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Synthesis, cytotoxic activities and molecular modeling studies of some 2-aminonaphtho[2,3-*d*][1,3]thiazole-4,9-dione derivatives

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Abstract: Quinones, especially 1,4-naphthoquinones, are one of the most significant and widely distributed phytochemical groups in nature. 1,4-Naphthoquinones and their synthetic derivatives are found to possess remarkable cytotoxic activities. In this study, a series of 2-aminonaphtho[2,3-*d*][1,3]thiazole-4,9-dione derivatives were synthesized and their structures were verified with spectral analysis. *In vitro* cytotoxic activities of the synthesized compounds were evaluated by using MTT assay against MKN-45 (Human Gastric cancer), MDA-MB-231 (Human Breast cancer) and HeLa (Human Cervical cancer) cell lines. Among the synthesized compounds, **3d** inhibited MDA-MB-cell proliferation with an IC_{50} value of 0.276 µM. Compound **3a** inhibited HeLa and MKN-45 cell proliferation with IC_{50} values of 0.336 µM and 8.769 µM, respectively. Compound **3b** inhibited HELA cell proliferation with an IC_{50} value of 0.269 µM. Molecular docking results suggest that the ligands may bind to the hDNA TopoIIβ binding pocket and partially exert their effects. These results propose that 2-aminonaphtho[2,3-*d*]thiazole-4,9-dion core has important biological effects and further explorations are worthwhile.

Keywords: 2-aminonaphtho[2,3-*d*][1,3]thiazole-4,9-dione, naphthoquinones, DNA topoisomerases, cytotoxic activity. ©2020 ACG Publication. All right reserved.

1. Introduction

Cancer, which is driven by genetic change, is the second most common disease worldwide, killing more than eight million people each year.[1] Radiotherapy,[2] chemotherapy,[3] surgery, and phototherapy are widely used strategies for cancer treatment.[4] Chemotherapy is one of the approaches that anticancer drugs are given systemically to the patients, meaning that drugs reach cancer cells via the blood stream to damage cancerous cells as well as healthy cells. Serious side effects of these

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chemotherapy drugs have been reduced thanks to the development of novel cancer-targeting formulations in clinics.[5] Unfortunately, continuous drug resistances against these agents have compelled the researchers to seek novel and more effective anticancer molecules.

In this regard, natural products have been a good source of molecules for researchers to utilize their various biological properties especially anticancer activities.[6-8] These natural compounds and their synthetic derivatives have widely been tested for their potential anticancer properties as well as their mechanisms of action[6-10]. Among these naturally occurring molecules, quinones especially 1,4-naphthoquinones are one of the most significant and widely distributed phytochemical groups. Naturally occurring 1,4-naphthoquinones and their synthetic derivatives were reported to possess remarkable therapeutic activities, such as; antibacterial,[11] antiallergic,[12] antiplatelet and antithrombotic,[13] antiviral, antifungal, and anticancer.[11]

Naphthoquinone based naturally occurring compounds such as griffithazanone A, laoticuzanone A,[14] lapachol,[15] alfa and β-lapachones,[16] lawsone,[17] juglone, plumbagin,[18] and shikonin[19] were reported to show cytotoxic activities with different mechanisms of action.[20]

Several mechanisms of action have been proposed for the anticancer activity of these naphthoquinone derivatives such as interaction with DNA topoisomerases I and II, ability to generate semiquinone radical anions and reactive oxygen species (ROS) and intercalation into DNA. However, mainly cytotoxic activity is associated with inhibition of human DNA topoisomerase IIβ (hDNA TopoIIβ).[20]

DNA topoisomerases catalyze the mutual transformation of various topological states of DNA,[21] and play the role in DNA replication, transcription, and chromosome separation.[22] They bind covalently to the phosphate groups of DNA, split DNA strands, and at last reunite them. Concerning their mechanism of action, DNA topoisomerases are divided into two main types; topoisomerase I and II, which are proved to be therapeutic targets of anticancer and antibacterial drugs.[13] In such studies, information on the interaction of the drug with DNA topoisomerase II (TopoII) activity was also provided.[23] Drugs targeting TopoII are divided into two main groups. The first group is called TopoII poisons and includes most of the active agents such as etoposide, doxorubicin, and mitoxantrone. It leads the increases in the levels of TopoII-DNA covalent complexes. These agents create lesions that contain DNA strand breaks and proteins covalently bound to DNA.[22] TopoII poisons are divided into two sub-groups as intercalating and non-intercalating poisons. TopoII poisons that do not intercalate include epipodophyllotoxins, etoposide, teniposide, and fluoroquinolones.[24] Compounds in the second group are called TopoII catalytic inhibitors. Although they inhibit TopoII catalytic activity, they do not increase the levels of TopoII covalent complexes. Agents in this second group are thought to kill cells by abolishing the essential enzymatic activity of TopoII by binding the ATPase site of enzyme resulted in mitotic failure. There are many studies in the literature regarding the use of TopoII inhibitors in cancer treatment.[22]

Anthracyclines such as doxorubicin, daunorubicin and idarubicin are naphthoquinone-containing compounds used for the treatment of various types of cancers. As anthracenediones do not contain a glycoside moiety, they differ from anthracyclines. The synthetic anthracenedione, mitoxantrone, is used to treat metastatic breast cancer, acute myeloid leukemia, and non-Hodgkin's lymphoma. Anticancer activities, exhibited by these 1,4-naphthoquinone carrying compounds prompted researchers to design novel derivatives with potent anticancer activity. Recently, various reports have appeared in the literature on the synthesis and anticancer activities of 1,4-naphthoquinone derivatives.[11,21,25-30]

Some small molecules were reported *via* HTS compound screening. It was stated that the proposed compounds are potential candidates for developing better anticancer drugs, among which 2-[(2-chlorophenyl)amino]naphtho[2,3-*d*]thiazole-4,9-dione (NSC631527) (Figure 1) was reported to be more cytotoxic toward *S. pombe* than mammalian cells.[31]

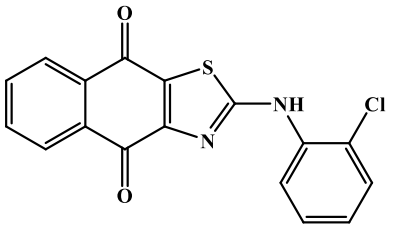


Figure 1. 2-[(2-chlorophenyl)amino]naphtho[2,3-*d*]thiazole-4,9-dione (NSC631527)[31]

On the other hand, heterocyclic thiazole ring exists in the structure of the many current anticancer drugs, indicating that thiazole fused rings and thiazole bearing hybrid structures are important for designing novel anticancer agents.[32-34]

