UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
Faculdade de Farmácia

Mariana Pies Gionbelli

Effects of inversion or substitution of the sulfonamide group at 5-position of 8-hydroxyquinoline derivatives against *Cryptococcus neoformans* and *C. gattii*.

Porto Alegre, 2019

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Trabalho de Conclusão de Curso apresentado à diretoria do curso de graduação em Farmácia da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de Farmacêutica, sob orientação do Prof. Saulo Fernandes de Andrade.

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Porto Alegre, 09 de Dezembro de 2019.

BANCA EXAMINADORA

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Prof. Saulo Fernandes de Andrade

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Prof.ª Juliana Caierão

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Msc. Débora Assumpção Rocha

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Title: Effects of inversion or substitution of the sulfonamide group at 5-position of 8-hydroxyquinoline derivatives against *Cryptococcus neoformans* and *C. gattii*

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ABSTRACT

Cryptococcosis is an important infectious disease, mainly because the increasing prevalence in recent decades and resistance treatment. Development of new drugs for the treatment of this disease is imperative. Recent studies from our research group revealed significant antimicrobial activity of 5-substituted 8-hydroxyquinoline derivatives as 8-hydroxy-5-quinolinesulfonic acid and 8-hydroxyquinoline-5-(*N*-4-chlorophenyl) sulfonamide. Therefore, in order to study the structure activity relationships (SAR) of these compounds, sulfonamide derivatives of 8-hydroxyquinoline were synthesized varying the substitution on the 5-sulfonamide and also inverting the sulfonamide group. Derivatives of 8-hydroxyquinoline-5-sulfonyl chloride and 5-aminoquinolin-8-ol were obtained for *in vitro* screening against *Cryptococcus neoformans* and *C. gattii* using broth microdilution method. Compound **3a** was the most active derivative of this series, demonstrating activity over 2-fold better than fluconazole against *C. neoformans*. Derivatives **3b** and **3c** were equally actives, but not as potent as **3a**. The position inversion of the sulfonamide resulted in reduced activity of derivatives **6a** and **6b**, emphasizing the importance of the sulfonyl group position in the molecule. Finally, the 3-series derivatives can be promising antifungal candidates to treat cryptococcosis.

KEYWORDS: 8-Hydroxyquinoline derivatives, cryptococcosis, antifungal activity, synthesis, sulfonamides.

INTRODUCTION

Fungal infections have been a cause of concern to human health, mainly because of resistance reports to current treatment. Cryptococcosis is an infectious disease that affects people worldwide and has advanced in recent decades due to increased occurrence of immunosuppressive diseases. It is caused predominantly by two species of encapsulated yeasts *Cryptococcus neoformans* and *C. gattii*, which are both cosmopolitan species of fungi. *C. neoformans* is commonly associated with bird droppings and soil and *C. gattii* is primarily found in tropical and subtropical regions [1,2].

In the current scenario, *C. neoformans* is responsible for over 90% of cryptococcosis cases, being a leading cause of meningoencephalitis and potentially fatal when affecting immunocompromised individuals, who are most affected by the disease [2]. Deaths caused by cryptococcosis have risen sharply in the last four decades and this fact is related to the AIDS pandemic, which is still a public health problem [3]. Otherwise, *C. gattii* is a major cause of infections in immunocompetent patients, accounting for about 10% of cases of cryptococcosis or 15% in tropical locations [3,4].

Current management of cryptococcosis is accomplished through induction therapy with amphotericin B *plus* 5-flucytosine for about 2 weeks (according to the host type) followed by consolidation of treatment for 8 weeks and maintenance therapy for 12 months with fluconazole [5]. The treatment is complex, time-consuming and must take into account toxicity from antifungal drugs used, since they generally have high toxicity, limiting their use for long periods. Also, reports of antimicrobial resistance have been increasing as the optimal fluconazole dose is still not known, ranging from 100 mg to 2000 mg/day for treatment or preemptive therapy [6]. The Minimum Inhibitory Concentration (MIC) of fluconazole against *Cryptococcus* has been increasing over time when comparing isolates from different years [7].

In this context, developing new pharmaceutical compounds aiming at the effective treatment of infectious diseases is essential. 8-Hydroxyquinoline (8HQ) or 8-quinolinol (**a**, Figure 1) derivatives have presented interesting biological effects such as antifungal, antibacterial and

antiparasitic activity and even oxidative stress against Alzheimer's disease has been reported [8,9,10]. Some halogenated derivatives, such as clioquinol (compound **b**, Figure 1), present a high antifungal activity and even clioquinol was already used as an oral antiparasitic agent, although, it is only found in topical formulations nowadays due to the reported subacute myelo-optic neuropathy (SMON) cases in Japan [11]. Furthermore, the antimicrobial activity of 8HQ is related to the nature of the substituent at 5 and 7-positions. Also, non-halogenated 5-substituted 8HQ compounds like nitroxoline (compound **c**, Figure 1), a drug used in Europe to treat urinary tract infections, displayed good antimicrobial potential with a good safety index [12,13,14]. Therefore, the search for non-halogenated active compounds is an effective alternative in the attempt to find possible new antimicrobial drugs.

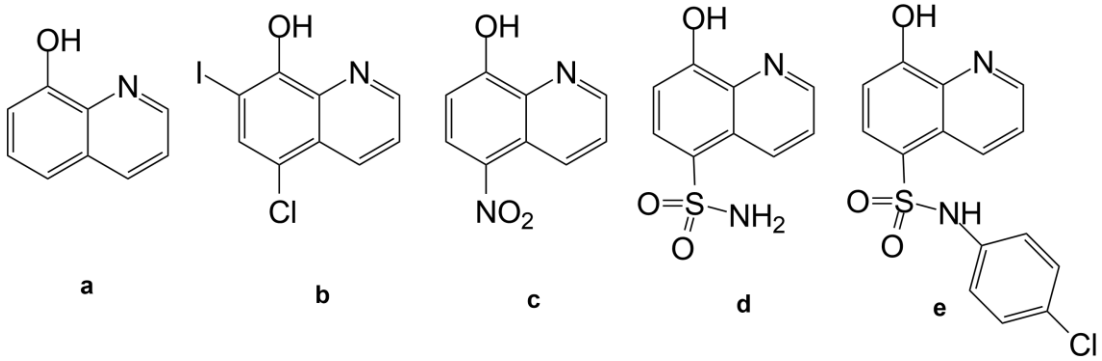


Figure 1. Structure of 8-Hydroxyquinoline **a**, clioquinol **b,** nitroxoline **c**, 8-hydroxy-5-sulfonamide **d** and 8-hydroxyquinoline-5-(*N*-4-chlorophenyl) sulfonamide **e**.

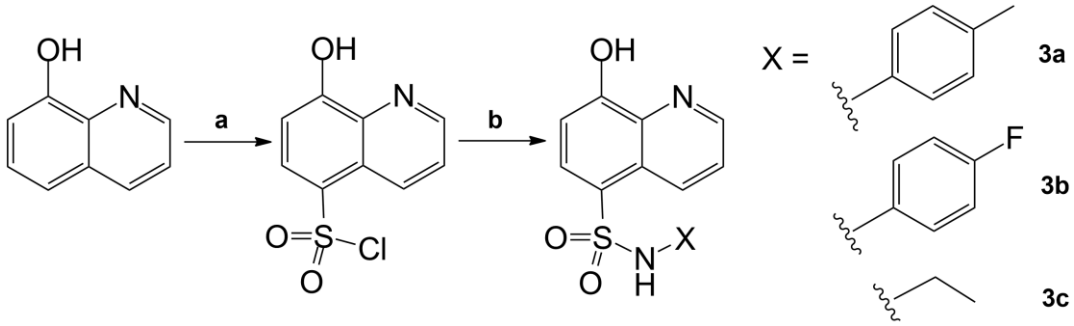
Studies from our research group revealed significant antimicrobial activity of 5-substituted 8HQ derivatives as 8-hydroxy-5-quinolinesulfonic acid [15], 8-hydroxyquinoline-5-sulfonamide (compound **d**, Figure 1, unpublished data) and 8-hydroxyquinoline-5-(*N*-4-chlorophenyl) sulfonamide (compound **e,** Figure 1) [16]. Thus, the purpose of this study was to synthesize new sulfonamide derivatives of 8-hydroxyquinoline aiming to study the importance of the substituent of the sulfonamide or its connectivity against strains of *C. neoformans* and *C. gattii*.

RESULTS AND DISCUSSION

Chemistry

Sulfonyl chloride was obtained as previously reported (Scheme 1) [16]. The 8-hydroxyquinoline chlorosulfonation, followed by treatment using brine and ice and extraction

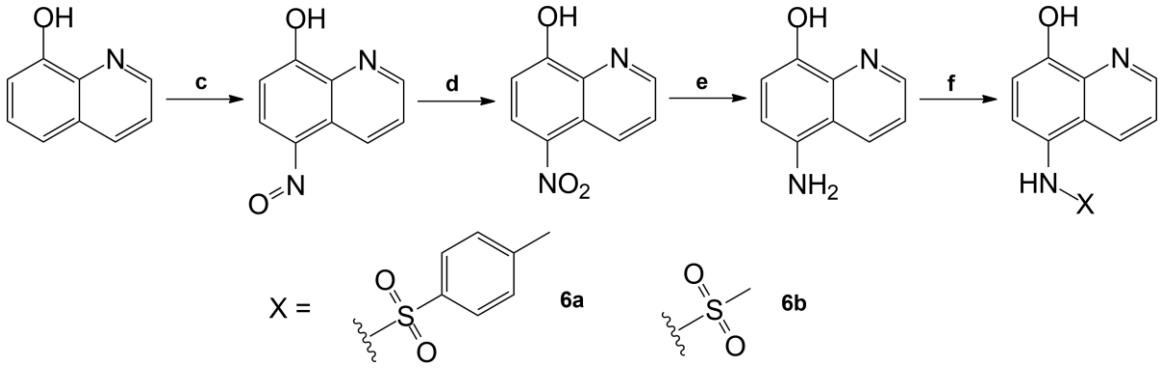
with dichloromethane, gave the sulfonyl chloride in 32% yield. Thereafter, the appropriate amine was added under the conditions also described in Scheme 1. Three different amines were used (4-fluoroaniline, ethylamine and 4-methylamine) resulting in derivatives **3a**-**3c** in 4%-21% yield. These derivatives were purified in column chromatography and the compounds purity was confirmed by ^{1}H and ^{13}C NMR spectra (Supporting Information).



Scheme 1. Chlorosulfonation of the 8-hydroxyquinoline and formation of derivatives **3a-3c.**
Reagents and conditions: **a)** $ClSO_3H$, r.t., 18 hr; **b)** Appropriate amine, acetonitrile, 80°C, 3 hr.

