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SYNTHESIS OF NOVEL 8-METHOXYQUINOLINE DERIVATIVES AND *IN VITRO* ANTIBACTERIAL ACTIVITY

Trabalho de Conclusão apresentado ao Curso de Farmácia da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de Farmacêutico.

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“In the desert there is no sign that says, Thou shalt not eat stones.”

Sufi proverb – The Handmaid’s Tale por Margaret Atwood

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SYNTHESIS OF NOVEL 8-METHOXYQUINOLINE DERIVATIVES AND *IN VITRO* ANTIBACTERIAL ACTIVITY

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Abstract

The resistance of bacteria to antibiotics has become a worldwide concern and the development of new agents is of particular interest. Quinolines derivatives have shown activity against several microorganism and the presence of substituent on the quinoline ring seems to modulate the activity and the toxicity. Thus, the purpose of this study was the synthesis and characterization of the novel series of 8-methoxyquinoline derivatives and evaluation of *in vitro* antibacterial activity against ATCC strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Shigella flexneri*, *Enterococcus faecalis*, and *Staphylococcus aureus*. The commercial available clioquinol **3** was used to prepare 5-chloro-7-amino-8-methoxyquinoline derivatives (**5a**-**5e**) with a two-step synthesis: methylation of **3** to 5-chloro-7-iodo-8-methoxyquinoline **4** and palladium-catalyzed cross-coupling reaction to amination at position 7 in 50-66% of overall yield. The compounds prepared were characterized by FT-IR, ^{1}H and ^{13}C NMR. The nitroxoline **2**, synthetized from 8-hydroxyquinoline in 54% yield, and **3** were used as positive controls for *in vitro* evaluation of antibacterial activity for the novel series by broth microdilution assay. The compounds **5a** and **5b** presented activity against two Gram-positive bacteria *E. faecalis* and *S. aureus*, including the Methicillin resistant strain of *S. aureus* (ATCC 33591). However just the compound **5b,** that bear an electron withdrawing group (EWG) at *para*-position of the 7-aniline, presented activity comparable to the positive controls, with value of minimal inhibitory concentration (MIC) of 4-8 µg/mL for those strains. Thus, this compound has the potential to be developed as an antibacterial agent.

Keywords: clioquinol, 8-methoxyquinoline, antibacterial

1. Introduction

The 8-hydroxyquinoline (8HQ) or quinolin-8-ol **1**, a planar bicycle molecule bearing a phenol and a pyridine fused rings (1, Fig. 1), is a quinoline derivative that has been studied for over a century due to its biological activity. This way, several synthesis methods are available for its preparation, such as the modified Skraup reaction that uses metal catalysis to give it in around 80% yield [1]. The 8HQ and their derivatives, nitroxoline **2** and clioquinol **3**, are mostly used as antiseptic nowadays, but they present a wide range of medical application since they have shown neuroprotective effects, anticancer activity, and antimicrobial activity against virus, parasitic disease, bacterial, and fungal [2] [3] [4]. The mechanisms of actions involved in these activities are not well stablished but are being related with chelation of metals like Mn^{2+}, Cu^{2+}, Fe^{3+}, Co^{2+}, and Zn^{2+}. In addition, the metal-complexes are also active against microorganism and in some cases when tested against bacteria it presents a better activity that the ligand alone [3]. In the other hand, the complexation could affect the drug pharmacokinetic since it increases molecular weight and changes the molecular polar surface area. This is well established for quinolones that have large decrease on bioavailability when co-administrated with metal ions [5]. It is also known that these complexes could be produced after distribution contributing with the compound activity.