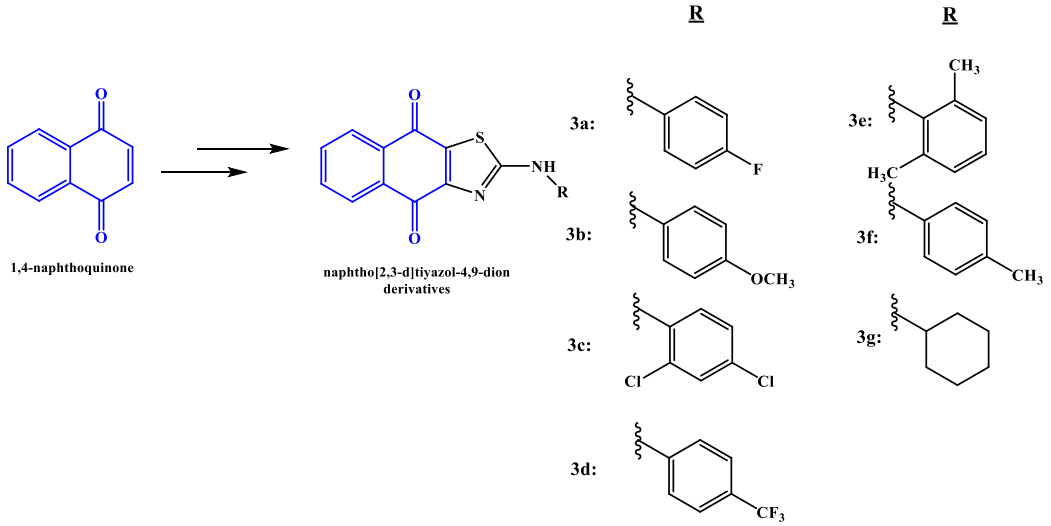


Figure 2. Molecular structures of synthesized compounds **3a-g**

In light of these observations, in this study, we aimed to synthesize potential cytotoxic 1,4-naphthoquinone derivatives. Structural modifications were made through 2-aminothiazole fusion to the ring. Also, derivatization was rationalized considering the amino group on the thiazole ring. The substituents are generally activity enhancing groups, such as chlorophenyl, methoxyphenyl, methylphenyl, fluorophenyl and cyclohexyl (Figure 2). Novel naphtho[2,3-*d*]thiazole-4,9-dione derivatives were synthesized by using two-step reactions. Their structures were evaluated and confirmed by elemental analysis, FT-IR and ^{1}H-NMR spectra. The synthesized compounds were evaluated on L929 (Mouse Fibroblast Cell line), MKN-45 (Human Gastric), MDA-MB-231 (Human Breast), and HeLa (Human Cervical) cancer cell lines by using MTT assay. Molecular modeling studies of the compounds were also performed against hDNA TopoIIβ active site by using molecular modeling tools to acquire insights into the binding mode.

2. Experimental

2.1. Chemical Material and Apparatus

4-(Trifluorophenyl) isothiocyanate, 4-fluorophenyl isothiocyanete, 4-(methoxyphenyl) isothiocyanate, 2,4-dichlorophenyl isothiocyanate, 2,6-dimethyl isothiocyanate, p-tolyl isothiocyanate and cyclohexyl isothiocyanate were purchased from Sigma Aldrich and Merck. The solvents were dried and distilled according to the standard procedures.

Infrared spectra were recorded on a Perkin-Elmer FT-IR System Spectra BX over the range 4000-500 cm^{-1}. All NMR measurements were carried out on a Varian 600 Spectrometer operating at

600 MHz. ^{13}C-NMR was acquired on an Agilent VNMRS Spectrometer at 125 MHz. The chemical shifts were reported in δ (ppm) with respect to the internal standard TMS. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy measurements were recorded using a Perkin-Elmer Spectrum BX FT-IR spectrometer over the range of 4000-500 cm^{-1}. Melting points (ºC) were measured using the Schmelzpunktbestimmer SMP II melting point apparatus.

2.2. Chemistry

*2.2.1. The Synthesis of Compound **2***

*(2-Amino-3-bromo-1,4- naphthoquinone):*A mixture of 2,3-dibromo-1,4-naphthoquinone (**1**) (8.0 g, 20.0 mmol), dioxane (130 mL), and 25% aqueous NH_3 (110 mL) was stirred at room temperature for 2.5 h, which was precipitated from water, filtered and dried in air to yield the title compound **2** (5.8 g, 91%), melting point is 204-205.[35]

*2.2.2 General Procedure for the Synthesis of **3a-3g***

2-((Substituted)amino)naphtho[2,3-d]thiazole-4,9-dions: Isothiocyanate derivative (1 mmol) was treated with 2-amino-3-bromo-1,4-naphthoquinone (1 mmol) (**2**) under neat conditions to obtain the corresponding thiourea, to which *in situ* generated thiourea, CuO nanoparticles (2.5 mol%) and K_2CO_3 (1.5 mmol) in H_2O (5 mL) was subsequently added. Then, the reaction mixture was subjected to refluxed. The progress of the reaction was monitored by TLC. After 24 h, on completion of the reaction, the mixture was cooled to room temperature and extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified on a column chromatography packed with silica gel eluting with a mixture of EtOAc-petroleum ether (2 : 8) to give the pure products **3a**-**3g**.[36]

*2-[(4-fluorophenyl)amino]naphtho[2,3-d][1,3]thiazole-4,9-dione (**3a**):* (Yield, 13%), Analysis for $C_{17}H_9FN_2O_2S$; calcd. (%): C, 62.96; H, 2.80; N, 5.86; S, 9.89. Found: C, 62.13; H, 2.37; N, 8.68; S, 9.41. ^{1}H-NMR (600 MHz, DMSO-d_6, δ, ppm): 11.59 (1H, s), 8.10-8.02 (2H, dd, *J*= 8.9 Hz), 7.92-7.91 (2H, d, *J*=8.5 Hz), 7.85-7.84 (2H, t, *J*=8.8 Hz), 7.77-7.76 (2H, d, *J*=8.6 Hz). FT-IR (ATR) v [cm^{-1}] 1476.70 (aromatic C=C), 1329.94, 1301.28, 1054.58 (amines C-N), 1376.99, 1024.85, 1009.53 (C-F). Melting point is above 300 ºC.[37, 38]

*2-[(4-methoxyphenyl)amino]naphtho[2,3-d][1,3]thiazole-4,9-dione (**3b**):* (Yield, 18%), Analysis for $C_{18}H_{12}N_2O_3S$; calcd. (%): C, 64.27; H, 3.60; N, 8.33; S, 9.53. Found: C, 64.22; H, 3.77; N, 8.37; S, 9.07. ^{1}H-NMR (600 MHz, DMSO-d_6, δ, ppm): 11.13 (s, 1H), 8.06-7.99 (dd, *J*= 8.8 Hz, 2H), 7.83-7.81 (t, J=8.8 Hz, 2H), 7.57-7.55 (d, *J*=8.9 Hz, 2H), 7.00-6.98 (d, *J=8.*9 Hz, 2H), 3.74 (s, 3H). FT-IR (ATR) v [cm^{-1}] 1601.48, 1474.3 (aromatic C=C), 1374.88, 1259.21 (C-N), 1258.73, 1086.88, 1055.12, 1013.53 (ether). Melting point is above 300 ºC.[38]

*2-[(2,4-dichlorophenyl)amino]naphtho[2,3-d][1,3]thiazole-4,9-dione (**3c**):* (Yield, 17%), Analysis for $C_{17}H_8Cl_2N_2O_2S$ H_2O; calcd. (%): C, 51.92; H, 2.56; N, 7.12; S, 8.15. Found: C, 52.38; H, 2.52; N, 7.11; S, 8.13. ^{1}H-NMR (600 MHz, DMSO-d_6, δ, ppm): 10.89 (1H, s), 8.24-8.23 (1H, m), 8.08-8.01 (2H, dd, *J*= 8.8 Hz,), 7.85-7.83 (2H, t, *J*=8.9 Hz), 7.75 (1H, s), 7.55-7.54 (1H, d, *J*=8.7 Hz). FT-IR (ATR) v [cm^{-1}] 1673.96, 1643.36 (C=C), 1324.60, 1259.21, 1027.53 (amines C-N), 716.77, 634.26, 603.91 (C-Cl). Melting point is above 300 ºC.[37]

*2-[(4-(trifluoromethyl)amino)methyl]naphtho[2,3-d][1,3]thiazole-4,9-dione (**3d**):* (Yield, 11%), Analysis for $C_{18}H_9F_3N_3O_2S$; calcd. (%): C, 57.75; H, 2.42; N, 7.48; S, 8.57. Found: C, 56.98; H, 2.68; N, 7.32; S, 8.45. ^{1}H-NMR (600 MHz, DMSO-d_6, δ, ppm): 11,62 (1 H, s), 8.11-8.03 (2H, dd, *J*= 8.9 Hz), 7.94-7.92 (2H, d, *J*=8.6 Hz), 7.86-7.85 (2H, t, *J*=8.9 Hz), 7.79-7.78 (2H, d, J=8.6 Hz). FT-IR (ATR) v [cm^{-1}] 1604.70, 1475.14 (aromatic C=C), 1334. 83, 1218, 1167.85 (amines C-N), 1011.97, 1334.80, 1381.89 (C-F), 824.17 (=C-H). Melting point is above 300 ºC.[38]

*2-[(2,6-dimethylphenyl)amino]naphtho[2,3-d][1,3]thiazole-4,9-dione (***3e***):* (Yield, 16%), Analysis for $C_{19}H_{14}N_2O_2S$; calcd. (%): C, 68.24; H, 4.22; N, 8.38; S, 9.59. Found: C, 68.82; H, 4.39; N, 7.94; S, 9.22. ^{1}H-NMR (600 MHz, DMSO-d_6, δ, ppm): 10.69 (1H, s), 8.03-7.95 (2H, dd, *J*= 8.4 Hz), 7.80-7.79 (2H, m), 7.21 (3H, s), 2.20 (6H, s). FT-IR (ATR) v [cm^{-1}] 1472.10 (aromatic C=C), 1372.43 (-CH_3 bending), 1292.10, 1189.87 (amines C-N). Melting point is above 300 ºC.