Similar to our previous study, the 8HQ chlorosulfonation yield was modest, possibly due to 8-hydroxyquinoline-5-sulfonyl chloride protonation under the acid condition during the reaction treatment, making the intermediate compound more soluble in the aqueous layer. [16]

The synthesis of 5-nitroquinolin-8-ol was performed as reported in Scheme 2 [17]. Mazumder et al. described the two steps obtaining process of 5-nitroquinolin-8-ol and also its purifying process which was done by recrystallization. The 8-hydroxyquinoline nitration process in adapted conditions gave the 5-nitroquinolin-8-ol in 26% yield. This process was performed in two steps, as a nitrosation followed by nitration of the 5-position, in an attempt to inhibit 5,7-dinitroquinolin-8-ol formation. However, it could still be formed, and this is the probable responsible for the lower yield since it is taken out in the recrystallization step. The 5-nitroquinolin-8-ol reduction to 5-aminoquinolin-8-ol was performed in adapted conditions [18] and the three-step synthesis of 5-aminoquinolin-8-ol yielded 22%. The reverse sulfonamides 8-hydroxyquinoline-5-(p-tolylsulfonylamino) **6a** and the 8-hydroxyquinoline-5-(N-methanesulfonylamino) **6b** were synthesized in adapted conditions [19] and purified by silica gel column, giving the derivatives in 17% and 14% yield respectively.



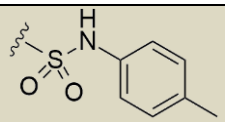
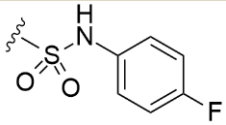
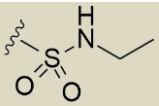
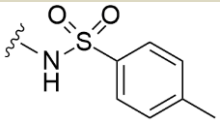
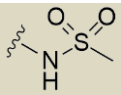
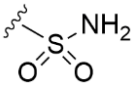
Scheme 2. Nitrosation, nitration, nitro group reduction to amine and obtainment of derivatives **6a** and **6b**. Reagents and conditions: c) $NaNO_2$, HCl, 0°C, 1 hr; d) $HNO_{3\,(aq)}$, 17°C, 1 hr 15 min; e) $N_2H_{4\,(sol)}$, Pd/C, isopropanol, 82°C, 3 hr; f) Appropriate sulfonyl group, pyridine, r.t., 18 hr.

All the compounds were characterized by ^{1}H and ^{13}C NMR spectra (Supporting information), FT-IR and melting point. It will be discussed as an example the characterization of the derivative **3a**, since it revealed to be the most potent compound of this series. The ^{1}H NMR spectrum of **3a** presented the 8HQ core signals at δ 8.94 (dd, 1H, H4), δ 8.81 (dd, 1H, H2), δ 8.15 (d, 1H, H6), δ 7.54 (dd, 1H, H3) and δ 7.09 (d, 1H, H7) and the methyl corresponding signal revealed at δ 2.19 (s, 3H). The signals of toluidine hydrogens *ortho* to sulfonamide appeared at δ 6.92 (d, 2H, H2) and hydrogens *meta* to sulfonamide appeared at δ 6.79 (d, 2H, H3). The ^{13}C NMR spectrum revealed the aromatic carbons signals at δ 157.2-108.4 ppm and the aliphatic one at δ 21.0 ppm.

Antimicrobial evaluation

The results of the antifungal evaluation are presented in Table 1 and are expressed as MIC in μg/mL. Compound **3a** was the most active derivative of this series, having activity over 2-fold better than fluconazole against *C. neoformans*. The compounds **3b** and **3c** showed to have the same activity, which was an interesting activity but a little less potent than compound **3a** and comparable to fluconazole activity against *C. neoformans*. Interestingly, derivatives **6a** and **6b** – both retro-sulfonamides - demonstrated to have poor activity. This suggests that the sulfonyl group be directly attached to the 8HQ core (especially at 5-position) is important. Besides, the compound 8-hydroxyquinoline-5-sulfonamide (**d**, Figure 1, previously synthesized by our research group) was also not effective in inhibiting *Cryptococcus.* This suggests that the 5-sulfonamide substituted is important for the activity.

Table 1. Antifungal activity of novel 8HQ derivatives against *Cryptococcus neoformans* and *C. gattii*.

Minimum Inhibitory Concentration (MIC) in µg/mL			
8HQ Derivatives	5-Substituent	*C. neoformans* H99	*C. gattii* MYA-4039
3a		4	2
3b		8	8
3c		8	8
6a		32	16
6b		32	32
d		32	16
FLZ	-	8	2
AMP B	-	1	0,125

Notes: FLZ: Fluconazole; AMP B: Amphotericin B

Other quinoline derivatives are reported to have good activity against *Cryptococcus neoformans* and *C. gattii*. 3-(Phenylthio)quinoline compounds demonstrated to have a better inhibition activity when the phenylthiol group was *m*-CH_3 or *p*-CH_3 substituted [20]. This may corroborate the fact that the toluene (as in **3a**) improves the activity. Derivatives of quinolines 5,7,8-substituted also showed interesting activity against *C. gattii* and *C. neoformans*, especially when having a trifluoromethyl benzene in the 8-substituent [21]. Also, some sulfonamides have shown activity against *C. neoformans* by inhibiting carbonic anhydrases, besides revealing no significant cytotoxicity [22,23,24]. At our best knowledge, it is the first time that sulfonamides derived from 8-hydroxyquinoline presented activity against *Cryptococcus* strains.

CONCLUSIONS

This study reports the synthesis and characterization of a novel series of sulfonamide derivatives of 8HQ in 4%-21% yield overall and also presents the evaluation of obtained compounds against fungal cells. Derivative **3a** was the most active compound against *C. neoformans* and *C. gattii,* presenting an activity close to the positive controls fluconazole and amphotericin B, both commonly used drugs in the treatment of cryptococcosis. Compounds **3b** and **3c** also demonstrated a good activity against both of the ATCC strains, being still comparable to fluconazole against *C. neoformans*. The position inversion of the sulfonamide resulted in reduced activity of derivatives **6a** and **6b**, highlighting the importance of the sulfonyl group position in the molecule. Thus, the **3**-series derivatives can be promising antifungal candidates for treatment of cryptococcosis.

METHODS AND MATERIALS

General

Melting points were determined on Kofler Melting Point Apparatus (Reichert, Austria). ^{1}H and ^{13}C RMN spectra were obtained on Bruker 400 (Billerica, USA) nuclear magnetic resonance spectrometer. Proton and carbon chemical shifts (δ) were referenced by TMS (tetramethylsinale). IR (Fourier-transform infrared spectroscopy) was performed on Perkin Elmer Spectrum BX (Shelton, USA). Reagents as 8-hydroxyquinoline were purchased from commercial suppliers (Sigma-Aldrich, Saint Louis, USA) and were used without further purification. Column chromatography was performed on silica gel Fluka (Sigma-Aldrich) 0.035-0.070 mm. The solvents were distilled before use.

Synthesis of 8-hydroxyquinoline-5-sulfonyl chloride 2

8-Hydroxyquinoline (0.500 g, 3.44 mmol) was added to chlorosulfonic acid (3.0 mL, 45.11 mmol) and the mixture was kept at room temperature for 18 hours. The resulting solution was added to a mixture of ice (50 g), brine (100 mL) and dichloromethane (150 mL). The organic fraction was dried over sodium sulfate anhydrous and filtered. The solvent was distilled off and the 8-hydroxyquinoline-5-sulfonyl chloride **2** was added to later reactions without further purification.

General procedure for synthesis of 8-hydroxyquinoline-5-sulfonamides 3a, 3b, and 3c

The 8-hydroxyquinoline-5-sulfonyl chloride 2 (0.317 g, 1.29 mmol) was added to a solution of an appropriate amine (0.279 g, 2.6 mmol) in acetonitrile (4.0 mL). This mixture was kept stirring at 80°C for 3 hr. The solvent of the resulting solution was distilled off under reduced pressure and the remaining concentrate was purified by silica gel column using hexanes/EtOAc in gradient as eluent.

8-Hydroxyquinoline-5-(N-4-tolyl)sulfonamide 3a

Eluent hexanes:EtOAc (80:20). Off-white solid. 14% yield (two steps): mp: 168-170°C. IR: 3,309, 3,252, 2,921, 2,851, 1,570, 1,497, 1,465, 1,331, 1,194, 1,146, 1,122, 795 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.94 (dd, 1H, J = 8.7 Hz, 1.6 Hz), 8.81 (dd, 1H, J = 4.3 Hz, 1.6 Hz), 8.15 (d, 1H, J = 8.2 Hz), 7.54 (dd, 1H, J = 8.7 Hz, 4.3 Hz), 7.09 (d, 1H, J = 8.2 Hz), 6.92 (d, 2H, J = 8.4 Hz), 6.79 (d, 2H, J = 8.4 Hz), 2.19 (s, 3H). ^{13}C NMR (100 MHz, CDCl3) δ (ppm): 157.2, 148.7, 138.1, 136.0, 133.9, 133.5, 130.0, 124.9, 124.2, 123.8, 122.9, 108.4, 21.0.

8-Hydroxyquinoline-5-(N-4-fluorophenyl)sulfonamide 3b

Eluent hexanes:EtOAc (70:30). Yellow solid. 21% yield (two steps): mp: 186-190°C. IR: 3,296, 3,227, 2,357, 2,340, 1,503, 1,186, 1,149, 1,126, 816, 785 cm-1. ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm): 10.45 (s, 1H), 9.00 (dd, 1H, J = 8.8 Hz, 1.3 Hz), 8.96 (dd, 1H, J = 4.3 Hz, 1.3 Hz), 8.08 (d, 1H, J = 8.4 Hz), 7.76 (dd, 1H, J = 8.8 Hz, 4.3 Hz), 7.10 (d, 1H, 8.4 Hz), 6.98 (m, 4H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ (ppm): 160.0, 158.5, 157.6, 149.0, 138.3, 133.7, 132.8, 124.6, 123.4, 123.2, 122.0, 115.8, 109.6.

8-Hydroxyquinoline-5-(N-ethyl)sulfonamide 3c

Eluent hexanes:EtOAc (70:30). Off-white solid. 4% yield (two steps): mp: 149-152°C. IR: 3,295, 2,984, 2,357, 1,505, 1,317, 1,197, 1,147, 1,114, 830, 785 cm-1. ^{1}H NMR (400 MHz, CDCl3) δ (ppm): 9.01 (dd, 1H, J = 8.7 Hz, 1.4 Hz), 8.85 (dd, 1H, J = 4.1 Hz, 1.4 Hz), 8.22 (d, 1H, J = 8.4 Hz), 7.60 (dd, 1H, J = 8.7 Hz, 4.1 Hz), 7.16 (d, 1H, J = 8.4 Hz), 4.74 (s, 1H), 2.95 (bq, 2H), 1.01 (t, 3H, J = 7.2). ^{13}C NMR (100 MHz, CDCl3) δ (ppm): 157.0 148.7, 138.4, 134.1, 132.8, 124.8, 124.7, 123.8, 108.2, 38.3, 15.3.

