many antimicrobial and anticancer agents, have an important role in cell proliferation and differentiation [1]. They are involved in all events related to DNA metabolism including replication, transcription, recombination, repair, chromatin assembly and chromosome segregation [1-3]. DNA topoisomerases are basically classified into two types; topoisomerase I (topo I) and topoisomerase II (topo II). They can change the topological state of DNA through the breaking and rejoining of DNA strands. Their catalytic cycle include DNA strand breakage (one strand by topo I or both strand by topo II), formation of a covalent complex with DNA, followed by DNA strand passage, and the final religation step of the initial DNA strand [4]. Several topoisomerase inhibitors such as camptothecin, doxorubicin, etoposide etc are widely used as active anticancer agents [5,6]. Due to severe side effects associated with currently available anticancer agents, extensive research is going on for the development of new derivatives and novel compounds with improved therapeutic efficacy and minimizing the toxicities [5-10]. Several naturally occurring biologically active polyphenolic compounds are reported to possess antioxidant, antiinflammatory, and anticancer activity [11,12]. Therefore they are viewed as an important pharmacophore in the medicinal chemistry or drug discovery area. Resveratrol, curcumin, epigallocatechin gallate etc are few polyphenolic phytochemicals that are well studied for their antioxidant and anticancer activity [13-16]. Similarly, some flavonoids are reported to interfere with human topoisomerases. It is observed that hydroxyl moiety present in the flavonoids have important role for the topoisomerase inhibitory acitivity [17-20]. Several other studies of synthetic compounds support that introduction of hydroxyl group enhances topoisomerase inhibitory activity [21-23]. Many clinically used topoisomerase inhibitors such as camptothecin, doxorubicin and etoposide also contain hydroxyl moieties in their structure.

All these promising reports on the importance of hydroxyl moiety and our previous results [24,25] motivated us to synthesize more compounds with hydroxyl groups. Our study is aimed at designing and synthesizing topoisomerase-targeting anticancer agents. As a part of the study, we have previously designed, synthesized and reported several 2,4,6-triaryl pyridine derivatives as bioisosteres of terpyridine for the topoisomerase inhibitory activity and cytotoxicity against several human cancer cell lines [26-34]. Furthermore, we have introduced hydroxyl group(s) [24,25] into phenyl moiety to improve topo I and/or II inhibitory activities, and cytotoxicity against several human cancer cell lines. In addition, positive correlation between substituted position of the hydroxyl moiety, topo II inhibition and cytotoxicity has been observed. Recently reported dihydroxylated 2,4,6-triphenyl pyridines also displayed significant topo II inhibitory activity, and cytotoxicity [25]. As an extension to this work, herein we designed and synthesized dihydroxylated 2,4-diphenyl-6-aryl pyridine derivatives possessing various aryl groups on 6-position of central pyridine as shown in Figure 1.

(Figure 1)

In this article, we present the synthesis and biological studies of novel dihydroxylated 2,4-diphenyl-6-aryl pyridine compounds. The bioevaluation of these compounds against topo I and II was carried out. Further, the ability of these compounds to inhibit proliferation of various human cancer cell lines were examined and structure-activity relationship (SAR) according to the number and position of hydroxyl group was established.

2. Chemistry

Dihydroxylated 2,4-diphenyl-6-aryl pyridine derivatives **15-59** were synthesized in three steps as illustrated in Scheme 1. The first step involves the synthesis of various dihydroxylated propenone intermediates (**3**, **4** and **5**), using *Claisen-Schmidt* condensation reaction [35-37]. Total nine compounds were synthesized in 36-90% yield that were reported previously [24,25]. In the second step, five pyridinium iodide salts **6** (R^3 = d-h) were synthesized in quantitative yield by the treatment of aryl ketone **1** (R^1 = d-h) with iodine in pyridine. Using modified Kröhnke pyridine synthesis [38,39], final compounds **7** (R^1 = a, R^2 = a-c, R^3 = d-h), **8** (R^1 = b, R^2 = a-c, R^3 = d-h) and **9** (R^1 = c, R^2 = a-c, R^3 = d-h) were synthesized by the reaction of appropriate dihydroxylated propenone intermediates **3**, **4** or **5** with pyridinium iodide salt **6** in the presence of ammonium acetate and glacial acetic acid in 20-94% yield. The compounds shown in Figure 2 are the non-substituted 2,4-diphenyl-6-aryl pyridines which were reported earlier [28]. All the compounds displayed weak topo II inhibitory activity as shown in Table 1. In this study the dihydroxylated derivatives of those compounds were synthesized. The reaction of nine intermediates and five pyridinium iodide salts gave total forty-five final compounds. The compounds were designed and synthesized in nine different series as shown in Figure 3. Each series contains five different compounds with various aryl groups at 6-position of central pyridine. All the prepared compounds contain two hydroxyl moieties at various positions (ortho, meta or para) of 2- and 4-phenyl ring of central pyridine. It was previously shown that introduction of hydroxyl moiety increased topo II inhibitory activity and cytotoxicity. Furthermore, increase in the number of hydroxyl group enhanced the activity [24,25]. Herein, more dihydroxylated compounds (**15-59**) with various aryl groups at 6-position of the central pyridine were synthesized as displayed in Figure 4.

(Scheme 1)

(Figure 2)

(Table 1)

(Figure 3)

(Figure 4)

3. Results and Discussion

*3.1. Biological activity of compounds **15-59***

All the synthesized compounds (**15-59**) were tested for topo I and II inhibitory activity and cytotoxicity against several human cancer cell lines. Camptothecin and etoposide, well known topo I and II inhibitors, respectively, were used as positive controls. Figure 5 and 6 respectively illustrate the topo I and II inhibitory activity of synthesized compounds **15-59**. Among the forty-five synthesized dihydroxylated 2,4-diphenyl-6-aryl pyridine compounds, only compounds **25**, **26** and **29** showed considerable topo I inhibitory activity as shown in Figure 5 and Table 2. Compounds **25** and **26** displayed 59.9% and 44.3% inhibition at 100 µM and 43.0% and 31.7% inhibition at 20 µM, respectively. Compound **29** showed 33.3% inhibition at 100 µM. It is interesting to note that all three compounds were from Series C, which contain hydroxyl group at *ortho* position of 2-phenyl and *para* position of 4-phenyl ring on central pyridine. Although a concrete SAR could not be established, it can be expected that hydroxyl at *ortho* position of 2-phenyl and *para* position of 4-phenyl might be important for topo I inhibitory activity.

The effect of synthesized compounds on human DNA topo II were observed in the relaxation assay using supercoiled pBR322 plasmid DNA in the presence of ATP. Figure 6 and Table 3-5 summarizes the topo II inhibitory activity of the synthesized compounds. Most of the compounds showed significant inhibitory activity. Compound **54** possessed the highest topo II inhibition (98.2%) at 100 µM. Compounds **35**, **36**, **40**, **41** and **49** showed more than 80% inhibition at 100 µM which was higher than the positive control etoposide. Compounds **15**, **19**, **20**, **25**, **26**, **36**, **45**, **49**, **54** and **56** displayed significant activity at both 100 and 20 µM. Compound **56** possessed 76.8% and 74.2% inhibition at 100 and 20 µM, respectively. These results suggest that dihydroxylated 2,4-diphenyl-6-aryl pyridines are selective for topo II inhibition, as most of the compounds were devoid of topo I inhibitory activity. Compounds **15**-**19**, **20**-**24** and **25**-**29** belong to Series A, Series B and Series C, respectively. Compounds from these three series possess *ortho*-hydroxyl group on 2-phenyl ring whereas *ortho*, *meta* or *para*-hydroxyl on 4-phenyl ring of central pyridine. All compounds from Series A (**15**–**19**) possessed significant topo II inhibitory activity, compound **15** being the most potent with 75.5% and 67.3% inhibition at 100 and 20 µM, respectively. Compounds **17**-**19** also displayed strong topo II inhibition at 100 µM which was comparable to etoposide. Compounds **20**, **21** and **24** from Series B displayed considerable inhibitory activity. Compounds **25**-**29** from Series C showed significant inhibitory activity that was comparable to etoposide. Compounds **30**-**34**, **35**-**39** and **40**-**44** belong to Series D, Series E and Series F, respectively, which all the compounds from these three series possess *meta*-hydroxyl group on 2-phenyl ring whereas *ortho*, *meta* or *para*-hydroxyl on 4-phenyl ring of central pyridine. Compounds **30**, **32** and **33** from Series D possessed significant topo II inhibitory activity. Similarly, compounds **35** and **36** from Series E displayed potent inhibitory activity (83.9% and 81.1%) which was higher than etoposide. All compounds **40**-**44** from Series F exhibited significant inhibitory activity (72-88%) at 100 µM that was higher than etoposide. Compound

40 showed the highest inhibition (88.6%) in this series. Compounds **45**-**49**, **50**-**54** and **55**-**59** belong to Series G, Series H and Series I, respectively, which all the compounds from these three series possess *para*-hydroxyl group on 2-phenyl ring whereas *ortho*, *meta* or *para*-hydroxyl on 4-phenyl ring of central pyridine. Compounds **45** and **49** from Series G displayed stronger inhibitory activity than etoposide whereas compounds **46** and **48** showed considerable activity. Compound **54** from Series H possessed 98.2% and 37.0% topo II inhibition at 100 and 20 µM, respectively. Compounds **50**, **51** and **53** from the same series possessed considerable activity. Similarly, compound **56** from Series I showed significant inhibitory activity of 76.8% and 74.2% at 100 and 20 µM, respectively. Compound **55** also possess significant activity at 100 µM. For the evaluation of cytotoxicity, five different human cancer cell lines were utilized: HCT15 (human colorectal adenocarcinoma cell line), K562 (human myeloid leukemic tumor cell line), DU145 (human prostate tumor cell line), MCF-7 (human breast adenocarcinoma cell line) and HeLa (human cervix tumor cell line). The inhibitory activity (IC_{50}) is expressed as micromolar concentration as illustrated in Table 2-5. The cytotoxicity results indicate that most of the compounds are active against HCT15 and K562 cell lines. Compounds from Series A, Series B and Series C were less cytotoxic compared to other series. Most of the compounds possessed considerable activity against HCT15 and K562 but were less cytotoxic (> 30 µM) against DU145, MCF-7 and HeLa. Compounds **17**, **19**, **24** and **29** displayed significant cytotoxicity against HCT15 and compounds **17**, **19** and **29** against K562 as compared to adriamycin and etoposide. Compounds from Series D, Series E and Series F displayed significant cytotoxicity (< 4 µM) against HCT15 and K562 whereas moderate cytotoxicity against DU145, MCF-7 and HeLa. Compounds **30**, **32**, **33**, **37** and **42** possessed stronger cytotoxicity than adriamycin and etoposide against HCT15. Similarly, compounds **30**, **31**, **40** and **43** displayed potent cytotoxicity against K562. Compounds **35**–**37** displayed significant cytotoxicity (< 3 µM)

against DU145. Most of these compounds displayed moderate cytotoxicity (< 15 µM) against MCF-7 and HeLa. The most cytotoxic compounds were from Series G, Series H and Series I. Compounds **45**, **46**, **48**, **53**–**56** from these three series displayed potent cytotoxicity (< 1 µM) against HCT15 which is stronger than adriamycin and etoposide. Compound **56** is the most cytotoxic among all the synthesized compounds against HCT15, with IC_{50} value 0.08 µM. Similarly, compounds **49**, **53**–**56** possessed significant cytotoxicity (< 2 µM) against K562 compared to adriamycin and etoposide. Also compounds **45**, **46** and **48** showed cytotoxicity comparable to adriamycin against K562. Most of the compounds from these three series displayed moderate cytotoxicity (< 10 µM) against DU145, MCF-7 and HeLa. Compounds **45**, **48**, **49**, **53** and **54** possessed considerable cytotoxicity (< 3 µM) against HeLa. It is observed that all the compounds contain hydroxyl moiety at *para* position on 2-phenyl ring of central pyridine.

(Figure 5)

(Figure 6)

(Table 2)

(Table 3)

(Table 4)

(Table 5)

3.2. Structure-activity relationship (SAR) study

It was found that the introduction of hydroxyl moiety is important for topo II inhibitory activity and cytotoxicity of 2,4-diphenyl-6-aryl pyridine compounds (**10**–**14**). The number and position of hydroxyl group strongly influenced the activities. Monohydroxylated 2,4-diphenyl-6-aryl pyridine compounds possessed stronger topo II inhibition and cytotoxicity [24] compared to non-hydroxylated compounds but less potent than the dihydroxylated compounds **15-59**. Several studies have indicated that introduction of hydroxyl group at certain position enhances topo II inhibitory activity [24,25]. In this study, the position of hydroxyl group at the phenyl ring have variable influence on the topoisomerase inhibitory activity and cytotoxicity. For the clear SAR study, biological activities of evaluated compounds in accordance to the position of phenyl ring on the central pyridine are explained in detail.

Substitution at 2-phenyl ring: Most of the compounds (**15**, **17-20**, **25-29**) with hydroxyl substitution at *ortho* position has shown significant topo II inhibition but are less cytotoxic to the tested cancer cell lines. Compounds **30**, **35**, **36**, **40-44** having hydroxyl moiety at *meta* position displayed stronger topo II inhibition than etoposide, and also possessed significant cytotoxicity. Similarly, compounds (**45**, **46**, **48**, **49**, **53-56**) with hydroxyl moiety at *para* position also possessed moderate to significant topo II inhibition and potent cytotoxicity. So the favorable order of substitution of hydroxyl group at 2-phenyl ring for the topo II inhibition and cytotoxicity is *meta* > *para* > *ortho*.

Substitution at 4-phenyl ring: Most of the compounds (**15**, **17-19**, **30**, **32**, **33**, **45**, **49**) having *ortho* hydroxyl moiety at 4-phenyl ring possessed significant topo II inhibition. However, for

better cytotoxicity *meta* or *para* hydroxyl at 2-phenyl ring is necessary in combination with *ortho* hydroxyl at 4-phenyl ring. *Para* hydroxyl substitution seems to be the most favorable for both topo II inhibition and cytotoxicity. Some compounds (**20**, **35**-**37**, **54**) with *meta* hydroxyl moiety at 4-phenyl ring also possess significant topo II inhibition as well as cytotoxicity. Therefore, the topo II inhibitory activity and cytotoxicity of compound depends on the combination of hydroxyl moiety at 2- and 4- phenyl ring of central pyridine.

Aryl substitution at 6-position: Five different aryl groups were introduced at 6 position of central pyridine. The bioactivity of the prepared compounds is not dependent on the aryl groups. However, all the compounds with 3-thienyl moiety displayed significant topo II inhibitory activity.

Figure 7A indicates some compounds having significant topo II inhibitory activity and cytotoxicity. Most of the compounds (**35**, **36**, **40, 41, 43**) possessed hydroxyl moiety at *meta* position of 2-phenyl ring while some compounds (**49**, **54**, **56**) at *para* position. Similarly, compounds (**40**, **41**, **43**, **56**) possessed hydroxyl moiety at *para* position of 4-phenyl ring and compounds (**35**, **36**, **54**) at *meta* position. Compound **54** which displayed the highest topo II inhibition contains *para*- and *meta*-hydroxyl substitution at 2- and 4-phenyl ring of central pyridine respectively. Compound **56** having 76.8% and 74.2% inhibition at 100 and 20 µM respectively and IC_{50} 0.08 µM against HCT15 possessed *para* hydroxyl substitution at both phenyl rings. All the compounds from series F (**40**-**44**), which contain *meta* and *para* hydroxyl moiety at 2- and 4-phenyl ring of central pyridine respectively, displayed stronger topo II inhibitory activity than the positive control etoposide. Taken together, *meta* or *para* substitution at 2-phenyl ring in combination with *ortho*, *meta* or *para* at 4-phenyl ring of

central pyridine is important for both topo II inhibition and cytotoxicity as shown in Figure 7B which further supports our previous result [25].

(Figure 7)

*3.3. Compound **56** functions as a Topo II poison*

Compounds **54** and **56** were further evaluated for the determination of their mode of action. Compound **54** showed the strongest topo II inhibitory activity at treatment of 100 μM among tested compounds and compound **56** exhibited the strongest topo II inhibitory activites at low concentration of 20 μM as well as the strongest cytoxicity againt HCT15 cells. First of all we checked whether compounds could stablize transiently-formed cleavage comlex with covalent bonding between DNA and topo II. It is well known that topo II poison induces a truncated DNA by preventing religation through stablilizing the cleavage complex [6,41]. The treatment of compound **56** formed a short linear DNA like etoposide while compound **54** did not as shown in Figure 8A. This clearly reflected compound **56** funtioned as a topo poison not as a catalytic inhibitor. We subsequently examined whether compounds **54, 56** and etoposide generated the accumulation of DNA strand breaks in HCT15 cells using comet assay. The more percentage of DNA in the tail implies more DNA strand breaks [42,43]. Figure 8B and C show that compound **56** (18.7 ± 5.2% at 10 μM and 46.7 ± 5.5% at 20 μM) induced significant tail at 20 μM treatment which is comparable to the extent induced by etoposide (43.0 ± 8.4% at 10 μM and 60.7 ± 8.4% at 20 μM). On the other hand, compound **54** did not induce the tail at both treated concentrations as much as compound **56**. The result

is consistent with no linear DNA form observed by compound **54** treatment in Figure 8A. Therefore, it is clear that compound **56** funtioned as a topo II poison like etoposide. Compound **56** was further performed for docking study to clearly compare its mode of action with that of etoposide. Currently there is only one human topo IIα cleavage core bound to DNA is available in the Protein Data Bank (PDB, PDB ID: 4FM9) [44]. However this structure was not appropriate for docking inhibitors, since there was no ligand bound. Therefore the structure of topo IIβ bound to etoposide and DNA (3QX3) was chosen for the template to generate the homology model of topo IIα [45]. Molecular docking were carried out for compound **56** with the topo IIα cleavage core bound to DNA (Figure 9). Compound **56** is bound to the catalytic core of topo IIα, intercalating into the cleaved bases of the DNA, which overlaps with etoposide bound to topo IIα. The compound has hydrophobic interactions with Gly462, Arg487, Gly488, T+1, G+2 on the cleaved strand of DNA and A+4 on the uncleaved strand. There are also π-π stacking interactions between the bases of T+1, A+4 and the 4-phenyl ring. The hydroxyl groups from 2, 4-phenyl rings form hydrogen bonds with Leu486/Gly462 and G+5, respectively, indicated with green dot lines in Figure 9. The site of the substitution of hydroxyl group on each phenyl ring is essential for the inhibitory activity of topo IIα in compound **56** since it was able to form hydrogen bonds. This can explain for the difference in the activity of the compounds depending on the hydroxyl group substitution sites.

(Figure 8)

(Figure 9)

4. Conclusions

We have synthesized a new series of dihydroxylated 2,4-diphenyl-6-aryl pyridines by efficient synthetic route and evaluated their topo I and II inhibition, and cytotoxicity. The activity of 2,4-diphenyl-6-aryl pyridines was strongly influenced by the number and position of hydroxyl group on the phenyl ring. Most of the compounds displayed significant topo II inhibition and cytotoxicity. SAR study suggests that introduction of hydroxyl moiety increased topo II inhibitory activity, and increase in the number of hydroxyl group further enhanced the activity. Hydroxyl group at *meta* or *para* position on 2-phenyl ring in combination with hydroxyl at *ortho*, *meta* or *para* position on 4-phenyl ring of central pyridine displayed significant topo II inhibitory activity and cytotoxicity. Compounds were particularly more cytotoxic to HCT15 and K562. Positive correlation between topo II inhibition and cytotoxicity was obtained for several compounds (**30**, **35**, **36**, **40**-**45**, **49**, **54**, **56**). Especially, compound **56** exhibited etoposide like inhibition by forming stable cleavage complex. These findings were confirmed by molecular docking study. The further study and optimization of these compounds may lead to a new potential antitumor agent.

5. Experimental Section

Compounds used as starting materials and reagents were obtained from Aldrich Chemical Co., Junsei or other chemical companies, and utilized without further purification. HPLC grade acetonitrile (ACN) and methanol were purchased from Burdick and Jackson, USA. Thin-layer chromatography (TLC) and column chromatography (CC) were performed with Kieselgel 60 F_{254} (Merck) and silica gel (Kieselgel 60, 230-400 mesh, Merck), respectively.

Since all the compounds prepared contain aromatic ring, they were visualized and detected on TLC plates with UV light (short wave, long wave or both). NMR spectra were recorded on a Bruker AMX 250 (250 MHz, FT) for ^{1}H NMR and 62.5 MHz for ^{13}C NMR, and chemical shifts were calibrated according to TMS. Chemical shifts (δ) were recorded in ppm and coupling constants (J) in hertz (Hz). Melting points were determined in open capillary tubes on electrothermal 1A 9100 digital melting point apparatus and were uncorrected.

HPLC analyses were performed using two Shimadzu LC-10AT pumps gradient-controlled HPLC system equipped with Shimadzu system controller (SCL-10A VP) and photo diode array detector (SPD-M10A VP) utilizing Shimadzu Class VP program. Sample volume of 10 µL was injected in Waters X- Terra® 5 µM reverse-phase C_{18} column (4.6 x 250 mm) with a gradient elutions of 50% to 100% of B in A for 15 min followed by 100% to 50% of B in A for 15 min at a flow rate of 1.0 mL/min at 254 nm UV detection, where mobile phase A was double distilled water with 20 mM ammonium formate (AF) and B was 90% ACN in water with 20 mM AF. In case of compounds of Series C (**25**-**29**) and Series G (**45**-**49**) gradient elutions of 85% to 100% of B in A for 10 min followed by 100% to 85% of B in A for 20 min was performed with the same mobile phase. Purity of compound is described as percent (%).

ESI LC/MS analyses were performed with a Finnigan LCQ Advantage® LC/MS/MS spectrometry utilizing Xcalibur® program. For ESI LC/MS, LC was performed with a 2 µL injection volume on Waters Atlantis® T3 reverse-phase C_{18} column (2.1 x 50 mm, 3 µm). The mobile phase consisted of 100% distilled water (A) and 100% ACN (B). A gradient program was used with a flow rate of 200 µL/min. The initial composition was 10% B and programmed linearly to 90% B after 5 min and finally 10% B after 10 min. MS ionization conditions were: Sheath gas flow rate: 70 arb, aux gas flow rate: 20 arb, I spray voltage: 4.5

KV, capillary temperature 215 °C, column temperature 40 °C, capillary voltage: 21 V, tube lens offset: 10 V. Retention time was given in minutes.

*5.1. General method for the preparation of compounds **3**, **4**, and **5***

Compounds **3**, **4**, and **5** were synthesized either by KOH/ NaOH or BF_3-Et_2O catalyzed *Claisen-Schmidt* condensation reaction.

KOH or NaOH catalyzed: To a solution of equimolar amounts of aryl ketone **1** (R^1 = a-c) and aryl aldehyde **2** (R^1 = a-c) in EtOH was added either 50% aqueous solution of KOH or 6 M aqueous NaOH solution and stirred for 2 to 24 h at 20 °C. The mixture was neutralized with 6 M aqueous HCl solution (pH adjusted to 2). The mixture was extracted with ethyl acetate, and washed with water and brine. It was further purified by either recrystallization or column chromatography to yield pure solid compounds.

BF_3-Et_2O catalyzed: To a solution of aryl ketone **1** (R^1 = c) and aryl aldehyde **2** (R^1 = c) in dioxane was added BF_3-Et_2O gradually at 20 °C and stirred for 2 h. The mixture was then extracted with ethyl acetate and washed with water and brine. It was further purified by column chromatography to yield pure solid compound.

*5.2. General method for the preparation of compound **6***

A mixture of aryl ketone **1** (R^3 = d-h), iodine (1.2 eq.) and pyridine (15 eq.) was refluxed at 140 °C for 3 h. Precipitate occurred during reaction which was cooled to room temperature. Then it was filtered and washed with cold pyridine to afford compound **6** (R^3 = d-h) in quantitative yield. Five different pyridinium iodide salts were prepared by this method.

*5.3. General method for the preparation of compounds **7**, **8**, and **9***

A mixture of propenone intermediate **3** (R^1 = a, R^2 = a-c), **4** (R^1 = b, R^2 = a-c), or **5** (R^1 = c, R^2 = a-c), pyridinium iodide salt **6** (R^3 = d-h) and anhydrous ammonium acetate in glacial acetic acid were heated at 90-100 °C for 12-24 h. The reaction mixture was then extracted with ethyl acetate, washed with water and brine. The organic layer was dried with magnesium sulfate and filtered. The filtrate was evaporated at reduced pressure, which was then purified by silica gel column chromatography with the gradient elution of ethyl acetate / *n*-hexane to afford solid compounds **7** (R^1 = a, R^2 = a-c, R^3 = d-h), **8** (R^1 = b, R^2 = a-c, R^3 = d-h), and **9** (R^1 = c, R^2 = a-c, R^3 = d-h) in 20–94% yield. Forty-five dihydroxylated 2,4-diphenyl-6-aryl pyridines (**15-59**) were synthesized by this method.

*5.3.1. 2,2'-[6-(Thiophen-3-yl)pyridine-2,4-diyl]diphenol (**15**)*

The general procedure was employed with **3** (R^1, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = d) (662.34 mg, 2.00 mmol) and acetic acid (2 mL) to yield a yellow solid (140.60 mg, 20.4%, 0.40 mmol). mp 211-212 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.37. LC/MS/MS: retention time: 8.93 min, $[MH]^+$: 346.23

purity by HPLC: 98%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 14.48 (s, 1H, 2-phenyl 2-OH), 10.01 (br, 1H, 4-phenyl 2-OH), 8.26 (dd, *J* = 2.6, 1.1 Hz, 1H, 6-thiophene H-2), 8.22 (s, 1H, pyridine H-3), 8.09 (d, *J* = 8.1 Hz, 1H, 2-phenyl H-6), 8.04 (s, 1H, pyridine H-5), 7.79-7.71 (m, 2H, 6-thiophene H-4, H-5), 7.57 (dd, *J* = 7.6, 1.1 Hz, 1H, 4-phenyl H-6), 7.35-7.27 (m, 2H, 2-phenyl H-4, 4-phenyl H-4), 7.04-6.90 (m, 4H, 2-phenyl H-3, H-5, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.33, 156.45, 155.04, 149.78, 149.74, 140.47, 131.54, 130.68, 128.23, 127.42, 125.99, 125.11, 125.04, 119.84, 119.49, 119.27, 119.14, 118.48, 118.01, 116.55.

*5.3.2. 2,2'-[6-(Furan-2-yl)pyridine-2,4-diyl]diphenol (**16**)*

The general procedure was employed with **3** (R^1, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = e) (630.22 mg, 2.00 mmol) and acetic acid (2 mL) to yield a yellow solid (510.90 mg, 77.6%, 1.55 mmol). mp 215-216 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.43, LC/MS/MS: retention time: 8.66 min, $[MH]^+$: 330.23 purity by HPLC: 100%, ^{1}H NMR (250 MHz, DMSO-d_6) δ 14.27 (s, 1H, 2-phenyl 2-OH), 10.05 (br, 1H, 2-phenyl 2-OH), 8.20 (d, *J* = 0.9 Hz, 1H, pyridine H-3), 8.10 (dd, *J* = 7.9, 1.1 Hz, 1H, 2-phenyl H-6), 7.99 (d, *J* = 1.0 Hz, 1H, pyridine H-5), 7.95 (d, *J* = 1.1 Hz, 1H, 6-furan H-5), 7.57 (dd, *J* = 7.6, 1.5 Hz, 1H, 4-phenyl H-6), 7.36-7.27 (m, 2H, 2-phenyl H-4, 4-phenyl H-4), 7.26 (d, *J* = 3.5 Hz, 1H, 6-furan H-3), 7.04-6.90 (m, 4H, 2-phenyl H-3, H-5, 4-phenyl H-3, H-5), 6.74 (dd, *J* = 3.4, 1.7 Hz, 1H, 6-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.40, 156.63, 155.11, 151.59, 149.58, 145.28, 145.11, 131.73, 130.87, 130.70, 127.51, 124.78, 119.94, 119.17, 119.09, 118.37, 118.19, 117.41, 116.61, 112.87, 110.16.

*5.3.3. 2,2'-[(2,2'-Bipyridine)-4,6-diyl]diphenol (**17**)*

The general procedure was employed with **3** (R^1, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = f) (652.26 mg, 2.00 mmol) and acetic acid (2 mL) to yield a greenish yellow solid (332.30 mg, 48.9%, 0.97 mmol). mp 201-202 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.34. LC/MS/MS: retention time: 8.43 min, $[MH]^+$: 341.27. purity by HPLC: 98%, ^{1}H NMR (250 MHz, DMSO-d_6) δ 14.04 (s, 1H, 6-phenyl 2-OH), 10.06 (br, 1H, 4-phenyl 2-OH), 8.78 (d, *J* = 4.1 Hz, 1H, 2-pyridine H-6'), 8.55 (s, 1H, pyridine H-3), 8.37 (s, 1H, pyridine H-5), 8.22 (d, *J* = 7.9 Hz, 1H, 2-pyridine H-3'), 8.15 (d, *J* = 7.8 Hz, 1H, 6-phenyl H-6), 8.06 (t, *J* = 7.7 Hz, 1H, 2-pyridine H-4'), 7.59 (d, *J* = 7.4 Hz, 1H, 4-phenyl H-6), 7.54 (d, *J* = 7.4 Hz, 1H, 2-pyridine H-5'), 7.37-7.29 (m, 2H, 6-phenyl H-4, 4-phenyl H-4), 7.05-6.93 (m, 4H, 6-phenyl H-3, H-5, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.00, 156.65, 155.14, 154.28, 152.62, 150.10, 149.58, 138.09, 131.62,

130.85, 130.70, 127.93, 124.96, 124.87, 120.67, 120.56, 120.03, 119.86, 119.32, 117.99, 116.66.