*2-[(4-methylphenyl)amino]naphtho[2,3-d][1,3]thiazole-4,9-dione (***3f***):* (Yield, 13%), Analysis for $C_{18}H_{12}N_2O_2S$; calcd. (%): C, 67.48; H, 3.78; N, 8.74; S, 10.01. Found: C, 66.88; H, 3.94; N, 8.63; S, 9.34. ^{1}H-NMR (600 MHz, DMSO-d_6, δ, ppm): 11.22 (1H, s), 8.08-8.00 (2H, dd, *J= 8.*9 Hz), 7.84-7.82 (2H, m), 7.56-7.55 (2H, d, *J*=8.3 Hz), 7.22-7.21 (2H, d, *J*=8.3 Hz), 2.28 (3H, s). ^{13}C-NMR (125 MHz, DMSO-d_6, δ, ppm): 23.57 (-CH_3), 121.86, 128.74, 129.78, 132.81, 135.10, 135.86, 136.02, 136.91, 137.05, 140.10, 156.57 (C-N), 171.02 (C=N), 180.48 and 180.23 (C=O). FT-IR (ATR) v [cm^{-1}] 1474.37 (aromatic C=C), 1376.64 (-CH_3 bending), 1335.16, 1319.00, 1024.39 (amines C-N), 1376.64 (-CH_3 bending). Melting point is above 300 ºC.

*2-(cyclohexylamino)naphtho[2,3-d][1,3]thiazole-4,9-dione (***3g***):* (Yield, 12%), Analysis for $C_{17}H_{16}N_2O_2S$; calcd. (%): C, 65.36; H, 5.16; N, 8.97; S, 10.26. Found: C, 64.48; H, 5.72; N, 8.49; S, 9.87. ^{1}H-NMR (600 MHz, DMSO-d_6, δ, ppm): 9.06 (1H, s), 8.02-7.95 (2H, dd, *J*= 8.8 Hz), 7.81-7.77 (2H, m), 1.94-1.92 (2H, d, *J*=12.0 Hz), 1.71-1.69 (2H, d, *J*=13.1 *Hz*), 1.57-1.55 (1H, d, *J*=13.1 Hz), 1.35-1.26 (4H, m), 1.20-1.16 (2H, m). FT-IR (ATR) v [cm^{-1}] 1472 (aromatic C=C), 1328.99, 1049.05, 1022.20, (amines C-N). Melting point is 245-246 ºC.

2.3. Biological Assay

2.3.1. Cell Culture Conditions

Human breast cancer epithelial (MDA-MB-231), human cervix cancer epithelial (HeLa), human stomach cancer (MKN-45) and mouse fibroblast (L929) cell lines were obtained from American Type Culture Collection (ATCC). L929 was used as healthy control cells. The cells were maintained in DMEM, containing 10% fetal bovine serum 100 units/mL penicilin/streptomycin, L-Glutamine and $NaHCO_3$ in an atmosphere having 5% CO_2 and 95% moisture at 37 ºC. After the cells had reached a state of 80 % confluency, cells were splitted with Trypsin-EDTA (0.05%) for passaging.

2.3.2. Cell Proliferation

Cell viability was measured using the MTT test.[39] Compounds were dissolved in DMSO and applied to the cells for 24 h in 96 well plates. Different concentrations of the compounds (0.1-1000 µM) were used to calculate IC_{50} values. MTT Cell Proliferation Assay Kit was used for cell viability according to the manufacture's protocol (Thermo Scientific, USA). After incubation, the cell mediums were removed, and the cells were washed with PBS. 5 mg/mL MTT solution was added to each well and incubated for 4 h at 37 °C in 5% CO_2. At the end of 4 h, to dissolve the purple-colored formazan crystals, 100 µL of SDS dissolved in 0.01 M HCl was added and incubated at 37 °C. The absorbance of the purple color formed after 4 h was measured with an Elisa plate reader (Epoch, Biotech) at 570 nm.

2.4. Molecular Modeling Studies

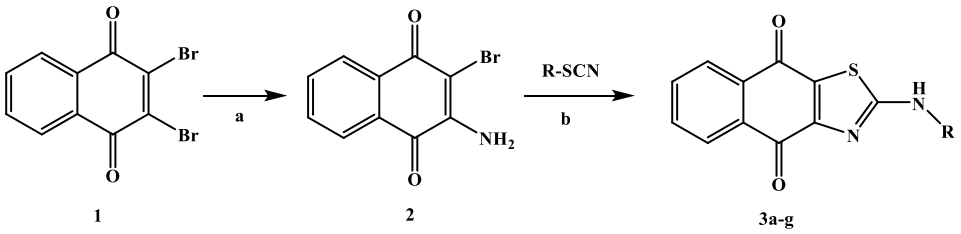
Three-dimensional structures of the investigated ligands were prepared with MOE (v2019.0102. Chemical Computing Group, Inc., Montreal) in low-energy conformations. The most prevalent protonation state of the ligands at pH 7 was calculated. Subsequently, the ligands were energy minimized using the MMFF94x force field.

The crystal structure of hDNA TopoIIβ in complex with etoposide (pdb: 3qx3, 2.16 Å) was obtained from RCSB Protein Data Bank. All ions and water molecules were removed as they were not required in the ligand-protein binding interactions. Hydrogen atoms were added with the Protonate3D functionality of MOE. Consequent minimizations with a stepwise release of the restraints were then performed. In the final step, the system was minimized without any restraints.

Docking studies were performed using a FlexX docking tool (v2.3.2; BioSolveIT GmbH, St. Augustin, Germany) within MOE. The binding pocket was described as all residues within 10 Å of the co-crystallized ligand etoposide. All the ligands were docked 50 times and the highest scoring three poses were subjected to refinement calculations. Finally, the docked ligand and the binding pocket (defined as all residues within 6.5 Å of the docked ligand) were energy minimized and rescored using the GBVI/WSA force field.[40]

3. Results and Discussion

2,3-Dibromo-1,4-naphthoquinone (**1**) was applied as a starting material to synthesize naphtho[2,3-*d*]thiazole-4,9-dion derivatives (compounds **3a-g**). 2-Amino-3-bromo-1,4-naphthoquinone (**2**) was synthesized with the reaction of 2,3-dibromo-1,4-naphthoquinone (**1**) with aqueous NH_3 at room temperature.[35]Several isothiocyanate derivatives were treated with 2-amino-3-bromo-1,4-naphthoquinone (**2**) to give the corresponding thiourea.[36] *In situ*, the thiourea was subsequently treated with CuO nanoparticles to obtain **3a-g**. The structures of the compounds were confirmed by elemental and spectral analysis such as ^{1}H-NMR, ^{13}C-NMR, and FT-IR. The first spectroscopic evidence of the synthesized compounds **3a-g** in ^{1}H-NMR spectra was the observation of -NH- proton at a range of 9.06-11.62 ppm as a singlet. The other aromatic protons and the substituent protons were consistent with the structures. ^{13}C-NMR data of compound **3f** shows consistency with data of similar derivatives. Chemical shift of C=N carbon in thiazole ring was seen at 171.02 ppm and consistent with previously reported data.[41,42]



Scheme 1. Synthetic route of compounds **3a-g** Conditions of reaction **a**: NH_3, H_2O, dioxane, 2.5 h, rt. Conditions of reaction **b**: isothiocyanate derivatives, CuO nanoparticles

Although among the synthesized compounds **3e-3g** are original, compounds **3a-3d**, are available in the literature.[37,38] Since any information about their cytotoxicities is not available, these compounds were synthesized to evaluate their anticancer activities.