Synthesis of nitroxoline/5-Nitroquinolin-8-ol 4

Sodium nitrite (8.2 mL, 71.64 mmol) was slowly added to a solution of 8-hydroxyquinoline (10 g, 68.90 mmol) in hydrochloric acid (28.8 mL). The reaction was kept stirring at room temperature. After 1 hr, the reaction precipitate was filtered and washed with cold distilled water. The resulting 5-nitrosoquinolin-8-ol (7.98 g, 45.82 mmol) was added to a solution of nitric acid (18.0 mL) with distilled water (10.5 mL). The solution was maintained at 17°C with occasional agitation and after 1 hr, cold distilled water (60 mL) was added. Sodium acetate was added to the mixture until it reached pH 6.0. The solution was filtered, washed with cold distilled water and then dried as previously described. The resulting product was purified by recrystallization with ethanol. Yellow solid. 26% yield (two steps): ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm): 9.09 (bd, 1H) , 8.97 (bd, 1H), 8.49 (d, 1H, J = 8.6 Hz), 7.83 (bd, 1H), 7.15 (d, 1H, J = 8.6 Hz). ^{13}C NMR (100 MHz, DMSO-*d6*) δ (ppm): 159.4, 148.3, 135.3, 135.1, 134.8, 129.6, 125.4, 122.8, 110.8.

Synthesis of 5-aminoquinolin-8-ol 5

Palladium on carbon (0.1 g) and a 16% hydrazine solution (3.0 mL) were added to a solution of nitroxoline (0.8 g, 4.21 mmol) in isopropanol (40 mL). The stirring mixture was maintained at 82°C under reflux. After 4 hr, the solution was hot filtered and concentrated under reduced pressure. Yellow solid. 22% yield (three steps): ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm): 8.75 (dd, 1H, J = 4.1 Hz, 1.4 Hz), 8.49 (dd, 1H, J = 8.5 Hz, 1.4 Hz), 7.41 (dd, 1H, J = 8.5 Hz, 4.1 Hz), 6.87 (d, 1H, J = 8.2), 6.64 (d, 1H, J = 8.2 Hz). ^{13}C NMR (100 MHz, DMSO-*d6*) δ (ppm): 147.7, 143.8, 138.6, 136.8, 131.6, 119.4, 118.7, 111.9, 108.6.

General procedure for synthesis of reverse sulfonamides 6a and 6b

The 8-hydroxyquinoline-5-amino (0.1 g, 0.624 mmol) was solubilized in ethanol (2.0 mL) and pyridine (0.2 mL, 2.50 mmol) and then the appropriate sulfonyl chloride (0.655 mmol) was added. The solution was kept at room temperature under agitation overnight. The resulting solution was added to a mixture of ethyl acetate (20 mL) and distilled water (20 mL). The organic layer was dried over sodium sulfate anhydrous, filtered and the solvent was distilled off under reduced pressure and the remaining concentrate was purified by silica gel column using hexanes/EtOAc in gradient as eluent.

8-Hydroxyquinoline-5-(p-tolylsulfonylamino) 6a

Eluent hexanes:EtOAc (75:25). Off-white solid. 17% yield (four steps): mp: 194-197°C. IR: 3,448, 2,923, 2,359, 2,340, 2,340, 1,572, 1,519, 1,392, 1,320, 1,150, 1,143, 1,076, 809 cm-1. ^{1}H NMR (400 MHz, CD3OD) δ (ppm): 8.73 (dd, 1H, J = 4.1 Hz, 1.5 Hz), 8.33 (dd, 1H, J = 8.6 Hz, 1.5 Hz), 7.49 (d, 2H, J = 8.2 Hz), 7.38 (dd, 1H, J = 8.6 Hz, 4.1 Hz), 7.22 (d, 2H, J = 8.2 Hz), 6.96 (d, 1H, J = 8.4 Hz), 6.90 (d, 1H, J = 8.4 Hz) , 2.35 (s, 3H). ^{13}C NMR (100 MHz, CD3OD) δ (ppm): 154.1, 149.5, 145.2, 140.0, 138.1, 134.1, 130.7, 128.6, 128.4, 124.4, 122.9, 111.1, 21.5.

8-hydroxyquinoline-5-(N-methanesulfonylamino) 6b

Eluent hexanes:EtOAc (60:40). Dark solid. 14% yield (four steps): mp: 180-184°C. IR: 3,230, 2,918, 2,849, 2,358, 1,475, 1,314, 1,185, 1,023, 974, 763, 667 cm-1. ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm): 9.46 (s, 1H), 8.88 (dd, 1H, J = 4.1 Hz, 1.6 Hz), 8.56 (dd, 1H, J = 8.5 Hz, 1.6 Hz), 7.63 (dd, 1H, J = 8.5 Hz, 4.1 Hz), 7.43 (d, 1H, J = 8.4 Hz), 7.08 (d, 1H, J = 8.4 Hz), 2.97 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ (ppm): 152.8, 148.5, 138.6, 132.8, 126.8, 126.6, 123.2, 122.1, 110.7, 39.2.

Antifungal evaluation

The antifungal activity of synthesized compounds was assayed on *Cryptococcus neoformans* H99 and *C. gattii* MYA-4039, both ATCC strains. The method for the determination of broth dilution minimum inhibitory concentrations of the derivatives was EUCAST 7.3.1 (2017) [25]. Results were determined after 48 hours incubation and MIC was considered to be the inhibition of 99% of visual growth (except for azoles whose MIC is considered as 50% of growth inhibition compared to positive control). Fluconazole and amphotericin B were assayed in the same conditions and were used as a comparative to the synthesized series against the *Cryptococcus* strains.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

[1] Selb, R. *et al*. Molecular typing and *in vitro* resistance of *Cryptococcus neoformans* clinical isolates obtained in Germany between 2011 and 2017. *Intern J Medical Microbiology*, Vol 309, 2019, DOI:10.1016/j.ijmm.2019.151336.

[2] Olszewski, M.A., et al. Mechanisms of cryptococcal virulence and persistence. *Future Microbiology*. Vol 5, 2010, DOI: 10.2217/fmb.10.93.

[3] Maziarz, E.K., Perfect JR. Cryptococccosis. *Infect Dis Clin North Am*, Vol 30, 2016, PMID: 26897067.

[4] Firacative, C. *et al*. The status of cryptoccosis in Latin America. *Mem Inst Oswaldo Cruz*, Vol 113, 2018, DOI: 10.1590/0074-02760170554.

[5] Chen, S.C. *et al*. Consensus guidelines for the treatment of yeast infections in the haematology, oncology and intensive care setting, 2014. *Intern Med J*, Vol 44, 2014, DOI: 10.1111/imj.12597.

[6] Chesdachai, S. *et al*. Minimum Inhibitory Concentration Distribution of Fluconazole against *Cryptococcus* Species and the Fluconazole Exposure Prediction Model. *Open F Infect Dis*, Vol 6, 2019, doi: 10.1093/ofid/ofz369.

[7] World Health Organization. Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Available at: http://www.who.int/hiv/pub/guidelines/cryptococcal-disease/en/. Accessed September 9, 2019.

[8] Scalese, G. *et al*. . Exploring oxidovanadium (IV) homoleptic complexes with 8-hydroxyquinoline derivatives as prospective antitrypanosomal agents. *New J Chem*, 2019, DOI: 10.1039/C9NJ02589H.

[9] El Faydy, M. *et al*. Synthesis and investigation of antibacterial and antioxidants properties of some new 5-subsituted-8-hydroxyquinoline derivatives. *JMES*, Vol 8, 2017.

[10] Yang, X. *et al*. Novel 8-hydroxyquinoline derivatives targeting β-amyloid aggregation, metal chelation and oxidative stress against Alzheimer's disease. *Bioorg Med Chem*, Vol 26, 2018, DOI: 10.1016/j.bmc.2018.04.043.

[11] Bareggi, S.R., Cornelli, U. Clioquinol: review of its mechanisms of action and clinical uses in neurodegenerative disorders. *CNS Neurosci Ther*, Vol 18, 2010, DOI: 10.1111/j.1755-5949.2010.00231.x.

[12] Laurie, M.T. et al. Functional Assessment of 2,177 U.S. and International Drugs Identifies the Quinoline Nitroxoline as a Potent Amoebicidal Agent against the Pathogen Balamuthia mandrillaris. *mBio*, Vol 9, 2018, DOI: 10.1128/mBio.02051-18.

[13] Cherdtrakulkiat, R. *et al*. Derivatives (halogen, nitro and amino) of 8-hydroxyquinoline with highly potent antimicrobial and antioxidant activities. *Biochemistry and Biophysics Reports*, Vol 6, 2016, DOI: 10.1016/j.bbrep.2016.03.014.

[14] Cherdtrakulkiat, R. *et al*. Nitroxoline: a potent antimicrobial agent against multidrug resistant Enterobacteriaceae. *EXCLI J*, published online, 2019, DOI: 10.17179/excli2019-1378.

[15] Pippi, B. *et al*. Evaluation of 8-hydroxyquinoline derivatives as hits for antifungal drug design. *Medical Mycology*, Vol 55, 2017, DOI: 10.1093/mmy/myx003.

[16] Joaquim, A.R. *et al*. Rapid tools to gain insights into the interaction dynamics of new 8-hydroxyquinolines with few fungal lines. *Chem Biol Drug Des.*, Vol 93, 2018, DOI: 10.1111/cbdd.13435.

[17] Mazumder, U.K. et al. Antineoplastic and Antibacterial Activity of some Mononuclear Ru(II) Complexes. *Journal of Enzyme Inhibition and Medicinal Chemistry*. Vol 19, 2004, DOI: 10.1080/14756360310001650192.

[18] Lu, S. et al. Synthesis, characterization, screening and docking analysis of 4-anilinoquinazoline derivatives as tyrosine kinase inhibitors. *European Journal of Medicinal Chemistry*. Vol 61, 2013, DOI: 10.1016/j.ejmech.2012.07.036.

[19] Belov A.V. and Nichvoloda V.M. Quinone Imines with a Fused Azine Ring: I. Synthesis and Hydrochlorination of 5-(*p*-Tolylsulfonilimino)quinolin-8-one. *Russian Journal of Organic* Chemistry. Vol 40, 2004, DOI: 1070-4280/04/4001-0093.

[20] Boateng, C.A. et al. Optimization of 3-(phenylthio)quinolinium compounds against opportunistic fungal pathogens. *European Journal of Medicinal Chemistry*. Vol 46, 2011, DOI: 10.1016/j.ejmech.2011.02.034.

[21] Mohammad, H. et al. Discovery of a Novel Dibromoquinoline Compound Exhibiting Potent Antifungal and Antivirulence Activity that Targets Metal ion Homeostasis. *ACS Infect Dis*. Vol 4, 2018, DOI: 10.1021/acsinfecdis.7b00215.

[22] Schlicker, C. et al. Structure and Inhibition of the CO2-Sensing Carbonic Anhydrase Can2 from the Pathogenic Fungus *Cryptococcus neoformans. Journal of Molecular Biology* Vol 385, 2009, DOI: 10.1016/j.jmb.2008.11.037.

[23] Annunziato, G. et al. Discovering a new class of antifungal agents that selectively inhibits microbial carbonic anhydrases. *J Enzyme Inhib Med Chem.* Vol 33, 2018, DOI: 10.1080/14756366.2018.1516652.