*5.3.4. 2,2'-[(2,3'-Bipyridine)-4,6-diyl]diphenol (**18**)*

The general procedure was employed with **3** (R^1, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = g) (652.26 mg, 2.00 mmol) and acetic acid (2 mL) to yield a greenish yellow solid (481.10 mg, 70.7%, 1.41 mmol). mp 268-269 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.26. LC/MS/MS: retention time: 7.69 min, $[MH]^+$: 341.26, purity by HPLC: 95%, ^{1}H NMR (250 MHz, DMSO-d_6) δ 13.87 (br, 1H, 6-phenyl 2-OH), 10.15 (br, 1H, 4-phenyl 2-OH), 9.23 (d, *J* = 1.8 Hz, 1H, 2-pyridine H-2'), 8.72 (dd, *J* = 4.7, 1.3 Hz, 1H, 2-pyridine H-6'), 8.42 (dt, *J* = 8.3, 1.8 Hz, 1H, 2-pyridine H-4'), 8.37 (s, 1H, pyridine H-3), 8.15 (s, 1H, pyridine H-5), 8.11 (d, *J* = 7.3 Hz, 1H, 6-phenyl H-6), 7.63-7.59 (m, 1H, 2-pyridine H-5'), 7.62 (d, *J* = 7.4 Hz, 1H, 4-phenyl H-6), 7.37-7.28 (m, 2H, 6-phenyl H-4, 4-phenyl H-4), 7.05-6.92 (m, 4H, 6-phenyl H-3, H-5, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.97, 157.01, 155.24, 151.69, 150.64, 149.77, 148.01, 134.46, 133.80, 131.69, 130.87, 127.90, 124.85, 124.40, 120.20, 119.91, 119.83, 119.40, 117.99, 116.64.

*5.3.5. 2,2'-[(2,4'-Bipyridine)-4,6-diyl]diphenol (**19**)*

The general procedure was employed with **3** (R^1, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = h) (652.26 mg, 2.00 mmol) and acetic acid (2 mL) to yield a greenish yellow solid (233.50 mg, 34.3%, 0.68 mmol). mp 234-235 °C; R_f (ethyl acetate/*n*-hexane 2:1 v/v): 0.24. LC/MS/MS: retention time: 7.68 min, $[MH]^+$: 341.28. purity by HPLC: 95%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 13.66 (s, 1H, 6-phenyl 2-OH), 10.07 (s, 1H, 4-phenyl 2-OH), 8.79 (d, *J* = 4.6 Hz, 2H, 2-pyridine H-2', H-6'), 8.40 (s, 1H,

pyridine H-3), 8.21 (s, 1H, pyridine H-5), 8.12 (d, *J* = 7.3 Hz, 1H, 6-phenyl H-6), 8.02 (dd, *J* = 4.6, 1.5 Hz, 2H, 2-pyridine H-3', H-5'), 7.62 (dd, *J* = 7.6, 1.5 Hz, 1H, 4-phenyl H-6), 7.37-7.29 (m, 2H, 6-phenyl H-4, 4-phenyl H-4), 7.05-6.93 (m, 4H, 6-phenyl H-3, H-5, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.82, 157.07, 155.15, 151.51, 150.92, 149.80, 145.13, 131.73, 130.93, 130.86, 128.07, 124.74, 121.10, 121.03, 120.47, 119.96, 119.94, 119.44, 117.94, 116.62.

*5.3.6. 2-[4-(3-Hydroxyphenyl)-6-(thiophen-3-yl)pyridin-2-yl]phenol (**20**)*

The general procedure was employed with **3** (R^1 = a, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = d) (496.75 mg, 1.5 mmol) and acetic acid (2 mL) to yield a yellow solid (471.00 mg, 90.90%, 1.36 mmol). mp 209-210 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.37. LC/MS/MS: retention time: 8.80 min, $[MH]^+$: 346.23. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 14.40 (br, 1H, 2-phenyl 2-OH), 9.80 (br, 1H, 4-phenyl 3-OH), 8.36 (dd, *J* = 2.7, 1.4 Hz, 1H, 6-thiophene H-2), 8.27 (s, 1H, pyridine H-3), 8.23 (d, *J* = 8.1 Hz, 1H, 2-phenyl H-6), 8.12 (d, *J* = 0.8 Hz, 1H, pyridine H-5), 7.81 (dd, *J* = 5.0, 1.3 Hz, 1H, 6-thiophene H-4), 7.77 (dd, *J* = 5.0, 2.8 Hz, 1H, 6-thiophene H-5), 7.45 (t, *J* = 7.8 Hz, 1H, 2-phenyl H-4), 7.39-7.30 (m, 3H, 4-phenyl H-5, H-6, H-2), 6.97-6.92 (m, 3H, 4-phenyl H-4, 2-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 150.36, 149.24, 148.28, 141.98, 141.60, 131.37, 129.83, 122.71, 121.34, 119.20, 118.87, 117.15, 116.52, 110.26, 110.13, 109.40, 108.99, 107.87, 107.80, 106.91, 105.52.

*5.3.7. 2-[6-(Furan-2-yl)-4-(3-hydroxyphenyl)pyridin-2-yl]phenol (**21**)*

The general procedure was employed with **3** (R^1 = a, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = e) (472.66 mg, 1.50 mmol) and acetic acid (2 mL) to yield a yellow solid (395.70 mg, 80.1%, 1.20 mmol). mp 229-230 °C; R_f (ethyl

acetate/*n*-hexane 1:1 v/v): 0.63. LC/MS/MS: retention time: 8.50 min, $[MH]^+$: 330.23. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 14.24 (br, 1H, 2-phenyl 2-OH), 9.74 (br, 1H, 4-phenyl 3-OH), 8.25 (d, *J* = 1.3 Hz, 1H, pyridine H-3), 8.23 (d, *J* = 7.9 Hz, 1H, 2-phenyl H-6), 8.00 (s, 1H, pyridine H-5), 7.96 (d, *J* = 1.2 Hz, 1H, 6-furan H-5), 7.43-7.30 (m, 5H, 2-phenyl H-4, 4-phenyl H-5, H-6, H-2, 6-furan H-3), 6.97-6.91 (m, 3H, 2-phenyl H-3, H-5, 4-phenyl H-4), 6.75 (dd, *J* = 3.4, 1.7 Hz, 1H, 6-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.44, 158.24, 157.33, 151.41, 150.77, 145.92, 145.36, 138.60, 131.83, 130.43, 127.82, 119.09, 119.01, 118.31, 118.14, 116.91, 115.78, 114.67, 114.34, 112.87, 110.67.

*5.3.8. 2-[4-(3-Hydroxyphenyl)-(2,2'-bipyridin)-6-yl]phenol (**22**)*

The general procedure was employed with **3** (R^1 = a, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = f) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield a yellow solid (448.20 mg, 87.8%, 1.31 mmol). mp 277-278 °C; R_f (ethyl acetate/*n*-hexane 1:2 v/v): 0.29. LC/MS/MS: retention time: 8.29 min, $[MH]^+$: 341.27. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 13.93 (s, 1H, 6-phenyl 2-OH), 9.75 (s, 1H, 4-phenyl 3-OH), 8.79 (d, *J* = 4.4 Hz, 1H, 2-pyridine H-6'), 8.50 (s, 1H, pyridine H-3), 8.43 (s, 1H, pyridine H-5), 8.28 (d, *J* = 6.8 Hz, 1H, 2-pyridine H-3'), 8.23 (d, *J* = 7.1 Hz, 1H, 6-phenyl H-6), 8.05 (td, *J* = 7.8, 1.6 Hz, 1H, 2-pyridine H-4'), 7.57 (dd, *J* = 6.5, 4.8 Hz, 1H, 2-pyridine H-5'), 7.43-7.32 (m, 4H, 6-phenyl H-4, 4-phenyl H-5, H-6, H-2), 6.99-6.93 (m, 3H, 4-phenyl H-4, 6-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.98, 158.30, 157.37, 154.02, 153.55, 150.66, 150.07, 138.77, 138.05, 131.72, 130.58, 128.26, 124.97, 120.79, 119.84, 119.25, 118.23, 118.15, 117.93, 117.00, 116.92, 114.15.

*5.3.9. 2-[4-(3-Hydroxyphenyl)-(2,3'-bipyridin)-6-yl]phenol (**23**)*

The general procedure was employed with **3** (R^1 = a, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = g) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield a light yellow solid (442.80 mg, 86.8%, 1.30 mmol). mp 249-250 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.29. LC/MS/MS: retention time: 7.52 min, $[MH]^+$: 341.26. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 13.79 (s, 1H, 6-phenyl 2-OH), 9.73 (s, 1H, 4-phenyl 3-OH), 9.30 (d, *J* = 1.8 Hz, 1H, 2-pyridine H-2'), 8.72 (d, *J* = 4.2 Hz, 1H, 2-pyridine H-6'), 8.49 (d, *J* = 8.0 Hz, 1H, 2-pyridine H-4'), 8.38 (s, 1H, pyridine H-3), 8.28 (d, *J* = 7.7 Hz, 6-phenyl H-6), 8.22 (s, 1H, pyridine H-5), 7.62 (dd, *J* = 7.9, 4.8 Hz, 1H, 2-pyridine H-5'), 7.46 (t, *J* = 7.6 Hz, 1H, 6-phenyl H-4), 7.40 (t, *J* = 7.2 Hz, 1H, 4-phenyl H-5), 7.37-7.32 (m, 2H, 4-phenyl H-2, H-6), 6.98-6.93 (m, 3H, 4-phenyl H-4, 2-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.94, 158.23, 157.74, 152.53, 150.98, 150.68, 148.16, 138.73, 134.55, 133.59, 131.77, 130.39, 128.27, 124.28, 119.80, 119.32, 118.54, 117.91, 117.65, 117.37, 116.88, 114.58.

*5.3.10. 2-[4-(3-Hydroxyphenyl)-(2,4'-bipyridin)-6-yl]phenol (**24**)*

The general procedure was employed with **3** (R^1 = a, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = h) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield a yellow solid (366.00 mg, 71.7%, 1.07 mmol). mp 269-270 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.23. LC/MS/MS: retention time: 7.53 min, $[MH]^+$: 341.28. purity by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 13.59 (br, 1H, 6-phenyl 2-OH), 9.75 (br, 1H, 4-phenyl 3-OH), 8.80 (dd, *J* = 4.4, 1.5 Hz, 2H, 2-pyridine H-2', H-6'), 8.43 (s, 1H, pyridine H-3), 8.27 (s, 1H, pyridine H-5), 8.25 (d, *J* = 5.4 Hz, 1H, 6-phenyl H-6), 8.10 (dd, *J* = 4.7, 1.6 Hz, 2H, 2-pyridine H-3', H-5'), 7.48 (t, *J* = 7.7 Hz, 1H, 6-phenyl H-4), 7.40 (t, *J* = 7.3 Hz, 1H, 4-phenyl H-5), 7.39-7.32 (m, 2H, 4-phenyl H-6, H-2), 6.99-6.94 (m, 3H, 4-phenyl H-4, 6-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.80, 158.26, 157.77,

152.38, 151.04, 150.84, 144.93, 138.61, 131.82, 130.42, 128.43, 121.20, 119.94, 119.37, 118.53, 117.92, 117.87, 116.93, 114.58.

*5.3.11. 2-[4-(4-Hydroxyphenyl)-6-(thiophen-3-yl)pyridin-2-yl]phenol (**25**)*

The general procedure was employed with **3** (R^1 = a, R^2 = c) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = d) (496.75 mg, 1.50 mmol) and acetic acid (2 mL) to yield a yellow solid (208.20 mg, 40.2%, 0.60 mmol). mp 233-234 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.58. LC/MS/MS: retention time: 8.65 min, $[MH]^+$: 346.23 purity by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 14.64 (br, 1H, 2-phenyl 2-OH), 9.95 (br, 1H, 4-phenyl 4-OH), 8.33 (dd, *J* = 2.4, 1.4 Hz, 1H, 6-thiophene H-2), 8.28 (s, 1H, pyridine H-3), 8.25 (d, *J* = 8.0 Hz, 1H, 2-phenyl H-6), 8.12 (s, 1H, pyridine H-5), 7.97 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.81 (dd, *J* = 5.4, 1.3 Hz, 1H, 6-thiophene H-4), 7.77 (dd, *J* = 4.9, 2.9 Hz, 1H, 6-thiophene H-5), 7.33 (t, *J* = 8.2 Hz, 1H, 2-phenyl H-4), 6.95-6.92 (m, 2H, 2-phenyl H-3, H-5), 6.96 (d, *J* = 8.49 Hz, 2H, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.52, 159.44, 157.25, 150.51, 150.41, 140.49, 131.65, 129.15, 128.17, 127.77, 127.62, 126.17, 125.33, 119.20, 119.05, 118.01, 116.08, 115.88, 114.66.

*5.3.12. 2-[6-(Furan-2-yl)-4-(4-hydroxyphenyl)pyridin-2-yl]phenol (**26**)*

The general procedure was employed with **3** (R^1 = a, R^2 = c) (288.30 mg, 1.20 mmol), dry ammonium acetate (924.96 mg, 12.00 mmol), **6** (R^3 = e) (378.13 mg, 1.20 mmol) and acetic acid (2 mL) to yield a yellow solid (278.60 mg, 70.5%, 0.84 mmol). mp 241-242 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.48. LC/MS/MS: retention time: 8.39 min, $[MH]^+$: 330.23. purity by HPLC: 98%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 14.46 (br, 1H, 2-phenyl 2-OH), 9.99 (br, 1H, 4-phenyl 4-OH), 8.28 (d, *J* = 8.1 Hz, 1H, 2-phenyl H-6), 8.26 (s, 1H, pyridine H-3), 8.02 (s, 1H, pyridine H-5), 7.96 (d, *J* = 0.8 Hz, 1H, 6-furan H-5), 7.94 (d, *J* = 8.6 Hz,

2H, 4-phenyl H-2, H-6), 7.37 (d, *J* = 3.1 Hz, 1H, 6-furan H-3), 7.33 (t, *J* = 8.3 Hz, 1H, 2-phenyl H-4), 6.96-6.92 (m, 2H, 2-phenyl H-3, H-5), 6.95 (d, *J* = 8.5 Hz, 2H, 4-phenyl H-3, H-5), 6.75 (dd, *J* = 3.3, 1.7 Hz, 1H, 6-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.60, 159.54, 157.28, 151.54, 150.34, 145.77, 145.28, 131.78, 129.09, 127.75, 127.42, 118.99, 118.18, 116.15, 114.57, 113.71, 112.86, 110.50.

*5.3.13. 2-[4-(4-Hydroxyphenyl)-(2,2'-bipyridin)-6-yl]phenol (**27**)*

The general procedure was employed with **3** (R^1 = a, R^2 = c) (240.25 mg, 1.00 mmol), dry ammonium acetate (770.80 mg, 10.00 mmol), **6** (R^3 = f) (326.13 mg, 1.00 mmol) and acetic acid (2 mL) to yield a light yellow solid (117.50 mg, 34.5%, 0.34 mmol). mp 259-260 °C; R_f (ethyl acetate/*n*-hexane 2:1 v/v): 0.13. LC/MS/MS: retention time: 8.14 min, $[MH]^+$: 341.27. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 14.27 (br, 1H, 6-phenyl 2-OH), 10.01 (br, 1H, 4-phenyl 4-OH), 8.79 (d, *J* = 4.0 Hz, 1H, 2-pyridine H-6'), 8.50 (s, 1H, pyridine H-3), 8.42 (s, 1H, pyridine H-5), 8.30 (d, *J* = 8.1 Hz, 1H, 2-pyridine H-3'), 8.23 (d, *J* = 7.9 Hz, 1H, 6-phenyl H-6), 8.06 (td, *J* = 7.8, 1.7 Hz, 1H, 2-pyridine H-4'), 7.92 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.54 (dd, *J* = 7.3, 4.8 Hz, 1H, 2-pyridine H-5'), 7.34 (td, *J* = 7.2, 1.3 Hz, 1H, 6-phenyl H-4), 6.98-6.93 (m, 2H, 6-phenyl H-3, H-5), 6.97 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.51, 159.25, 157.36, 154.14, 153.32, 150.45, 150.10, 138.07, 131.71, 129.04, 128.10, 127.69, 124.95, 120.80, 119.62, 119.17, 118.01, 116.85, 116.28, 116.20.

*5.3.14. 2-[4-(4-Hydroxyphenyl)-(2,3'-bipyridin)-6-yl]phenol (**28**)*

The general procedure was employed with **3** (R^1 = a, R^2 = c) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = g) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield a light yellow solid (190.00 mg, 37.2%, 0.55 mmol). mp 324-325 °C; R_f

(ethyl acetate: *n*-hexane 1:1 v/v): 0.10. LC/MS/MS: retention time: 7.41 min, $[MH]^+$: 341.26. purity by HPLC: 95%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 14.11 (br, 1H, 6-phenyl 2-OH), 9.99 (br, 1H, 4-phenyl 4-OH), 9.29 (d, *J* = 1.7 Hz, 1H, 2-pyridine H-2'), 8.70 (dd, *J* = 4.7, 1.2 Hz, 1H, 2-pyridine H-6'), 8.46 (d, *J* = 8.1 Hz, 1H, 2-pyridine H-4'), 8.38 (s, 1H, pyridine H-3), 8.29 (d, *J* = 7.8 Hz, 1H, 6-phenyl H-6), 8.22 (s, 1H, pyridine H-5), 8.00 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.62 (dd, *J* = 7.9, 4.7 Hz, 1H, 2-pyridine H-5'), 7.34 (t, *J* = 7.0 Hz, 1H, 6-phenyl H-4), 6.99-6.92 (m, 2H, 6-phenyl H-3, H-5), 6.96 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.57, 159.22, 157.78, 152.31, 150.69, 150.65, 148.17, 134.54, 133.70, 131.79, 129.33, 128.12, 127.55, 124.34, 119.60, 119.27, 118.00, 116.73, 116.16, 115.99.

*5.3.15. 2-[4-(4-Hydroxyphenyl)-(2,4'-bipyridin)-6-yl]phenol (**29**)*

The general procedure was employed with **3** (R^1 = a, R^2 = c) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = h) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield a light yellow solid (233.40 mg, 45.7%, 0.68 mmol). mp 358-359 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.10. LC/MS/MS: retention time: 7.40 min, $[MH]^+$: 341.29. purity by HPLC: 95%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 13.84 (br, 1H, 6-phenyl 2-OH), 9.82 (br, 1H, 4-phenyl 4-OH), 8.80 (dd, *J* = 4.6, 1.4 Hz, 2H, 2-pyridine H-2', H-6'), 8.42 (s, 1H, pyridine H-3), 8.28-8.26 (m, 2H, 6-phenyl H-6, pyridine H-5), 8.07 (dd, *J* = 4.6, 1.5 Hz, 2H, 2-pyridine H-3', H-5'), 7.99 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 9.37 (td, *J* = 7.8, 0.9 Hz, 1H, 6-phenyl H-4), 7.00-6.93 (m, 2H, 6-phenyl H-3, H-5), 6.97 (d, *J* = 8.5 Hz, 2H, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.52, 158.95, 157.71, 152.09, 150.73, 144.94, 131.66, 129.16, 128.11, 127.34, 121.06, 119.64, 119.17, 117.83, 116.98, 116.87, 116.07, 115.54.

*5.3.16. 2-[2-(3-Hydroxyphenyl)-6-(thiophen-3-yl)pyridin-4-yl]phenol (**30**)*

The general procedure was employed with **4** (R^1 = b, R^2 = a) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = d) (496.75 mg, 1.50 mmol) and acetic acid (2 mL) to yield a white solid (299.30 mg, 57.8%, 0.86 mmol). mp 162-163 °C; R_f (ethyl acetate/*n*-hexane 1: 1 v/v): 0.56. LC/MS/MS: retention time: 7.81 min, $[MH]^+$: 346.22. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.88 (br, 1H, 4-phenyl 2-OH), 9.60 (br, 1H, 2-phenyl 3-OH), 8.28 (dd, *J* = 2.9, 1.0 Hz, 1H, 6-thiophene H-2), 7.94 (s, 1H, pyridine H-3), 7.90 (d, *J* = 1.2 Hz, 1H, pyridine H-5), 7.89 (dd, *J* = 4.9, 1.0 Hz, 1H, 6-thiophene H-4), 7.69 (dd, *J* = 5.0, 3.0 Hz, 1H, 6-thiophene H-5), 7.62 (t, *J* = 1.9 Hz, 1H, 2-phenyl H-2), 7.59 (d, *J* = 7.9 Hz, 1H, 2-phenyl H-6), 7.52 (dd, *J* = 7.6, 1.5 Hz, 1H, 4-phenyl H-6), 7.30 (t, *J* = 7.9 Hz, 1H, 2-phenyl H-5), 7.26 (td, *J* = 7.6, 1.5 Hz, 1H, 4-phenyl H-4), 7.02 (d, *J* = 8.1 Hz, 1H, 4-phenyl H-3), 6.95 (td, *J* = 7.8, 1.1 Hz, 1H, 4-phenyl H-5), 6.85 (dd, *J* = 7.8, 2.0 Hz, 1H, 2-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 157.97, 155.66, 155.01, 152.56, 148.34, 142.54, 140.56, 130.60, 130.29, 129.97, 127.16, 126.82, 125.50, 124.33, 119.85, 119.26, 118.96, 117.66, 116.52, 116.26, 113.72.

*5.3.17. 2-[2-(Furan-2-yl)-6-(3-hydroxyphenyl)pyridin-4-yl]phenol (**31**)*

The general procedure was employed with **4** (R^1 = b, R^2 = a) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = e) (472.66 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (296.30 mg, 60.0%, 0.89 mmol). mp 199-200 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.55. LC/MS/MS: retention time: 7.50 min, $[MH]^+$: 330.21. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.93 (s, 1H, 4-phenyl 2-OH), 9.58 (s, 1H, 6-phenyl 3-OH), 7.88 (s, 2H, pyridine H-3, H-5), 7.86 (d, *J* = 0.8 Hz, 1H, 2-furan H-5), 7.60 (d, *J* = 1.6 Hz, 1H, 6-phenyl H-2), 7.58 (d, *J* = 8.3 Hz, 1H, 6-phenyl H-6), 7.53 (dd, *J* = 7.6, 1.4 Hz, 1H, 4-phenyl H-6), 7.30 (t, *J* = 7.9 Hz, 1H, 6-phenyl H-5), 7.27 (td, *J* = 7.7, 1.6 Hz,

1H, 4-phenyl H-4), 7.22 (d, *J* = 3.3 Hz, 1H, 2-furan H-3), 7.03 (d, *J* = 8.1 Hz, 1H, 4-phenyl H-3), 6.95 (t, *J* = 7.7 Hz, 1H, 4-phenyl H-5), 6.86 (dd, *J* = 7.8, 2.0 Hz, 1H, 6-phenyl H-4), 6.69 (dd, *J* = 3.3, 1.7 Hz, 1H, 2-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 157.95, 156.17, 155.00, 153.65, 148.39, 148.16, 144.33, 140.24, 130.45, 129.96, 125.10, 119.96, 119.00, 117.72, 117.26, 116.59, 116.40, 113.74, 112.52, 109.14.

*5.3.18. 2-[6-(3-Hydroxyphenyl)-(2,2'-bipyridin)-4-yl]phenol (**32**)*

The general procedure was employed with **4** (R^1 = b, R^2 = a) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = f) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield a yellow solid (377.60 mg, 74.0%, 1.10 mmol). mp 198-199 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.47. LC/MS/MS: retention time: 7.28 min, $[MH]^+$: 341.28. purity by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.86 (br, 1H, 4-phenyl 2-OH), 9.61 (br, 1H, 6-phenyl 3-OH), 8.71 (d, *J* = 3.9 Hz, 1H, 2-pyridine H-6'), 8.59-8.57 (m, 2H, 2-pyridine H-3', pyridine H-3), 8.06 (s, 1H, pyridine H-5), 8.00 (t, *J* = 7.7 Hz, 1H, 2-pyridine H-4'), 7.69 (s, 1H, 6-phenyl H-2), 7.65 (d, *J* = 7.7 Hz, 1H, 6-phenyl H-6), 7.54 (t, *J* = 8.3 Hz, 1H, 2-pyridine H-5'), 7.48 (d, *J* = 7.1 Hz, 1H, 4-phenyl H-6), 7.35 (t, *J* = 7.9 Hz, 1H, 6-phenyl H-5), 7.29 (t, *J* = 8.0 Hz, 1H, 4-phenyl H-4), 7.04 (d, *J* = 8.1 Hz, 1H, 4-phenyl H-3), 6.97 (t, *J* = 7.5 Hz, 1H, 4-phenyl H-5), 6.88 (d, *J* = 7.7 Hz, 1H, 6-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 157.97, 155.77, 155.72, 154.96, 154.88, 149.43, 148.34, 140.33, 137.45, 130.35, 129.95, 125.33, 124.33, 120.81, 120.59, 119.91, 119.49, 117.67, 116.58, 116.34, 113.71.

*5.3.19. 2-[6-(3-Hydroxyphenyl)-(2,3'-bipyridin)-4-yl]phenol (**33**)*

The general procedure was employed with **4** (R^1 = b, R^2 = a) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = g) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (265.60 mg, 52.0%, 0.78 mmol). mp 250-251 °C; R_f (ethyl

acetate/*n*-hexane 1:1 v/v): 0.23. LC/MS/MS: retention time: 6.66 min, $[MH]^+$: 341.25. purity by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.94 (br, 1H, 4-phenyl 2-OH), 9.59 (br, 1H, 6-phenyl 3-OH), 9.40 (d, *J* = 1.7 Hz, 1H, 2-pyridine H-2'), 8.66 (dd, *J* = 4.7, 1.4 Hz, 1H, 2-pyridine H-6'), 8.57 (dt, *J* = 8.0, 1.7 Hz, 1H, 2-pyridine H-4'), 8.12 (d, *J* = 0.9 Hz, 1H, pyridine H-3), 8.06 (d, *J* = 0.8 Hz, 1H, pyridine H-5), 7.68 (t, *J* = 1.8 Hz, 1H, 6-phenyl H-2), 7.62 (d, *J* = 7.4 Hz, 1H, 6-phenyl H-6), 7.59 (d, *J* = 7.5 Hz, 1H, 4-phenyl H-6), 7.56 (dd, *J* = 7.9, 5.0 Hz, 1H, 2-pyridine H-5'), 7.31 (t, *J* = 7.8 Hz, 1H, 6-phenyl H-5), 7.28 (td, *J* = 7.8, 1.5 Hz, 1H, 4-phenyl H-4), 7.04 (d, *J* = 8.1 Hz, 1H, 4-phenyl H-3), 6.96 (td, *J* = 7.9, 1.0 Hz, 1H, 4-phenyl H-5), 6.87 (dd, *J* = 7.9, 2.1 Hz, 1H, 6-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.02, 156.11, 155.03, 153.61, 150.06, 148.63, 148.23, 140.33, 134.64, 134.36, 130.69, 130.44, 130.04, 125.22, 124.00, 119.94, 119.90, 119.62, 117.70, 116.55, 116.44, 113.74.

*5.3.20. 2-[6-(3-Hydroxyphenyl)-(2,4'-bipyridin)-4-yl]phenol (**34**)*

The general procedure was employed with **4** (R^1 = b, R^2 = a) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = h) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (204.60 mg, 40.0%, 0.60 mmol). mp 263-264 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.13. LC/MS/MS: retention time: 6.69 min, $[MH]^+$: 341.27. purity by HPLC: 95%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.97 (br, 1H, 4-phenyl 2-OH), 9.61 (br, 1H, 6-phenyl 3-OH), 8.74 (d, *J* = 4.5 Hz, 2H, 2-pyridine H-2', H-6'), 8.20-8.18 (m, 3H, 2-pyridine H-3', H-5', pyridine H-3), 8.11 (s, 1H, pyridine H-5), 7.67 (s, 1H, 6-phenyl H-2), 7.63-7.56 (m, 2H, 6-phenyl H-6, 4-phenyl H-6), 7.35-7.27 (m, 2H, 6-phenyl H-5, 4-phenyl H-4), 7.05 (d, *J* = 8.7 Hz, 1H, 4-phenyl H-3), 6.97 (t, *J* = 7.5 Hz, 1H, 4-phenyl H-5), 6.89 (d, *J* = 7.3 Hz, 1H, 6-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.03, 156.24, 155.03, 153.25, 150.55, 148.77, 146.14, 140.19, 130.69, 130.53, 130.07, 125.07, 121.16, 120.94, 120.02, 119.92, 117.76, 116.55, 113.76.

*5.3.21. 3,3'-[6-(Thiophen-3-yl)pyridine-2,4-diyl]diphenol (**35**)*

The general procedure was employed with **4** (R^1, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = d) (496.75 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (317.00 mg, 61.2%, 0.91 mmol). mp 206-207 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.50. LC/MS/MS: retention time: 7.54 min, $[MH]^+$: 346.22. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.65 (br, 2H, 2-phenyl 3-OH, 4-phenyl 3-OH), 8.40 (dd, *J* = 2.9, 1.1 Hz, 1H, 6-thiophene H-2), 8.04 (d, *J* = 0.9 Hz, 1H, pyridine H-3), 7.97 (dd, *J* = 5.0, 1.1 Hz, 6-thiophene H-4), 7.93 (d, *J* = 0.9 Hz, 1H, pyridine H-5), 7.70-7.66 (m, 3H, 2-phenyl H-2, H-6, 6-thiophene H-5), 7.40-7.28 (m, 4H, 2-phenyl H-5, 4-phenyl H-5, H-6, H-2), 6.91 (td, *J* = 8.2, 2.3 Hz, 2H, 2-phenyl H-4, 4-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.24, 158.01, 156.56, 153.29, 149.81, 142.42, 140.35, 139.40, 130.41, 130.00, 127.20, 126.98, 124.80, 118.22, 117.88, 116.78, 116.45, 116.24, 114.24, 113.90.