In the MTT assay, the potency of the cells in mitochondria changes as a result of the treatment of the cells with the compounds for 24 h, and the concentration at which the viability 50% was determined. Table 1 shows the mean average IC_{50} values obtained at 24 h treatment in L929, MDA-MB231, MKN-45 and HeLa cell lines. In general, an anticancer drug candidate is expected to have some effects on healthy cells and be effective at low doses in cancer cells. Compounds **3a-g** inhibited cell proliferation with mild to moderate IC_{50} values are shown in Table 1. Among the compounds, **3d** inhibited MDA-MB-231 (Human Breast cancer) cell proliferation with an IC_{50} value of 0.276 µM. Compound **3a** inhibited HeLa and MKN-45 cell proliferation with IC_{50} values of 0.336 µM and 8.769 µM, respectively. Compound **3b** inhibited HeLa cell proliferation with an IC_{50} value of 0.269 µM. Compound **3c** inhibited MKN-45 cell proliferation with an IC_{50} value of 0.363 µM. Compound **3f** inhibited MDA-MB-231 (Human Breast cancer cell with an IC_{50} value of 0.935 µM. On the other hand, compounds **3e** and **3g** did not show any remarkable activities. The selectivity index (SI) of the compounds was calculated by obtaining the ratio of IC_{50} in the healthy cell line/IC_{50} in the cancer line.[43] SI values representing the ratio of the IC_{50} value for healthy cell lines to the IC_{50} value for cancer cell lines after 24 h of treatment of the compounds **3a-g** are summarized in Table 2.

Table 1. The IC_{50} values (μM) of compounds in cells at 24h treatment

Comp.	R	Normal cell line	Cancer cell lines		
		L929	MKN-45	MDA-MB-231	HeLa
3a	4-fluorophenyl	301.379±12.028	8.769±1.065	96.200±2.038	0.336±0.037
3b	4-methoxyphenyl	305.080±11.024	51.743±2.149	59.591±1.503	0.269±0.011
3c	2,4-dichlorophenyl	129.822±3.871	0.363±0.024	31.159±3.062	3389.633±19.827
3d	4-(trifluoromethyl)phenyl	341.555±6.325	591.496±7.742	0.276±0.011	1408.279±18.025
3e	2,6-dimethylphenyl	857.212±10.305	200.416±5.387	15.837±1.207	363.274±10.294
3f	4-methylphenyl	516.586±6.341	16.509±1.542	0.935±0.039	26.570±5.774
3g	cyclohexyl	461.710±3.846	366.181±4.068	81.683±7.036	96.404±3.689

Table 2. The selectivity index of compounds at 24h treatment (IC_{50} in healthy cells/ IC_{50} in cancer cells)

Compound	Selectivity Index (SI)		
	L929/MKN-45	L929/MDA-MB-231	L9297/HeLa
3a	34.36	3.12	0.89
3b	5.98	5.16	1133
3c	355	4.16	0.03
3d	0.57	1235	0.24
3e	4.28	54	2.36
3f	31	555	20
3g	1.25	5.69	4.80

The activity of compounds **3a-g** against human DNA TopoIIβ was predicted by molecular docking. All the ligands adopted a similar docked pose in the hDNA TopoIIβ binding pocket. Also, their tricyclic scaffold formed hydrophobic interactions with the DNA base pairs (Figure 3). The NH group located between the two ring systems hydrogen-bonded with a DNA base. The positively charged Arg503 side chain was located within 5.5 Å of the aromatic ring of the ligand forming cation-π interactions. Finally, the substituted phenyl ring was located closer to Gly776, Gln778, and Ala779. The docking results suggested that the ligands may bind to hDNA TopoIIβ and, at least, may partially exert some effects *via* this investigated enzyme.

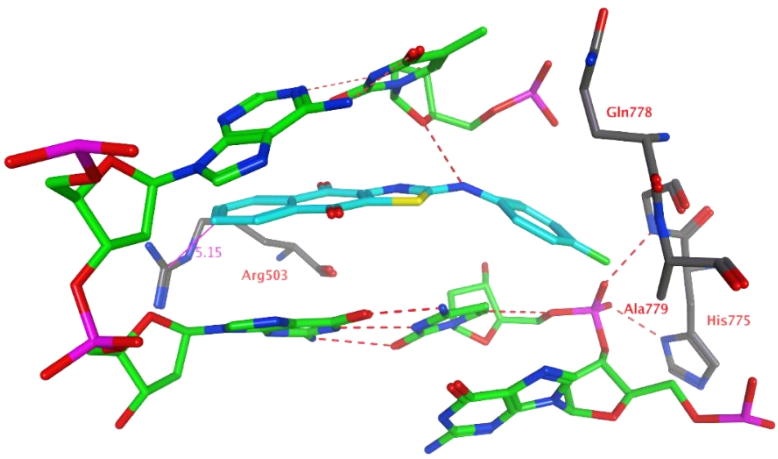


Figure 3. The docked pose of representative compound **3a** (turqoise) with the binding pocket of hDNA TopoIIβ. Hydrogen bonds are indicated in red dashed lines. The distance between the ligand's aromatic ring and Arg503 is shown in purple. Amino acids carbon atoms are shown in grey, while DNA carbon atoms are shown in green.

4. Conclusion

A series of 2,3-dibromo-1,4-naphthoquinone derivatives were synthesized and their structures were verified with spectral analysis. Their in vitro cytotoxic activities were evaluated by using MTT assay. While the compounds, except **3e** and **3g**, did not present any remarkable cytotoxicity in MTT assay, compound **3d** inhibited MDA-MB-231 (Human Breast cancer) cell proliferation with an IC_{50} value of 0.276 µM. **3a** inhibited HeLa and MKN-45 cell proliferation with IC_{50} values of 0.336 µM and 8.769 µM, respectively. **3b** inhibited HeLa cell proliferation with an IC_{50} value of 0.269 µM and **3c** inhibited MKN-45 cell proliferation with an IC_{50} value of 0.363 µM. Docking results suggested that the ligands may bind to the hDNA TopoIIβ binding pocket. As some of these compounds exhibited some promising activities, they are currently under further investigation and the results will be disclosed in future reports.