[24] Guzel, O. et al. Carbonic anhydrase inhibitors. The β-carbonic anhydrases from the fungal pathogens *Cryptococcus neoformans* and *Candida albicans* are strongly inhibited by substituted-phenyl-1H-indole-5-sulfonamides. *Bio & Med Chemistry Let.* Vol 20, 2010, DOI: 10.1016/j.bmcl.2010.02.103.

[25] European Comittee on Antimicrobial Susceptibility Testing, v 7.3.1. Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_7_3_1_Yeast_testing__definitive.pdf. Accessed November 17, 2019.

Title: Effects of inversion or substitution of the sulfonamide group at 5-position of 8-hydroxyquinoline derivatives against *Cryptococcus neoformans* and *C. gattii*.

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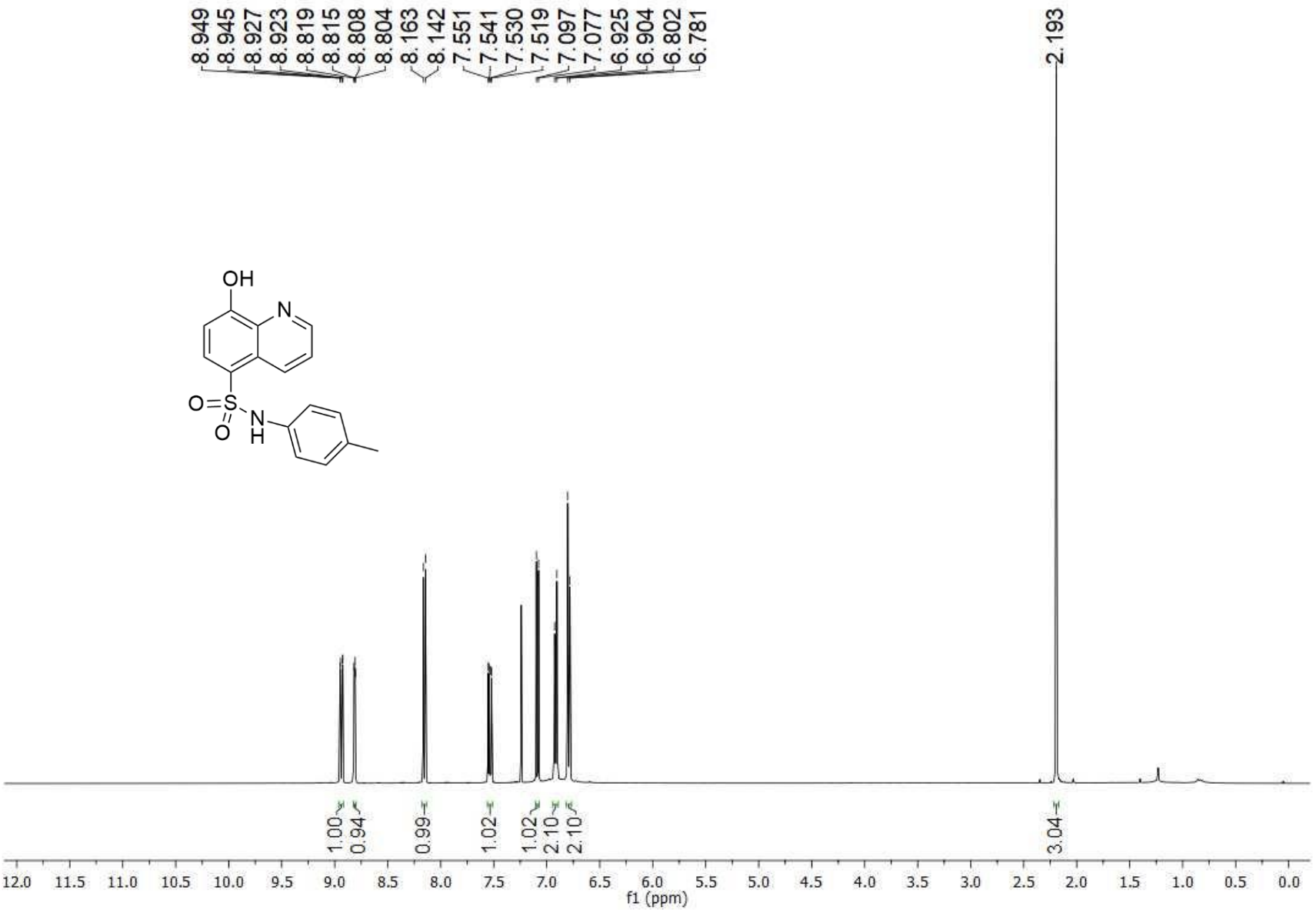

Figure S1. ^{1}H NMR spectrum in $CDCl_3$ of compound **3a.**

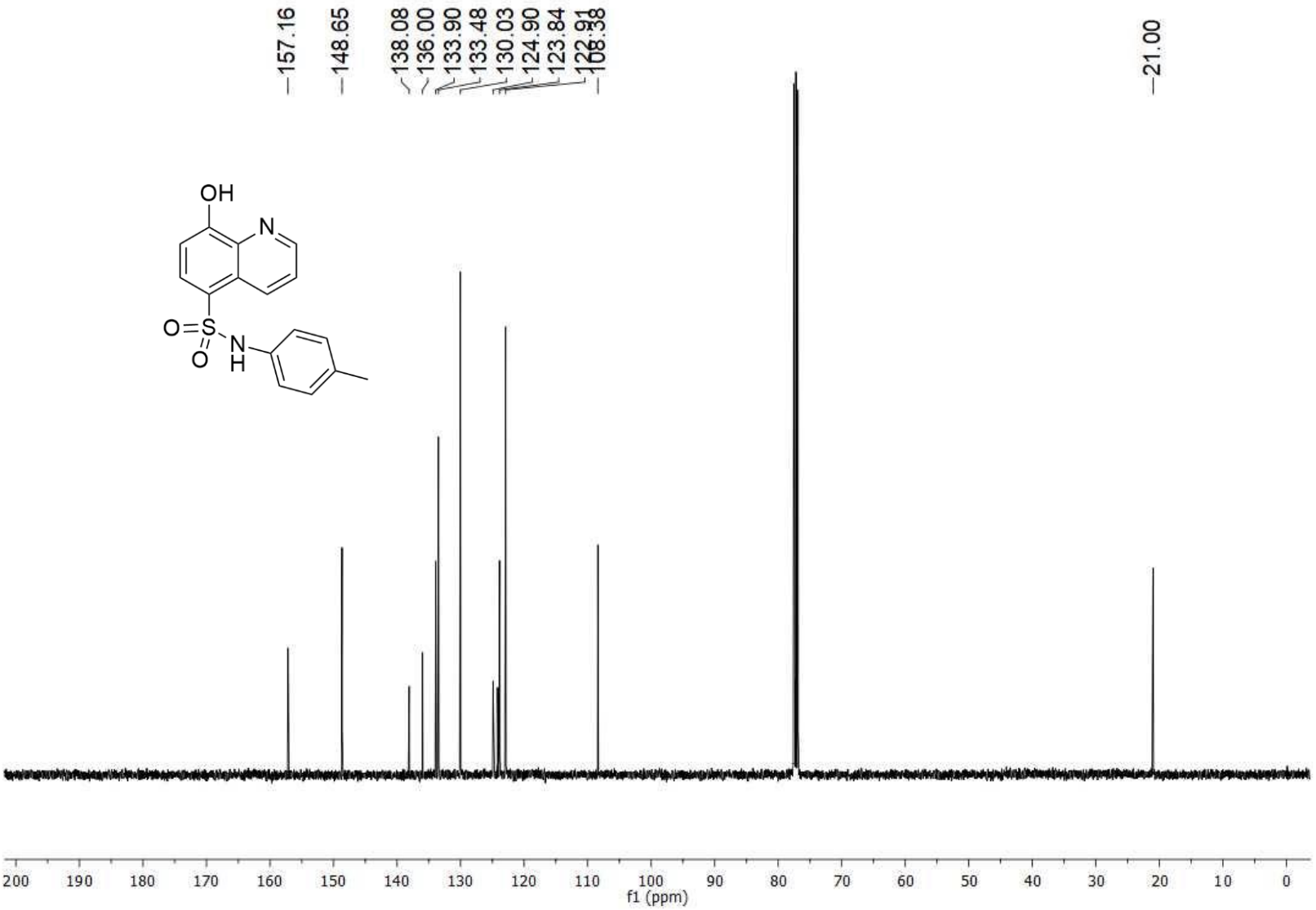

Figure S2. ^{13}C NMR spectrum in $CDCl_3$ of compound 3a.

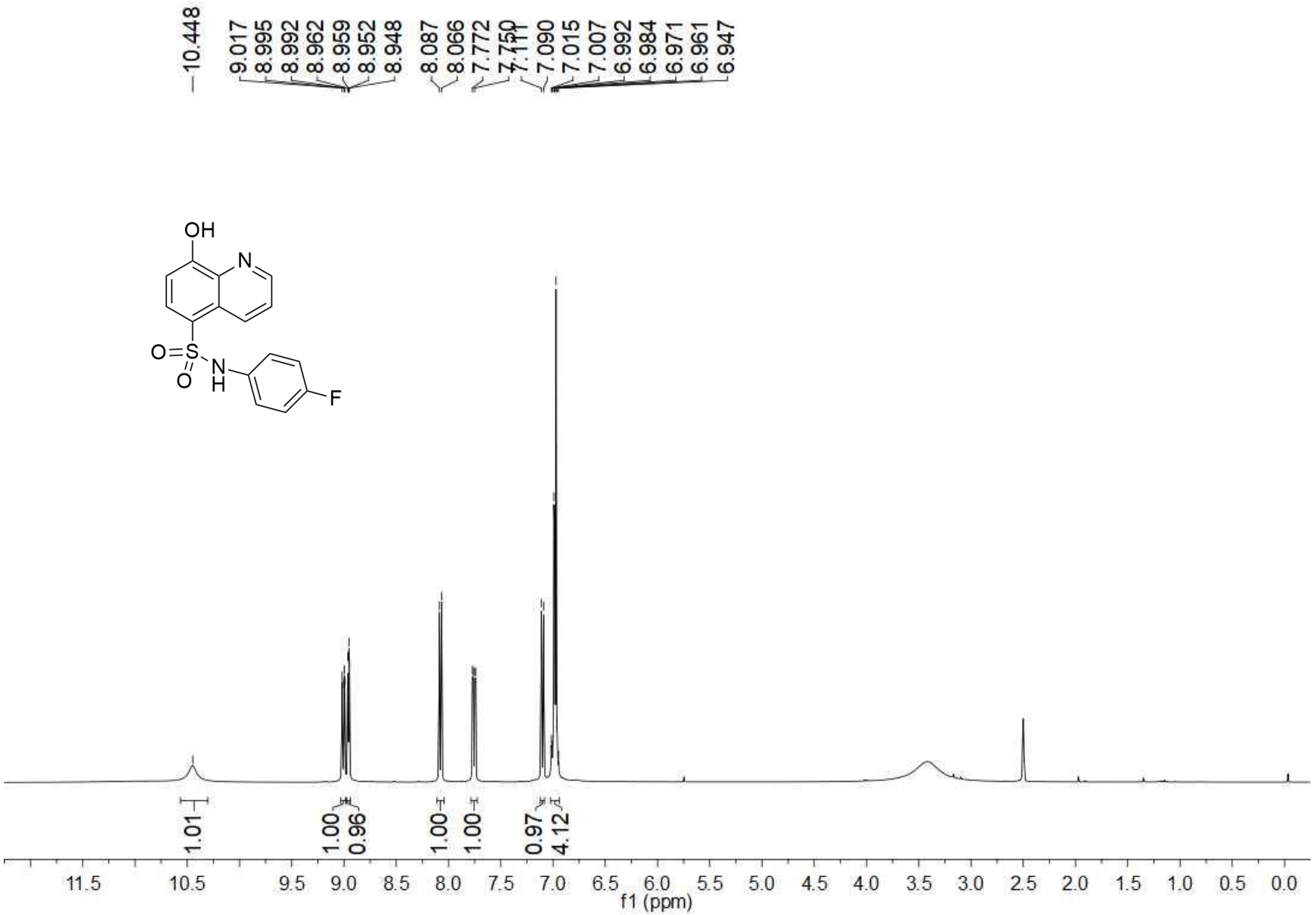

Figure S3. ^{1}H NMR spectrum in DMSO-*d6* of compound **3b**.