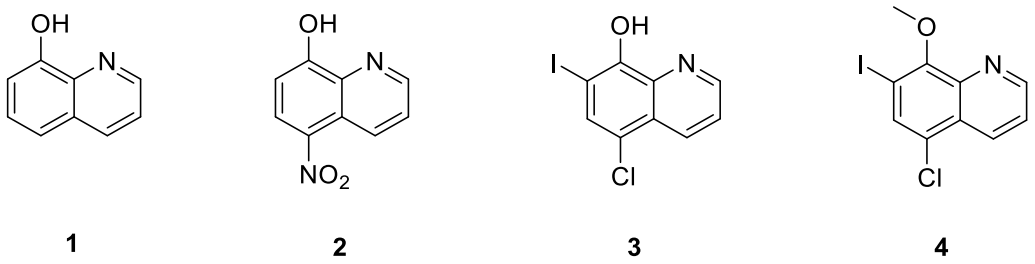


Fig. 1 - Chemical structure of quinolin-8-ol or 8-hydroxyquinoline **1**, nitroxoline or 5-nitro-8-hydroxyquinoline **2**, clioquinol or 5-chloro-7-iodo-8-hydroxyquinoline **3**, and 5-chloro-7-iodo-8-methoxyquinoline **4**.

The increasing number of severe cases of bacterial infections has become a worldwide concern. The abuse of commercial antibiotic contributes to the rapidly increase of multidrug resistant bacteria that cause infection difficult to treat with currently used antibiotic. These infections lead to treatment failure, increase of medical expenses and use of restrict and more potent antibiotics. Therefore, alternatives to solve the multi-resistant infections are being attempted and the introduction of novel compounds is an important topic of current research [6]. Quinolines derivatives have shown activity against several microorganism and the presence of substituent on the quinoline ring seems to modulate the toxicity. Substitution at position 5 and 7 of 8HQ seems to increase the activity against bacteria [7] [8]. Thus, the

antibacterial interest increases toward those quinolines, like clioquinol, a 5-chloro and 7-iodo substituted (3, Fig. 1).

The clioquinol **3** is a versatile molecule that initially was used as oral antiprotozoal medicine, nowadays besides the remaining use as antimicrobial in topical formulation, it has been studied as an alternative to treat Alzheimer's disease [2], and cancer [9]. However, it is also associated with severe cases of neurotoxicity in Japan, and this had being the reason of oral formulations banishment in the 70's. Some estimates shown that over 10,000 people had suffered from clioquinol neurotoxicity. The side effects reported by the patients in Japan were sensory, motor and visual disorders, abdominal pain, and diarrhea. Then, later toxicological studies demonstrated the relationship between clioquinol and the development of subacute myelo-optico-neuropathy (SMON) [10]. Otherwise, more recently, studies are relating the reducing of vitamin B_{12} in serum and brain caused by oral administration of clioquinol with the neurotoxicity cases [11]. Therefore, the clioquinol is a good option to synthesis of new derivatives seeking better activity and less side effects. The 8-hydroxyl group together with the nitrogen, from the quinoline ring, provide the chelate propriety of 8HQ, which participate of the antimicrobial activity. So modifications at those positions could affect the compound bioactivity, as in the case of methylated clioquinol, 5-chloro-7-iodo-8-methoxyquinoline (4, Fig. 1), that despite of having few published works, it seems to maintain the antimicrobial activity [12]. Furthermore, aryl halides undergoes palladium-catalyzed cross-coupling reactions making possible the preparation of several substituted compounds.