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Supporting Information

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Supporting Information

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Synthesis, cytotoxic activities and molecular modeling studies of some 2-aminonaphtho[2,3-*d*][1,3]thiazole-4,9-dione derivatives

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	Table of Contents	Page
Figure S1:	^{1}H-NMR Spectrum of Compound **3a** (DMSO- d_6, 600 MHz)	2
Figure S2:	^{1}H-NMR Spectrum of Compound **3b** (DMSO- d_6, 600 MHz)	3
Figure S3:	^{1}H-NMR Spectrum of Compound **3c** (DMSO- d_6, 600 MHz)	4
Figure S4:	^{1}H-NMR Spectrum of Compound **3d** (DMSO- d_6, 600 MHz)	5
Figure S5:	^{1}H-NMR Spectrum of Compound **3e** (DMSO- d_6, 600 MHz)	6
Figure S6:	^{1}H-NMR Spectrum of Compound **3f** (DMSO- d_6, 600 MHz)	7
Figure S7:	^{1}H-NMR Spectrum of Compound **3g** (DMSO- d_6, 600 MHz)	8
Figure S8:	^{13}C-NMR Spectrum of Compound **3f** (DMSO- d_6, 150 MHz)	9

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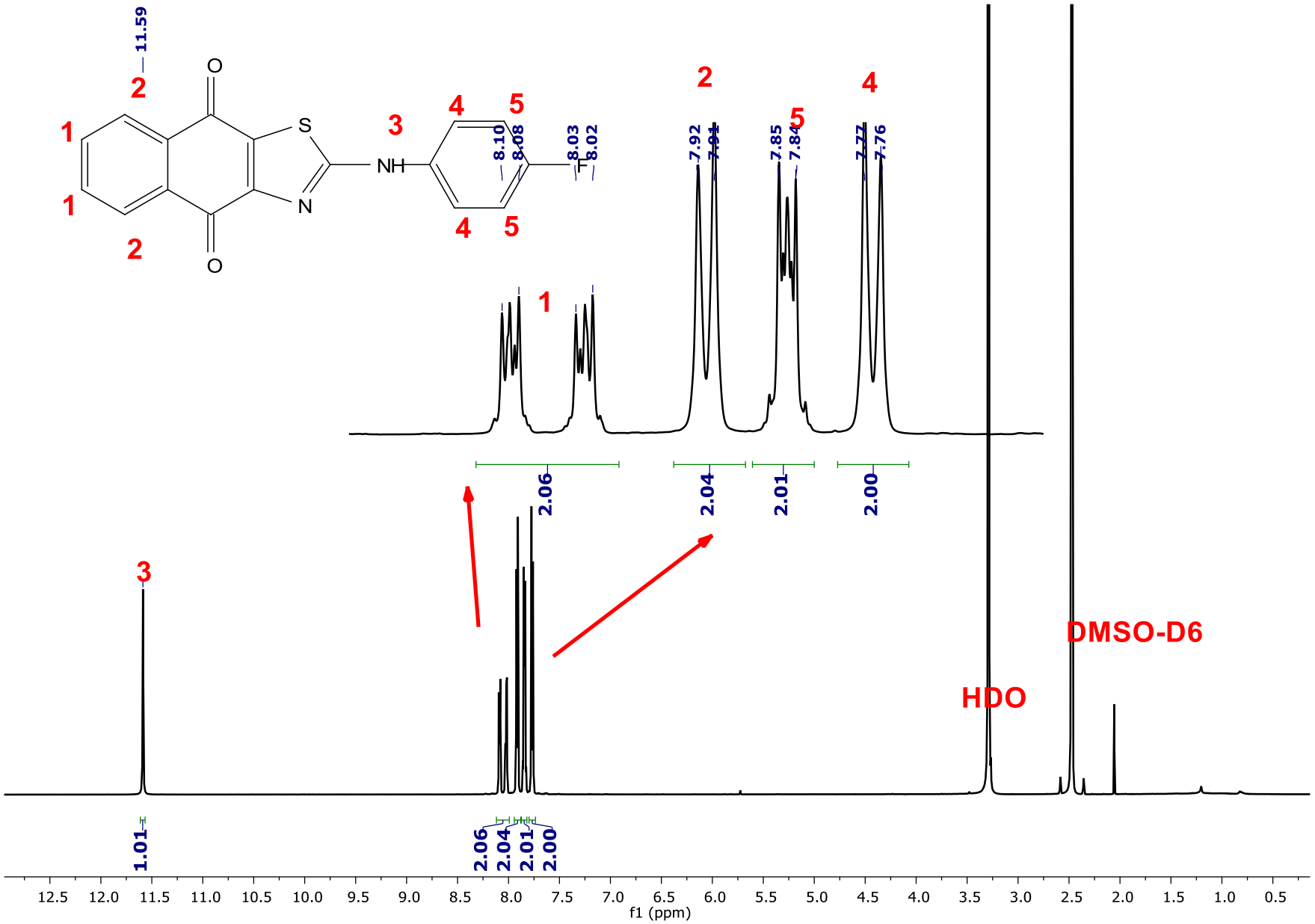


Figure S1: [1]H-NMR spectrum of compound **3a** (DMSO- d_6, 600 MHz)

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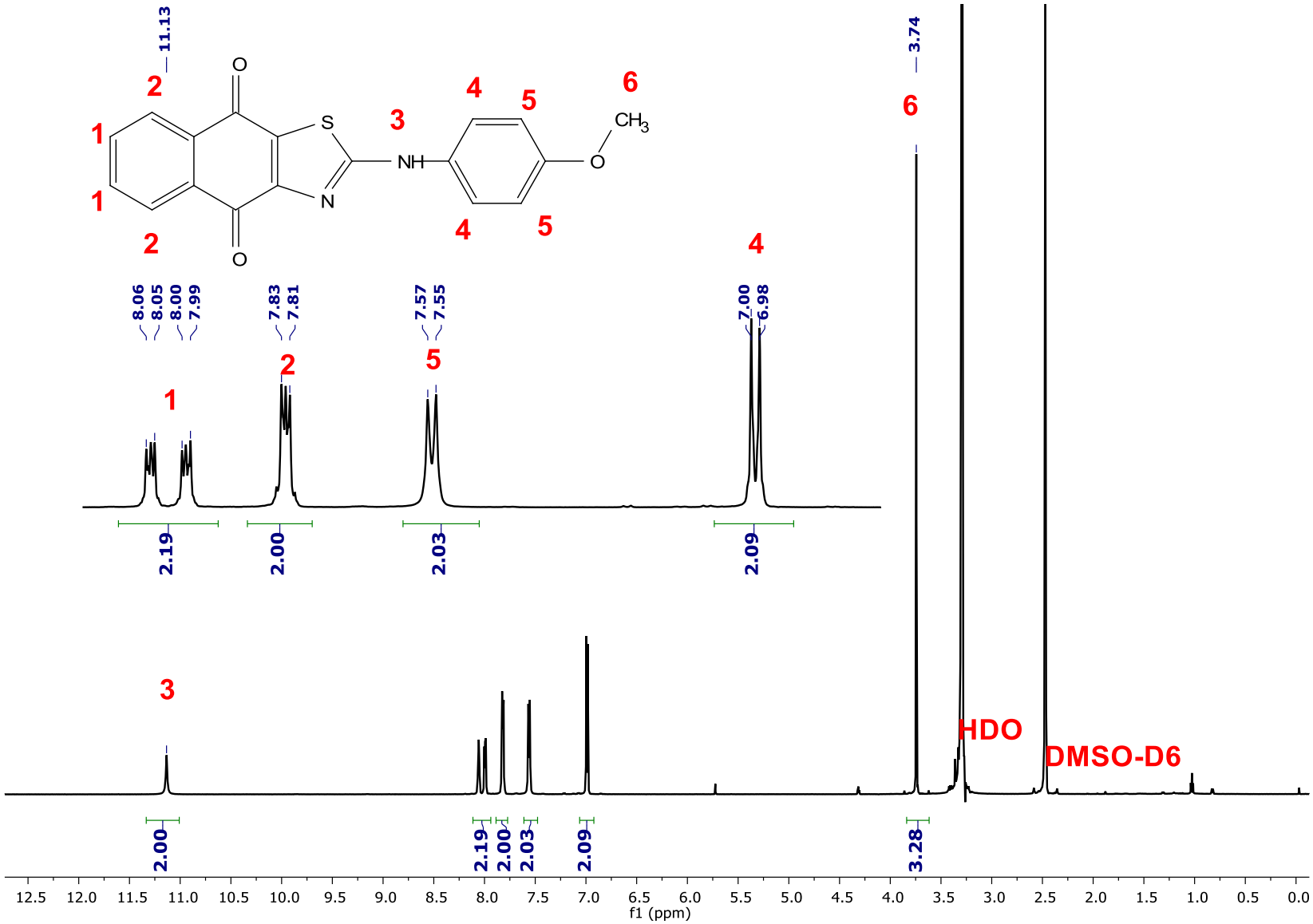


Figure S2: ^{1}H-NMR spectrum of compound **3b** (DMSO- d_6, 600 MHz)