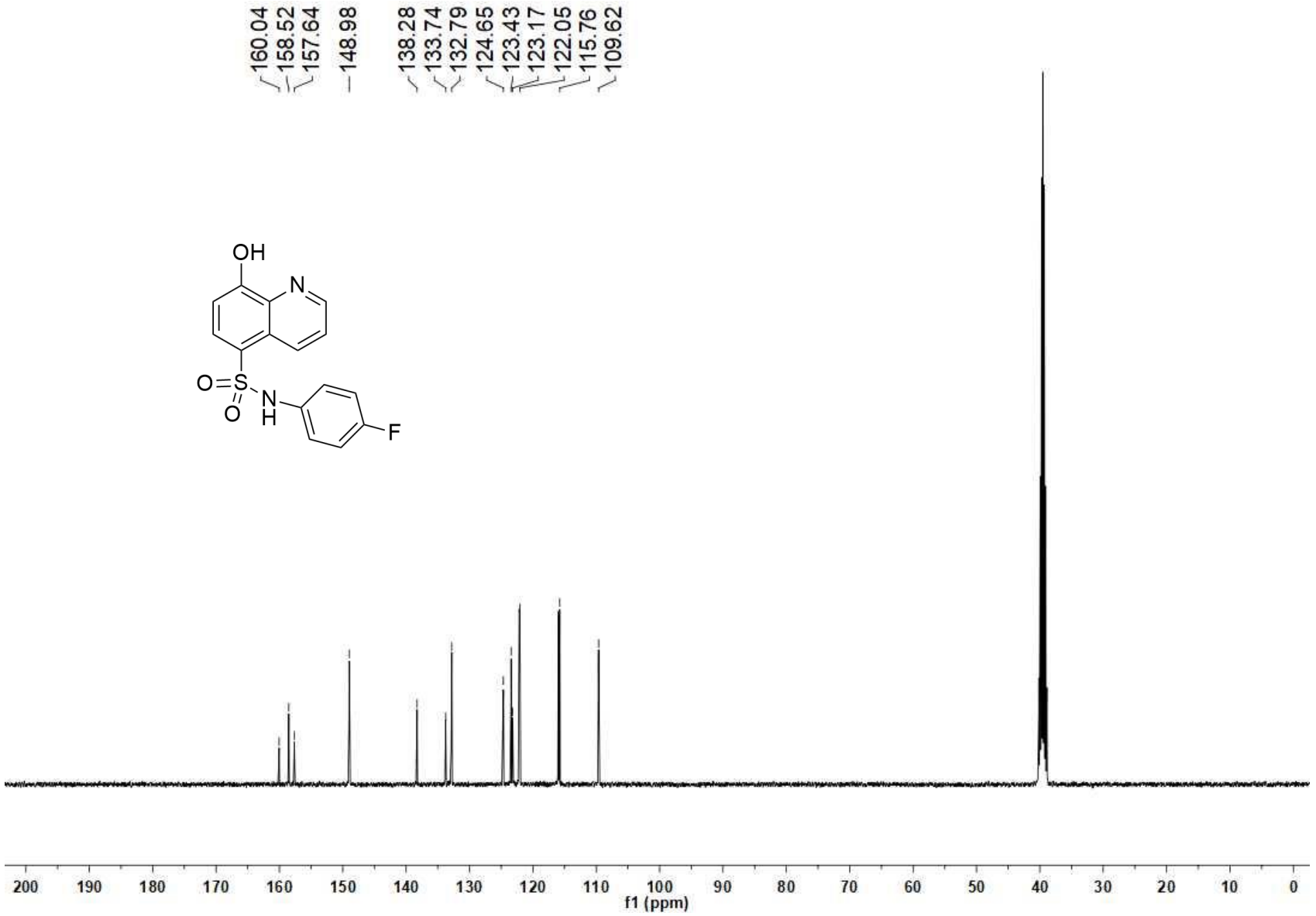

Figure S4. ^{13}C NMR spectrum in DMSO-*d6* of compound **3b**

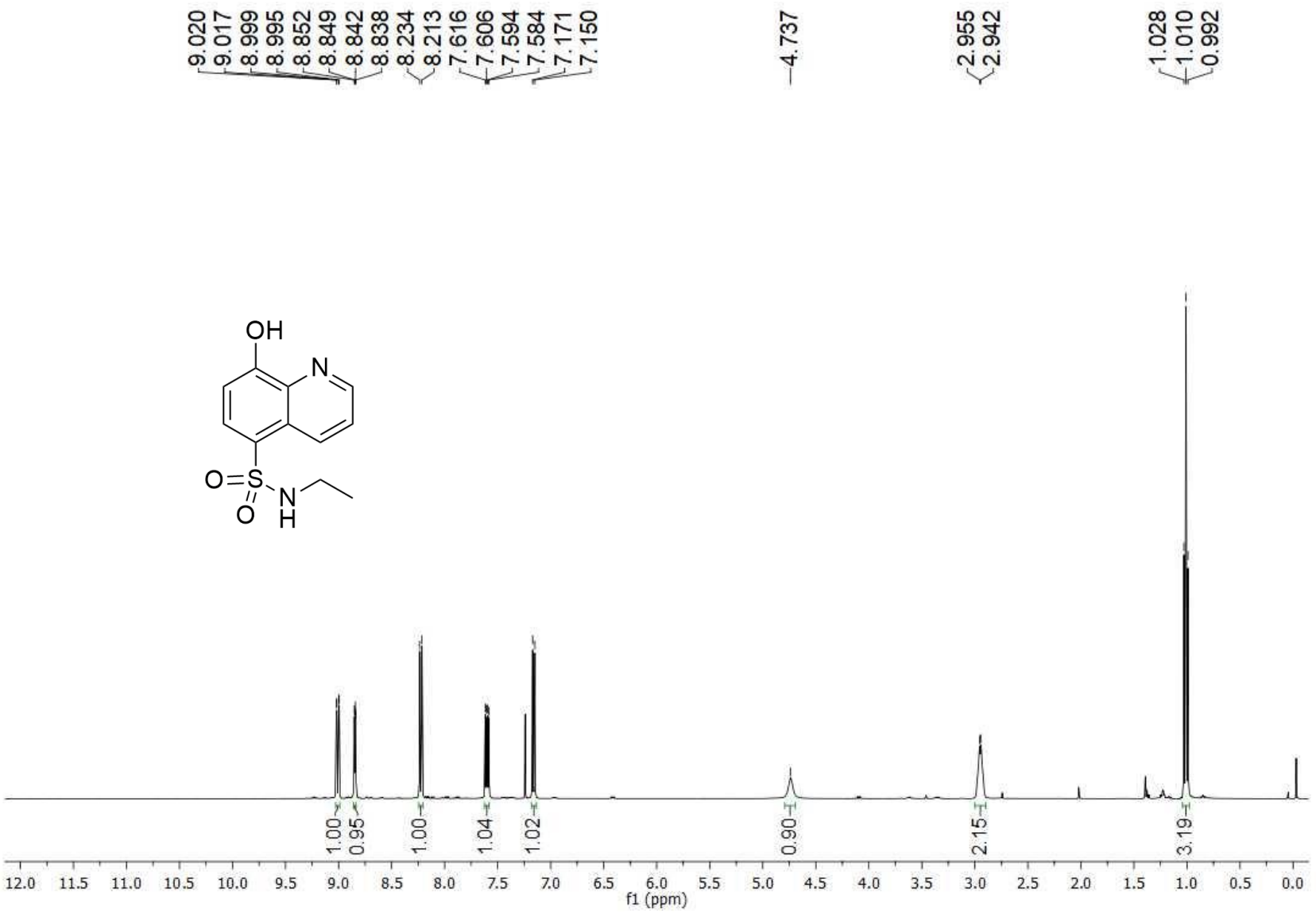

Figure S5. ^{1}H NMR spectrum in $CDCl_3$ of compound **3c.**

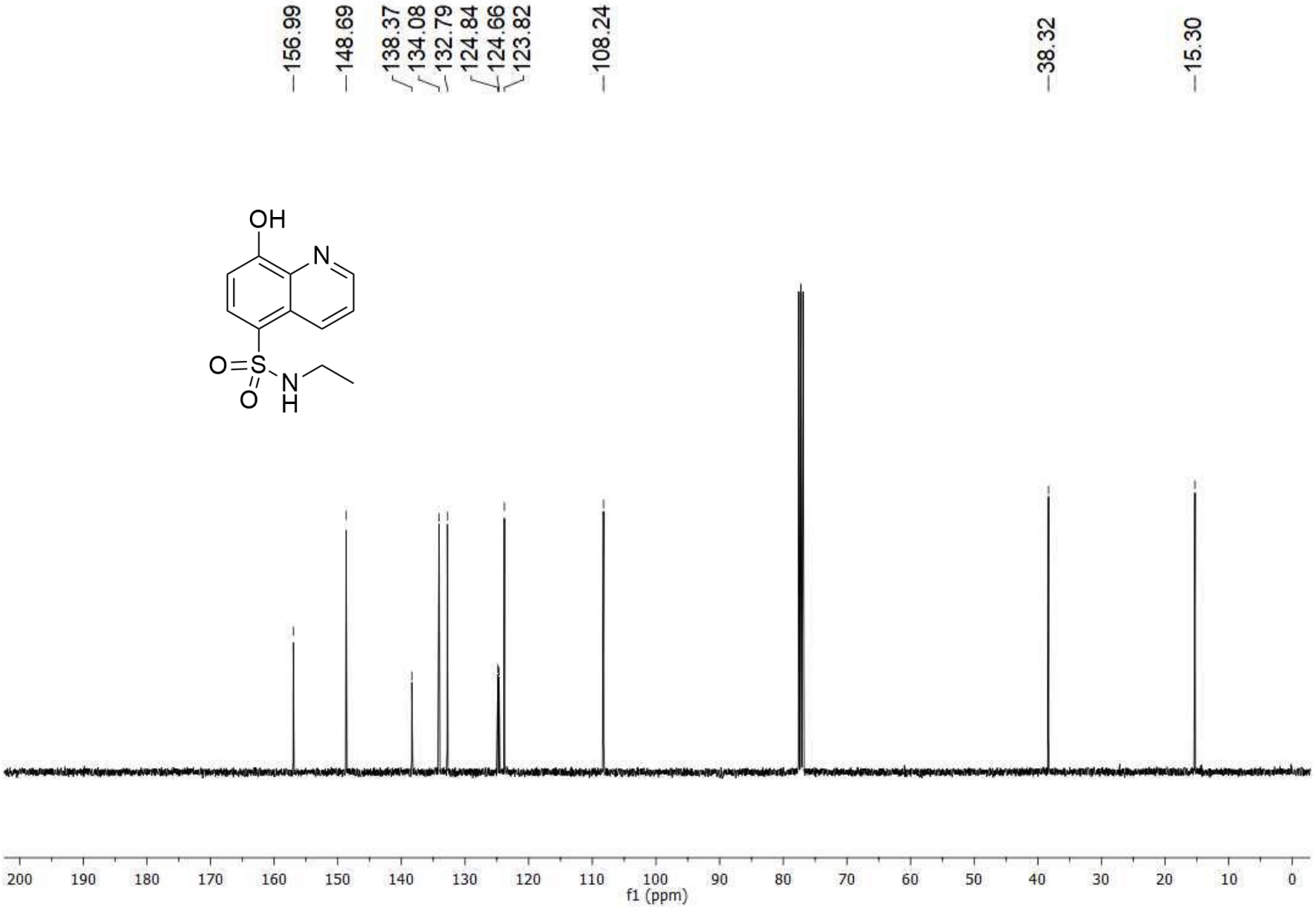

Figure S6. ^{13}C NMR spectrum in $CDCl_3$ of compound **3c.**