*5.3.22. 3,3'-[6-(Furan-3-yl)pyridine-2,4-diyl]diphenol (**36**)*

The general procedure was employed with **4** (R^1, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = e) (472.66 mg, 1.50 mmol) and acetic acid (2 mL) to yield a light yellow solid (465.30 mg, 94.2%, 1.41 mmol). mp 222-223 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.52. LC/MS/MS: retention time: 7.27 min, $[MH]^+$: 330.22. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.67 (br, 2H, 2-phenyl 3-OH, 4-phenyl 3-OH), 7.94 (s, 1H, pyridine H-3), 7.89 (dd, *J* = 1.7, 0.7 Hz, 1H, 6-furan H-5), 7.85 (s, 1H, pyridine H-5), 7.66 (d, *J* = 1.1 Hz, 1H, 2-phenyl H-2), 7.63 (d, *J* = 6.5 Hz, 1H, 2-phenyl H-6), 7.36-7.27 (m, 4H, 2-phenyl H-5, 4-phenyl H-5, H-6, H-2), 7.31 (d, *J* = 3.7 Hz, 1H, 6-furan H-3), 6.93-6.84 (m, 2H, 2-phenyl H-4, 4-phenyl H-4), 6.70 (dd, *J* = 3.3, 1.7 Hz, 1H, 6-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.29, 158.00, 157.00, 153.45, 149.65,

149.18, 144.61, 140.04, 139.08, 130.56, 130.02, 118.07, 117.95, 116.63, 116.50, 114.62, 114.00, 113.93, 112.66, 109.68.

*5.3.23. 3,3'-[(2,2'-Bipyridine)-4,6-diyl]diphenol (**37**)*

The general procedure was employed with **4** (R^1, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = f) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (411.20 mg, 80.6%, 1.20 mmol). mp 164-165 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.43. LC/MS/MS: retention time: 7.11 min, $[MH]^+$: 341.27. purity by HPLC: 98%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.69 (br, 2H, 6-phenyl 3-OH, 4-phenyl 3-OH), 8.74 (ddd, *J* = 5.6, 1.7, 0.9 Hz, 1H, 2-pyridine H-6'), 8.60 (d, *J* = 7.9 Hz, 1H, 2-pyridine H-3'), 8.55 (d, *J* = 1.4 Hz, 1H, pyridine H-3), 8.13 (d, *J* = 1.4 Hz, 1H, pyridine H-5), 8.02 (td, *J* = 7.8, 1.8 Hz, 1H, 2-pyridine H-4'), 7.75 (d, *J* = 1.9 Hz, 1H, 6-phenyl H-2), 7.71 (d, *J* = 8.2 Hz, 1H, 6-phenyl H-6), 7.50 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H, 2-pyridine H-5'), 7.38-7.30 (m, 4H, 6-phenyl H-5, 4-phenyl H-5, H-6, H-2), 6.93-6.90 (m, 2H, 6-phenyl H-4, 4-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.35, 158.08, 156.71, 155.74, 155.43, 149.77, 149.63, 140.12, 139.21, 137.71, 130.66, 130.08, 124.73, 120.98, 118.17, 118.01, 117.97, 116.63, 113.94, 113.88.

*5.3.24. 3,3'-[(2,3'-Bipyridine)-4,6-diyl]diphenol (**38**)*

The general procedure was employed with **4** (R^1, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = g) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (416.60 mg, 81.6%, 1.22 mmol). mp 294-295 °C; R_f (ethyl acetate/*n*-hexane 2:1 v/v): 0.32. LC/MS/MS: retention time: 6.47 min, $[MH]^+$: 341.27. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.67 (br, 2H, 6-phenyl 3-OH, 4-phenyl 3-OH), 9.48 (s, 1H, 2-pyridine H-2'), 8.67-8.63 (m, 2H, 2-pyridine H-6', H-4'), 8.21 (s, 1H,

pyridine H-3), 8.07 (s, 1H, pyridine H-5), 7.75 (s, 1H, 6-phenyl H-2), 7.73 (d, *J* = 8.04 Hz, 1H, 6-phenyl H-6), 7.57 (dd, *J* = 7.81, 4.86 Hz, 1H, 2-pyridine H-5'), 7.45-7.29 (m, 4H, 6-phenyl H-5, 4-phenyl H-5, H-6, H-2), 6.90 (t, *J* = 8.44 Hz, 2H, 6-phenyl H-4, 4-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.26, 158.06, 156.96, 154.38, 150.27, 150.16, 148.45, 140.13, 139.20, 134.58, 134.46, 130.43, 130.08, 124.04, 118.36, 117.95, 117.30, 116.65, 116.58, 114.37, 113.93.

*5.3.25. 3,3'-[(2,4'-Bipyridine)-4,6-diyl]diphenol (**39**)*

The general procedure was employed with **4** (R^1, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = h) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (335.60 mg, 65.7%, 0.98 mmol). mp 306-307 °C; R_f (ethyl acetate/*n*-hexane 2:1 v/v): 0.28, LC/MS/MS: retention time: 6.45 min, $[MH]^+$: 341.29. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.72 (br, 2H, 6-phenyl 3-OH, 4-phenyl 3-OH), 8.74 (s, 2H, 2-pyridine H-2', H-6'), 8.28 (d, *J* = 5.6 Hz, 2H, 2-pyridine H-3', H-5'), 8.26 (s, 1H, pyridine H-3), 8.12 (s, 1H, pyridine H-5), 7.73 (s, 1H, 6-phenyl H-2), 7.70 (d, *J* = 8.3 Hz, 1H, 6-phenyl H-6), 7.45-7.30 (m, 4H, 6-phenyl H-5, 4-phenyl H-5, H-6, H-2), 6.90 (t, *J* = 8.1 Hz, 2H, 6-phenyl H-4, 4-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.27, 158.08, 157.08, 154.04, 150.59, 150.31, 145.96, 140.01, 139.04, 130.45, 130.10, 121.37, 118.38, 118.01, 117.68, 116.74, 116.65, 114.40, 113.96.

*5.3.26. 3-[4-(4-Hydroxyphenyl)-6-(thiophen-3-yl)pyridin-2-yl]phenol (**40**)*

The general procedure was employed with **4** (R^1 = b, R^2 = c) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = d) (496.75 mg, 1.50 mmol) and acetic acid (2 mL) to yield a white solid (455.72 mg, 88.0%, 1.31 mmol). mp 258-259 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.48. LC/MS/MS: retention time: 7.50 min, $[MH]^+$: 346.21. purity

by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.82 (br, 1H, 4-phenyl 4-OH), 9.55 (br, 1H, 2-phenyl 3-OH), 8.36 (dd, *J* = 3.1, 1.0 Hz, 1H, 6-thiophene H-2), 8.03 (s, 1H, pyridine H-3), 7.96 (dd, *J* = 4.9, 0.9 Hz, 1H, 6-thiophene H-4), 7.94 (s, 1H, pyridine H-5), 7.88 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.69 (s, 1H, 2-phenyl H-2), 7.68 (d, *J* = 7.9 Hz, 1H, 2-phenyl H-6), 7.67 (dd, *J* = 4.9, 3.0 Hz, 1H, 6-thiophene H-5), 7.30 (t, *J* = 7.8 Hz, 1H, 2-phenyl H-5), 6.94 (d, *J* = 8.5 Hz, 2H, 4-phenyl H-3, H-5), 6.86 (dd, *J* = 7.7, 2.1 Hz, 1H, 2-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.96, 157.90, 156.46, 153.15, 149.27, 142.56, 140.50, 129.83, 128.71, 128.26, 126.99, 126.93, 124.47, 117.82, 116.28, 116.04, 115.79, 115.29, 113.87.

*5.3.27. 3-[6-(Furan-2-yl)-4-(4-hydroxyphenyl)pyridin-2-yl]phenol (**41**)*

The general procedure was employed with **4** (R^1 = b, R^2 = c) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = e) (472.66 mg, 1.50 mmol) and acetic acid (2 mL) to yield a light yellow solid (463.90 mg, 93.9%, 1.40 mmol). mp 226-227 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.47. LC/MS/MS: retention time: 7.14 min, $[MH]^+$: 330.22. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.84 (br, 1H, 4-phenyl 4-OH), 9.61 (br, 1H, 2-phenyl 3-OH), 7.94 (s, 1H, pyridine H-3), 7.88 (dd, *J* = 1.5, 0.8 Hz, 1H, 6-furan H-5), 7.86 (s, 1H, pyridine H-5), 7.84 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.65 (s, 1H, 2-phenyl H-2), 7.63 (d, *J* = 6.5 Hz, 1H, 2-phenyl H-6), 7.30 (t, *J* = 7.6 Hz, 1H, 2-phenyl H-5), 7.26 (d, *J* = 3.4 Hz, 1H, 6-furan H-3), 6.93 (d, *J* = 8.5 Hz, 2H, 4-phenyl H-3, H-5), 6.86 (dd, *J* = 7.2, 1.7 Hz, 1H, 2-phenyl H-4), 6.70 (dd, *J* = 3.3, 1.7 Hz, 1H, 6-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.11, 157.94, 156.86, 153.62, 149.24, 149.09, 144.41, 140.22, 129.91, 128.68, 128.02, 117.91, 116.47, 116.17, 115.64, 113.92, 113.75, 112.57, 109.43.

*5.3.28. 3-[4-(4-Hydroxyphenyl)-(2,2'-bipyridin)-6-yl]phenol (**42**)*

The general procedure was employed with **4** (R^1 = b, R^2 = c) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = f) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield a white solid (370.60 mg, 72.6%, 1.08 mmol). mp 296-297 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.31. LC/MS/MS: retention time: 6.93 min, $[MH]^+$: 341.29. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.84 (br, 1H, 4-phenyl 4-OH), 9.60 (br, 1H, 6-phenyl 3-OH), 8.74 (d, *J* = 4.0 Hz, 1H, 2-pyridine H-6'), 8.59 (d, *J* = 7.9 Hz, 1H, 2-pyridine H-3'), 8.54 (d, *J* = 1.0 Hz, 1H, pyridine H-3), 8.11 (s, 1H, pyridine H-5), 8.01 (td, *J* = 7.7, 1.4 Hz, 1H, 2-pyridine H-4'), 7.84 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.75-7.70 (m, 2H, 6-phenyl H-2, H-6), 7.49 (dd, *J* = 6.6, 5.0 Hz, 1H, 2-pyridine H-5'), 7.33 (t, *J* = 7.9 Hz, 1H, 6-phenyl H-5), 6.95 (d, *J* = 8.5 Hz, 2H, 4-phenyl H-3, H-5), 6.89 (dd, *J* = 8.0, 1.8 Hz, 1H, 6-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.04, 157.97, 156.53, 155.61, 155.59, 149.46, 149.43, 140.28, 137.53, 129.91, 128.58, 128.23, 124.49, 120.89, 117.88, 117.28, 116.43, 116.22, 115.76, 113.89.

*5.3.29. 3-[4-(4-Hydroxyphenyl)-(2,3'-bipyridin)-6-yl]phenol (**43**)*

The general procedure was employed with **4** (R^1 = b, R^2 = c) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = g) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (456.90 mg, 89.5%, 1.34 mmol). mp 299-300 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.20. LC/MS/MS: retention time: 6.31 min, $[MH]^+$: 341.26. purity by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.75 (br, 2H, 4-phenyl 4-OH, 6-phenyl 3-OH), 9.47 (s, 1H, 2-pyridine H-2'), 8.67-8.61 (m, 1H, 2-pyridine H-6'), 8.65 (dt, *J* = 7.9, 2.1 Hz, 1H, 2-pyridine H-4'), 8.21 (d, *J* = 0.9 Hz, 1H, pyridine H-3), 8.08 (d, *J* = 1.0 Hz, 1H, pyridine H-5), 7.94 (d, *J* = 8.7 Hz, 2H, 4-phenyl H-2, H-6), 7.75 (d, *J* = 1.9 Hz, 1H, 6-phenyl H-2), 7.72 (d, *J* = 7.9 Hz, 1H, 6-phenyl H-6), 7.56 (dd, *J* = 7.8, 4.9 Hz, 1H, 2-pyridine H-5'), 7.32 (t, *J* = 7.9 Hz, 1H, 6-phenyl H-5), 6.94 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-3, H-5), 6.88 (dd,

J = 8.0, 1.7 Hz, 1H, 6-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.75, 158.61, 157.46, 154.83, 150.76, 150.29, 149.01, 140.91, 135.20, 135.10, 130.58, 129.56, 128.69, 124.58, 118.51, 117.11, 116.94, 116.88, 116.70, 114.53.

*5.3.30. 3-[4-(4-Hydroxyphenyl)-(2,4'-bipyridin)-6-yl]phenol (**44**)*

The general procedure was employed with **4** (R^1 = b, R^2 = c) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = h) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (396.30 mg, 77.6%, 1.16 mmol). mp 304-305 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.29. LC/MS/MS: retention time: 6.35 min, $[MH]^+$: 341.28. purity by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.79 (br, 2H, 4-phenyl 4-OH, 6-phenyl 3-OH), 8.75 (d, *J* = 4.5 Hz, 2H, 2-pyridine H-2', H-6'), 8.28-8.26 (m, 3H, 2-pyridine H-3', H-5', pyridine H-3), 8.13 (s, 1H, pyridine H-5), 7.94 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.74 (d, *J* = 2.0 Hz, 6-phenyl H-2), 7.73 (d, *J* = 8.6 Hz, 1H, 6-phenyl H-6), 7.32 (t, *J* = 7.8 Hz, 1H, 6-phenyl H-5), 6.95 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-3, H-5), 6.88 (dd, *J* = 7.9, 2.2 Hz, 1H, 6-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.25, 158.04, 156.98, 153.89, 150.54, 149.85, 146.13, 140.19, 130.01, 129.00, 127.92, 121.31, 117.97, 117.36, 116.69, 116.61, 116.13, 113.95.

*5.3.31. 2-[2-(4-Hydroxyphenyl)-6-(thiophen-3-yl)pyridin-4-yl]phenol (**45**)*

The general procedure was employed with **5** (R^1 = c, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = d) (662.34 mg, 2.00 mmol) and acetic acid (2 mL) to yield a yellow solid (140.30 mg, 20.3%, 0.40 mmol). mp 242-243 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.51. LC/MS/MS: retention time: 7.71 min, $[MH]^+$: 346.21. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.85 (br, 1H, 4-phenyl 2-OH), 9.75 (br, 1H, 6-phenyl 4-OH), 8.28 (dd, *J* = 3.1, 1.2 Hz, 1H, 6-thiophene H-2), 8.06 (d, *J* = 8.6 Hz, 2H,

2-phenyl H-2, H-6), 7.88-7.85 (m, 3H, 6-thiophene H-4, pyridine H-3, H-5), 7.65 (dd, *J* = 4.9, 3.00 Hz, 1H, 6-thiophene H-5), 7.50 (dd, *J* = 7.6, 1.3 Hz, 1H, 4-phenyl H-6), 7.26 (td, *J* = 8.6, 1.6 Hz, 1H, 4-phenyl H-4), 7.01 (d, *J* = 8.2 Hz, 1H, 4-phenyl H-3), 6.94 (t, *J* = 7.8 Hz, 1H, 4-phenyl H-5), 6.90 (d, *J* = 8.6 Hz, 2H, 2-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.62, 155.73, 154.86, 152.26, 148.12, 142.62, 130.42, 130.05, 130.00, 128.21, 126.87, 126.72, 125.72, 124.03, 119.70, 118.14, 117.74, 116.43, 115.60.

*5.3.32. 2-[2-(Furan-2-yl)-6-(4-hydroxyphenyl)pyridine-4-yl]phenol (**46**)*

The general procedure was employed with **5** (R^1 = c, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = e) (630.22 mg, 2.00 mmol) and acetic acid (2 mL) to yield a red solid (409.30 mg, 62.2%, 1.24 mmol). mp 218-219 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.51. LC/MS/MS: retention time: 7.42 min, $[MH]^+$: 330.22. purity by HPLC: 97%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.88 (br, 1H, 4-phenyl 2-OH), 9.78 (br, 1H, 6-phenyl 4-OH), 8.04 (dd, *J* = 8.6, 1.2 Hz, 2H, 6-phenyl H-2, H-6), 7.84 (d, *J* = 1.3 Hz, 1H, 2-furan H-5), 7.83 (s, 1H, pyridine H-5), 7.79 (s, 1H, pyridine H-3), 7.50 (d, *J* = 7.6 Hz, 1H, 4-phenyl H-6), 7.26 (t, *J* = 8.0 Hz, 1H, 4-phenyl H-4), 7.21 (d, *J* = 3.3 Hz, 1H, 2-furan H-3), 7.01 (d, *J* = 8.2 Hz, 1H, 4-phenyl H-3), 6.94 (t, *J* = 7.5 Hz, 1H, 4-phenyl H-5), 6.89 (dd, *J* = 8.5, 1.2 Hz, 2H, 4-phenyl H-3, H-5), 6.67 (dd, *J* = 3.3, 1,7 Hz, 1H, 2-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.85, 156.23, 154.99, 153.82, 148.18, 148.05, 144.21, 130.45, 130.34, 129.74, 128.40, 125.36, 119.91, 117.89, 116.55, 116.26, 115.69, 112.50, 109.01.

*5.3.33. 2-[6-(4-Hydroxyphenyl)-(2,2'-bipyridin)-4-yl]phenol (**47**)*

The general procedure was employed with **5** (R^1 = c, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = f) (652.26 mg, 2.00 mmol) and acetic acid (2 mL) to yield a red solid (202.30 mg, 29.8%, 0.59 mmol). mp 255-256 °C; R_f (ethyl

acetate/*n*-hexane 2:1 v/v): 0.37. LC/MS/MS: retention time: 7.17 min, $[MH]^+$: 341.27. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.88 (br, 1H, 4-phenyl 2-OH), 9.79 (br, 1H, 6-phenyl 4-OH), 8.70 (d, *J* = 3.9 Hz, 1H, 2-pyridine H-6'), 8.60 (d, *J* = 7.9 Hz, 1H, 2-pyridine H-3'), 8.49 (d, *J* = 1.1 Hz, 1H, pyridine H-3), 8.12 (d, *J* = 8.6 Hz, 2H, 6-phenyl H-2, H-6), 8.01 (d, *J* = 1.3 Hz, 1H, pyridine H-5), 7.99 (td, *J* = 7.8, 1.7 Hz, 1H, 2-pyridine H-4'), 7.52 (dd, *J* = 7.6, 1.4 Hz, 1H, 4-phenyl H-6), 7.46 (ddd, *J* = 7.4, 4.9, 0.9 Hz, 1H, 2-pyridine H-5'), 7.28 (td, *J* = 8.2, 1.5 Hz, 1H, 4-phenyl H-4), 7.03 (d, *J* = 8.2 Hz, 1H, 4-phenyl H-3), 6.95 (t, *J* = 8.1 Hz, 1H, 4-phenyl H-5), 6.92 (d, *J* = 8.7 Hz, 2H, 6-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.85, 155.94, 155.89, 155.00, 154.68, 149.46, 148.29, 137.54, 130.49, 130.32, 129.93, 128.43, 125.62, 124.35, 120.94, 119.95, 119.59, 118.60, 116.56, 115.77.

*5.3.34. 2-[6-(4-Hydroxyphenyl)-(2,3'-bipyridin)-4-yl]phenol (**48**)*

The general procedure was employed with **5** (R^1 = c, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = g) (652.26 mg, 2.00 mmol) and acetic acid (2 mL) to yield a red solid (138.10 mg, 20.2%, 0.40 mmol). mp 251-252 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.17. LC/MS/MS: retention time: 6.55 min, $[MH]^+$: 341.25. purity by HPLC: 96%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.85 (br, 2H, 4-phenyl 2-OH, 6-phenyl 4-OH), 9.37 (s, 1H, 2-pyridine H-2'), 8.65 (d, *J* = 4.5 Hz, 1H, 2-pyridine H-6'), 8.57 (d, *J* = 7.9 Hz, 2-pyridine H-4'), 8.09 (d, *J* = 8.3 Hz, 2H, 6-phenyl H-2, H-6), 8.02 (s, 1H, pyridine H-3), 7.99 (s, 1H, pyridine H-5), 7.56-7.51 (m, 2H, 2-pyridine H-5', 4-phenyl H-6), 7.27 (t, *J* = 7.7 Hz, 1H, 4-phenyl H-4), 7.03 (d, *J* = 8.1 Hz, 1H, 4-phenyl H-3), 6.95 (t, *J* = 7.7 Hz, 1H, 4-phenyl H-5), 6.91 (d, *J* = 8.4 Hz, 2H, 6-phneyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.92, 156.31, 155.06, 153.39, 150.01, 148.55, 148.22, 134.83, 134.42, 130.72, 130.37, 129.92, 128.48, 125.50, 124.06, 119.89, 118.92, 118.66, 116.55, 115.80.

*5.3.35. 2-[6-(4-Hydroxyphenyl)-(2,4'-bipyridin)-4-yl]phenol (**49**)*

The general procedure was employed with **5** (R^1 = c, R^2 = a) (480.50 mg, 2.00 mmol), dry ammonium acetate (1.54 g, 20.00 mmol), **6** (R^3 = h) (652.26 mg, 2.00 mmol) and acetic acid (2 mL) to yield a brown solid (287.10 mg, 42.1%, 0.84 mmol). mp 279-280 °C; R_f (ethyl acetate/*n*-hexane 2:1 v/v): 0.11. LC/MS/MS: retention time: 6.57 min, $[MH]^+$: 341.27. purity by HPLC: 95%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.91 (br, 1H, 4-phenyl 2-OH), 9.81 (br, 1H, 6-phenyl 4-OH), 8.72 (dd, *J* = 4.6, 1.4 Hz, 2H, 2-pyridine H-2', H-6'), 8.18 (dd, *J* = 4.6, 1.4 Hz, 2H, 2-pyridine H-3', H-5'), 8.11 (d, *J* = 8.6 Hz, 2H, 6-phenyl H-2, H-6), 8.09 (d, *J* = 0.9 Hz, 1H, pyridine H-3), 8.05 (s, 1H, pyridine H-5), 7.56 (dd, *J* = 7.6, 1.5 Hz, 1H, 4-phenyl H-6), 7.28 (td, *J* = 8.5, 1.5 Hz, 1H, 4-phenyl H-4), 7.03 (d, *J* = 8.1 Hz, 1H, 4-phenyl H-3), 6.96 (t, *J* = 7.5 Hz, 1H, 4-phenyl H-5), 6.92 (d, *J* = 8.7 Hz, 2H, 6-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.97, 156.37, 155.01, 152.99, 150.52, 148.65, 146.28, 130.67, 130.40, 129.74, 128.47, 125.33, 121.15, 119.87, 119.01, 116.52, 115.78.

*5.3.36. 3-[2-(4-Hydroxyphenyl)-6-(thiophen-3-yl)pyridin-4-yl]phenol (**50**)*

The general procedure was employed with **5** (R^1 = c, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = d) (496.75 mg, 1.50 mmol) and acetic acid (2 mL) to yield a white solid (430.80 mg, 83.1%, 1.24 mmol). mp 246-247 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.50. LC/MS/MS: retention time: 7.55 min, $[MH]^+$: 346.22. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.70 (br, 2H, 2-phenyl 4-OH, 4-phenyl 3-OH), 8.38 (dd, *J* = 2.9, 1.0 Hz, 1H, 6-thiophene H-2), 8.15 (d, *J* = 8.6 Hz, 2H, 2-phenyl H-2, H-6), 7.96-7.93 (m, 2H, 6-thiophene H-4, pyridine H-3), 7.89 (s, 1H, pyridine H-5), 7.66 (dd, *J* = 4.9, 3.0 Hz, 1H, 6-thiophene H-5), 7.36-7.33 (m, 2H, 4-phenyl H-5, H-6), 7.29 (s, 1H, 4-phenyl H-2), 6.90 (dd, *J* = 8.7, 1.8 Hz, 3H, 2-phenyl H-3, H-5, 4-phenyl H-4). ^{13}C NMR

(62.5 MHz, DMSO-d_6) δ 158.86, 158.13, 156.58, 153.06, 149.61, 142.50, 139.55, 130.25, 129.82, 128.48, 126.96, 126.94, 124.55, 118.10, 116.27, 115.64, 115.63, 115.03, 114.15.

*5.3.37. 3-[2-(Furan-2-yl)-6-(4-hydroxyphenyl)pyridin-4-yl]phenol (**51**)*

The general procedure was employed with **5** (R^1 = c, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = e) (472.66 mg, 1.50 mmol) and acetic acid (2 mL) to yield a white solid (437.60 mg, 88.6%, 1.32 mmol). mp 219-220 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.48. LC/MS/MS: retention time: 7.24 min, $[MH]^+$: 330.22. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.73 (br, 2H, 4-phenyl 3-OH, 6-phenyl 4-OH), 8.13 (dd, *J* = 8.7, 1.8 Hz, 2H, 6-phenyl H-2, H-6), 7.90 (s, 1H, pyridine H-5), 7.87 (dd, *J* = 1.6 Hz, 0.6 Hz, 1H, 2-furan H-5), 7.75 (s, 1H, pyridine H-3), 7.34 (d, *J* = 3.6 Hz, 1H, 2-furan H-3), 7.33-7.27 (m, 2H, 4-phenyl H-5, H-6), 7.26 (s, 1H, 4-phenyl H-2), 6.92 (dd, *J* = 8.6, 1.7 Hz, 3H, 6-phenyl H-3, H-5, 4-phenyl H-4), 6.69 (dd, *J* = 3.3, 1.6 Hz, 1H, 2-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.99, 158.20, 156.97, 153.58, 149.45, 148.98, 144.36, 139.24, 130.42, 129.49, 128.56, 117.96, 116.46, 115.65, 115.28, 113.91, 113.42, 112.52, 109.41.

*5.3.38. 3-[6-(4-Hydroxyphenyl)-(2,2'-bipyridin)-4-yl]phenol (**52**)*

The general procedure was employed with **5** (R^1 = c, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = f) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (445.00 mg, 87.2%, 1.30 mmol). mp 236-237 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.42. LC/MS/MS: retention time: 7.01 min, $[MH]^+$: 341.28. purity by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.74 (br, 2H, 4-phenyl 3-OH, 6-phenyl 4-OH), 8.73 (d, *J* = 4.6 Hz, 1H, 2-pyridine H-6'), 8.61 (d, *J* = 7.4 Hz, 1H, 2-pyridine H-3'), 8.47 (s, 1H, pyridine H-3), 8.21 (d, *J* = 8.4 Hz, 6-phenyl H-2, H-6), 8.09 (s, 1H, pyridine H-5),

8.00 (td, *J* = 7.8, 1.7 Hz, 1H, 2-pyridine H-4'), 7.48 (ddd, *J* = 7.4, 4.9, 1.1 Hz, 1H, 2-pyridine H-5'), 7.37-7.35 (m, 2H, 4-phenyl H-5, H-6), 7.30 (s, 1H, 4-phenyl H-2), 6.93 (d, *J* = 8.4 Hz, 3H, 6-phenyl H-3, H-5, 4-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.99, 158.24, 156.73, 155.55, 155.53, 149.54, 149.46, 139.36, 137.56, 130.50, 129.63, 128.59, 124.50, 120.96, 117.90, 116.94, 116.48, 115.71, 115.51, 113.80.

*5.3.39. 3-[6-(4-Hydroxyphenyl)-(2,3'-bipyridin)-4-yl]phenol (**53**)*

The general procedure was employed with **5** (R^1 = c, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = g) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (423.30 mg, 82.9%, 1.24 mmol). mp 269-270 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.26. LC/MS/MS: retention time: 6.38 min, $[MH]^+$: 341.27. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.73 (br, 2H, 4-phenyl 3-OH, 6-phenyl 4-OH), 9.46 (br, 1H, 2-pyridine H-2'), 8.65 (m, 2H, 2-pyridine H-6', H-4'), 8.18 (d, J = 8.5 Hz, 2H, 6-phenyl H-2, H-6), 8.10 (s, 1H, pyridine H-3), 8.03 (s, 1H, pyridine H-5), 7.54 (t, *J* = 4.9 Hz, 1H, 2-pyridine H-5'), 7.43-7.31 (m, 2H, 4-phenyl H-5, H-6), 7.34 (s, 1H, 4-phenyl H-2), 6.92 (m, 3H, 6-phenyl H-3, H-5, 4-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.02, 158.17, 157.04, 154.15, 150.07, 149.95, 148.35, 139.35, 134.48, 130.29, 129.66, 128.62, 118.25, 116.40, 116.13, 116.07, 115.72, 114.29.

*5.3.40. 3-[6-(4-Hydroxyphenyl)-(2,3'-bipyridin)-4-yl]phenol (**54**)*

The general procedure was employed with **5** (R^1 = c, R^2 = b) (360.37 mg, 1.50 mmol), dry ammonium acetate (1.15 g, 15.00 mmol), **6** (R^3 = h) (489.19 mg, 1.50 mmol) and acetic acid (2 mL) to yield an off-white solid (375.40 mg, 73.5%, 1.10 mmol). mp 304-305 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.31. LC/MS/MS: retention time: 6.35 min, $[MH]^+$: 341.28. purity by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.76 (br, 2H, 4-phenyl 3-OH, 6-phenyl 4-OH), 8.74 (d, *J* = 4.6 Hz, 2H, 2-pyridine H-2', H-6'), 8.27 (dd, *J* = 4.6, 1.5 Hz, 2H, 2-pyridine H-3', H-5'), 8.19 (d, *J* = 8.4 Hz, 2H, 6-phenyl H-2, H-6), 8.16 (s, 1H, pyridine H-3), 8.09 (s, 1H, pyridine H-5), 7.40 (td, *J* = 7.8, 1.0 Hz, 1H, 4-phenyl H-5), 7.38 (dd, *J* = 7.7, 1.0 Hz, 1H, 4-phenyl H-6), 7.34 (s, 1H, 4-phenyl H-2), 6.93 (dd, *J* = 8.3, 1.0 Hz, 3H, 6-phenyl H-3, H-5, 4-phenyl H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.12, 158.19, 157.11, 153.79, 150.48, 150.09, 146.05, 139.19, 130.32, 129.50, 128.65, 121.27, 118.26, 117.15, 116.48, 115.74, 114.31.