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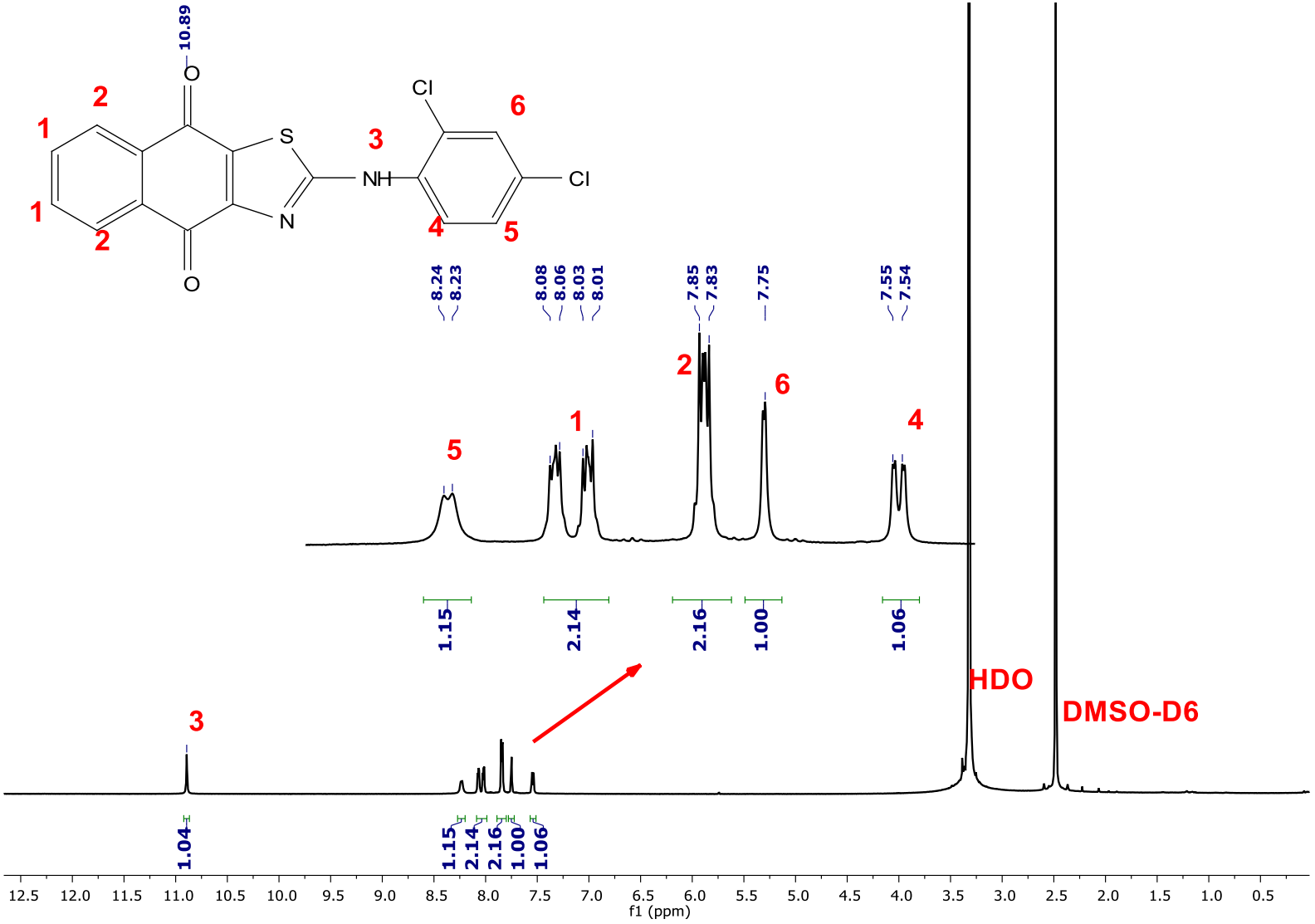


Figure S3: ^{1}H-NMR spectrum of compound **3c** (DMSO- d_6, 600 MHz)

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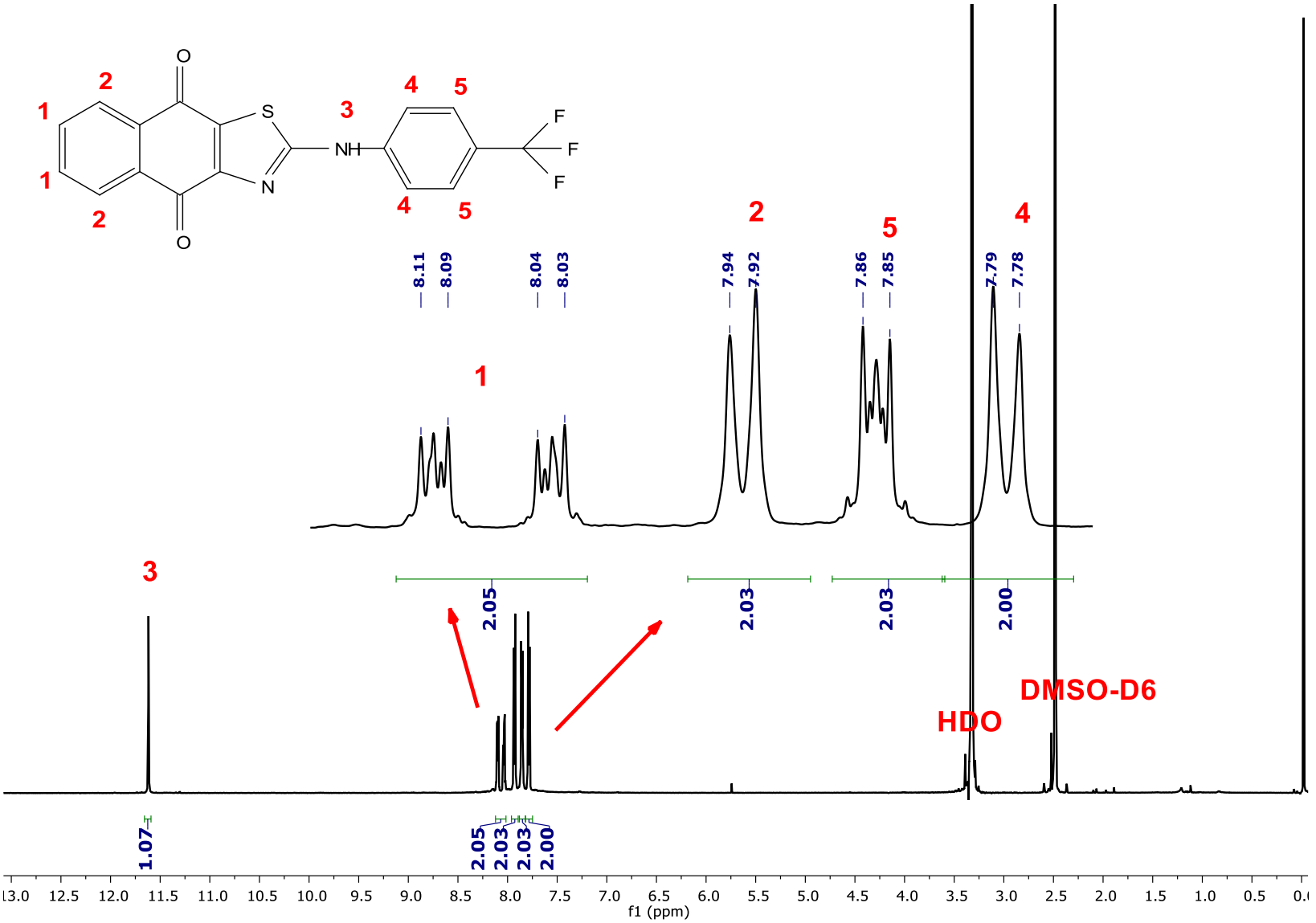


Figure S4: ^{1}H-NMR spectrum of compound **3d** (DMSO- d_6, 600 MHz)