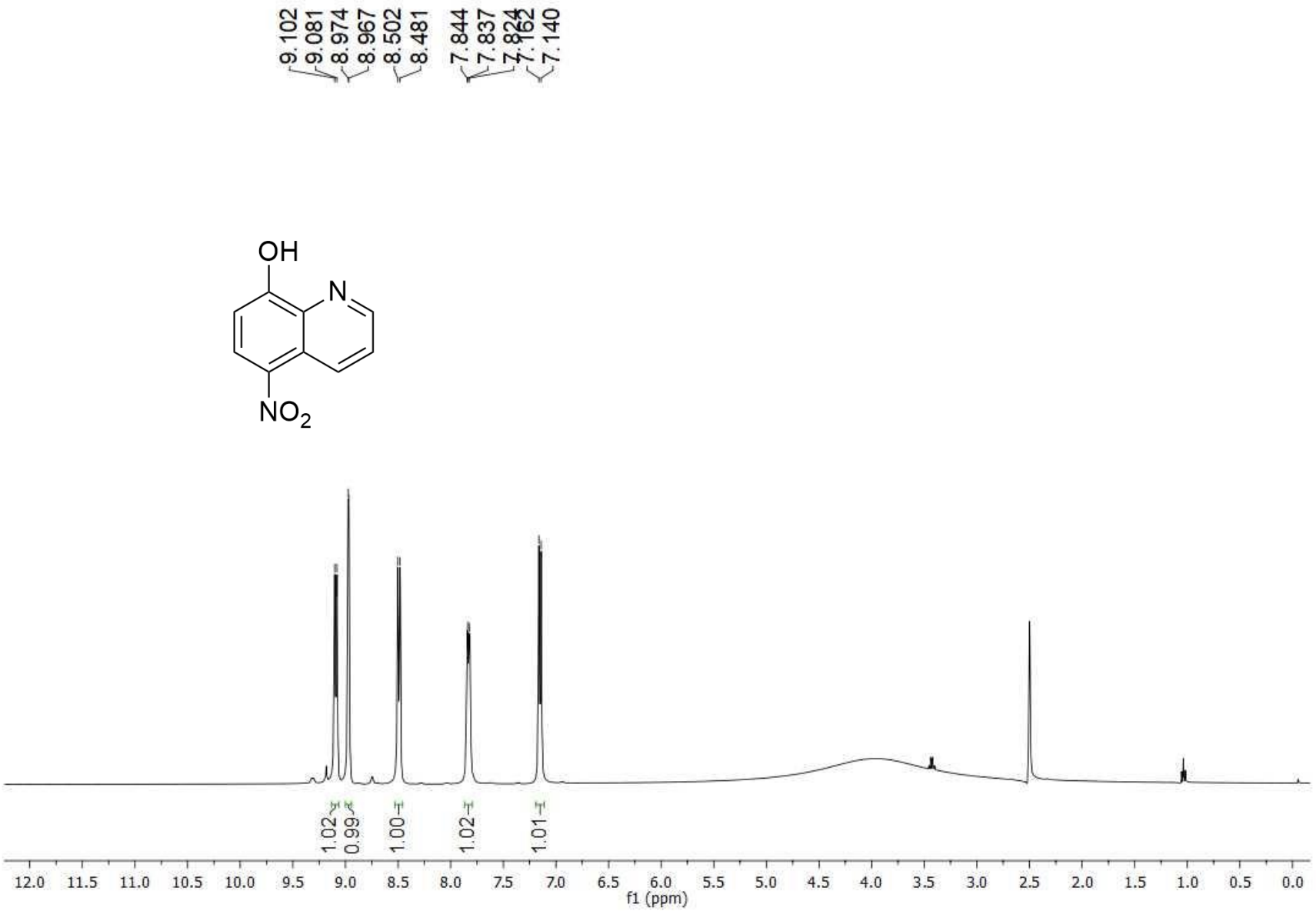

Figure S7. [1]H NMR spectrum in DMSO-*d6* of nitroxoline**.**

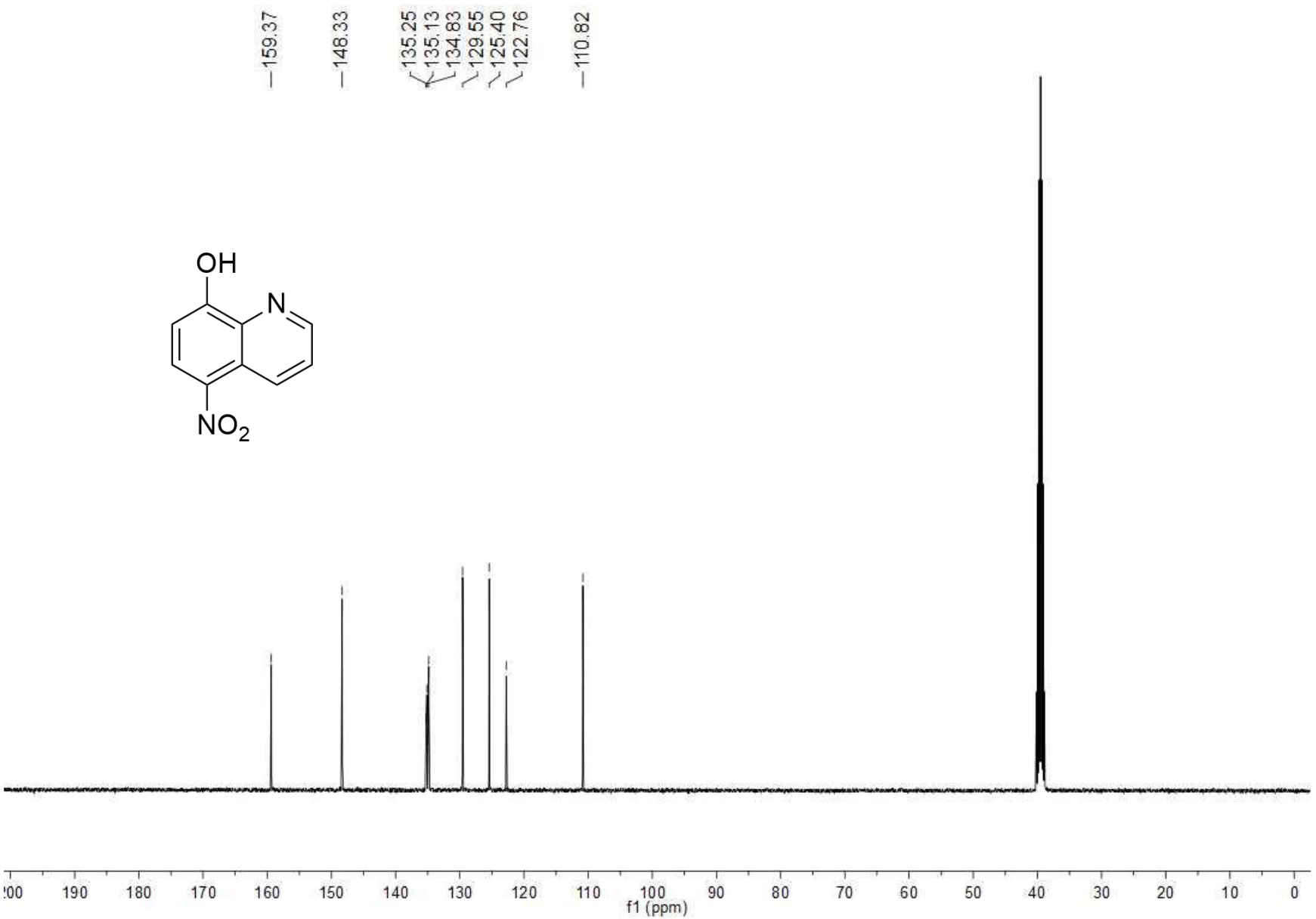

Figure S8. ^{13}C NMR spectrum in DMSO-*d6* of nitroxoline**.**

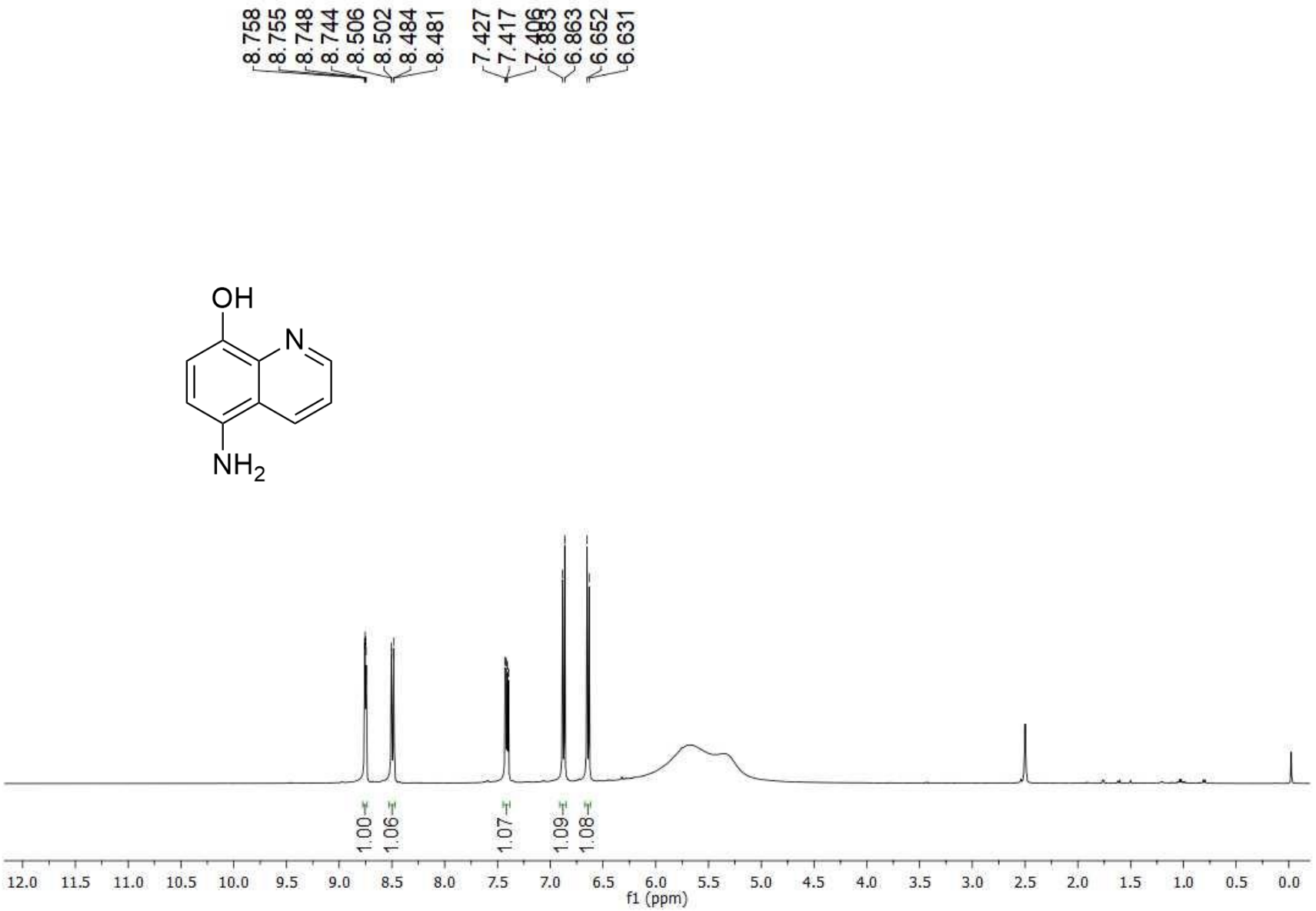