*5.3.41. 4,4'-[6-(Thiophen-3-yl)pyridine-2,4-diyl]diphenol (**55**)*

The general procedure was employed with **5** (R^1, R^2 = c) (240.25 mg, 1.00 mmol), dry ammonium acetate (770.80 mg, 10.00 mmol), **6** (R^3 = d) (331.17 mg, 1.00 mmol) and acetic acid (2 mL) to yield a light yellow solid (206.30 mg, 59.7%, 0.59 mmol). mp 239-240 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.40. LC/MS/MS: retention time: 7.40 min, $[MH]^+$: 346.21. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.80 (br, 2H, 2-phenyl 4-OH, 4-phenyl 4-OH), 8.36 (dd, *J* = 2.9, 1.1 Hz, 6-thiophene H-2), 8.15 (d, *J* = 8.7 Hz, 2H, 2-phenyl H-2, H-6), 7.96 (dd, *J* = 5.0, 1.1 Hz, 1H, 6-thiophene H-4), 7.94 (s, 1H, pyridine H-3), 7.91 (s, 1H, pyridine H-5), 7.87 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.66 (dd, *J* = 5.0, 3.0 Hz, 1H, 6-thiophene H-5), 6.93 (d, *J* = 8.5 Hz, 2H, 2-phenyl H-3, H-5), 6.90 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 158.90, 158.80, 156.50, 153.00, 149.13,

142.73, 130.03, 128.70, 128.50, 128.46, 127.01, 126.95, 124.41, 116.02, 115.62, 114.76, 114.16.

*5.3.42. 4,4'-[6-(Furan-2-yl)pyridine-2,4-diyl]diphenol (**56**)*

The general procedure was employed with **5** (R^1, R^2 = c) (240.25 mg, 1.00 mmol), dry ammonium acetate (770.80 mg, 10.00 mmol), **6** (R^3 = e) (315.11 mg, 1.00 mmol) and acetic acid (2 mL) to yield a yellow solid (264.40 mg, 80.3%, 0.80 mmol). mp 231-232 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.36. LC/MS/MS: retention time: 7.07 min, $[MH]^+$: 330.22. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.83 (br, 2H, 2-phenyl 4-OH, 4-phenyl 4-OH), 8.12 (d, *J* = 8.6 Hz, 2H, 2-phenyl H-2, H-6), 7.91 (s, 1H, pyridine H-3), 7.86 (dd, *J* = 1.6, 0.9 Hz, 1H, 6-furan H-5), 7.82 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.77 (s, 1H, pyridine H-5), 7.26 (d, *J* = 3.3 Hz, 1H, 6-furan H-3), 6.93 (d, *J* = 8.3 Hz, 2H, 2-phenyl H-3, H-5), 6.90 (d, *J* = 8.4 Hz, 2H, 4-phenyl H-3, H-5), 6.68 (d, *J* = 3.3, 1.8 Hz, 1H, 6-furan H-4). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.01, 158.92, 156.85, 153.78, 149.07, 148.94, 144.26, 129.69, 128.61, 128.56, 128.22, 116.12, 115.62, 114.47, 112.65, 112.52, 109.26.

*5.3.43. 4,4'-[(2,2'-Bipyridine)-4,6-diyl]diphenol (**57**)*

The general procedure was employed with **5** (R^1, R^2 = c) (240.25 mg, 1.00 mmol), dry ammonium acetate (770.80 mg, 10.00 mmol), **6** (R^3 = f) (326.13 mg, 1.00 mmol) and acetic acid (2 mL) to yield a yellow solid (187.50 mg, 57.0%, 0.55 mmol). mp 304-305 °C; R_f (ethyl acetate/*n*-hexane 1:1 v/v): 0.13. LC/MS/MS: retention time: 6.84 min, $[MH]^+$: 341.27. purity by HPLC: 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.85 (br, 2H, 6-phenyl 4-OH, 4-phenyl 4-OH), 8.72 (d, *J* = 3.9 Hz, 1H, 2-pyridine H-6'), 8.60 (d, *J* = 7.9 Hz, 1H, 2-pyridine H-3'), 8.46 (d, *J* = 0.9 Hz, 1H, pyridine H-3), 8.20 (d, *J* = 8.6 Hz, 2H, 6-phenyl H-2, H-6), 8.08 (d, *J* = 0.9 Hz, 1H, pyridine H-5), 7.99 (td, *J* = 7.9, 1.7 Hz, 1H, 2-pyridine H-4'), 7.83 (d,

J = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 7.48 (dd, *J* = 7.2, 5.6 Hz, 1H, 2-pyridine H-5'), 6.95 (d, *J* = 8.5 Hz, 2H, 6-phenyl H-3, H-5), 6.93 (d, *J* = 8.5 Hz, 2H, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.00, 158.94, 156.61, 155.78, 155.44, 149.45, 149.29, 137.57, 129.83, 128.58, 128.45, 124.46, 120.98, 116.21, 116.16, 115.70, 114.76.

*5.3.44. 4,4'-[(2,3'-Bipyridine)-4,6-diyl]diphenol (**58**)*

The general procedure was employed with **5** (R^1, R^2 = c) (240.25 mg, 1.00 mmol), dry ammonium acetate (770.80 mg, 10.00 mmol), **6** (R^3 = g) (326.13 mg, 1.00 mmol) and acetic acid (2 mL) to yield an off-white solid (269.50 mg, 79.2%, 0.79 mmol). mp 275-276 °C; R_f (ethyl acetate/*n*-hexane 2:1 v/v): 0.13. LC/MS/MS: retention time: 6.21 min, $[MH]^+$: 341.24. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.84 (br, 2H, 6-phenyl 4-OH, 4-phenyl 4-OH), 9.46 (d, *J* = 1.2 Hz, 2-pyridine H-2'), 8.65 (d, *J* = 4.6 Hz, 1H, 2-pyridine H-6'), 8.64 (dd, *J* = 7.9, 2.0 Hz, 1H, 2-pyridine H-4'), 8.18 (d, *J* = 8.6 Hz, 2H, 6-phenyl H-2, H-6), 8.11 (s, 1H, pyridine H-3), 8.04 (s, 1H, pyridine H-5), 7.92 (d, *J* = 8.5 Hz, 2H, 4-phenyl H-2, H-6), 7.55 (dd, *J* = 7.9, 4.9 Hz, 1H, 2-pyridine H-5'), 6.93 (d, *J* = 8.4 Hz, 2H, 6-phenyl H-3, H-5), 6.92 (d, *J* = 8.5 Hz, 2H, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.06, 158.97, 156.96, 154.05, 150.05, 149.51, 148.37, 134.75, 134.49, 129.86, 128.90, 128.63, 128.28, 123.97, 116.05, 115.70, 115.21.

*5.3.45. 4,4'-[(2,4'-Bipyridine)-4,6-diyl]diphenol (**59**)*

The general procedure was employed with **5** (R^1, R^2 = c) (240.25 mg, 1.00 mmol), dry ammonium acetate (770.80 mg, 10.00 mmol), **6** (R^3 = h) (326.13 mg, 1.00 mmol) and acetic acid (2 mL) to yield a light yellow solid (198.66 mg, 58.4%, 0.58 mmol). mp 324-325 °C; R_f (ethyl acetate/*n*-hexane 2:1 v/v): 0.13. LC/MS/MS: retention time: 6.19 min, $[MH]^+$: 341.27. purity by HPLC: 100%. ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.86 (br, 2H, 6-phenyl 4-OH, 4-

phenyl 4-OH), 8.73 (dd, *J* = 4.4, 1.2 Hz, 2H, 2-pyridine H-2', H-6'), 8.27 (dd, *J* = 4.5, 1.2 Hz, 2H, 2-pyridine H-3', H-5'), 8.19 (d, *J* = 8.4 Hz, 2H, 6-phenyl H-2, H-6), 8.17 (s, 1H, pyridine H-3), 8.10 (s, 1H, pyridine H-5), 7.93 (d, *J* = 8.6 Hz, 2H, 4-phenyl H-2, H-6), 6.94 (d, *J* = 8.5 Hz, 2H, 6-phenyl H-3, H-5), 6.92 (d, *J* = 8.5 Hz, 2H, 4-phenyl H-3, H-5). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 159.12, 159.05, 157.03, 153.70, 150.49, 149.65, 146.27, 129.72, 128.92, 128.67, 128.13, 121.29, 116.21, 116.08, 115.73, 115.61.

5.4. Pharmacology

5.4.1. DNA topoisomerase I inhibition assay in vitro

DNA topo I inhibition assay was determined following the method reported by Fukuda *et al*. with minor modifications [40]. The test compounds were dissolved in DMSO at 20 mM as stock solution. The activity of DNA topo I was determined by assessing the relaxation of supercoiled DNA pBR322. The mixture of 100 ng of plasmid pBR322 DNA and 1 unit of recombinant human DNA topoisomerase I (TopoGEN INC., USA) was incubated without and with the prepared compounds at 37 °C for 30 min in the relaxation buffer (10 mM Tris-HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin, 1 mM spermidine, 5% glycerol). The reaction in the final volume of 10 μL was terminated by adding 2.5 μL of the stop solution containing 5% sarcosyl, 0.0025% bromophenol blue and 25% glycerol. DNA samples were then electrophoresed on a 1% agarose gel at 15 V for 7 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and were quantitated using AlphaImager™ (Alpha Innotech Corporation).

5.4.2. DNA Topoisomerase II Inhibition assay in vitro

DNA topo II inhibitory activity of compounds were measured as follows [42]. The mixture of 200 ng of supercoiled pBR322 plasmid DNA and 1 unit of human DNA topoisomerase IIα (Usb Corp., USA) was incubated without and with the prepared compounds in the assay buffer (10 mM Tris-HCl (pH 7.9) containing 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP, and 15 μg/mL bovine serum albumin) for 30 min at 30 °C. The reaction in a final volume of 20 μL was terminated by the addition of 3 μL of 7 mM EDTA. Reaction products were analyzed on 1% agarose gel at 25 V for 4 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and supercoiled DNA was quantitated using AlphaImager^{TM} (Alpha Innotech Corporation).

5.4.3. Cytotoxicity assay

Cancer cells were cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of 2~4 x 10^4 cells per well and incubated for overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Hyclone, USA) in 5% CO_2 incubator at 37 °C. On day 2, after FBS starvation for 4 h, culture medium in each well was exchanged with 0.1 mL aliquots of medium containing graded concentrations of compounds. On day 4, each well was added with 5 μL of the cell counting kit-8 solution (Dojindo, Japan) then incubated for additional 4 h under the same condition. The absorbance of each well was determined by an Automatic Elisa Reader System (Bio-Rad 3550) at 450 nm wavelength. For determination of the IC_{50} values, the absorbance readings at 450 nm were fitted to the four-parameter logistic equation. The compounds like adriamycin, etoposide, and camptothecin were purchased from Sigma and used as positive controls.

5.4.4. Comet Assay

To evaluate DNA damage, comet assay was performed using single-cell gel electrophoresis with a Trevigen kit (Gaithersburg, MD) according to the method previously reported [41] Briefly, HCT15 cells, seeded in a density of 1 x 10^5 cells per well in six-well plates were treated with 10 μM and 20 μM of each compound and etoposide for 24 h and harvested by trypsinization followed by resuspending cells in 1 mL of ice-cold PBS. Then, 8 μL of resuspened cells were mixed with 80 μL of low-melting agarose at 37 ˚C, spread on slides and solidified in the dark for 40 min at 4 ˚C. Slides were lysed in ice-cold lysis solution in the dark for 30 min at 4 ˚C and then submerged in a fresh alkaline solution (pH>13) at room temperature for 30 min to allow alkaline unwinding. Electrophoresis was performed under alkaline conditions for 20 min for 15 V. Slides were rinsed twice with distilled water, once with 70 % ethanol and stained with SYBR Green (Trevigen, Gaithersburg) in a TE buffer for 5 min in the dark at 4 ˚C. Comet images were obtained using an inverted fluorescence microscope (Zeiss, Axiovert 200) at 10X magnification and percentage DNA in tail was analyzed by Komet 5.0 software (Kinetic imaging Ltd, UK). Data were represented both by imaging and graphically by randomly selecting comet lengths of HCT15 cells.

5.5. Statistical Analysis

The data of DNA percentage in the tail obtained from comet assay are expressed as the mean ± standard deviation, with each experiment performed in triplicate. Comparison of the differences was conducted with an unpaired, two-tailed Student's t-test. The differences were considered statistically significant when the p value was ≤ 0.05.

5.6. *Molecular Docking Study*

For the molecular docking studies, the receptor and the ligands were prepared. Homology model of topo IIα bound to etoposide and DNA were prepared by Modeller using topo IIβ, etoposide and DNA complex structure (3QX3) as template [46]. Compounds **56** were sketched using Sybyl X-2.0 [47]. Gasteiger-Hückel charges were applied to the molecule and energy minimization was performed using the Tripos force field. Molecular docking was carried out by Surflex-Dock, where the compounds were docked into the topo IIα. The receptor was prepared by removing the etoposide. The program generates protomol based on the ligand binding site of the receptor where etoposide was removed. The default parameters in Surflex-Dock were used for the docking and the highest score conformation were examined. For the graphical representation of the docked structure, ADT software was used [48].

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Scheme caption

Scheme 1. General synthetic scheme of dihydroxylated 2,4-diphenyl-6-aryl pyridines. Reagents and conditions: (i) aryl aldehydes 2 (R^1 = a-c) (1.0 equiv), KOH/ NaOH/ BF_3Et_2O, EtOH/ Dioxane, 2–24 h, 20 °C, 36–90% yield; (ii) NH_4OAc (10.0 equiv), glacial acetic acid, 12–24 h, 90–100 °C, 20–94% yield.

Figure captions

Figure 1. Structures of α-terpyridine, 2,4,6-trisubstituted pyridine and dihydroxylayed 2,4-diphenyl-6-aryl pyridines.

Figure 2. Strucutres of non-substitutued 2,4-diphenyl-6-aryl pyridines.

Figure 3. Strategy for the design of dihydroxylated 2,4-diphenyl-6-aryl pyridines.

Figure 4. Structures of dihydroxylated 2,4-diphenyl-6-aryl pyridines.

Figure 5. Human DNA topo I inhibitory activity of compounds **25**, **26**, **29** at the concentration of 100 μM (A) and 20 μM (B). Lane D: pBR322 DNA only; lane T: pBR322 DNA + Topo I; lane C: pBR322 DNA + Topo I + Camptothecin; lane **25**, **26**, **29**: pBR322 DNA + Topo I + each compound of **25**, **26**, **29**.

Figure 6. Human DNA topo II inhibitory activity of compounds **15-59** at the concentration of 100 μM (A) and 20 μM (B). Lane D: pBR322 DNA only; lane T: pBR322 DNA + Topo II; lane E: pBR322 DNA + Topo II + Etoposide; lane **15-59**: pBR322 DNA + Topo II + each compound of **15-59**.

Figure 7. (A) Structure of compounds having significant topo II inhibitory activity. (B) Favorable order of substitution for topo II inhibitory activity and cytotoxicity in dihydroxylated 2,4-diphenyl-6-aryl pyridines.

Figure 8. (A) The result of cleavage complex assay. A linear band indicating the formation of cleavage complex was detected with 200 μM treatment of compound **56** as well as etoposide, an well know topo II poison. (B) The result of comet assay. The images of control (non-treated), etoposide (topo poison) and compounds **54** and **56** treated HCT15 cells ahowing comet formation. (C) Graphical representation of the selected comet tail lengths of untreated- and treated HCT15 cells using Komet 5.0 software. Columns and error bars indicate mean ± SD (n = 50). *** $P < 0.001$, for significant differences from the vehicle control.

Figure 9. Molecular docking between compound **56** and cleavage core domain of human topo IIα. The topo IIα is shown in light pink surface representation and compound and DNA (blue) in stick representation. The residues and DNA bases involved in the interactions are labeled. The stick representation of the complex is colored by the atom type (carbon, green; oxygen, red; hydrogen, white). The residues of topo IIα that have interaction with compound **56** are colored by the atom type (carbon, blue; oxygen, red; phosphorus, orange). The dotted green lines indicate the hydrogen bonding interactions.

Table captions

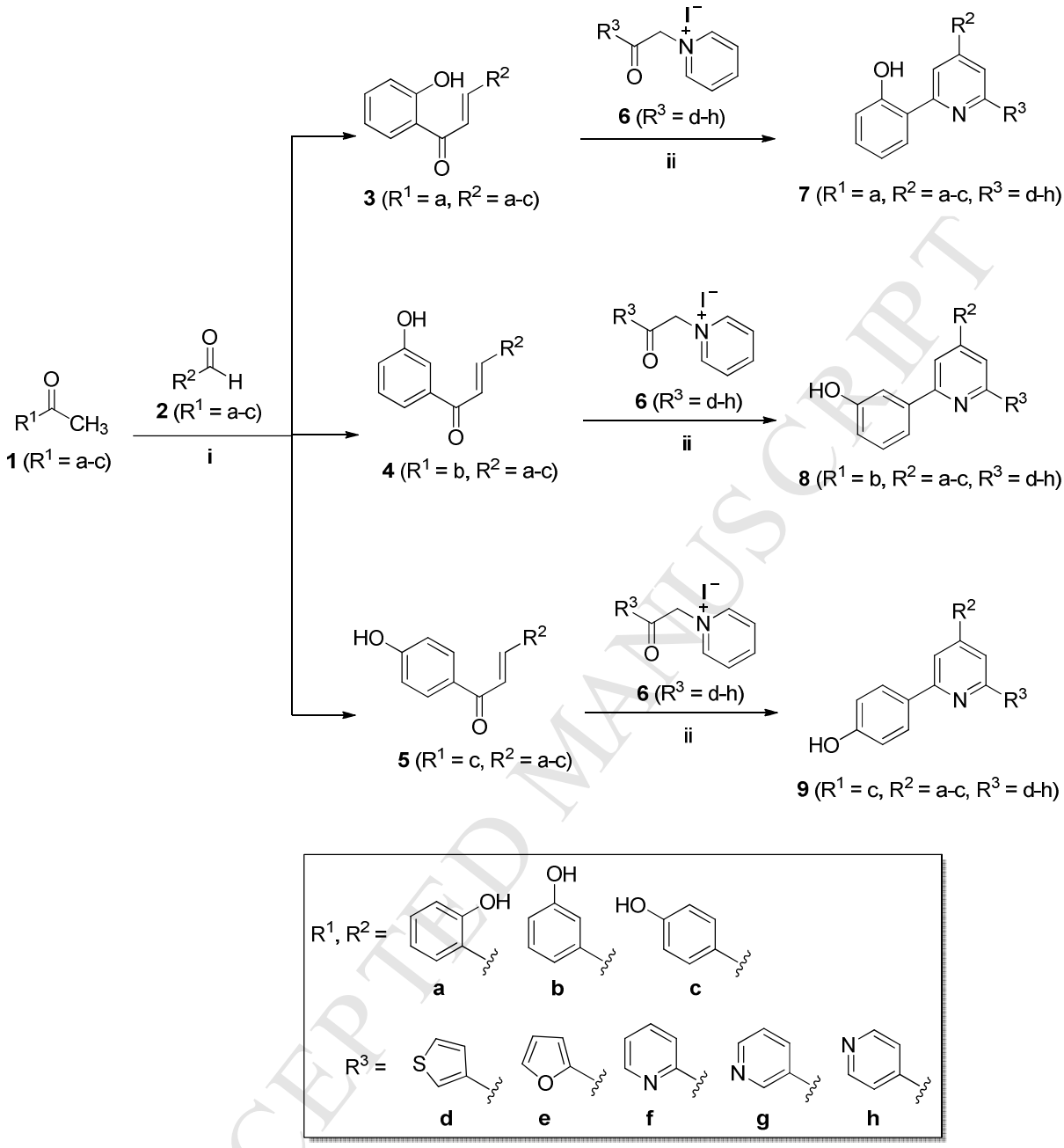
Table 1. Topo I and II inhibitory activity of compounds **10-14**.

Table 2. Topo I inhibitory activity and cytotoxicity of compounds **25, 26, 29**.

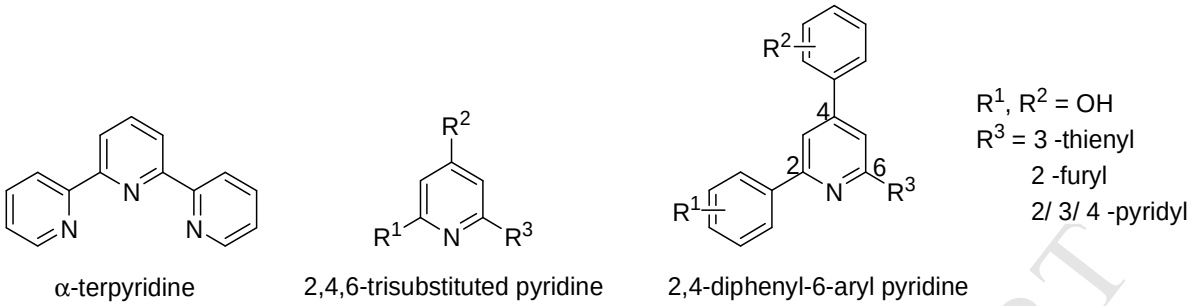
Table 3. Topo II inhibitory activity and cytotoxicity of compounds from Series A, B and C.

Table 4. Topo II inhibitory activity and cytotoxicity of compounds from Series D, E and F.

Table 5. Topo II inhibitory activity and cytotoxicity of compounds from Series G, H and I.



Scheme 1. General synthetic scheme of dihydroxylated 2,4-diphenyl-6-aryl pyridines. Reagents and conditions: (i) aryl aldehydes 2 (R^1 = a-c) (1.0 equiv), KOH/ NaOH/ BF_3Et_2O, EtOH/ Dioxane, 2–24 h, 20 °C, 36–90% yield; (ii) NH_4OAc (10.0 equiv), glacial acetic acid, 12–24 h, 90–100 °C, 20–94% yield.



α-terpyridine 2,4,6-trisubstituted pyridine 2,4-diphenyl-6-aryl pyridine

Figure 1. Structures of α-terpyridine, 2,4,6-trisubstituted pyridine and dihydroxylayed 2,4-diphenyl-6-aryl pyridines.

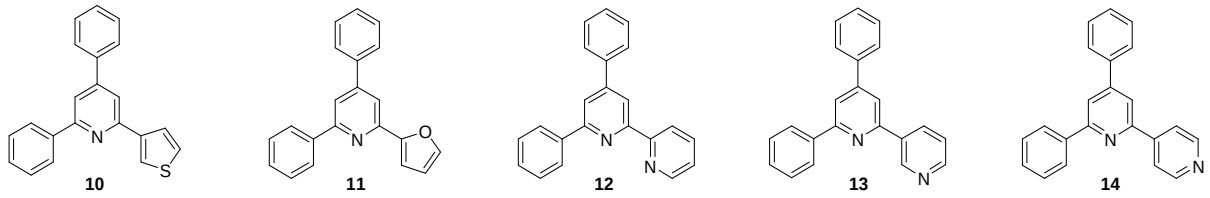


Figure 2. Strucutres of non-substitutued 2,4-diphenyl-6-aryl pyridines.

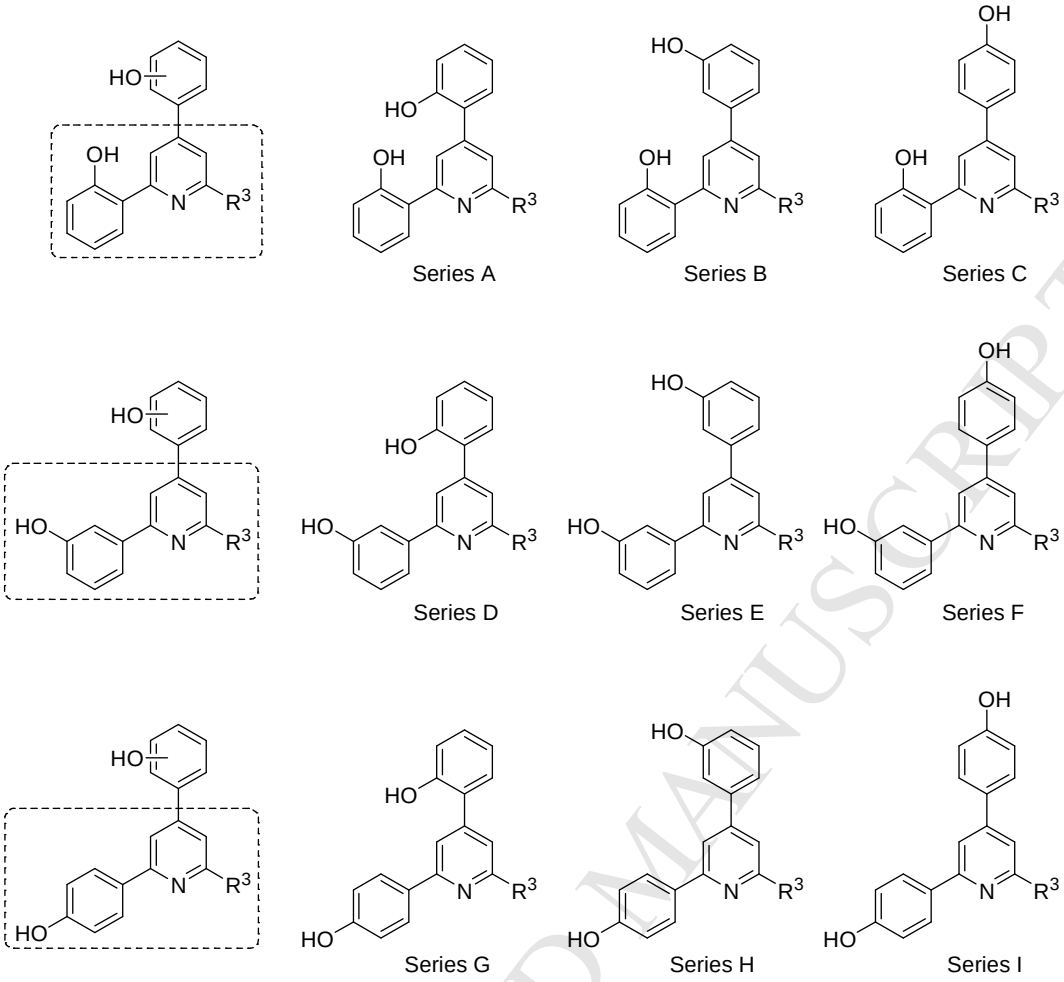


Figure 3. Strategy for the design of dihydroxylated 2,4-diphenyl-6-aryl pyridines.

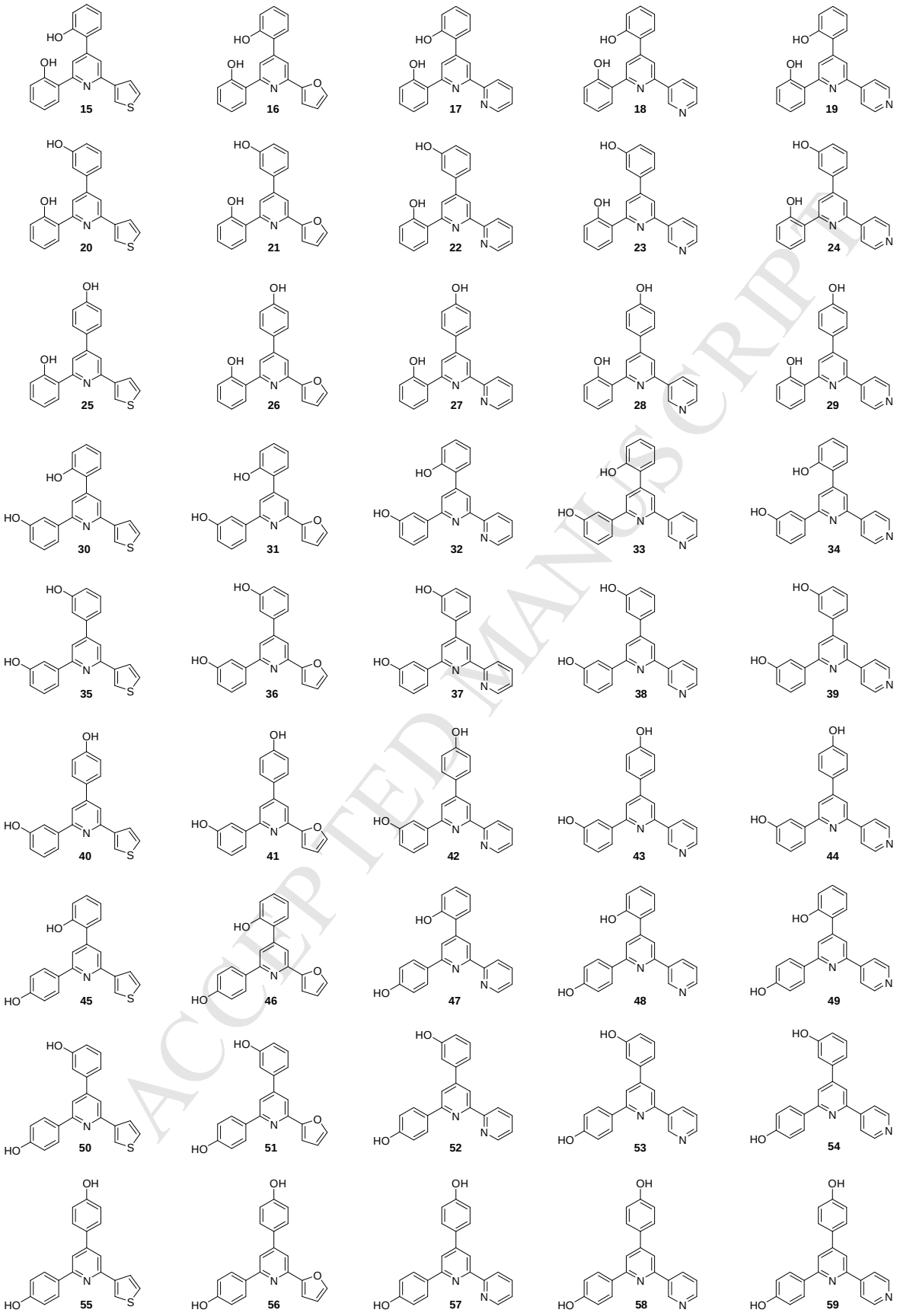


Figure 4. Structures of dihydroxylated 2,4-diphenyl-6-aryl pyridines.