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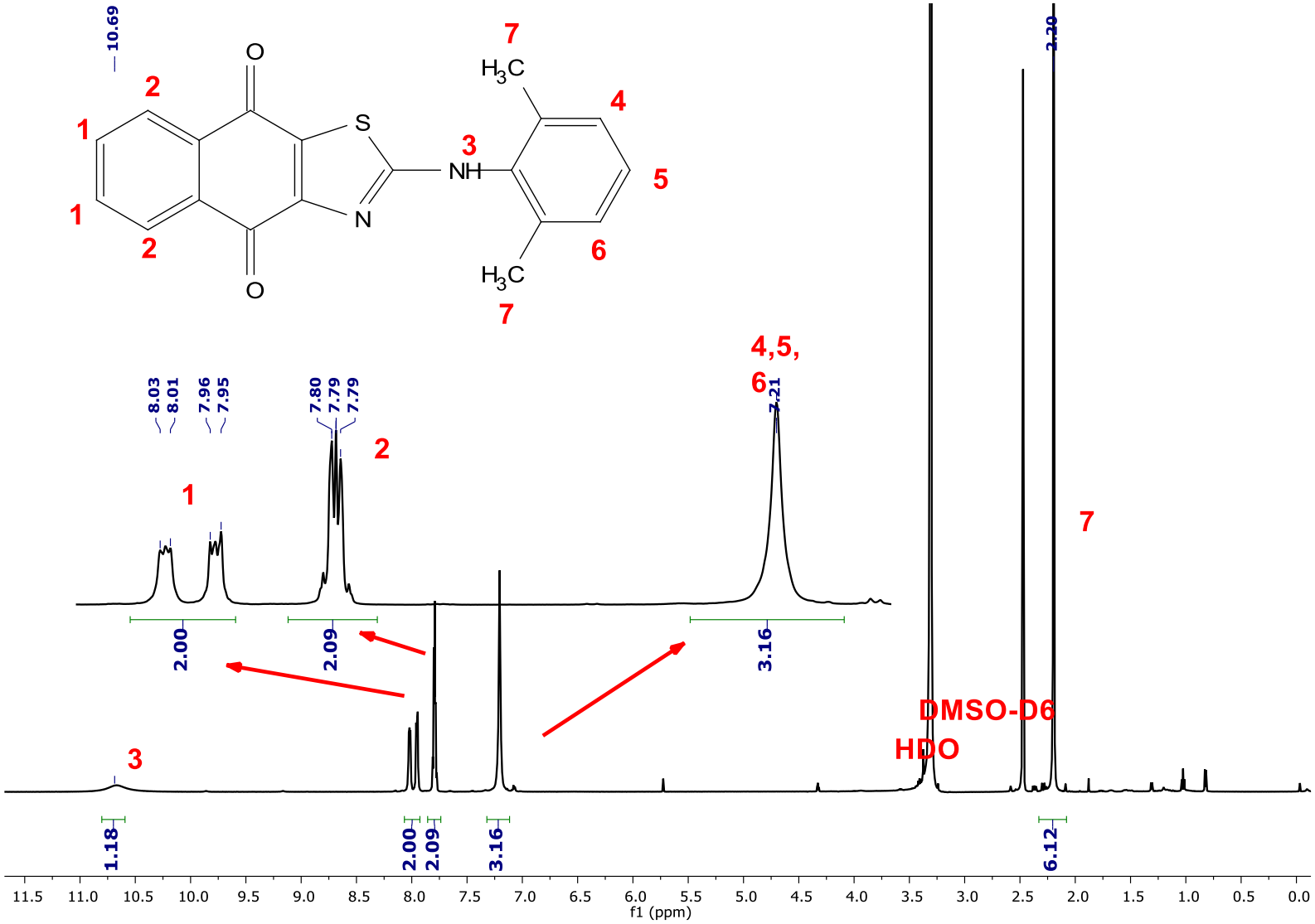


Figure S5: [1]H-NMR spectrum of compound **3e** (DMSO- d_6, 600 MHz)

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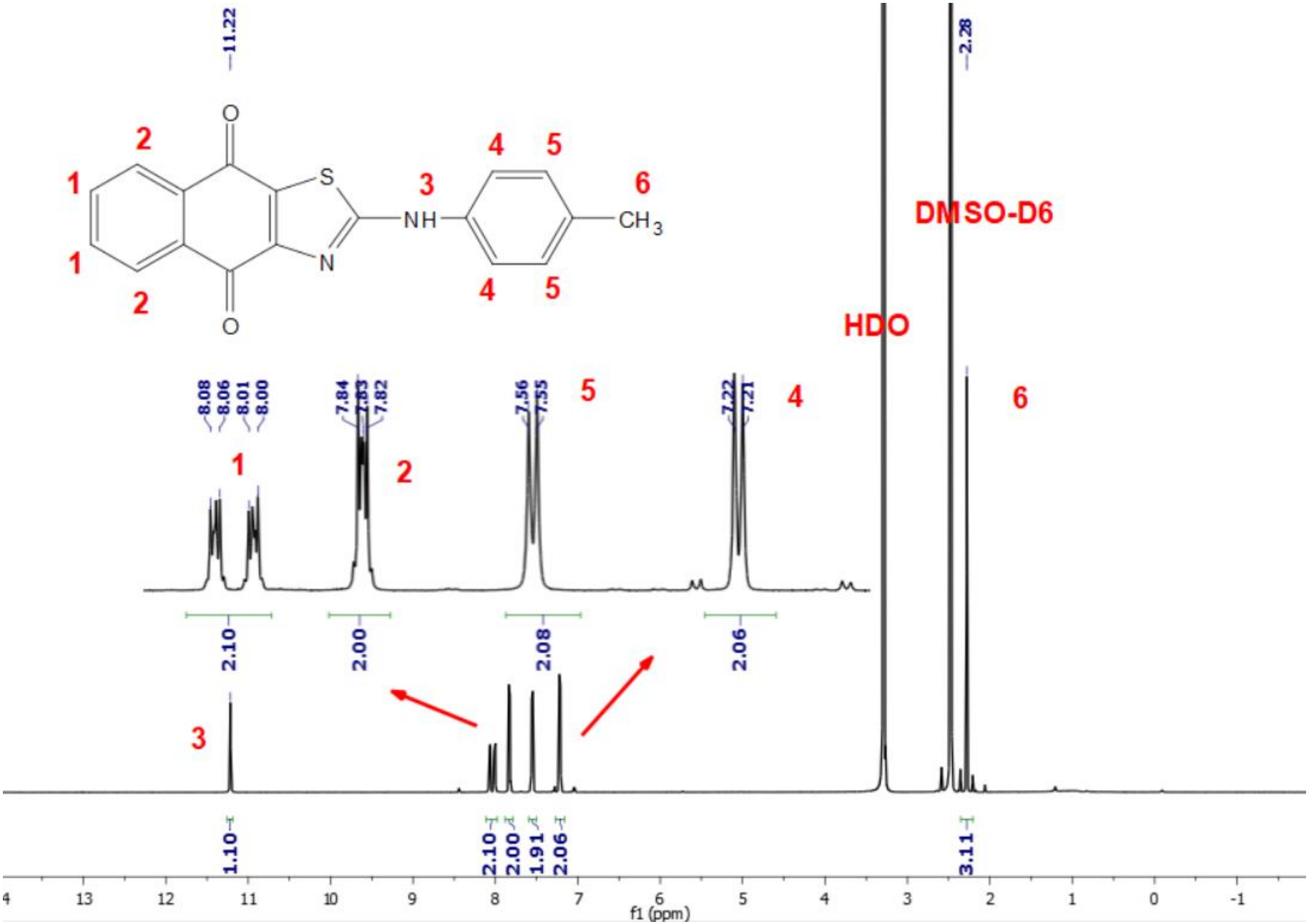


Figure S6: [1]H-NMR spectrum of compound **3f** (DMSO- d_6, 600 MHz)