Figure S9. ^{1}H NMR spectrum in DMSO-*d6* of 5-Aminoquinolin-8-ol**.**

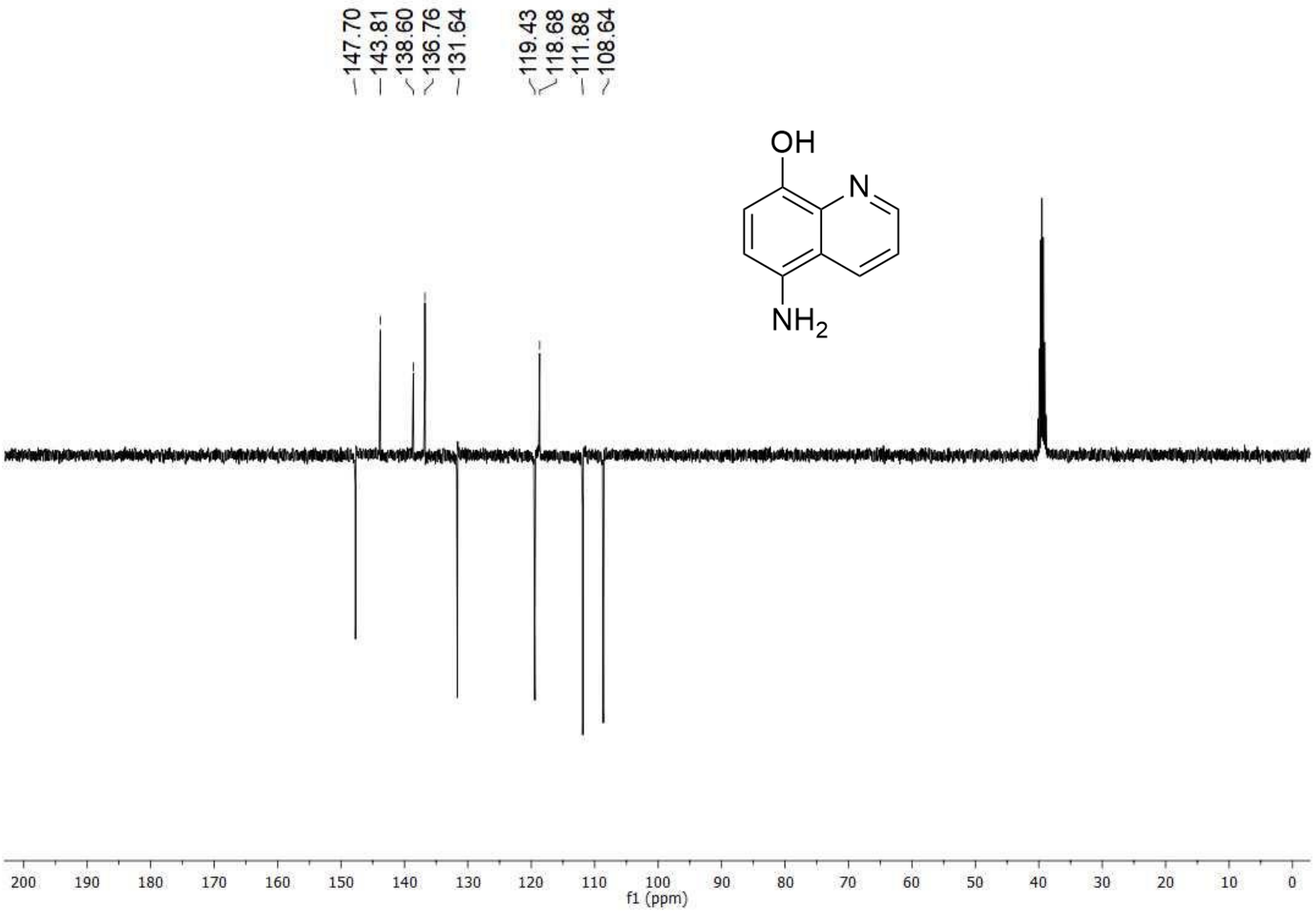

Figure S10. ^{13}C APT spectrum in DMSO-*d6* of 5-Aminoquinolin-8-ol**.**

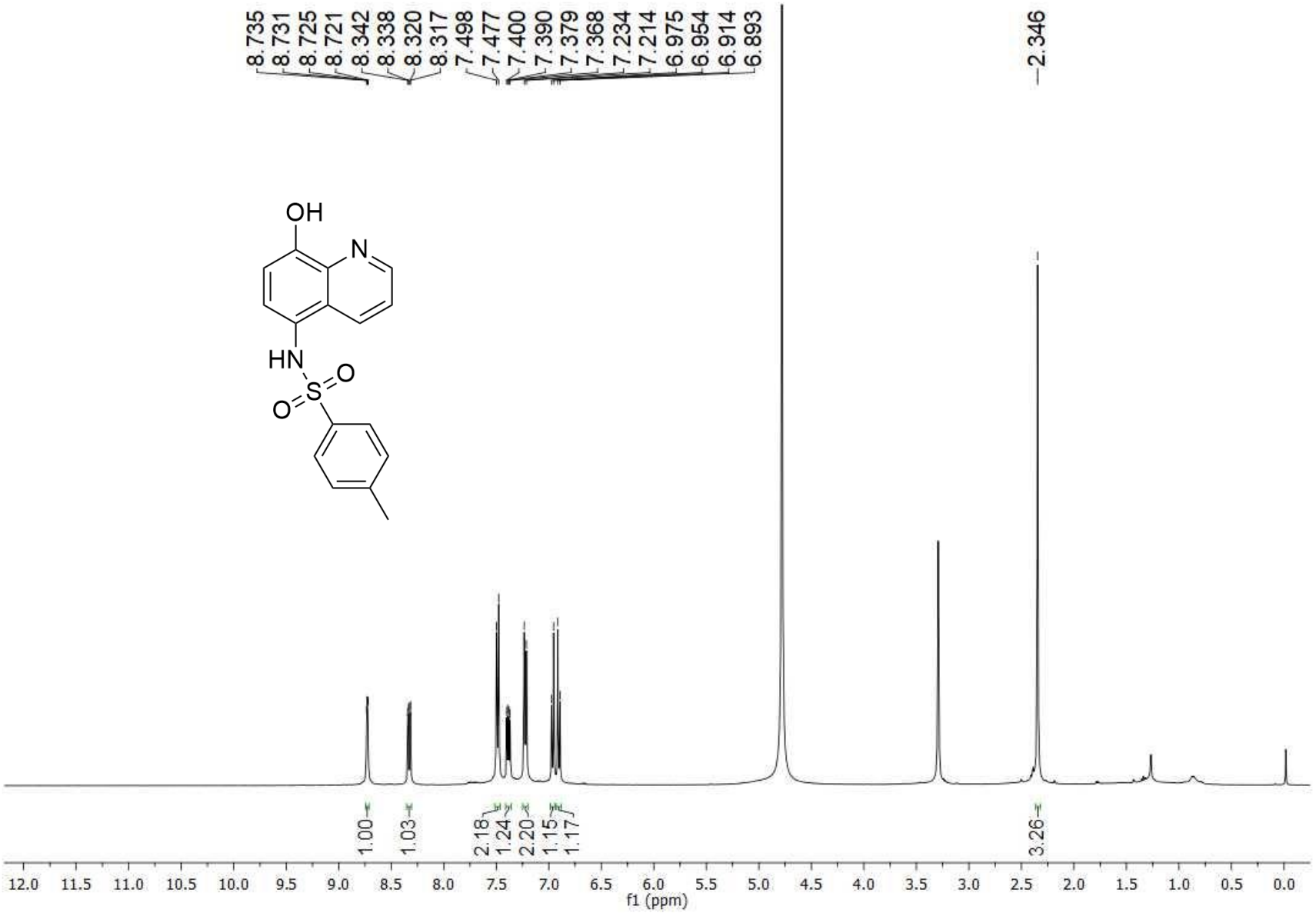

Figure S11. ^{1}H NMR spectrum in CD_3OD of compound **6a.**

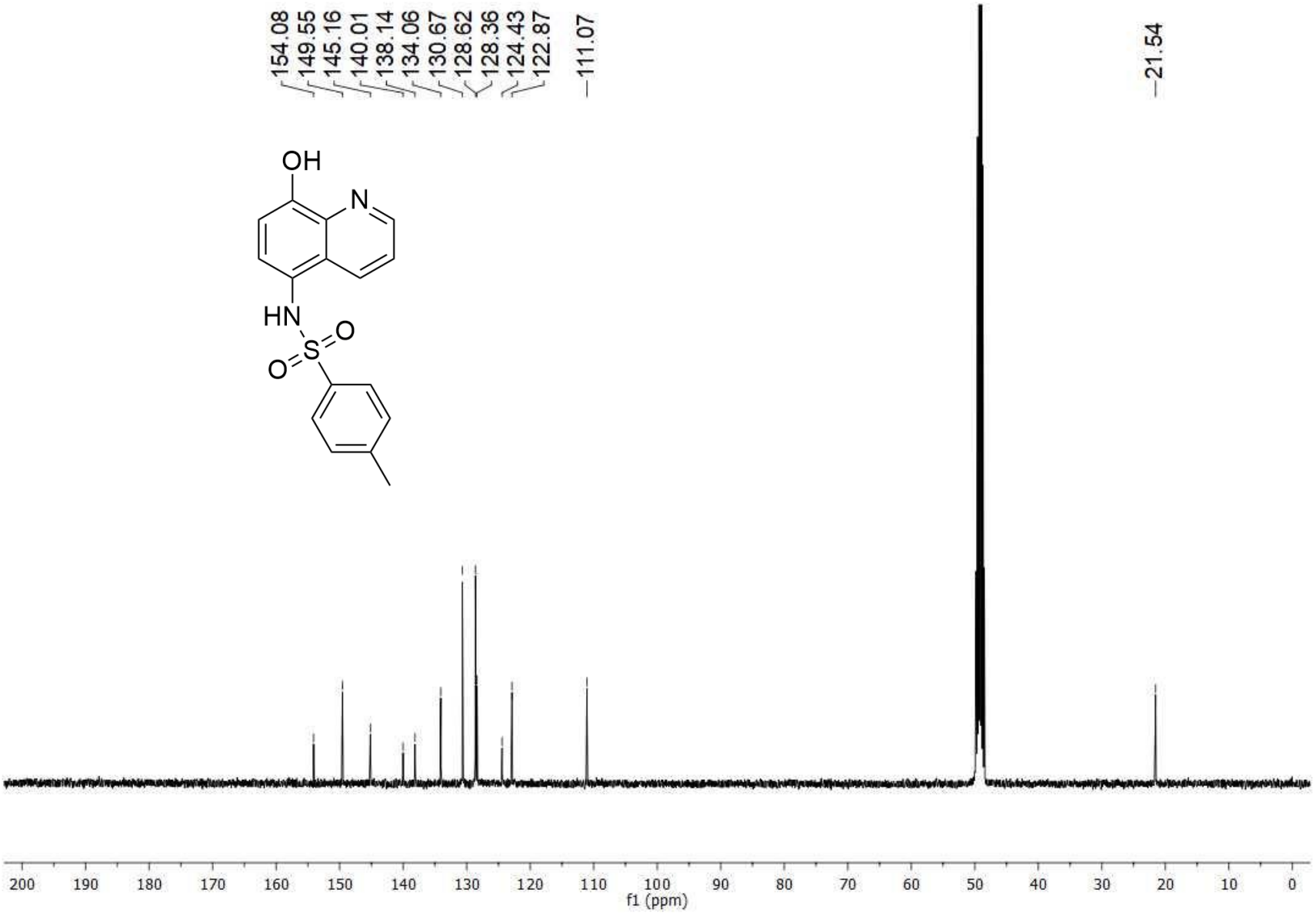