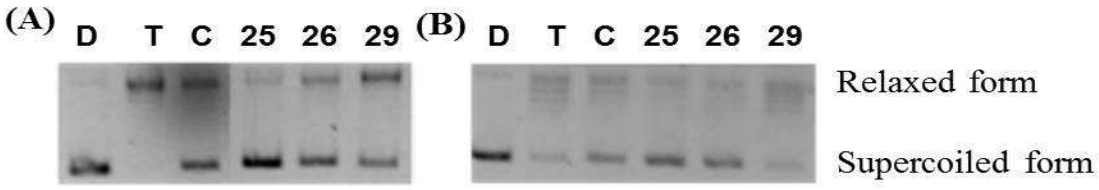


Figure 5. Human DNA topo I inhibitory activity of compounds **25**, **26**, **29** at the concentration of 100 μM (A) and 20 μM (B). Lane D: pBR322 DNA only; lane T: pBR322 DNA + Topo I; lane C: pBR322 DNA + Topo I + Camptothecin; lane **25**, **26**, **29**: pBR322 DNA + Topo I + each compound of **25**, **26**, **29**.

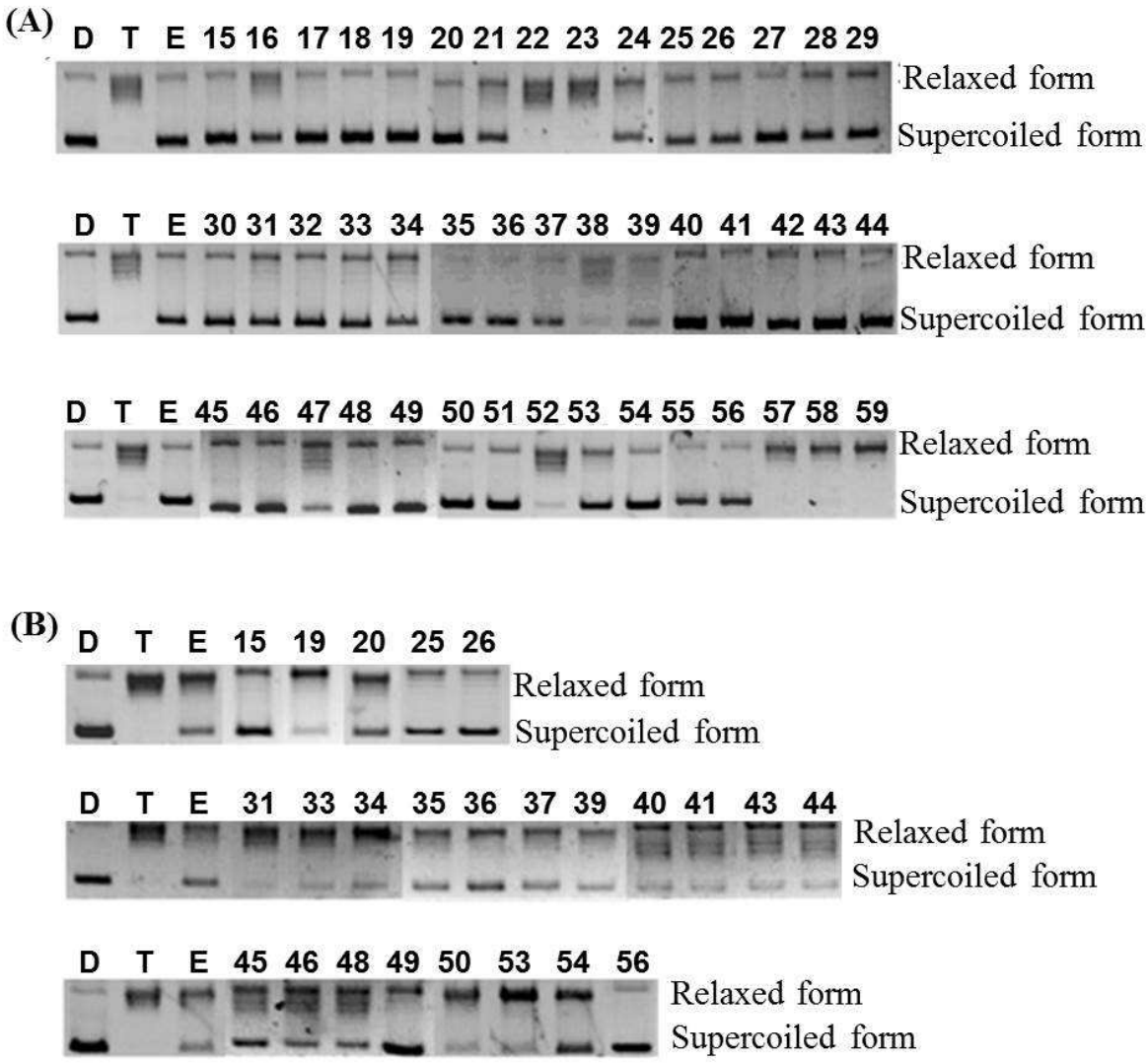
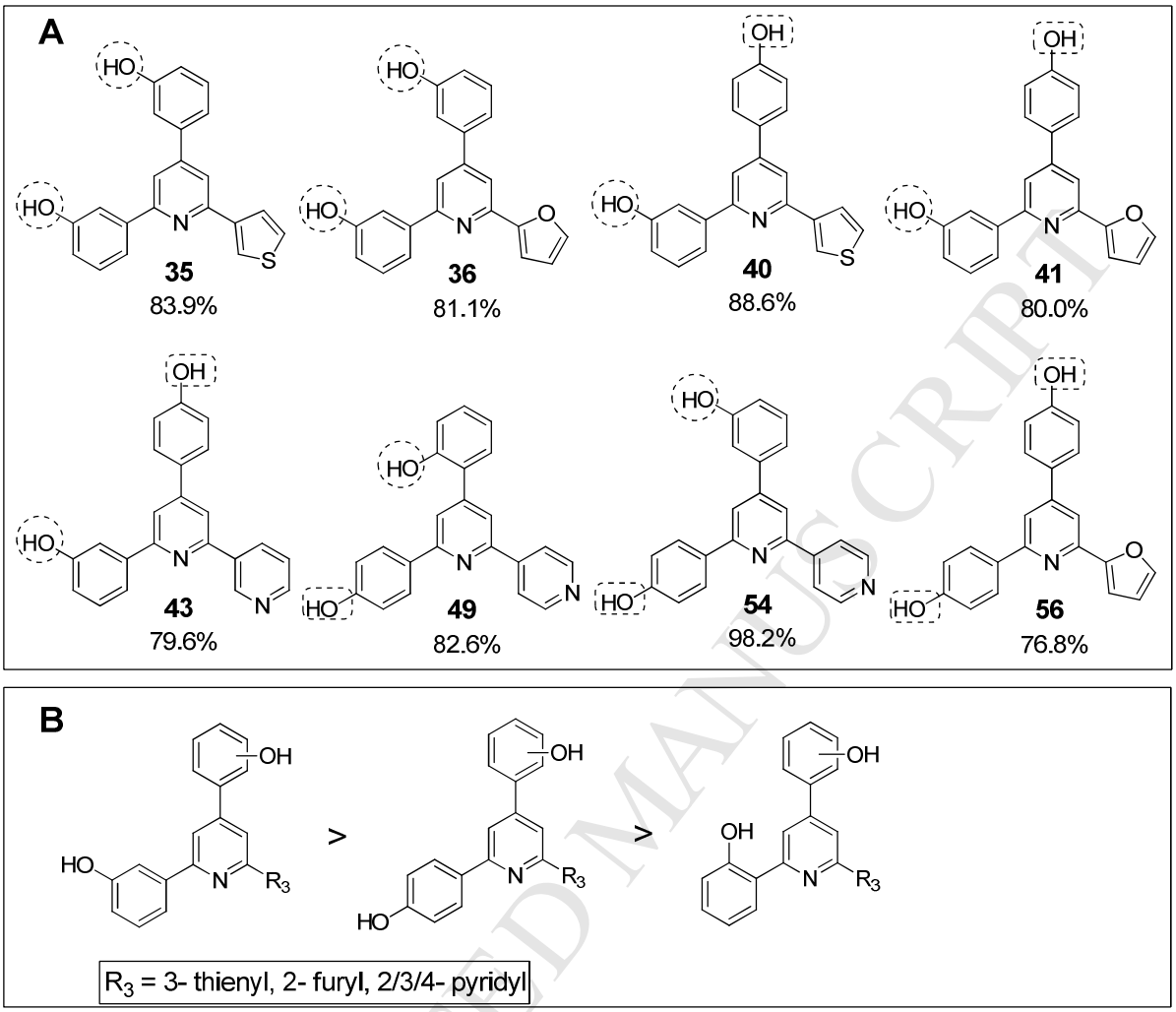


Figure 6. Human DNA topo II inhibitory activity of compounds **15-59** at the concentration of 100 μM (A) and 20 μM (B). Lane D: pBR322 DNA only; lane T: pBR322 DNA + Topo II; lane E: pBR322 DNA + Topo II + Etoposide; lane **15-59**: pBR322 DNA + Topo II + each compound of **15-59**.



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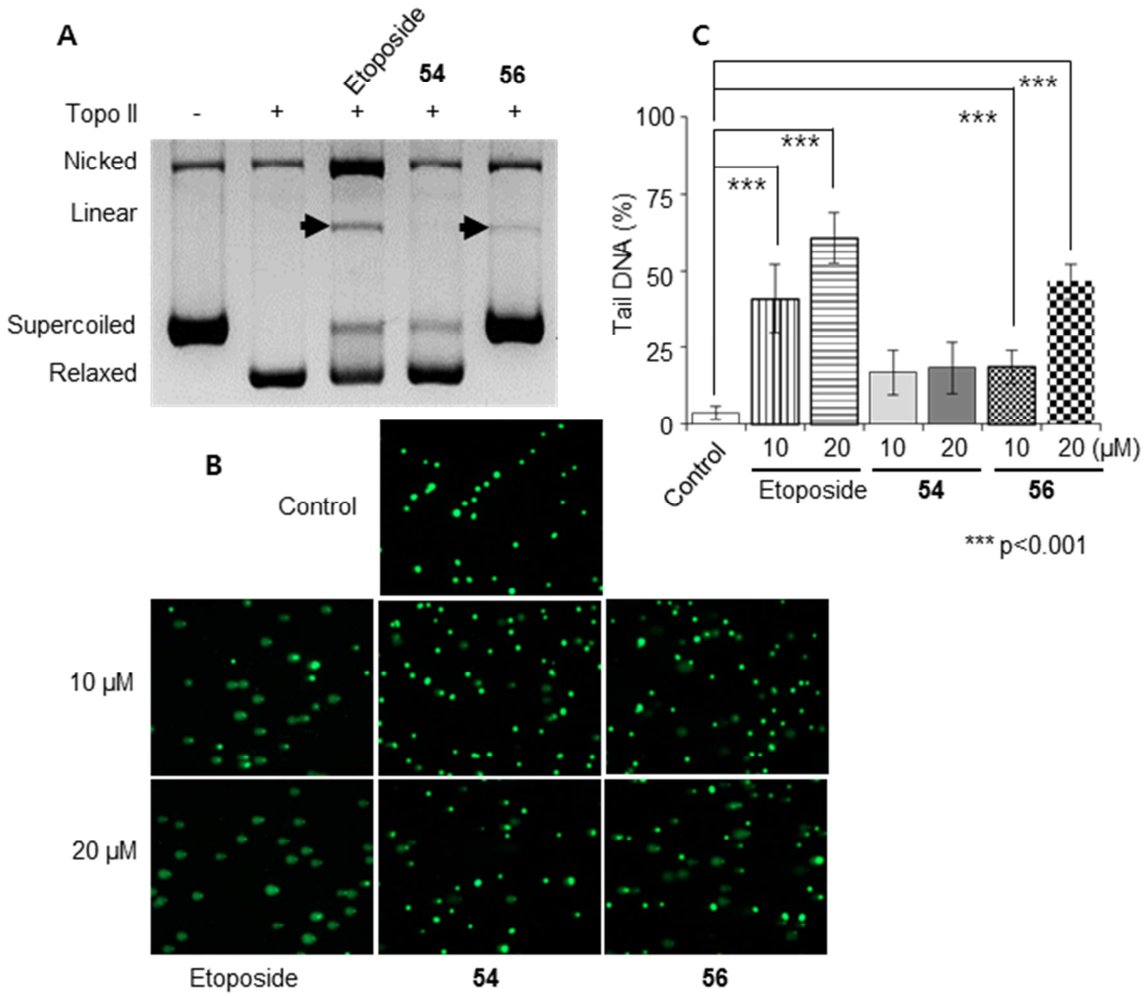

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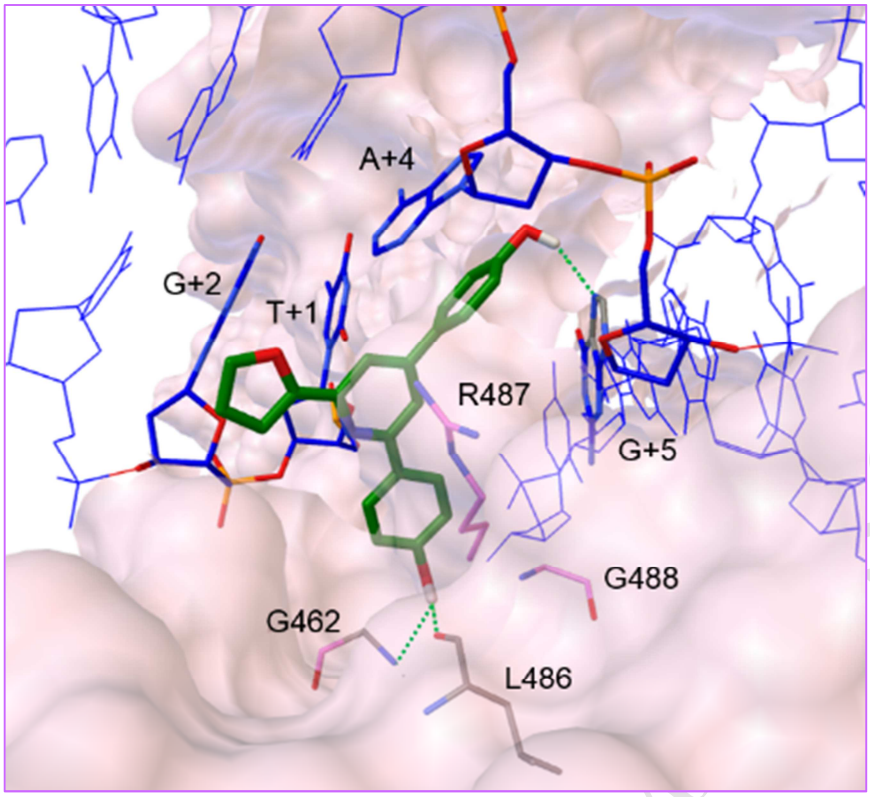


Figure 9. Molecular docking between compound **56** and cleavage core domain of human topo IIα. The topo IIα is shown in light pink surface representation and compound and DNA (blue) in stick representation. The residues and DNA bases involved in the interactions are labeled. The stick representation of the complex is colored by the atom type (carbon, green; oxygen, red; hydrogen, white). The residues of topo IIα that have interaction with compound **56** are colored by the atom type (carbon, blue; oxygen, red; phosphorus, orange). The dotted green lines indicate the hydrogen bonding interactions.

Table 1. Topo I and II inhibitory activity of compounds **10**-**14**.

Compounds	%Inhibition	
	Topo I at 100 μM	Topo II at 100 μM
Camptothecin	**69.0**	
Etoposide		**65.3**
10	0.0	11.1
11	0.0	9.1
12	2.0	3.0
13	0.0	5.0
14	0.0	4.0

Table 2. Topo I inhibitory activity and cytotoxicity of compounds **25, 26, 29**.

Compounds	%Inhibition		IC_{50}[a] (μM)				
	Topo I		HCT15	K562	DU145	MCF-7	HeLa
	100 μM	20 μM					
Adriamycin			1.28 ± 0.07	2.85 ± 1.38	0.86 ± 0.04	3.69 ± 0.13	1.45 ± 0.01
Camptothecin	**54.6**	**36.8**	0.26 ± 0.04	1.18 ± 0.14	2.37 ± 0.55	3.91 ± 0.66	1.01 ± 0.01
Etoposide			1.33 ± 0.10	2.09 ± 0.55	2.94 ± 0.04	3.25 ± 0.04	3.20 ± 0.57
25	59.9	43.0	4.52 ± 0.07	5.04 ± 1.17	>50	>50	>50
26	44.3	31.7	27.37 ± 6.22	>50	>50	>50	>50
29	33.3	0.0	1.68 ± 0.62	2.92 ± 1.66	34.46 ± 2.27	41.30 ± 1.39	22.58 ± 4.01

Table 3. Topo II inhibitory activity and cytotoxicity of compounds from Series A, B and C.

Compounds	%Inhibition		IC_{50}[a] (μM)				
	Topo II		HCT15	K562	DU145	MCF-7	HeLa
	100 μM	20 μM					
Adriamycin			1.28 ± 0.07	2.85±1.38	0.86±0.04	3.69±0.13	1.45±0.01
Camptothecin			0.26 ± 0.04	1.18±0.14	2.37±0.55	3.91±0.66	1.01±0.01
Etoposide	**72.6**	**31.6**	1.33 ± 0.10	2.09±0.55	2.94±0.04	3.25±0.04	3.20±0.57
15	75.5	67.3	13.64 ± 0.66	14.01±0.72	36.82±0.83	>50	45.88±0.92
16	49.5	5.2	>50	30.00±1.11	>50	>50	>50
17	73.7	6.5	1.45 ± 0.31	2.62±0.24	33.01±2.45	>50	7.65±0.30
18	72.9	2.1	20.73 ± 1.61	30.88±2.81	>50	>50	44.40±1.10
19	74.6	32.4	1.03 ± 0.06	1.75±0.35	>50	>50	23.50±0.09
20	63.8	29.4	3.23 ± 0.29	5.31±1.47	11.82±2.44	23.55±1.73	3.25±0.34

21	36.6	0	3.89 ± 0.48	>50	>50	>50	>50
22	6.7	NA	ND	ND	ND	ND	ND
23	6.1	NA	2.68 ± 0.62	>50	>50	>50	>50
24	34.4	0	1.70±0.01	>50	>50	>50	>50
25	66.0	42.4	4.52±0.07	5.04±1.17	>50	>50	>50
26	63.0	33.6	27.37±6.22	>50	>50	>50	>50
27	68.5	3.2	15.15±3.39	8.27±1.75	>50	>50	30.66±5.27
28	65.5	0	10.62±0.69	5.03±1.06	15.17±1.94	37.30±5.33	23.22±2.65
29	72.4	0	1.68±0.62	2.92±1.66	34.46±2.27	41.30±1.39	22.58±4.01

Table 4. Topo II inhibitory activity and cytotoxicity of compounds from Series D, E and F.

Compounds	%Inhibition		IC_{50}[a] (μM)				
	Topo II		HCT15	K562	DU145	MCF-7	HeLa
	100 μM	20 μM					
Adriamycin			1.28±0.07	2.85±1.38	0.86±0.04	3.69±0.13	1.45±0.01
Camptothecin			0.26±0.04	1.18±0.14	2.37±0.55	3.91±0.66	1.01±0.01
Etoposide	**72.1**	**28.5**	1.33±0.10	2.09±0.55	2.94±0.04	3.25±0.04	3.20±0.57
30	75.9	6.2	0.96±0.02	0.96±0.01	41.75±0.97	6.60±0.34	1.56±0.18
31	51.6	10.4	1.85±0.30	1.74±0.55	>50	9.80±0.04	5.65±0.07
32	68.8	2.4	1.07±0.04	3.89±0.10	>50	14.86±0.52	6.04±0.31
33	66.0	13.0	1.32±0.09	4.20±0.33	12.03±1.73	8.02±0.15	6.67±0.39
34	47.5	17.3	1.81±0.06	2.40±0.10	>50	>50	30.45±2.60

35	83.9	10.9	2.95±0.14	4.17±0.88	2.53±0.17	8.39±1.78	23.92±1.48
36	81.1	26.2	2.12±0.95	9.58±2.74	2.83±0.18	11.33±0.35	14.90±2.75
37	64.9	11.8	1.26±0.49	6.99±0.22	2.33±0.19	8.01±1.62	13.01±1.06
38	20.6	NA	ND	ND	ND	ND	ND
39	45.7	10.7	6.49±1.18	>50	>50	>50	>50
40	88.6	11.5	2.80±0.84	1.95±0.88	5.72±0.13	2.78±0.88	12.13±1.55
41	80.0	14.4	1.91±0.84	4.38±0.67	6.30±0.08	4.75±0.69	11.54±0.27
42	72.6	6.6	1.06±0.29	3.51±1.10	7.38±0.20	10.99±0.03	13.45±0.86
43	79.6	14.3	2.76±0.05	1.01±0.16	>50	44.55±4.34	14.61±0.04
44	74.0	14.9	4.93±1.07	25.31±3.40	>50	>50	31.12±5.83

Table 5. Topo II inhibitory activity and cytotoxicity of compounds from Series G, H and I.

Compounds	%Inhibition		IC_{50}[a] (μM)				
	Topo II		HCT15	K562	DU145	MCF-7	HeLa
	100 μM	20 μM					
Adriamycin			1.28±0.07	2.85±1.38	0.86±0.04	3.69±0.13	1.45±0.01
Camptothecin			0.26±0.04	1.18±0.14	2.37±0.55	3.91±0.66	1.01±0.01
Etoposide	**69.6**	**53.7**	1.33±0.10	2.09±0.55	2.94±0.04	3.25±0.04	3.20±0.57
45	71.6	25.3	0.85±0.03	2.20±0.73	8.13±0.32	5.49±0.38	1.88±0.23
46	59.8	15.4	0.84±0.02	2.70±0.11	4.50±0.47	6.99±1.17	3.24±0.34
47	9.9	NA	ND	ND	ND	ND	ND
48	55.0	16.2	0.83±0.06	2.09±0.08	2.28±0.38	5.95±0.67	1.89±0.09
49	82.6	71.5	1.04±0.04	0.86±0.02	5.64±0.52	3.46±0.10	0.87±0.04

50	38.1	23.1	1.82±0.27	6.41±0.61	10.5±1.08	2.56±0.27	5.31±0.15
51	36.2	8.5	2.08±0.16	6.58±0.23	10.59±1.03	3.98±0.10	8.07±1.02
52	0	NA	ND	ND	ND	ND	ND
53	54.5	27.0	0.67±0.01	0.85±0.03	>50	19.81±0.26	2.51±0.08
54	98.2	37.0	0.72±0.01	1.29±-0.27	1.85±0.20	>50	2.85±0.63
55	68.3	7.6	0.74±0.03	1.15±0.18	3.32±0.21	8.25±1.52	4.25±0.41
56	76.8	74.2	0.08±0.01	0.92±0.04	5.06±0.63	7.91±0.78	7.26±0.24
57	0.0	NA	1.3±0.025	>50	>50	>50	>50
58	7.5	NA	1.17±0.009	2.93±0.45	1.96±0.12	11.86±0.37	0.65±0.01
59	0.0	NA	2.72±0.038	>50	>50	>50	>50

HCT15: human colorectal adenocarcinoma cell line; K562: human myeloid leukemic tumor cell line; DU145: human prostate tumor cell line; MCF-7: human breast adenocarcinoma cell line; HeLa: human cervix tumor cell line; [a] Each data represents mean ± S.D. from three different experiments performed in triplicate, NA: not applicable; ND: not determined

Highlights

► We designed and synthesized 45 dihydroxylated 2,4-diphenyl-6-aryl pyridines

► Evaluated for topo I and II inhibitory activity and cytotoxicity

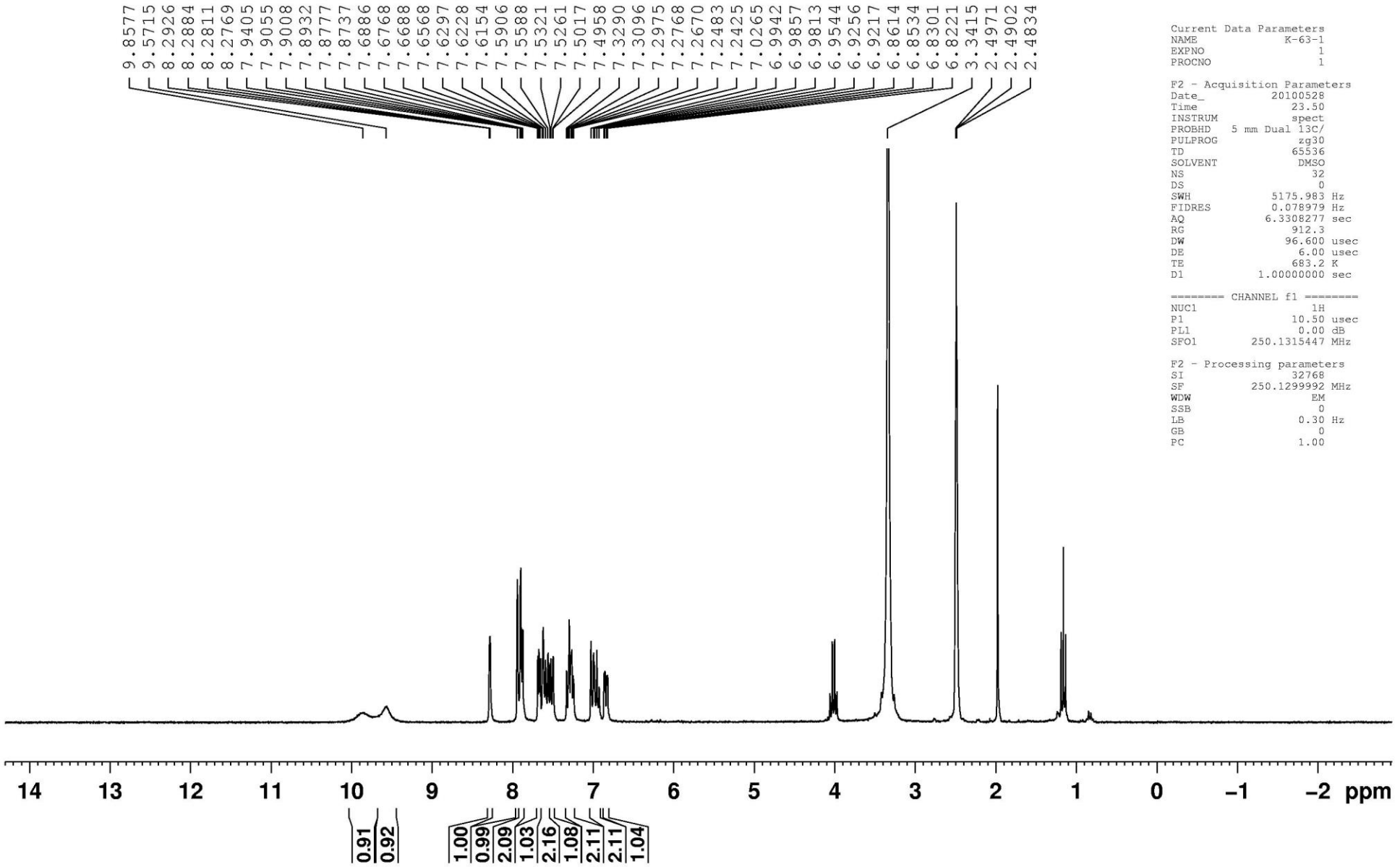
► Most of compounds had potent antiproliferative activity on human colon cancer cells

► Most of compounds inhibited topo II comparably to or more strongly than etoposide

► Compound **56** inhibited topo II most strongly and functioned as a topo II poison