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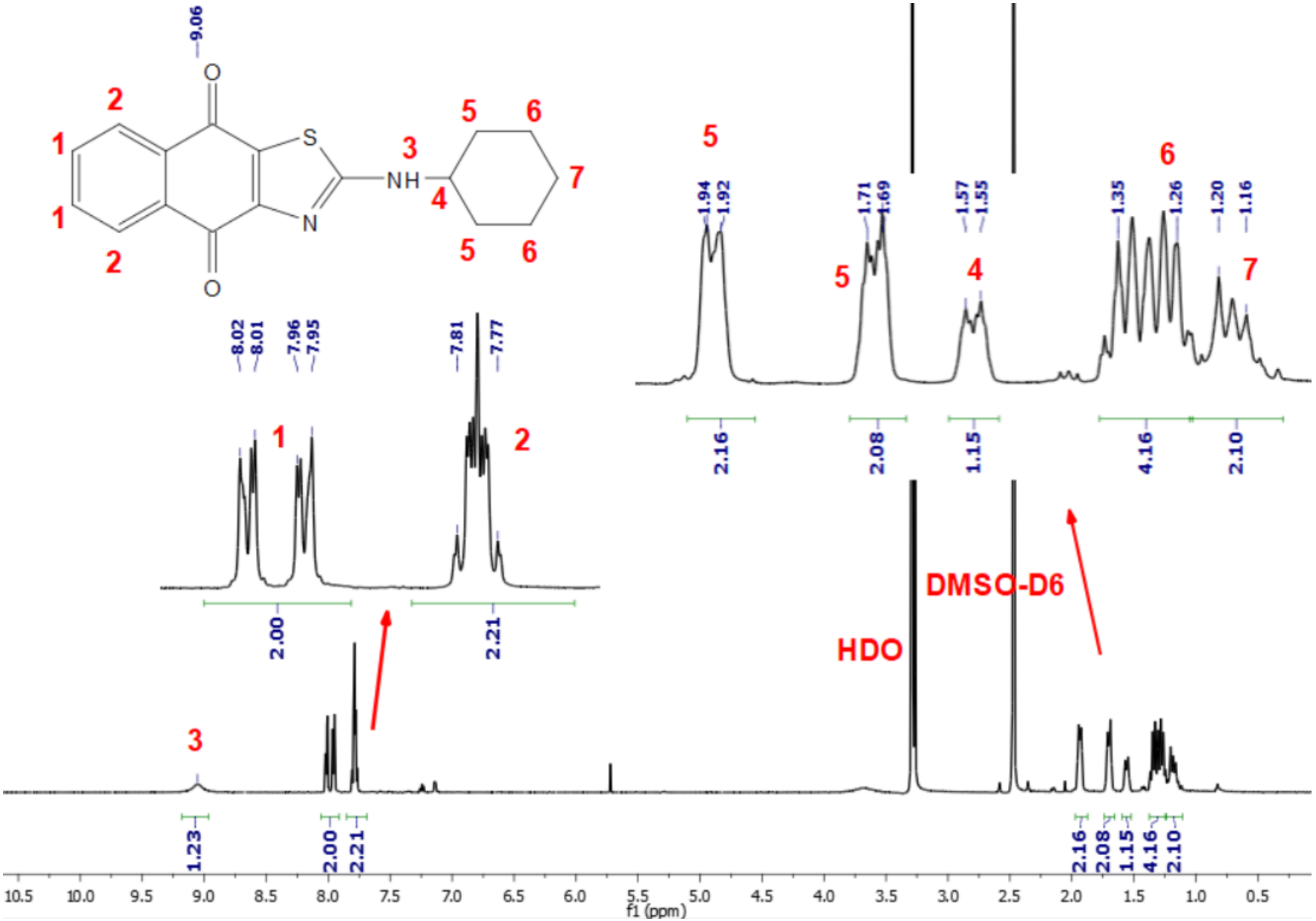


Figure S7: ^{1}H-NMR spectrum of compound **3g** (DMSO- d_6, 600 MHz)

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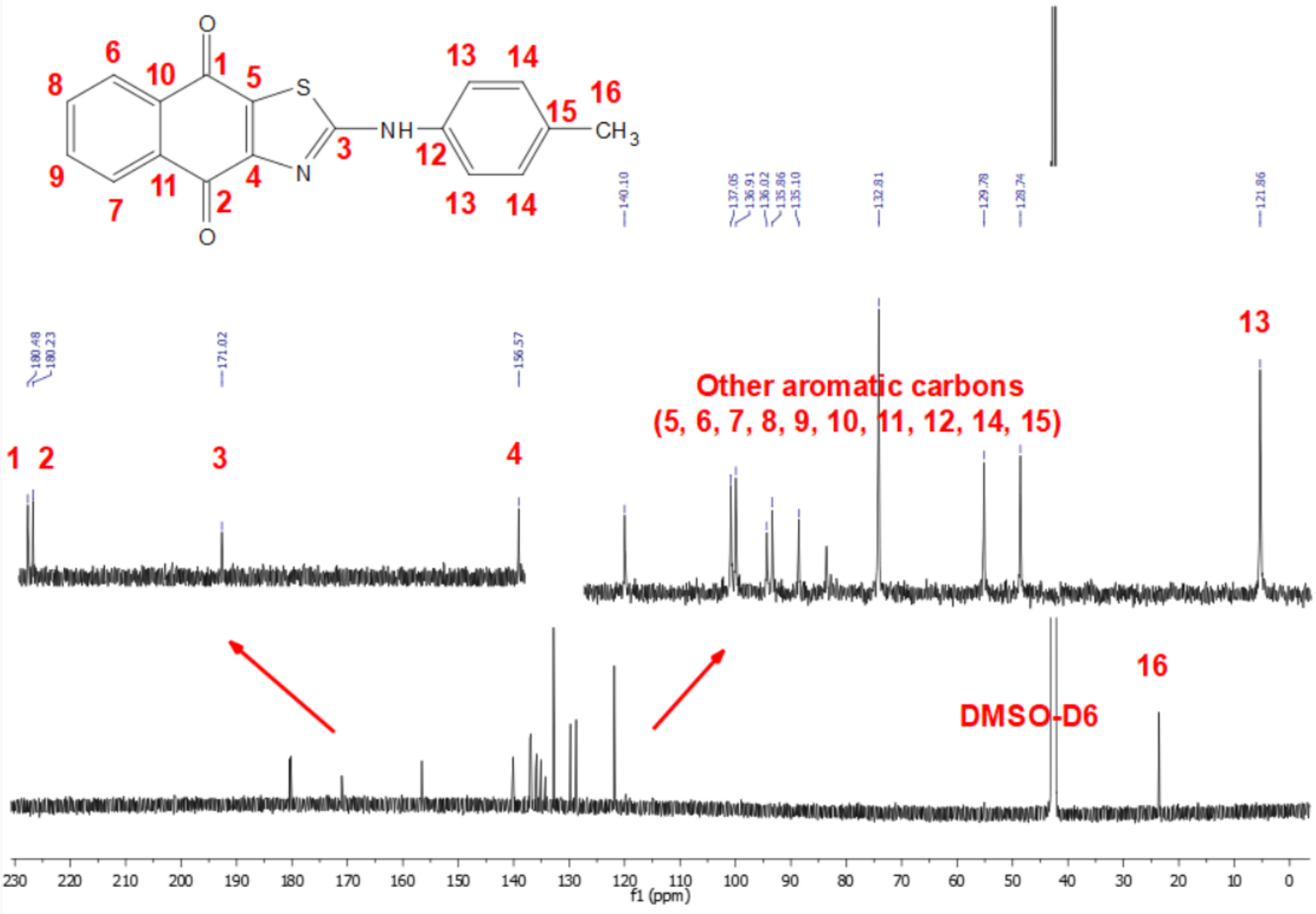


Figure S8: ^{13}C-NMR spectrum of compound **3f** (DMSO- d_6, 150 MHz)

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