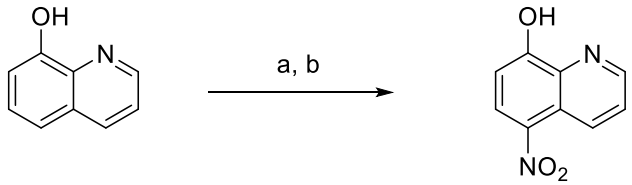
Those reactions have already been tested with quinolines to formation of C_{sp2}-C_{sp2} bound, as in the case presented by M. Brad Nolt et al. that cross-coupling halogenated quinolines with organoboronic acids in around 80% yield. The reaction is catalyzed by $Cl_2Pd(dppf)$ that selectively substitute the bromine over the chlorine for aryl and heteroaryl compounds [13]. Another palladium-catalyzed cross-coupling uses amine to substitute aryl halide, and since its development, it has become a powerful tool to C_{sp2}-N bond formation, especially when it comes to anilines due its nucleophilicity. Production of secondary [14] and tertiary [15] amines can be achieved using $Pd(OAc)_2$, $Pd_2(dba)_3$, or $PdCl_2(PhCN)_2$, and a phosphine ligand, such as Xantphos, BINAP, or DPPE. To the best of our knowledge, this chemistry was never used before with clioquinol derivatives. Thus, the purpose of this study was to provide the synthesis and characterization of the novel 5-chloro-7-amino-8-methoxyquinoline derivatives. *In vitro* evaluation of this novel series against several strains of bacteria such as, *E. coli*, *P. aeruginosa*, *S. epidermidis*, *K. pneumoniae*, *E. aerogenes*, *S. flexneri*, *E. faecalis* and *S. aureus* aiming the development of new drug candidates for the treatment of microorganism infections.

2. Results and discussion

2.1 Chemistry

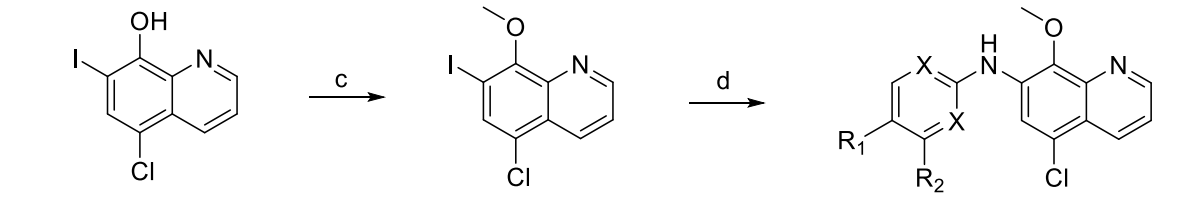
The nitroxoline **2** was prepared as previously described by Mazumder et al. (**Scheme 1**) [16]. In brief, commercially available 8-hydroxyquinoline **1** was dissolved in HCl (37%) and treated with $NaNO_2$ to give 5-nitroso-8HQ that was oxidized with HNO_3 to afford **2**. The mono-substitution at 5-position was confirmed by ^1H NMR spectrum with the presence of five ^1H signals at the aromatic region (around 7 to 9 ppm). The FT-IR spectrum present strong bands at 1283 and 1503 cm^{-1} from stretching of nitro group ($-NO_2$).

Synthesis of nitroxoline (5-nitro-8-hydroxyquinoline)



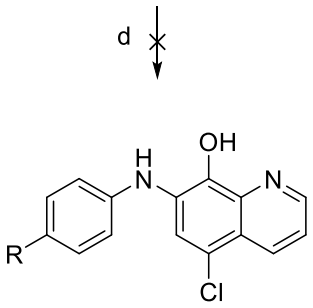
1 **2**

Synthesis of the **5a-5e** derivatives



3 **4**

5a: X= CH, R_1= OCH_3, R_2= H
5b: X= CH, R_1= Cl, R_2= H
5c: X= CH, R_1= Cl, R_2= Cl
5d: X= CH, R_1= CH_3, R_2= H
5e: X= N, R_1= H, R_2= H



R = $-CH_3$ or $-OCH_3$

Scheme 1 – Synthesis of the nitroxoline **2** and the designed derivatives **5a-5e**. Reagents and conditions: (a) HCl, $NaNO_2$, 0 °C, 40 min; (b) HNO_3, 17 °C, 75 min; (c) CH_3I, K_2CO_3, DMF, r.t., 16h; (d) $Pd(OAc)_2$, Xantphos, Cs_2CO_3, appropriate aniline, dioxane, argon atm., 100 °C, 16h.