Compound-30,^{1}H-NMR

K-63-1

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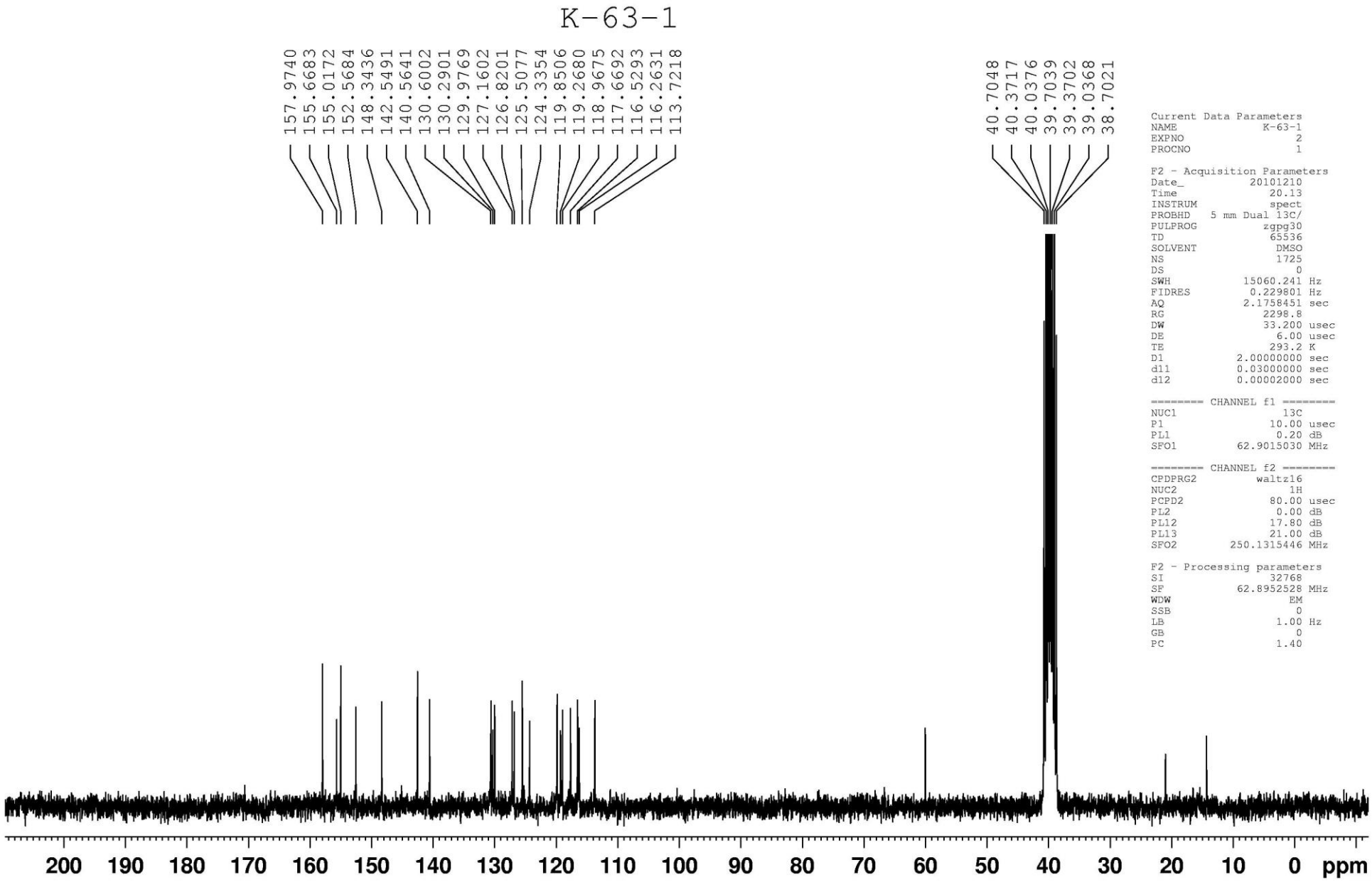

Compound-35,^{1}H-NMR

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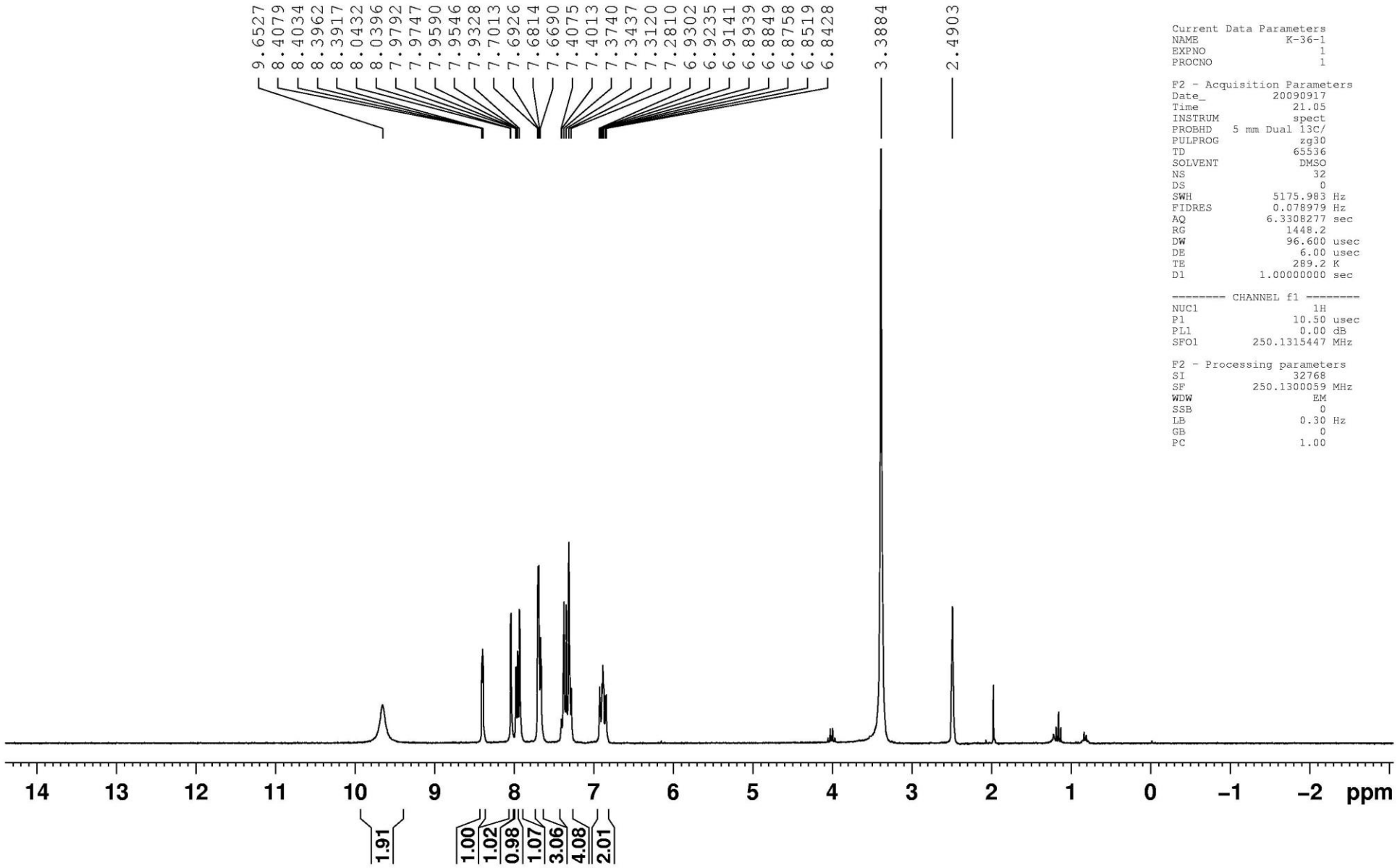

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INSTRUM	spect
PROBHD	5 mm Dual 13C/
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	32
DS	0
SWH	5175.983 Hz
FIDRES	0.078979 Hz
AQ	6.3308277 sec
RG	1448.2
DW	96.600 usec
DE	6.00 usec
TE	289.2 K
D1	1.00000000 sec

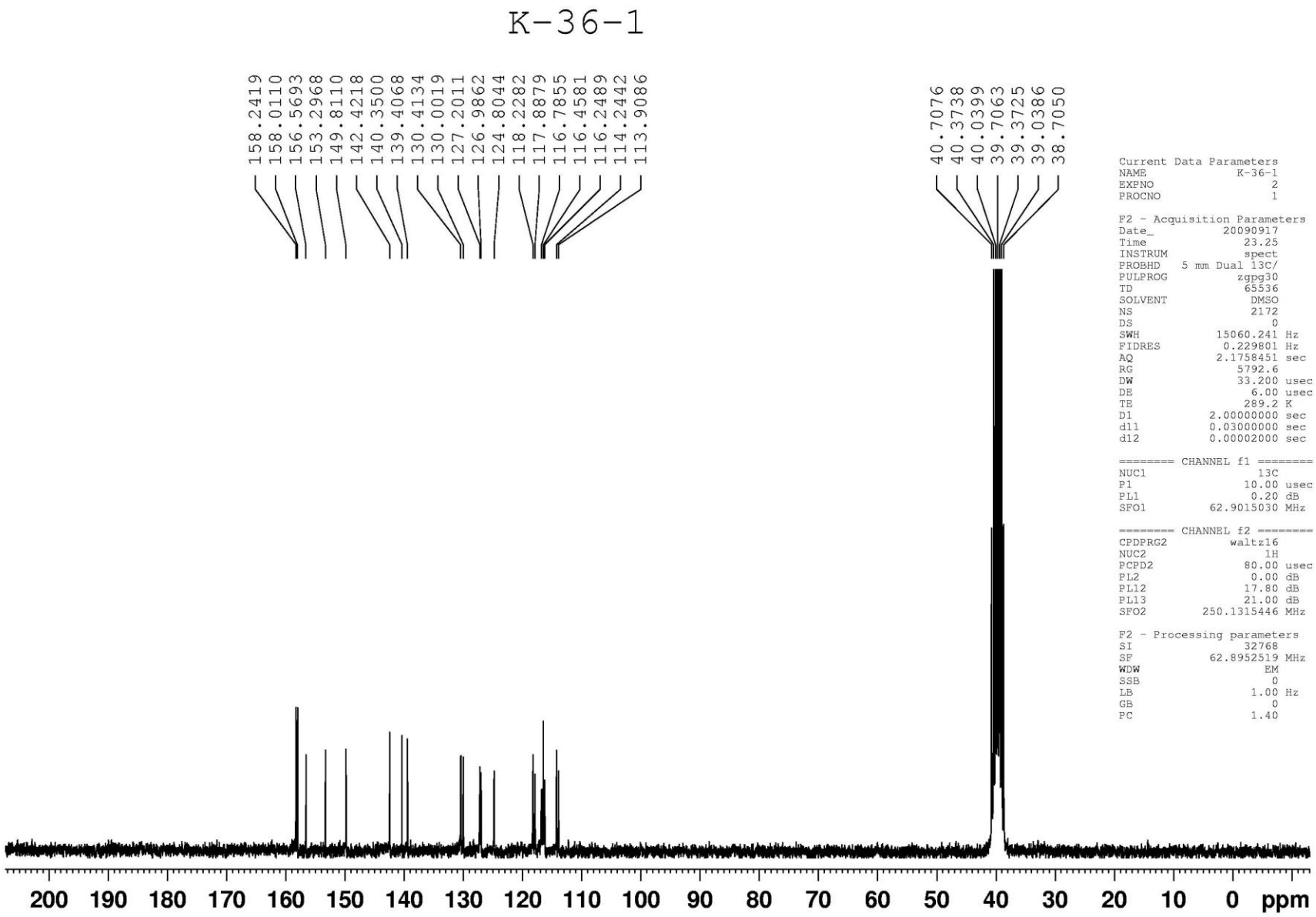
======== CHANNEL f1 ========

NUC1	1H
P1	10.50 usec
PL1	0.00 dB
SFO1	250.1315447 MHz

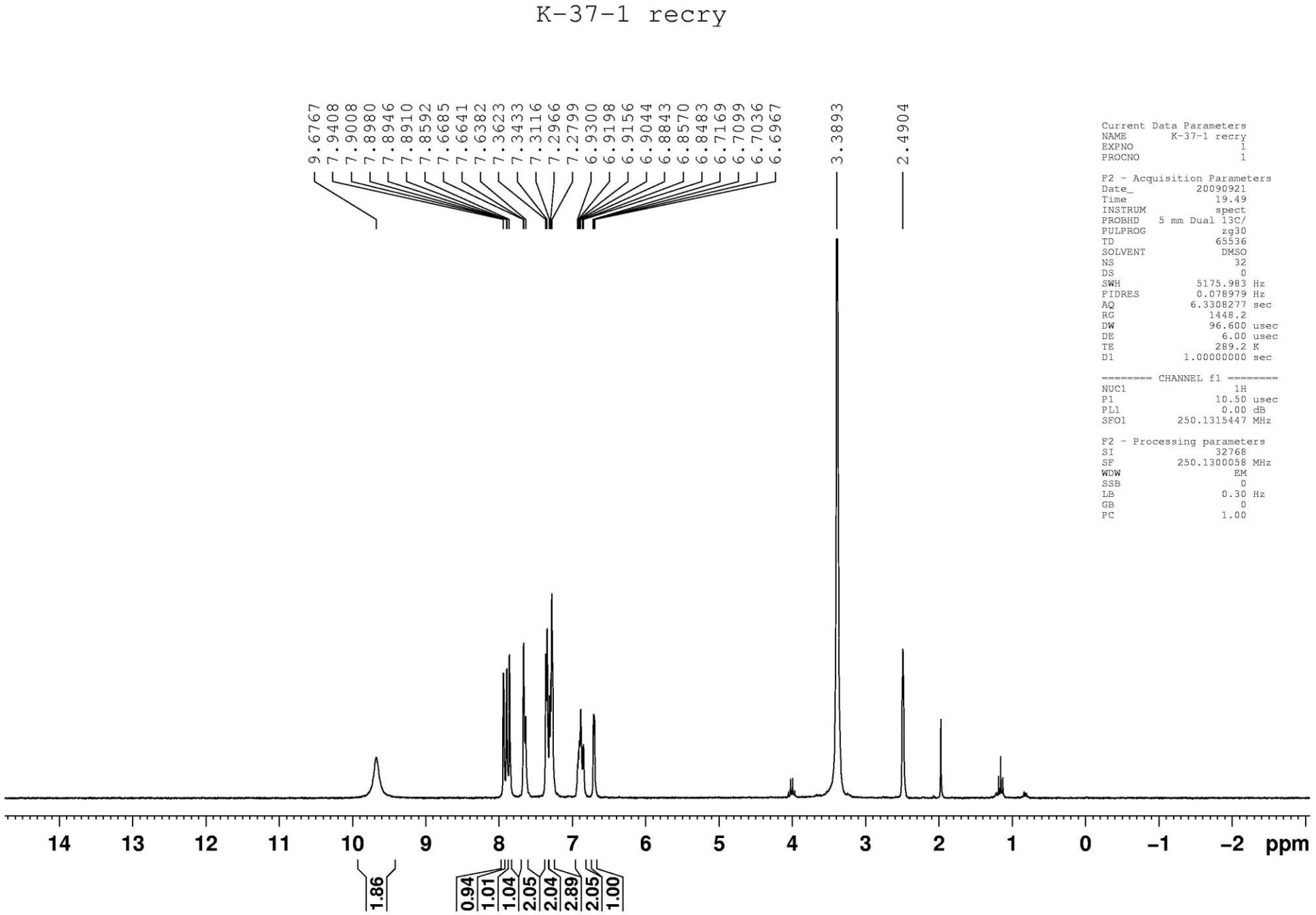
F2 - Processing parameters

SI	32768
SF	250.1300059 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

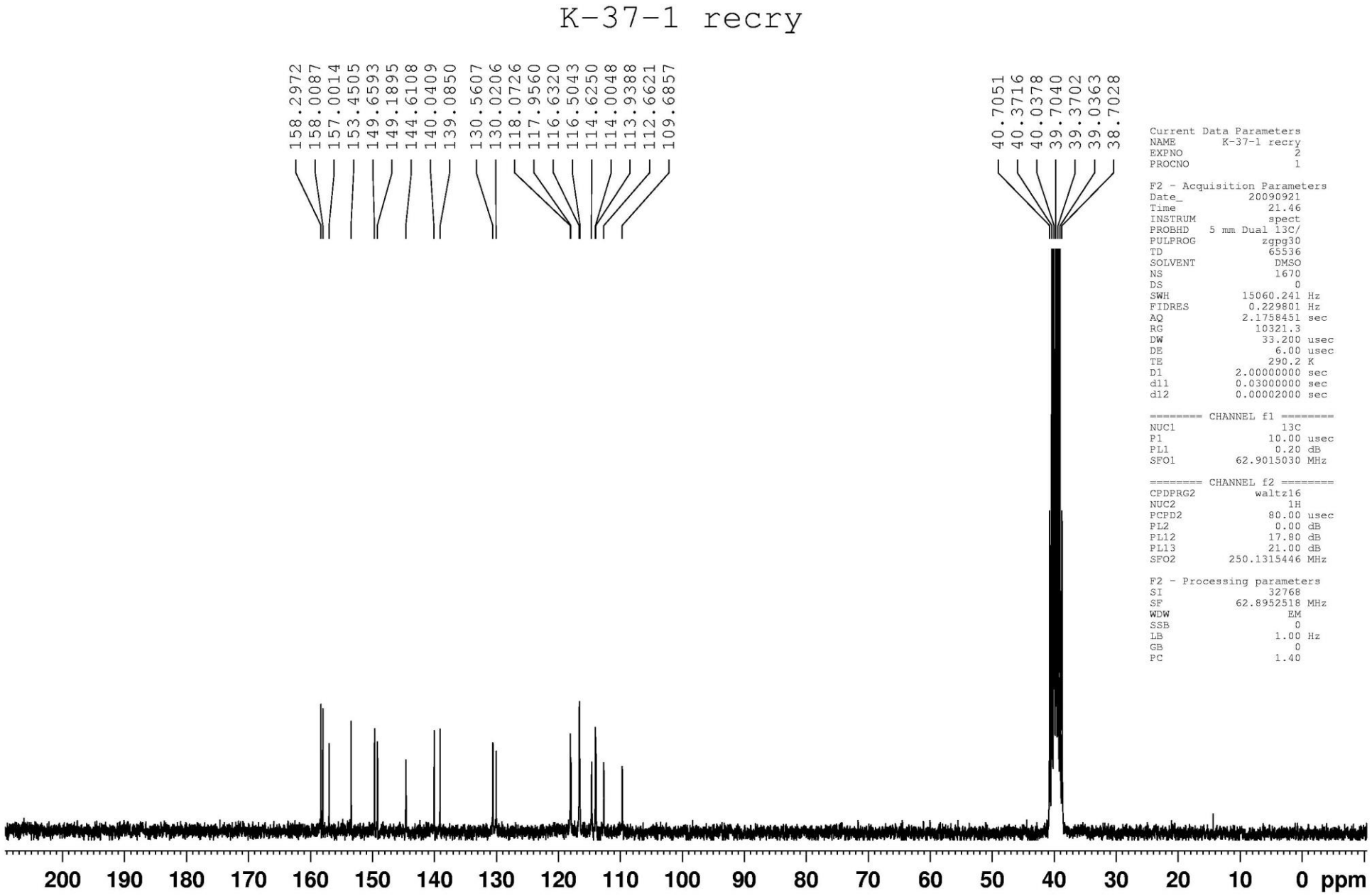
Compound-35, ^{13}C-NMR



Compound-36, ^{1}H-NMR

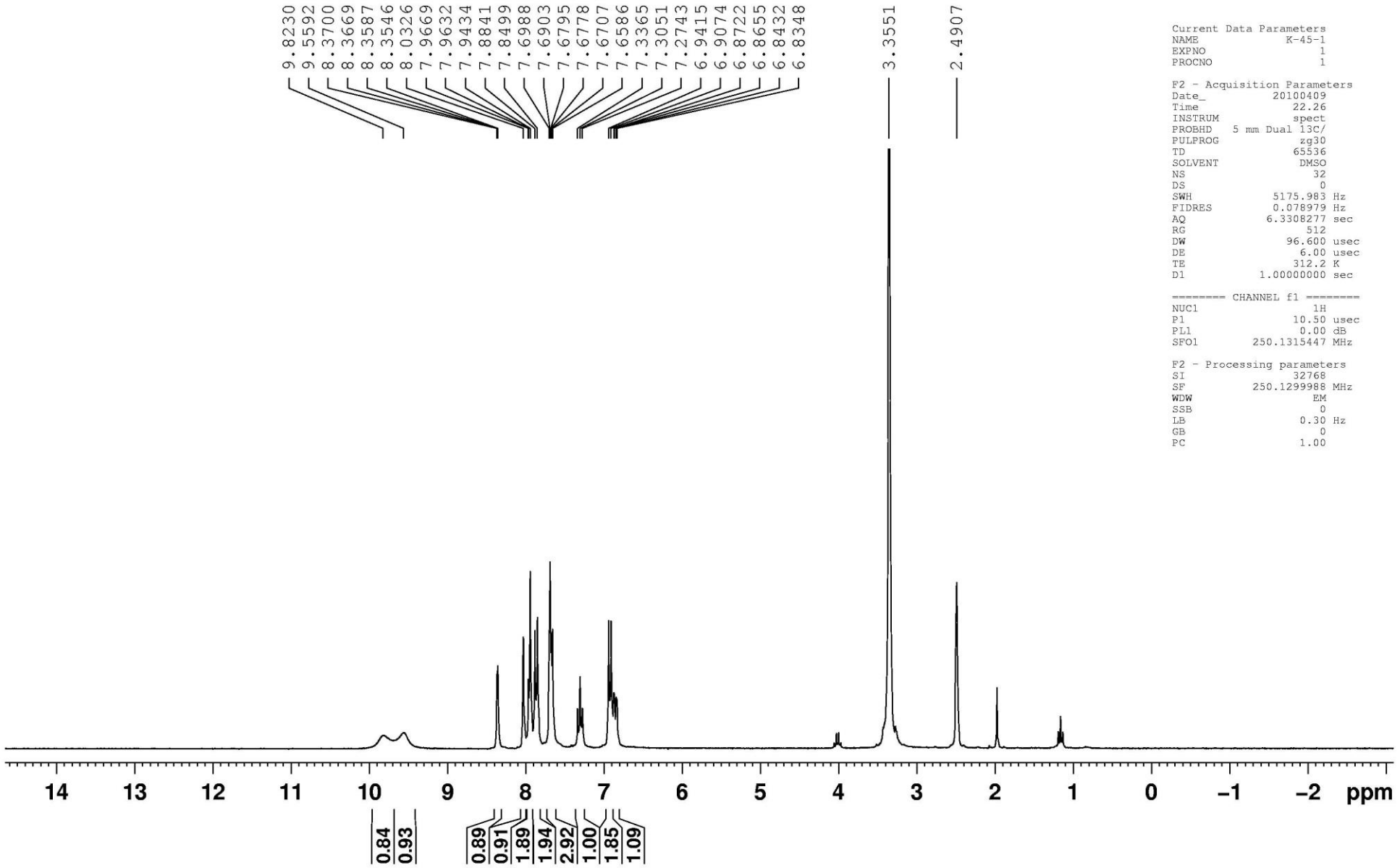


Compound-36, ^{13}C-NMR



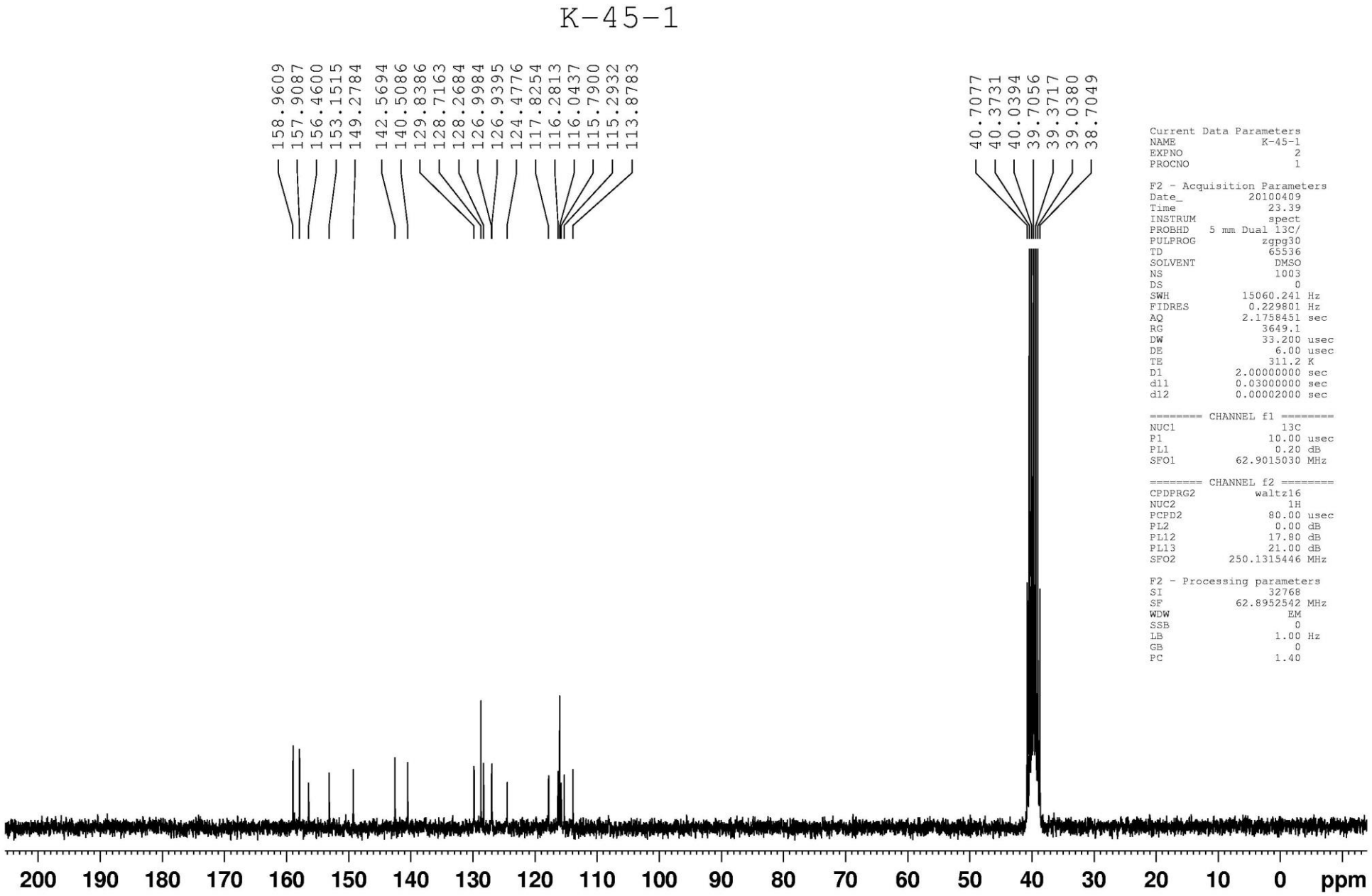
Compound-40, ^{1}H-NMR

K-45-1

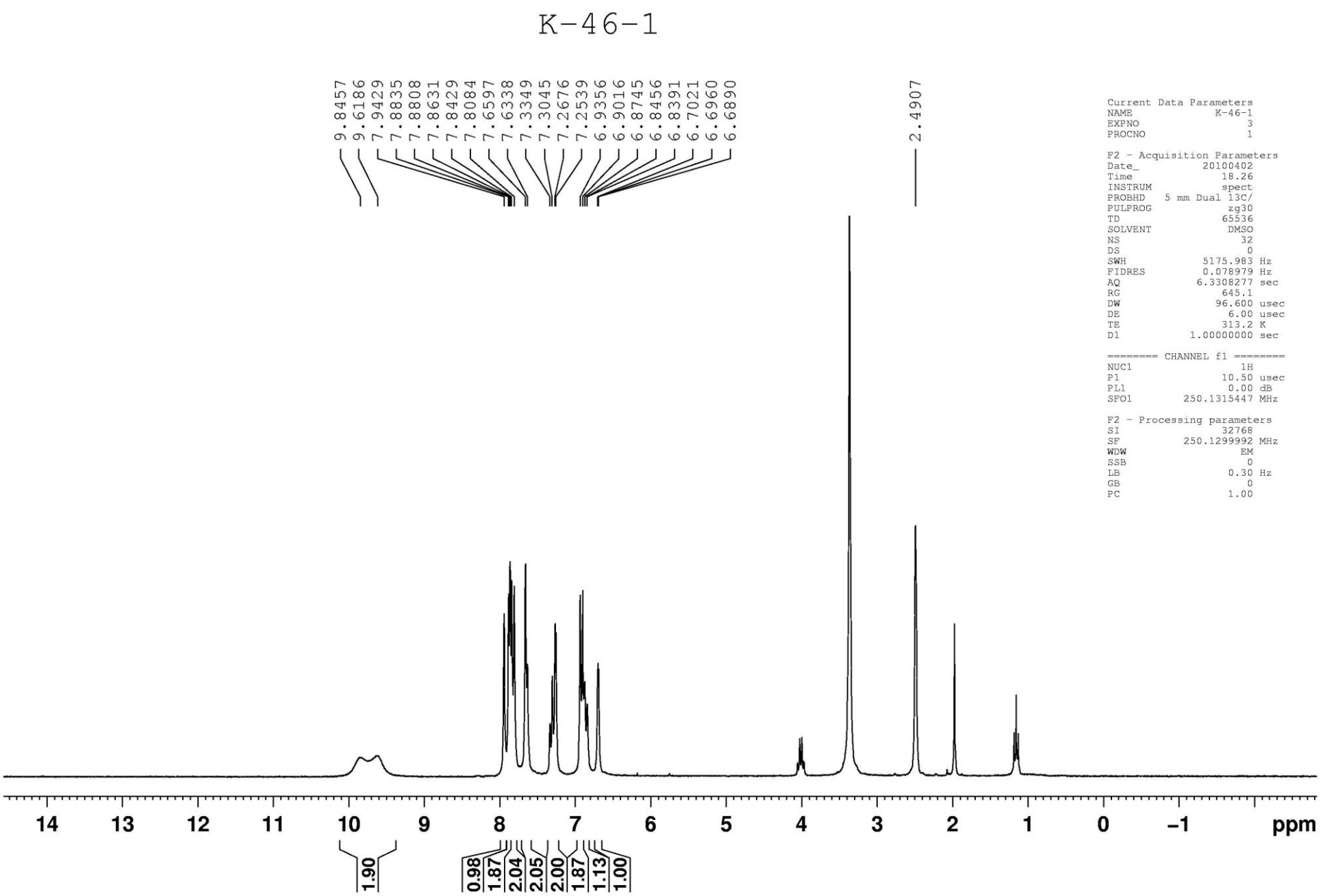



```
Current Data Parameters
NAME                  K-45-1
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_              20100409
Time                  22.26
INSTRUM               spect
PROBHD    5 mm Dual 13C/
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       32
DS                        0
SWH                5175.983 Hz
FIDRES            0.078979 Hz
AQ                6.3308277 sec
RG                      512
DW                   96.600 usec
DE                    6.00 usec
TE                   312.2 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   10.50 usec
PL1                   0.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                      32768
SF            250.1299988 MHz
WDW                        EM
SSB                        0
LB                    0.30 Hz
GB                         0
PC                     1.00
```