Figure S12. ^{13}C NMR spectrum in CD_3OD of compound **6a.**

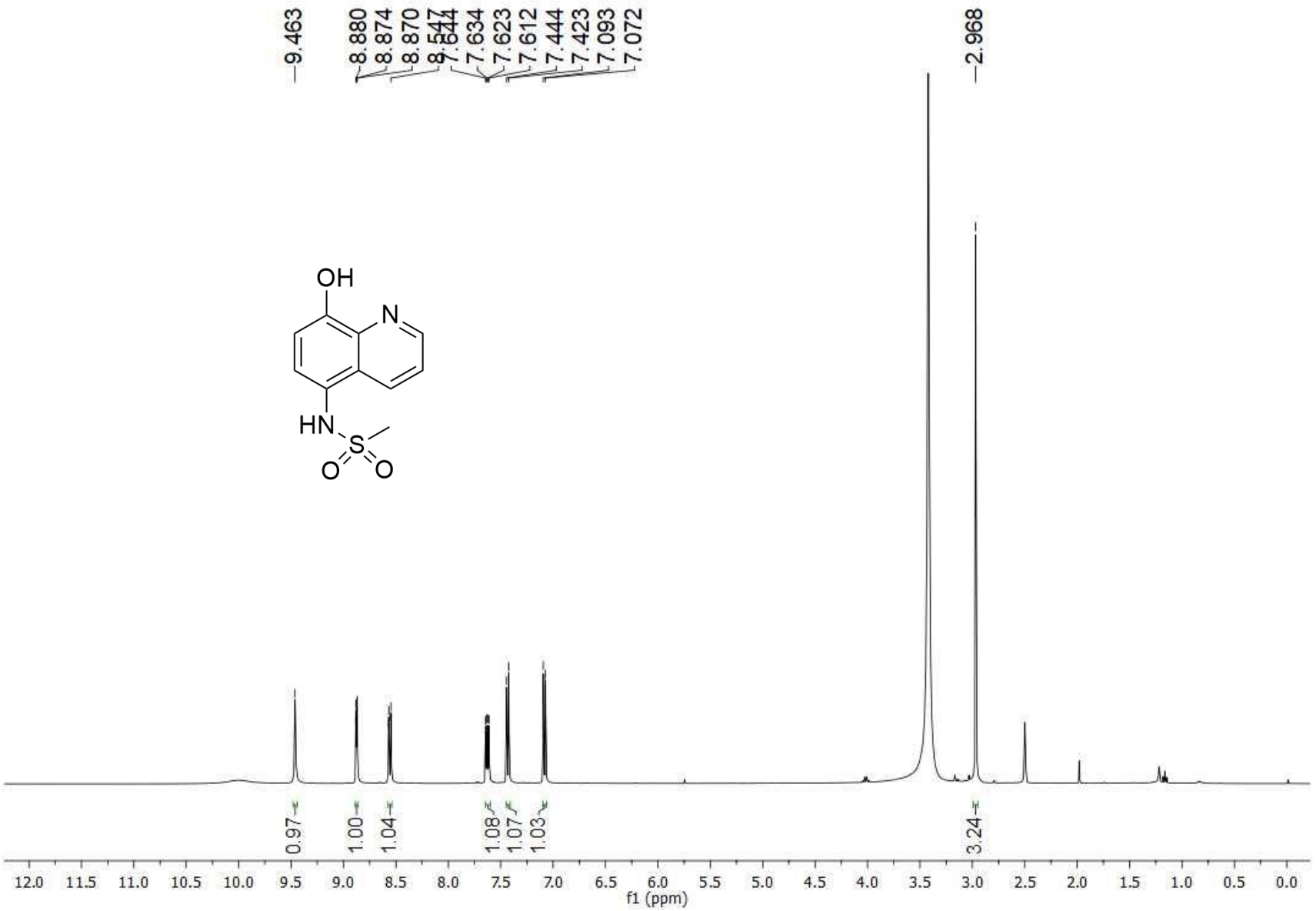

Figure S13. ^{1}H NMR spectrum in DMSO-*d6* of compound **6b.**

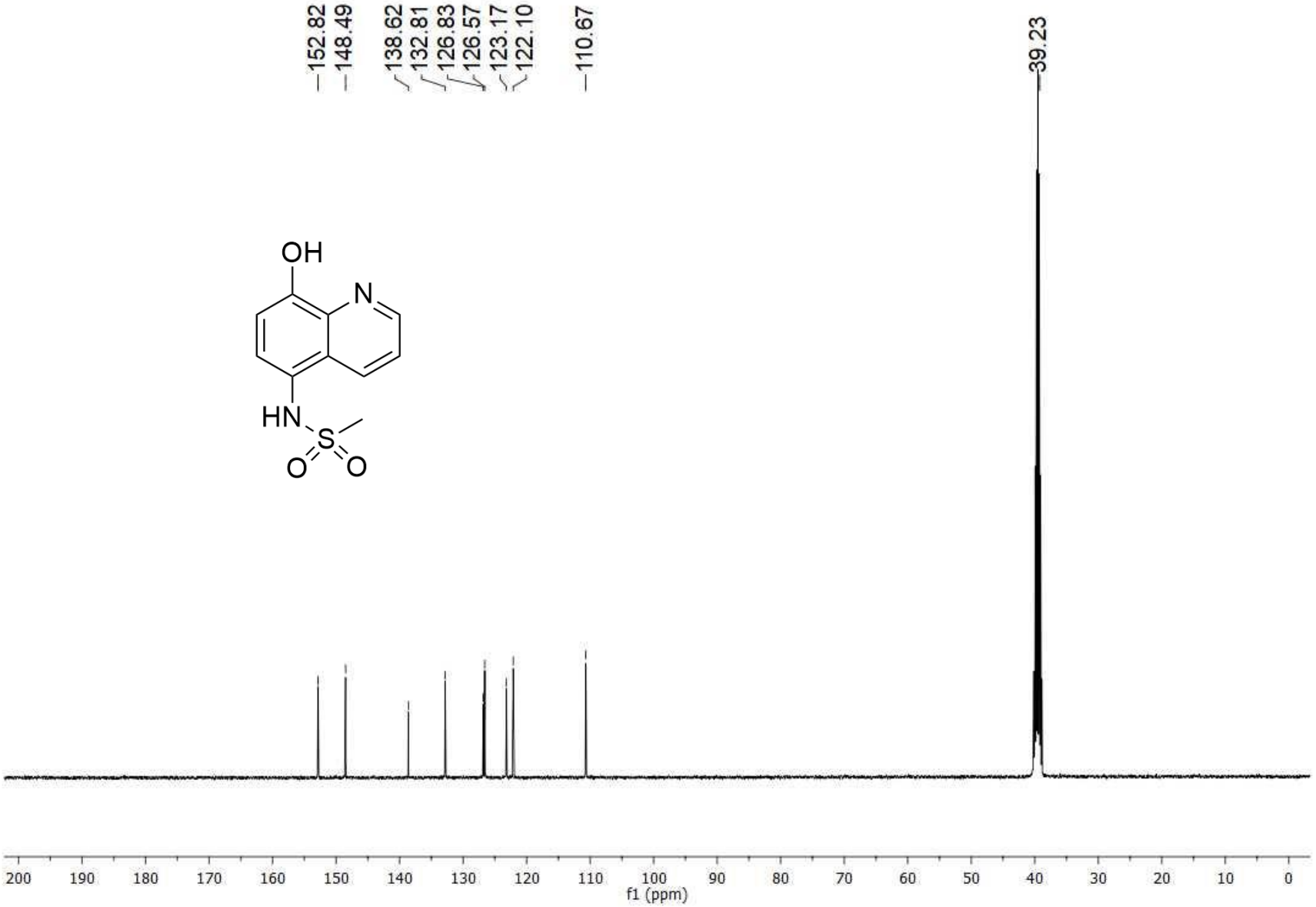

Figure S14. ^{13}C NMR spectrum in DMSO-*d6* of compound **6b.**