In order to modify the 7-position with amines, an initial attempt to couple clioquinol **3** with amines was carried out using $Pd(OAc)_2$/Xantphos system (**Scheme 1**). However, we just recovered the starting material in this condition for the two anilines tested: 4-methoxyaniline and 4-methylaniline. The possible cause for this could be due to hydroxyl deprotonation under alkaline condition, and consequential electron enrichment of the aromatic ring. Based on the literature reports, we decided to introduce the methyl group at OH-8 before the cross-coupling reaction to avoid the deprotonation [17] [18]. Then, the clioquinol **3** was treated with potassium carbonate and methyl iodide in DMF to provide 5-chloro-7-iodo-8-methoxiquinoline **4** in 96% yield [19]. The reaction was followed by FT-IR with the disappearance of broad O-H absorption at 3069 cm^{-1} and appearance of alkane C-H stretching at 2932 cm^{-1} and bending at 1454 cm^{-1}. The ^{1}H and ^{13}C NMR spectra confirm the methylation by the methyl peak at 4.153 ppm (3H integral) and at 62.45 ppm respectively.

The next step, palladium-catalyzed cross-coupling reaction, promotes the formation of secondary amine by replacing aryl iodide with anilines. It uses palladium acetate ($Pd(OAc)_2$) as source of Pd^0, Xantphos as ligand, and Cs_2CO_3 as alkalizing agent. The catalysis mechanism, schematized by Hartwig in a Nature review published in 2008, starts with the oxidative addition of the palladium-xantphos complex to the aryl iodide, and it continues with the replacement of iodine by amine with releasing of hydrogen iodide (HI) that is neutralized by the Cs_2CO_3. Finally, the reductive elimination generate the aryl amine (**Fig. 2**) [20]. The integration values obtained from ^{1}H spectra confirm the mono-substitution of compound **4**, because the cross-coupling reactions can be also used with aryl chloride. However, as shown by Mphahlele and Lesenyeho the aryl chloride has a much lower reactivity than iodine once the C-Cl (96 kcal/mol) bound is strong than C-I (65 kcal/mol) [21]. The analysis of ^{13}C NMR confirms the selectivity toward the iodide by the missing carbon peak from C-I that resonate with lower frequency due to shield effect from iodine vast electrosphere with peak at 90.30 ppm for the starting material **4**. The single band from secondary amine can be observed by FT-IR spectra around 3400 cm^{-1}. Five different anilines was used, 4-methoxyaniline, 4-chloroaniline, 3,4-dichloroaniline, 4-methylaniline, and 2-aminopyrimidine to give derivatives **5a** to **5e** in 50-66% of overall yield (**Scheme 1**).

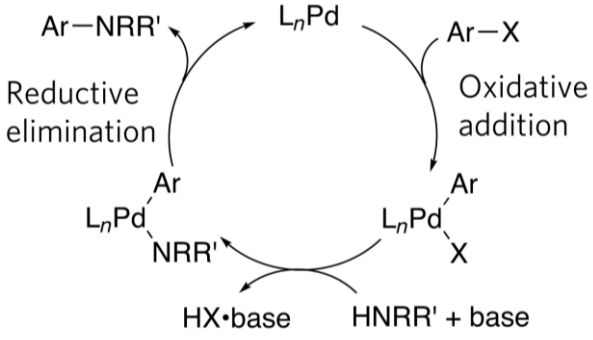
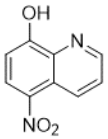
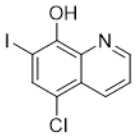
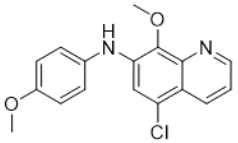
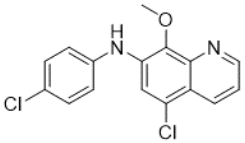
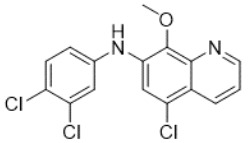
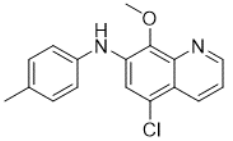
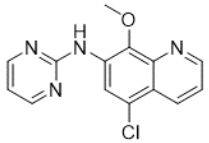


Fig. 2 – Catalysis mechanism of palladium-catalysed amination of aryl halides schematized by Hartwig in 2008 [20].