Compound-40, ^{13}C-NMR

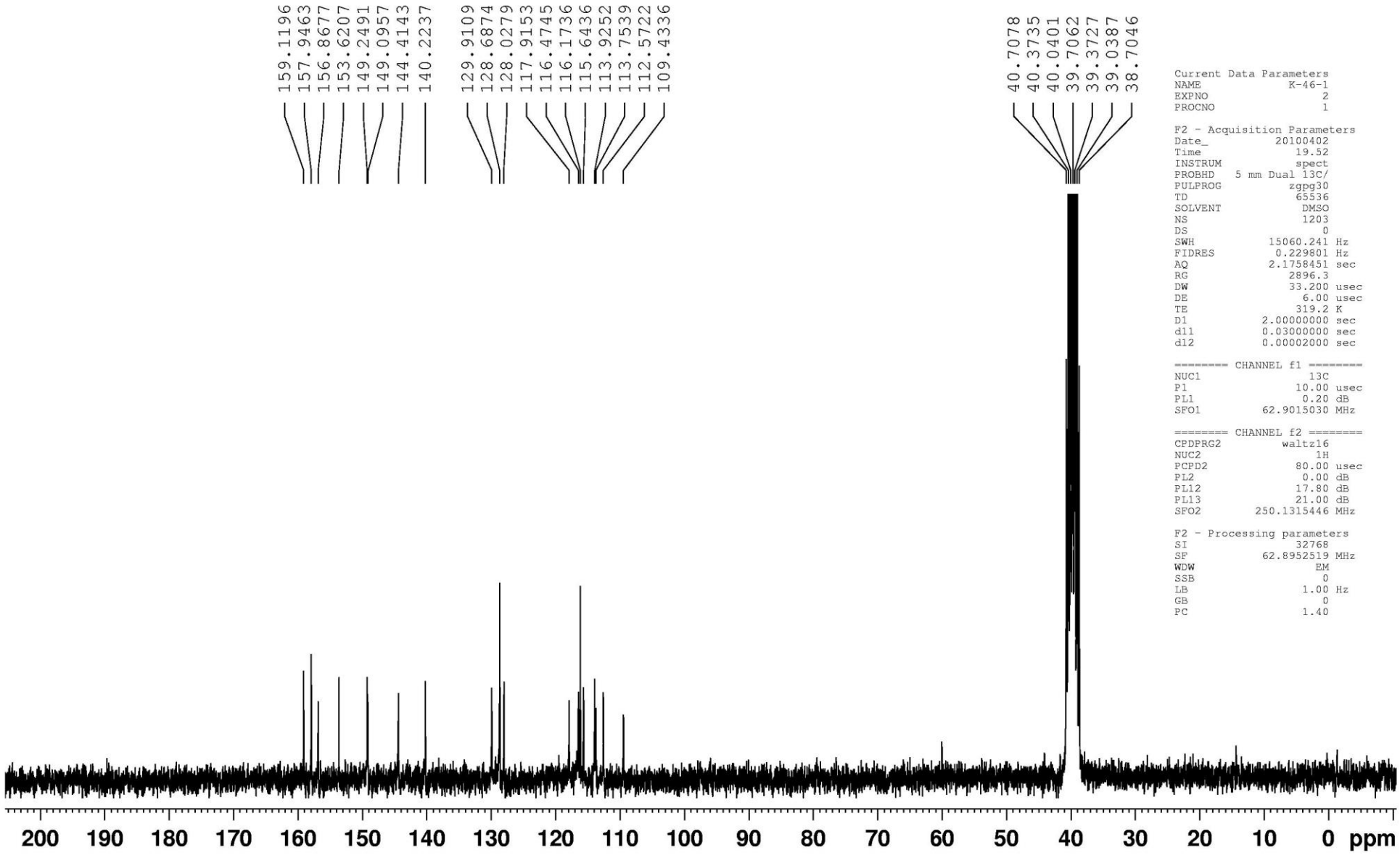


Compound-41, ^{1}H-NMR



Compound-41,^{1}H-NMR

K-46-1



Current Data Parameters

NAME	K-46-1
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

Date_	20100402
Time	19.52
INSTRUM	spect
PROBHD	5 mm Dual 13C/
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	1203
DS	0
SWH	15060.241 Hz
FIDRES	0.229801 Hz
AQ	2.1758451 sec
RG	2896.3
DW	33.200 usec
DE	6.00 usec
TE	319.2 K
D1	2.00000000 sec
d11	0.03000000 sec
d12	0.00002000 sec

======== CHANNEL f1 ========

NUC1	13C
P1	10.00 usec
PL1	0.20 dB
SFO1	62.9015030 MHz

======== CHANNEL f2 ========

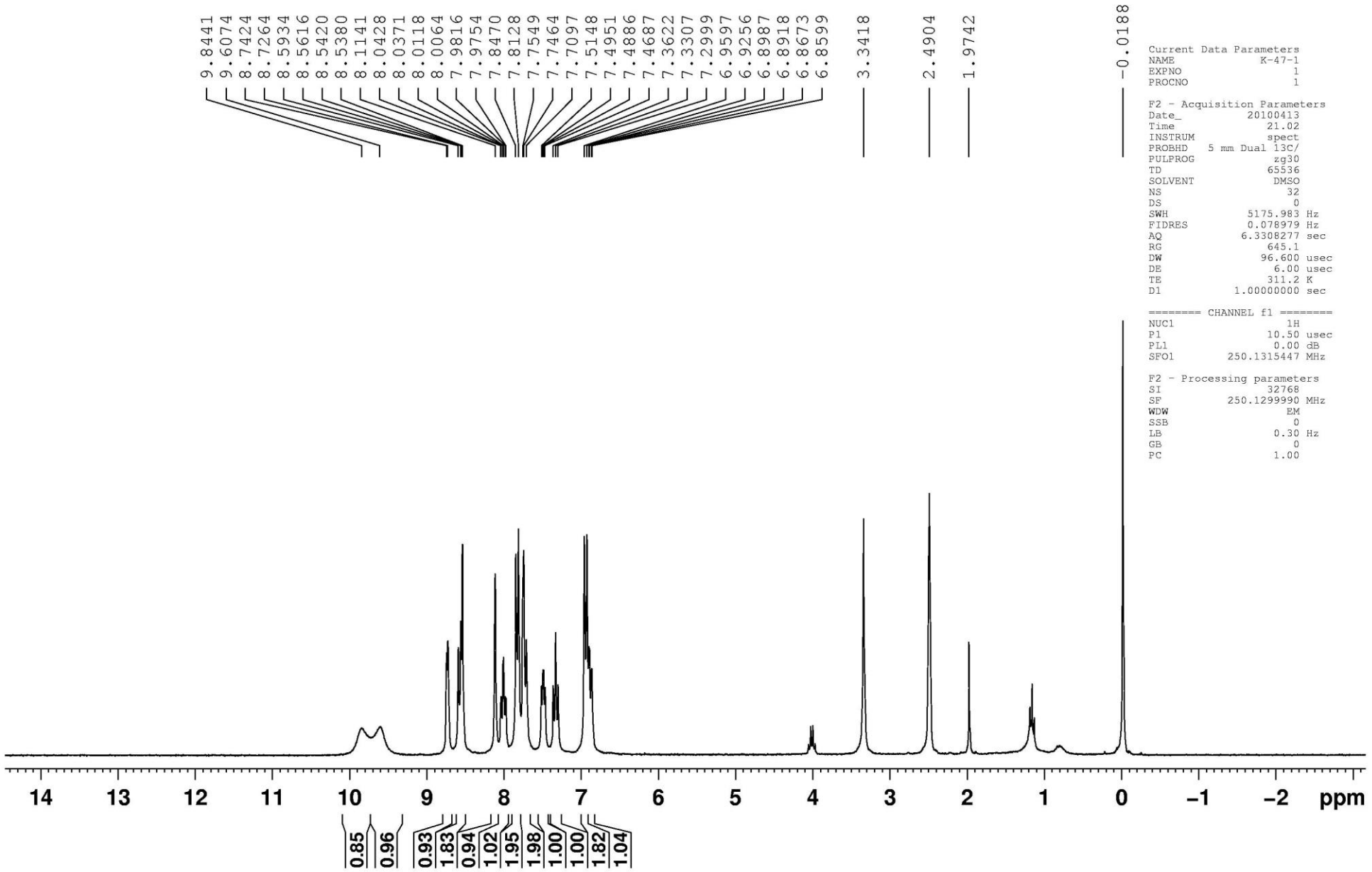
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	0.00 dB
PL12	17.80 dB
PL13	21.00 dB
SFO2	250.1315446 MHz

F2 - Processing parameters

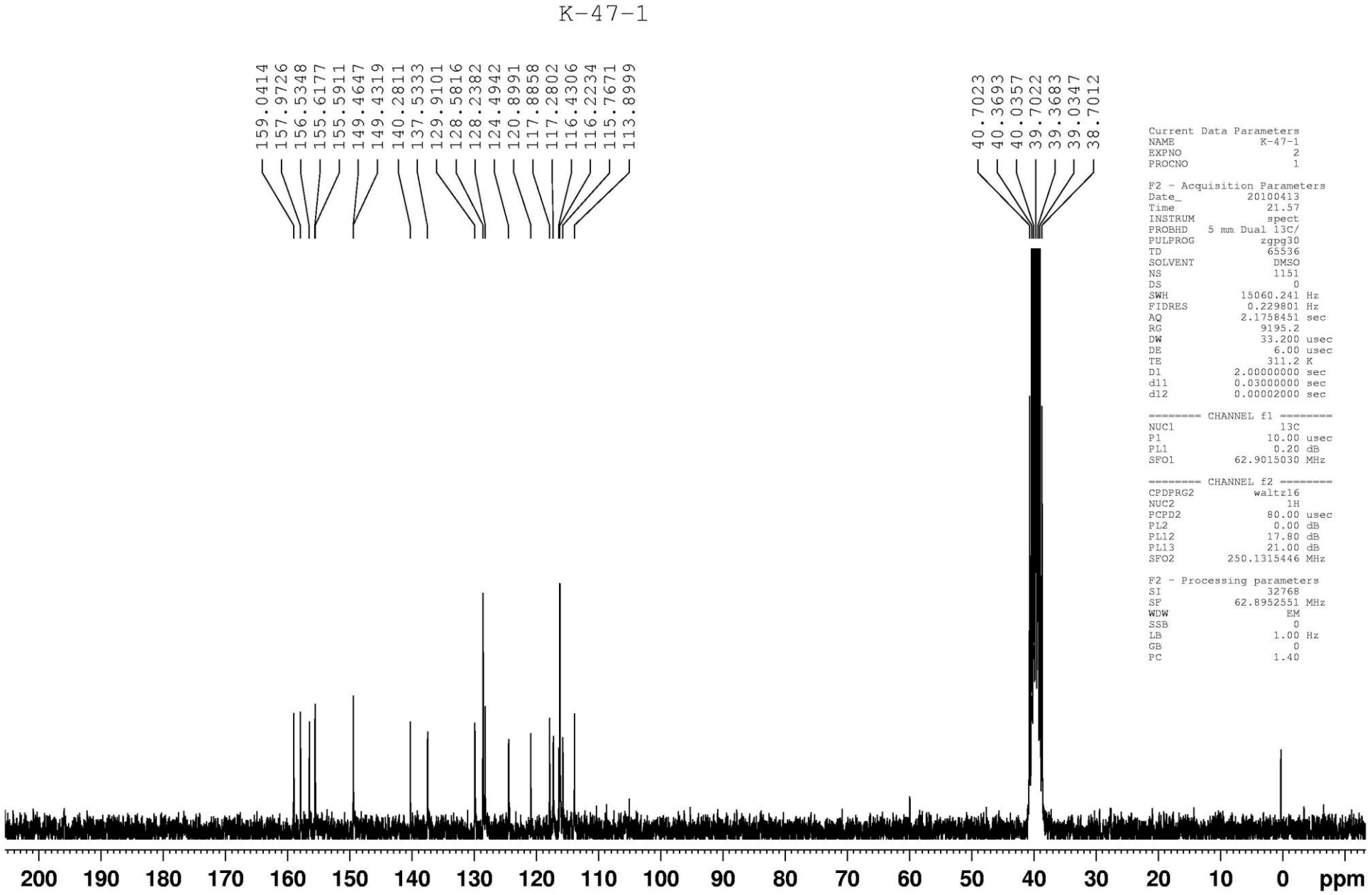
SI	32768
SF	62.8952519 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

Compound-42,^{1}H-NMR

K-47-1

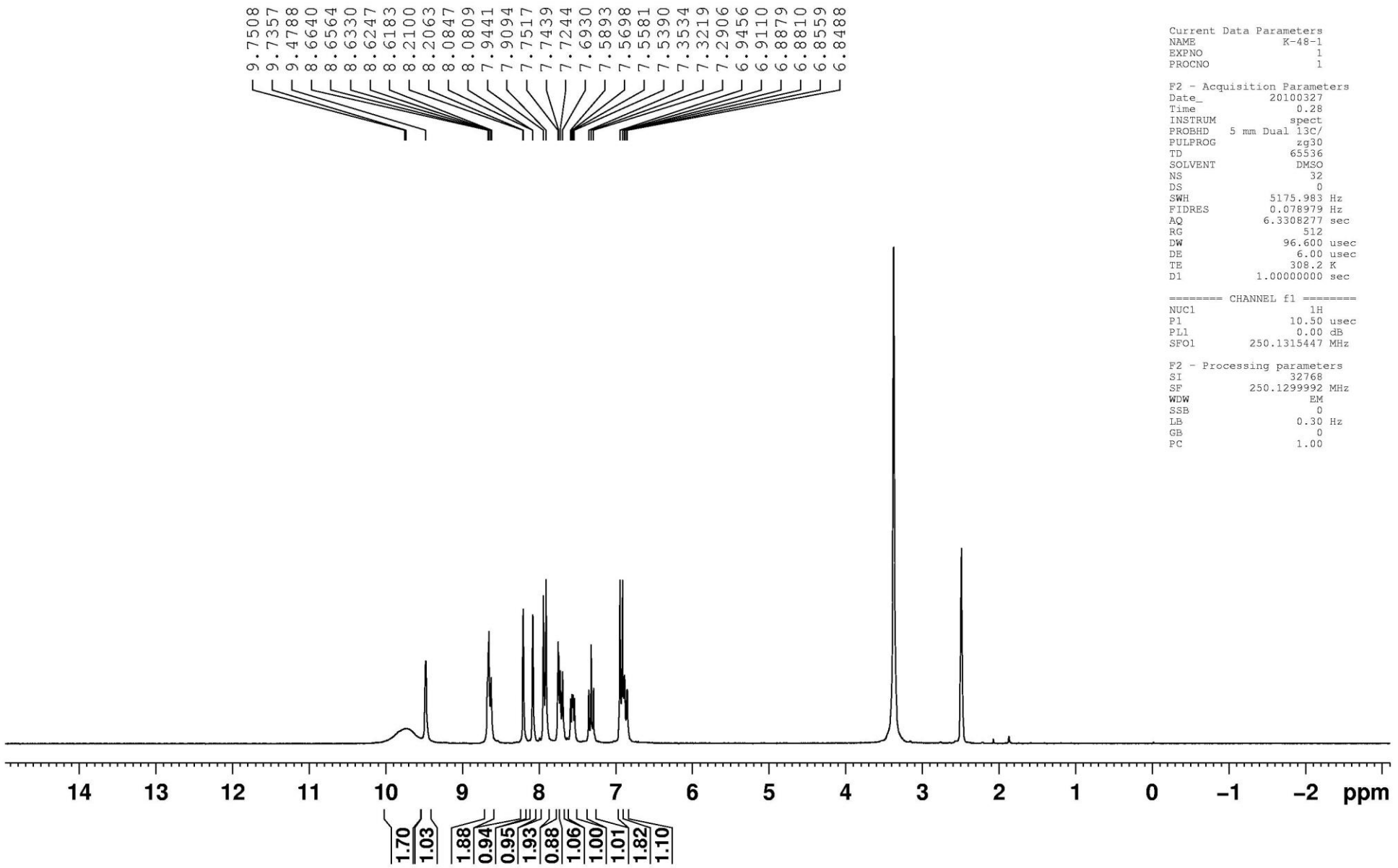


Compound-42, ^{13}C-NMR



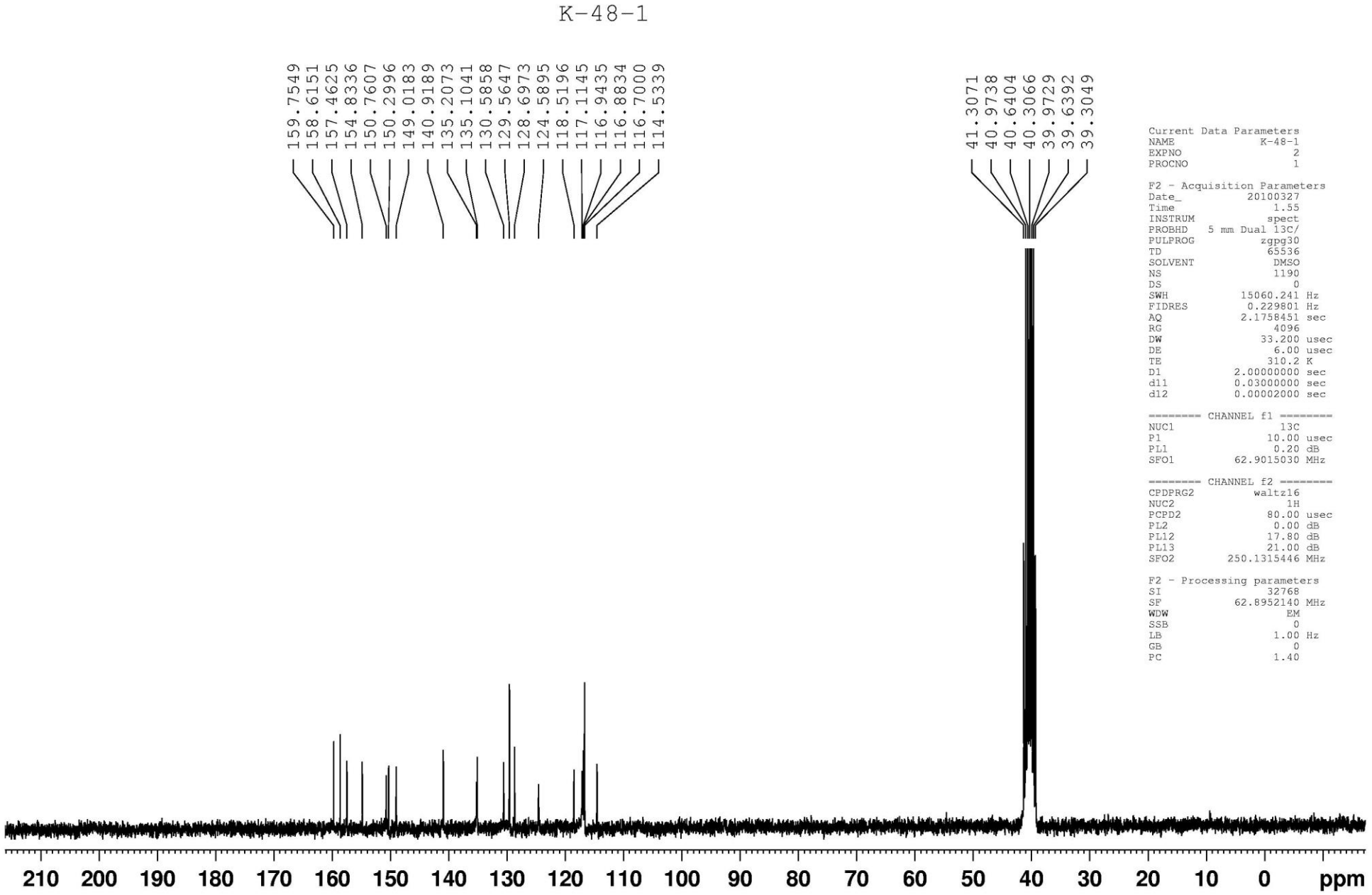
Compound-43,^{1}H-NMR

K-48-1

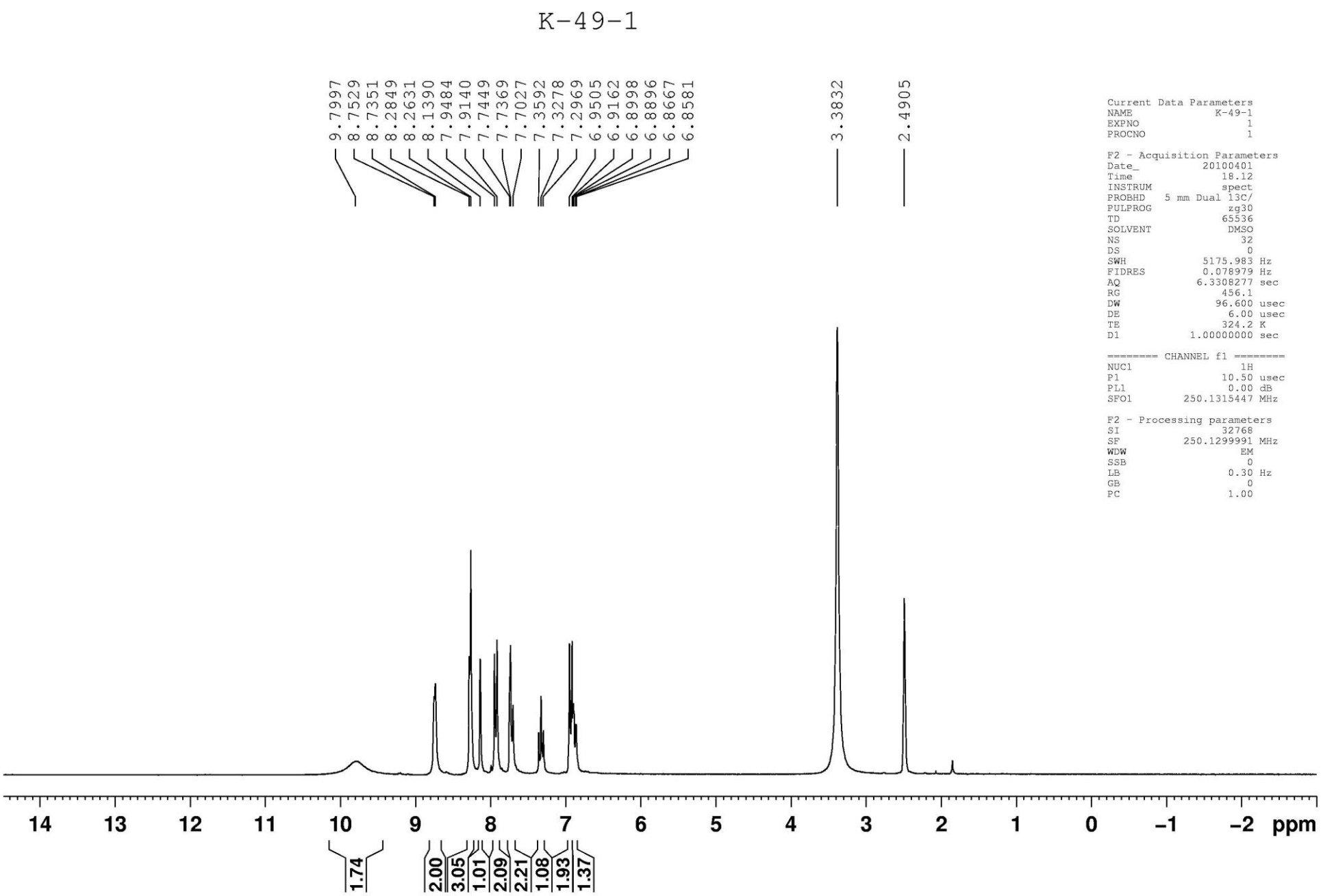



```
Current Data Parameters
NAME                  K-48-1
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_              20100327
Time                   0.28
INSTRUM               spect
PROBHD    5 mm Dual 13C/
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       32
DS                        0
SWH                5175.983 Hz
FIDRES            0.078979 Hz
AQ                6.3308277 sec
RG                       512
DW                  96.600 usec
DE                   6.00 usec
TE                   308.2 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                   10.50 usec
PL1                   0.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                     32768
SF            250.1299992 MHz
WDW                       EM
SSB                        0
LB                    0.30 Hz
GB                         0
PC                      1.00
```

Compound-43, ^{13}C-NMR

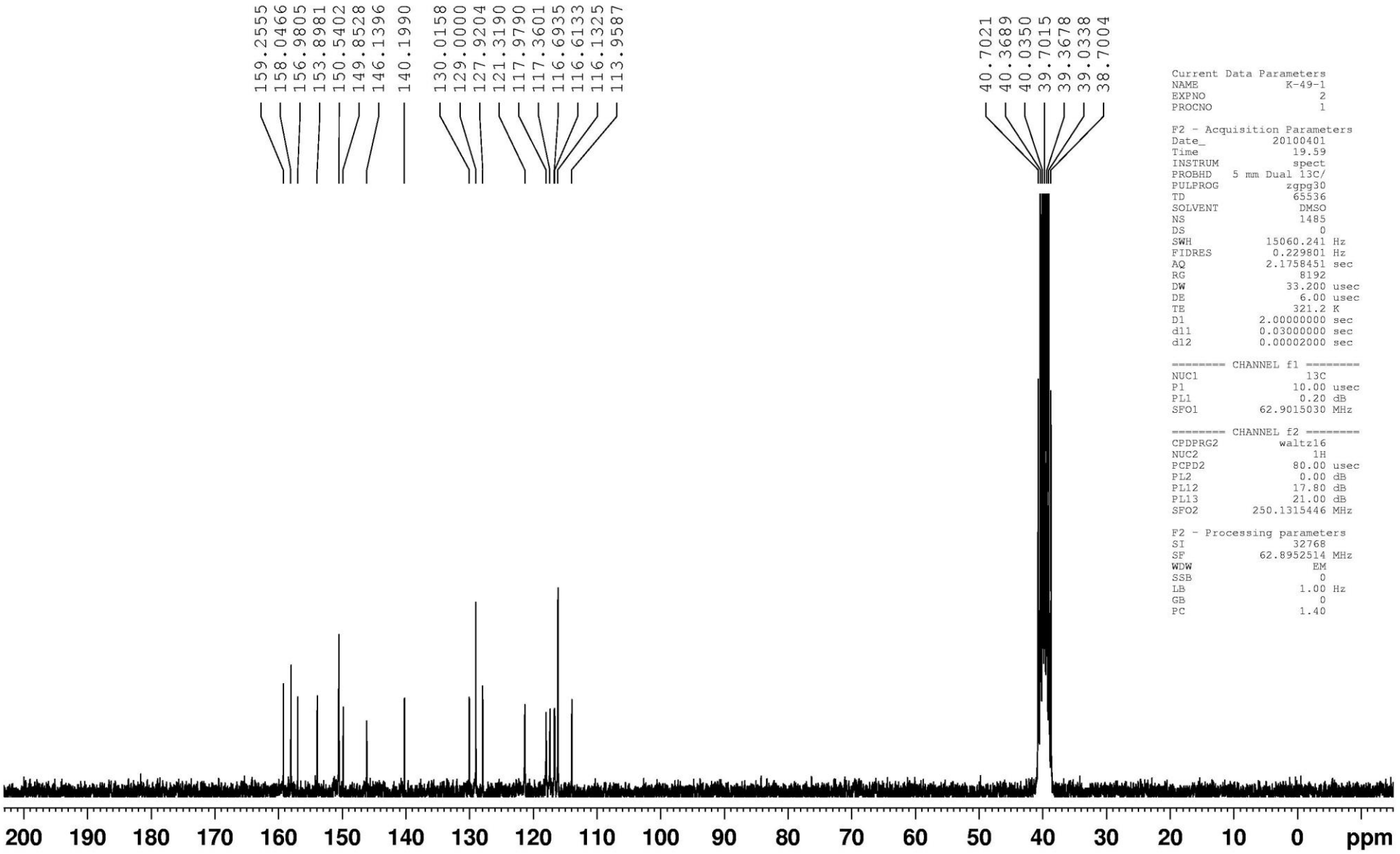


Compound-44,^{1}H-NMR



Compound-44, ^{13}C-NMR

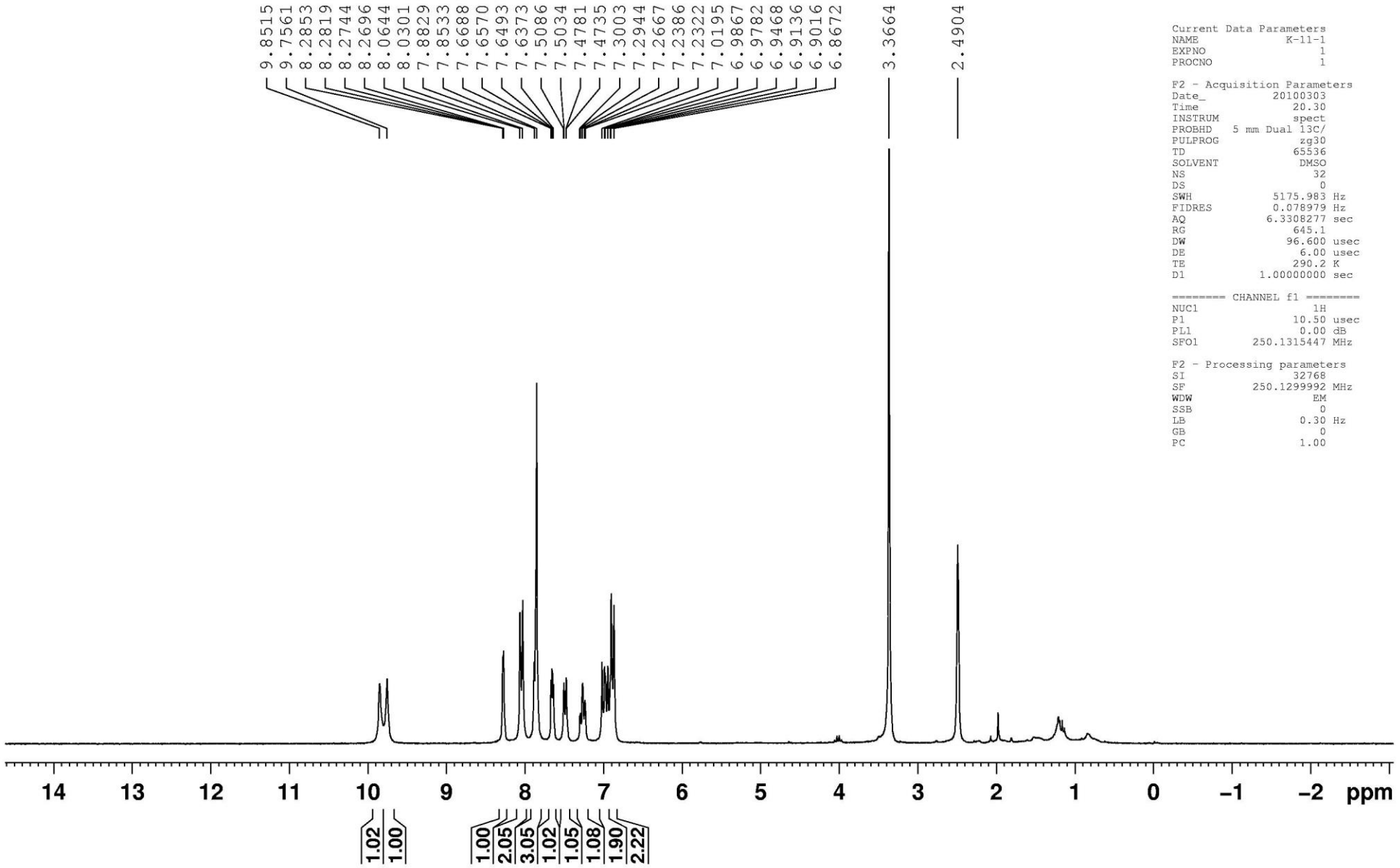
K-49-1




```
Current Data Parameters
NAME                  K-49-1
EXPNO                      2
PROCNO                     1

F2 - Acquisition Parameters
Date_              20100401
Time                  19.59
INSTRUM               spect
PROBHD    5 mm Dual 13C/
PULPROG             zgpg30
TD                   65536
SOLVENT                DMSO
NS                     1485
DS                        0
SWH               15060.241 Hz
FIDRES            0.229801 Hz
AQ                2.1758451 sec
RG                     8192
DW                  33.200 usec
DE                   6.00 usec
TE                   321.2 K
D1              2.00000000 sec
d11             0.03000000 sec
d12             0.00002000 sec

======== CHANNEL f1 ========
NUC1                    13C
P1                   10.00 usec
PL1                   0.20 dB
SFO1            62.9015030 MHz

======== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                    1H
PCPD2                80.00 usec
PL2                   0.00 dB
PL12                 17.80 dB
PL13                 21.00 dB
SFO2           250.1315446 MHz

F2 - Processing parameters
SI                     32768
SF              62.8952514 MHz
WDW                       EM
SSB                        0
LB                    1.00 Hz
GB                         0
PC                     1.40
```