Table 1 - *In vitro* evaluation of MIC (minimal inhibitory concentration) values in μg/mL for **2**, **3**, and derivatives **5a-5e** against bacteria strains.

Compound	2 	3 	5a 	5b 	5c 	5d 	5e 
E. coli (35218)	4	>64	>64	>64	>64	>64	>64
P. aeruginosa (27853)	32	>64	>64	>64	>64	>64	>64
K. pneuminiae (70060)	32	>64	>64	>64	>64	>64	>64
E. aerogenes (13048)	16	>64	>64	>64	>64	>64	>64
S. flexneri (12022)	2	16	>64	>64	>64	>64	>64
S. epidermitis (35984)	8	4	64	>64	>64	>64	>64
E. faecalis (29212)	16	REP	>64	>64	>64	>64	>64
E. faecalis (51299)	16	4	32	8	>64	>64	>64
S. aureus (33591)	4	1	32	8	>64	>64	>64
S. aureus (29213)	2	2	16	4	>64	>64	>64
S. aureus (25923)	8	2	32	>64	>64	>64	>64

2.2 Biological activity

The minimal inhibitory concentration (MIC, μg/mL) against several strains of Gram-positive and Gram-negative bacteria was determined for the five novel synthetized quinoline-derivatives **5a**-**5e** (**Table 1**). The clioquinol **3** and the nitroxoline **2** were used as positive controls. The compounds **5a** and **5b** were active against two species of Gram-positive bacteria, *E. faecalis* (ATCC 51299) and *S. aureus* (ATCC 29213 and ATCC 33591 the Methicillin resistant strain). However, only the compound **5b** had values of MIC comparable with the controls for those strains, and with lower MIC than **2** for *E. faecalis*. Therefore, substituent at the 7-aniline ring modulate the activity.

Three substituent at *para*-position of 7-aniline ring were tested, and the activity against bacteria increase as follow: methyl (**5d**) << methoxy (**5a**) < chlorine (**5b**). The chlorine substituent increases the compound hydrophobicity while methoxy group lead to a slight decrease, what could explain the activity improvement of compound **5b** against **5a**. However, the methyl group also increases the hydrophobicity and the compound **5d** presented the lowest activity between the *para*-substituted suggesting that the difference of activity is not related to hydrophobicity. In spite of that, among the tested compounds the chorine is the only electron withdrawing group (EWG), which indicates that the introduction of a substituent that withdraw electron density from the 7-aniline ring could increase the activity of 5-chloro-7-amino-8-methoxyquinoline toward bacteria. The introduction of a second chlorine at *meta*-position (**5c**) was not tolerated for the activity. The replacement of the phenyl ring by 2-pyrimidinyl ring (**5e**) also did not prove to be advantageous.

3. Conclusions

The synthesis of the 5-chloro-7-amino-8-methoxyquinoline derivatives was achieved using clioquinol **3** as starting material. In order to undergo cross-coupling reaction **3** was successfully methylated by methyl iodide to give **4** in 96% yield. The palladium-catalyzed cross-coupling reaction, using $Pd(OAc)_2$ and Xantphos as catalysts, lead to iodine replacement selectively over chlorine for anilines affording a secondary amine. This reaction was tested for five different anilines (4-methoxyaniline, 4-chloroaniline, 3,4-dichloroaniline, 4-methylaniline, and 2-aminopyrimidine) with success, to give the derivatives **5a-5e** in 50-66% of overall yield.

Among all the prepared compounds, **5a** and **5b** presented activity against the strain ATCC 51299 of *E. faecalis* and two strains of *S. aureus*, ATCC 29213 and ATCC 33591 the Methicillin resistant strain. The compound **5b** is the most potent derivative with MIC value between 4-8 μg/mL for those strains. The compound **5a** bears an electron donating group (-OCH_3) at *para*-position of the 7-aniline while **5b** an electron withdrawing group (-Cl) leading to the observation that EWG at that position increases the activity toward *E. faecalis* and *S. aureus*. Thus, the compound **5b** has the potential to be studied and developed as an antibacterial agent.