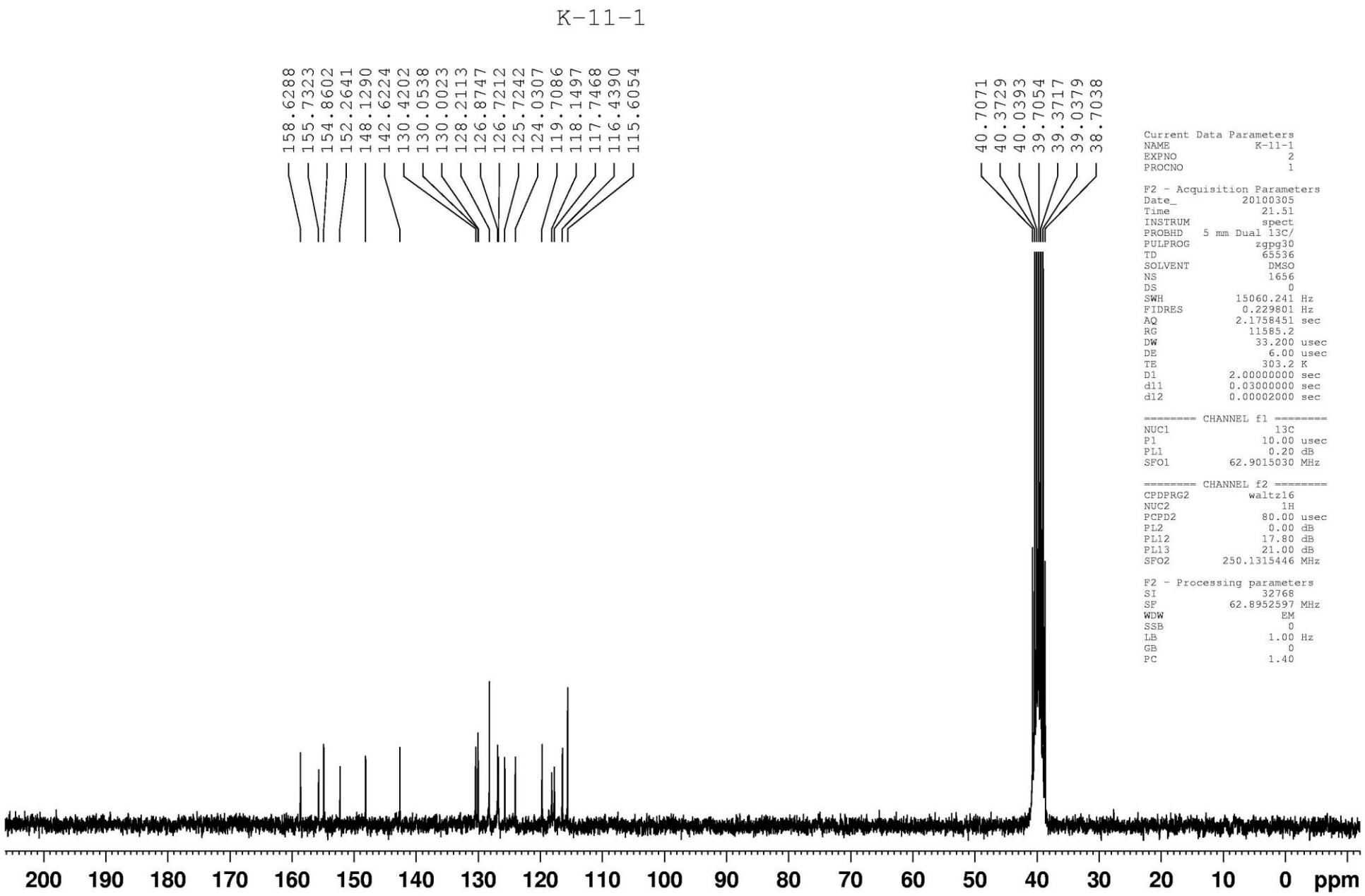
Compound-45, ^{1}H-NMR

K-11-1



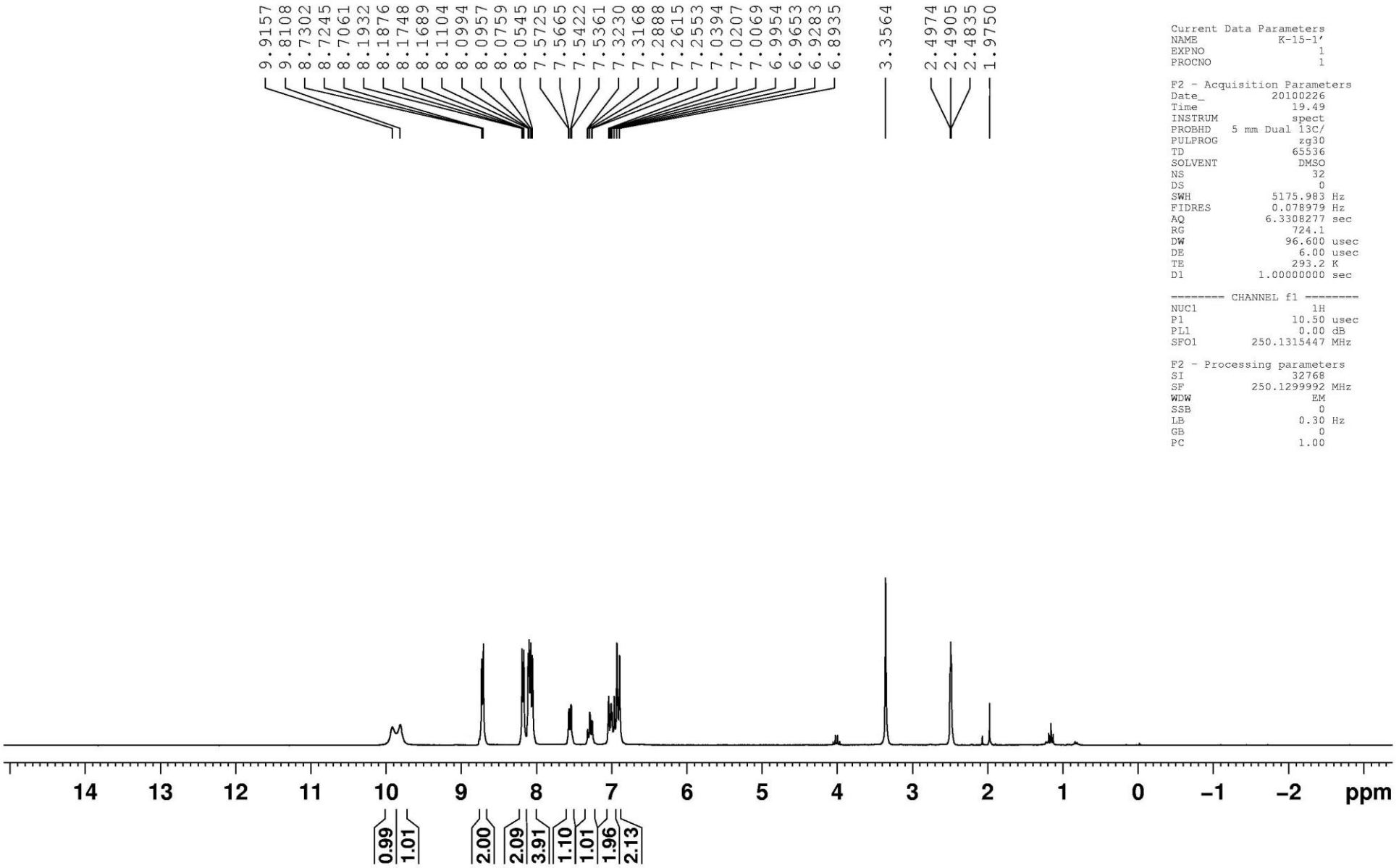

```
Current Data Parameters
NAME                  K-11-1
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_              20100303
Time                  20.30
INSTRUM               spect
PROBHD    5 mm Dual 13C/
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       32
DS                        0
SWH                5175.983 Hz
FIDRES            0.078979 Hz
AQ                6.3308277 sec
RG                    645.1
DW                  96.600 usec
DE                   6.00 usec
TE                   290.2 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                    10.50 usec
PL1                    0.00 dB
SFO1          250.1315447 MHz

F2 - Processing parameters
SI                     32768
SF            250.1299992 MHz
WDW                       EM
SSB                        0
LB                    0.30 Hz
GB                         0
PC                      1.00
```

Compound-45, ^{13}C-NMR



Compound-49, ^{1}H-NMR

K-15-1′



Current Data Parameters

NAME	K-15-1′
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20100226
Time	19.49
INSTRUM	spect
PROBHD	5 mm Dual 13C/
PULPROG	zg30
TD	65536
SOLVENT	DMSO
NS	32
DS	0
SWH	5175.983 Hz
FIDRES	0.078979 Hz
AQ	6.3308277 sec
RG	724.1
DW	96.600 usec
DE	6.00 usec
TE	293.2 K
D1	1.00000000 sec

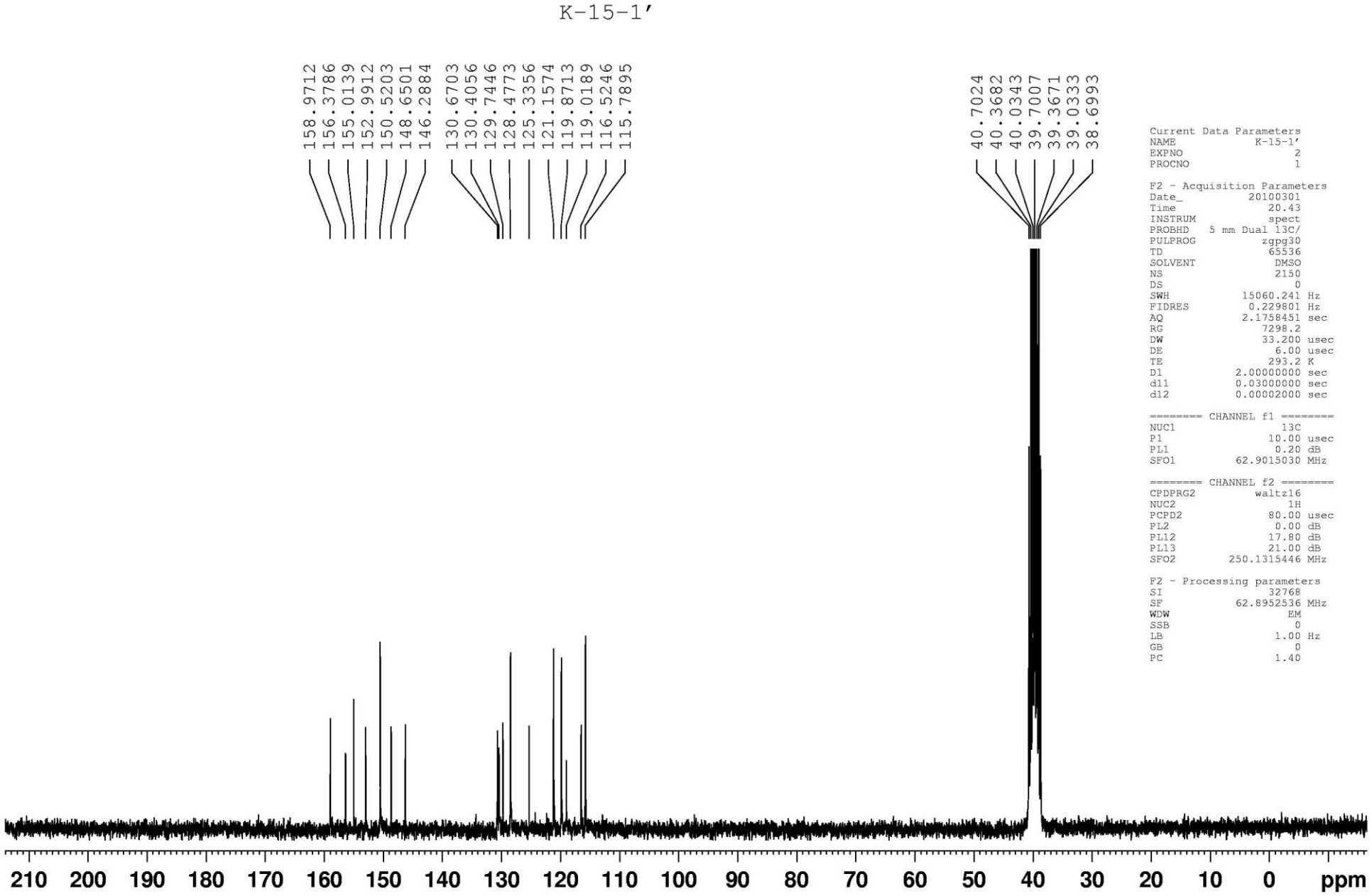
======== CHANNEL f1 ========

NUC1	1H
P1	10.50 usec
PL1	0.00 dB
SFO1	250.1315447 MHz

F2 - Processing parameters

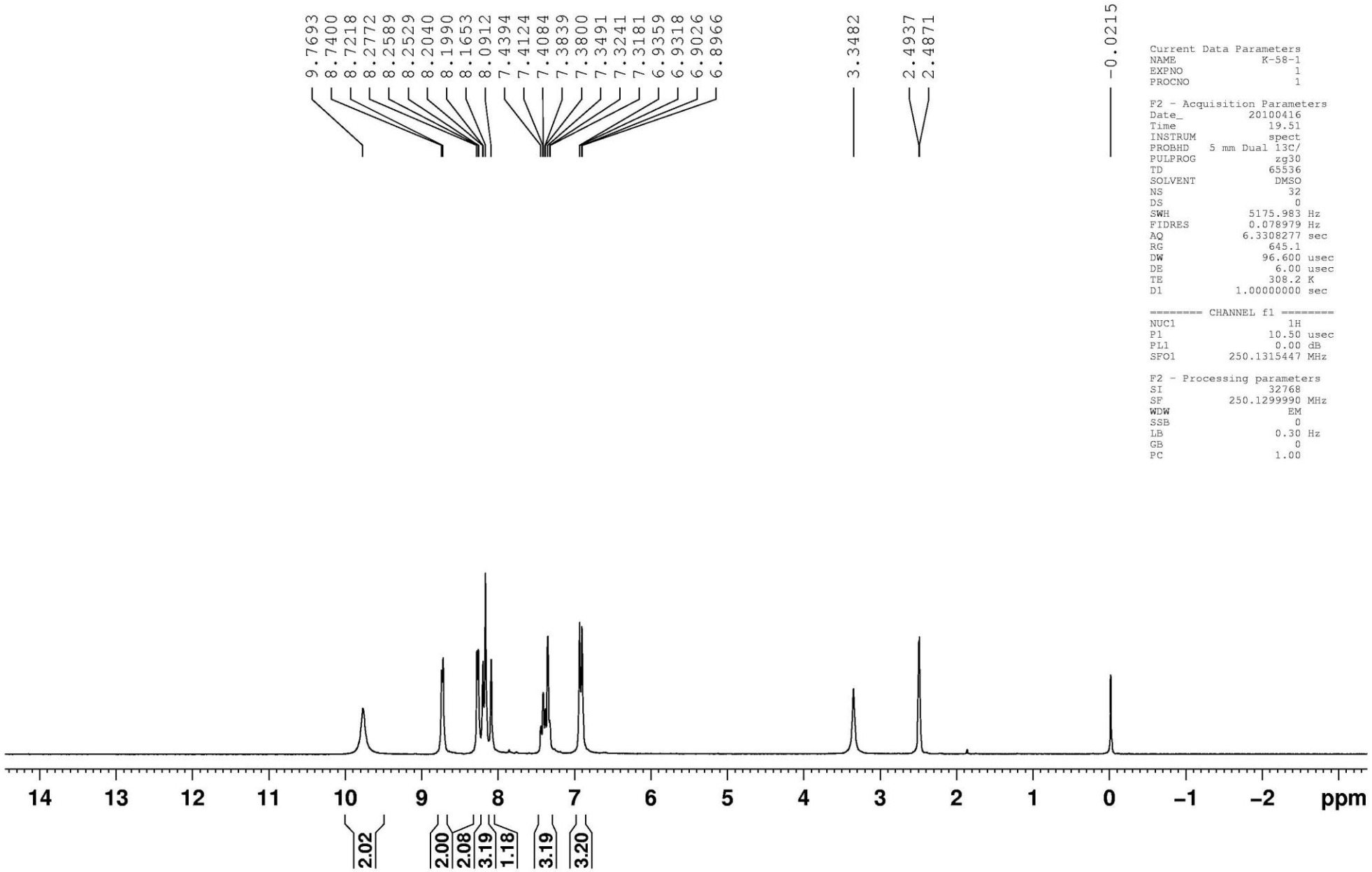
SI	32768
SF	250.1299992 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

Compound-49, ^{13}C-NMR



Compound-54,^{1}H-NMR

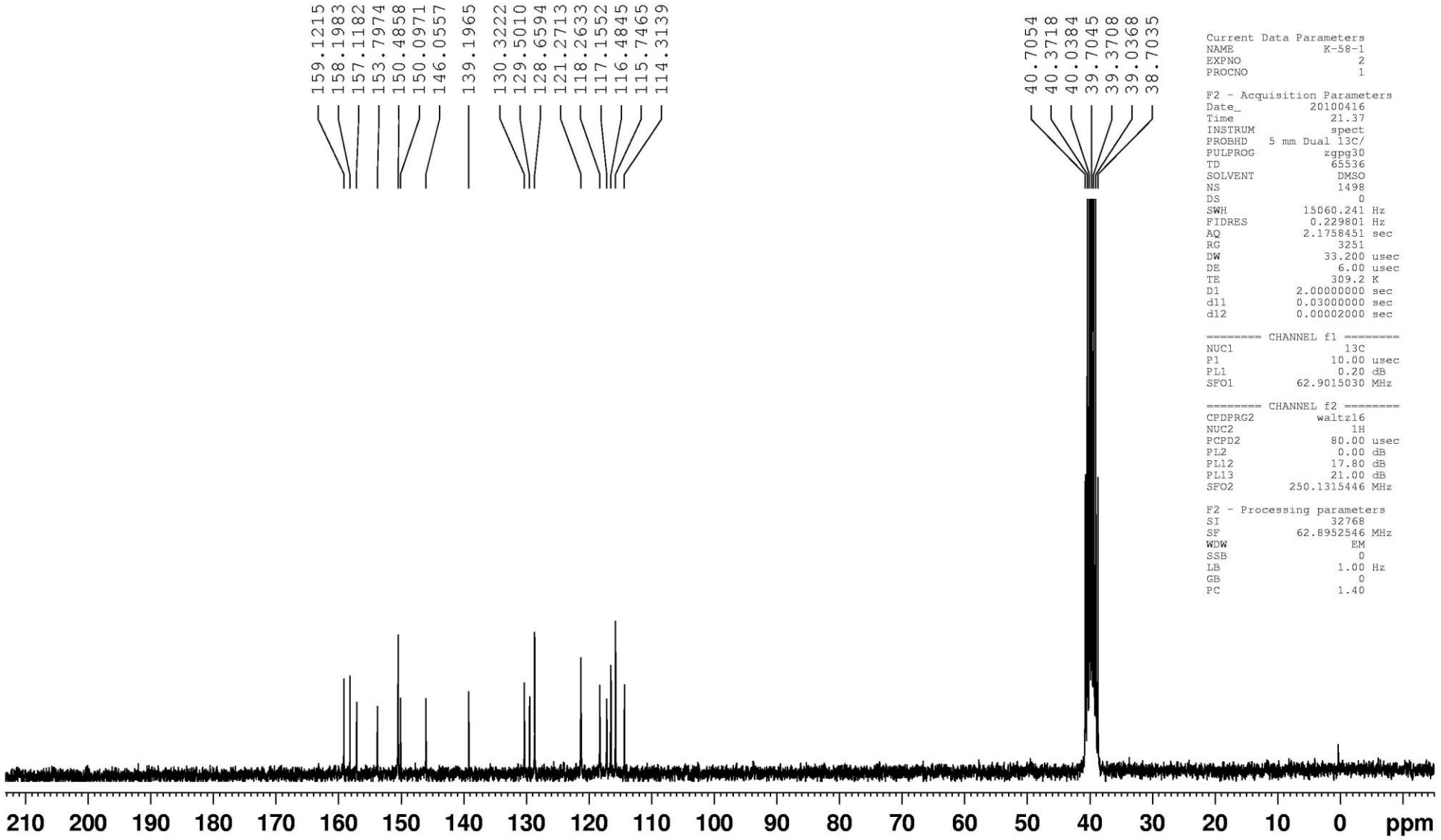
K-58-1




```
Current Data Parameters
NAME                  K-58-1
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_               20100416
Time                   19.51
INSTRUM                spect
PROBHD    5 mm Dual 13C/
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       32
DS                        0
SWH                5175.983 Hz
FIDRES             0.078979 Hz
AQ                 6.3308277 sec
RG                     645.1
DW                    96.600 usec
DE                     6.00 usec
TE                     308.2 K
D1                1.00000000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                     10.50 usec
PL1                     0.00 dB
SFO1           250.1315447 MHz

F2 - Processing parameters
SI                        32768
SF                250.1299990 MHz
WDW                        EM
SSB                        0
LB                      0.30 Hz
GB                         0
PC                      1.00
```

Compound-54, ^{13}C-NMR

K-58-1



Current Data Parameters

NAME	K-58-1
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

Date_	20100416
Time	21.37
INSTRUM	spect
PROBHD	5 mm Dual 13C/
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	1498
DS	0
SWH	15060.241 Hz
FIDRES	0.229801 Hz
AQ	2.1758451 sec
RG	3251
DW	33.200 usec
DE	6.00 usec
TE	309.2 K
D1	2.00000000 sec
d11	0.03000000 sec
d12	0.00002000 sec

======== CHANNEL f1 ========

NUC1	13C
P1	10.00 usec
PL1	0.20 dB
SFO1	62.9015030 MHz

======== CHANNEL f2 ========

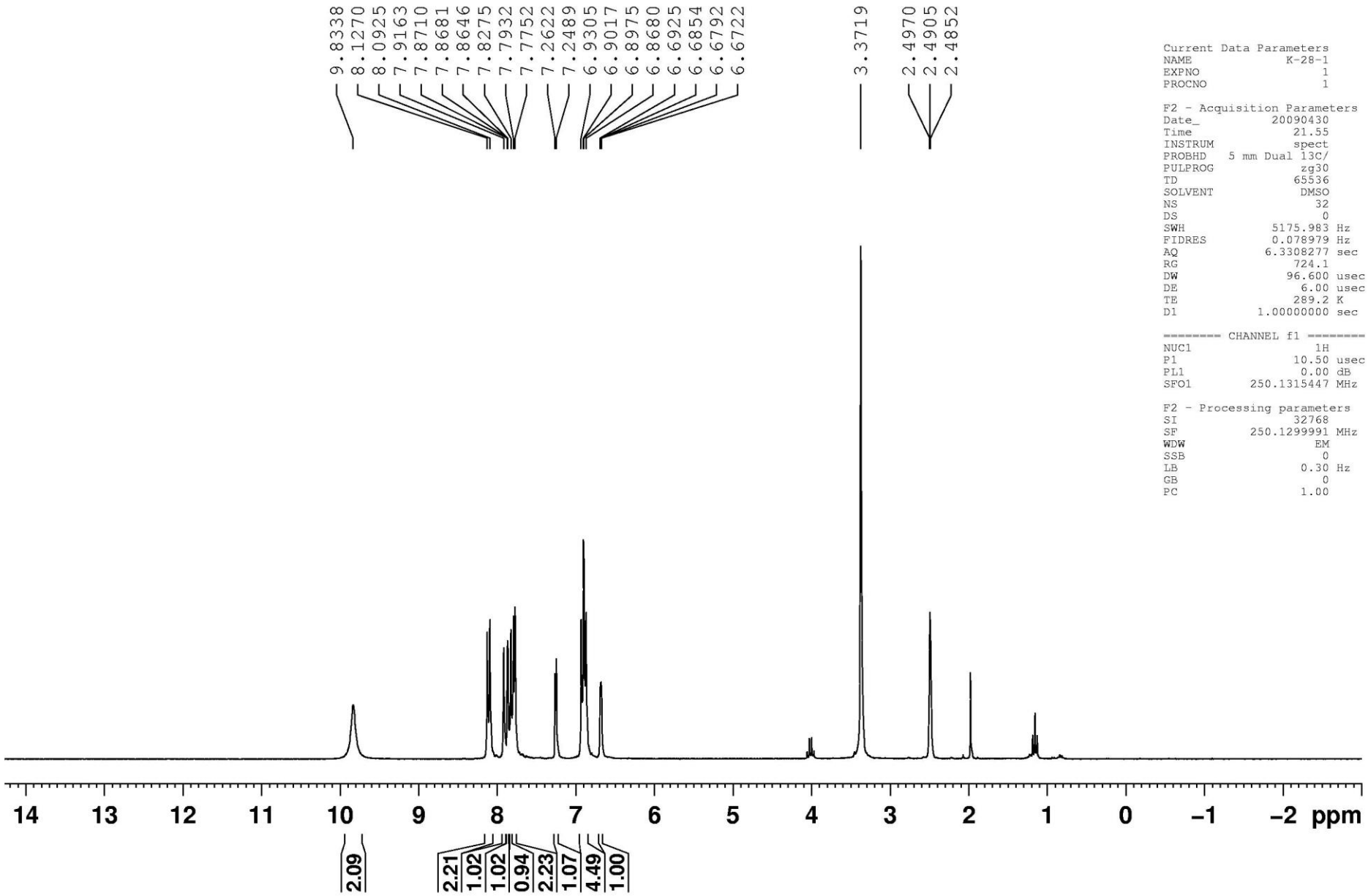
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	0.00 dB
PL12	17.80 dB
PL13	21.00 dB
SFO2	250.1315446 MHz

F2 - Processing parameters

SI	32768
SF	62.8952546 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

Compound-56,^{1}H-NMR

K-28-1



Compound-56, ^{13}C-NMR