4. Experimental section

4.1. Materials and instruments

Reactants were obtained from commercial suppliers. Column chromatography was performed on silica gel Fluka (Sigma-Aldrich) 0.035-0.070 mm. Proton and carbon NMR was carried out on Bruker 400 or Varian 400 nuclear magnetic resonance spectrometer. The proton and carbon shifts (δ) are given with respect to TMS. FT-IR was performed on PerkinElmer Spectrum BX. Melting points were determined on Buchi B-545 apparatus.

4.1.1. Synthesis of 5-nitro-8-hydroxyquinoline (2)

It was obtained as described previously [16]. Bright yellow crystal. 54% overall yield: mp 177 °C, lit: mp 180ºC. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.20 (dd, 1H, J = 1.4, 9.0 Hz), 9.02 (dd, 1H, J = 1.4, 4.2 Hz), 8.54 (d, 1H, J = 9.0 Hz), 7.92 (dd, 1H, J = 4.2, 9.0 Hz), 7.21 (d, 1H, J = 9.0 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 159.4, 148.3, 135.3, 135.1, 134.8, 129.6, 125.4, 122.8, 110.8. IR ν (cm^{-1}): 3111, 3064, 2747, 1600, 1551, 1503, 1444, 1283, 1193, 1150, 867, 815, 792, 739, 721, 633.

4.1.2. Synthesis of 5-chloro-7-iodo-8-methoxyquinoline (4)

To a stirred solution of clioquinol (2.0 g, 6.5 mmol) in DMF (15 mL) was added K_2CO_3 (1.81 g, 13 mmol) at room temperature. After 30 minutes CH_3I (1.23 mL, 13 mmol) was added dropwise. After 16 hours, the addition of ethyl acetate (300 mL) stopped the reaction. The organic layer was washed with 0.5 M NaOH (200 mL), water (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 5-chloro-7-iodo-8-methoxyquinoline **4** as a purple solid that was used in the next step without further purification (96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.93 (dd, 1H, *J* = 1.8, 4.2 Hz), 8.47 (dd, 1H, *J* = 1.8, 8.6 Hz), 7.91 (s, 1H), 7.51 (dd, 1H, *J* = 4.2, 8.6 Hz), 4.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 156.2, 150.8, 142.5, 135.2, 133.7, 127.7, 126.9, 122.5, 90.3, 62.5. IR ν (cm^{-1}): 3408, 3010, 2974, 2932, 2836, 1659, 1597, 1570, 1481, 1454, 1370, 1365, 1350, 1216, 1084, 981, 932, 844, 790, 708, 665.

4.1.3. General procedure for the synthesis of 5a-5e

To a stirred solution of 5-chloro-7-iodo-8-methoxyquinoline (300 mg, 0.94 mmol) in dioxane (3 mL) was added, in order, $Pd(OAc)_2$ (5 mol%), Xantphos (5 mol%), appropriate aniline (1.41 mmol), and Cs_2CO_3 (613 mg, 1.88 mmol) under argon atmospheric in a sealed tube. After 16 h at 100 ºC, the solution was allowed to cool at room temperature, concentrated under reduced pressure, and purified by silica gel column chromatography (hexane/EtOAc).

4.1.3.1. 5-chloro-7-(4-methoxyphenylamino)-8-methoxyquinoline (5a)

Eluent hexane:EtOAc (8:2). Dark yellow wax. 50% overall yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.83 (dd, 1H, J = 1.4, 4.1 Hz), 8.32 (bd, 1H, J = 8.4 Hz), 7.40 (s, 1H), 7.20 (dd, 1H, J = 4.1, 8.4 Hz), 7.14 (d, 2H, J = 8.8 Hz), 6.90 (d, 2H, J = 8.8 Hz), 6.46 (s, 1H), 4.09 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 156.7, 150.8, 143.4, 138.8, 138.7, 133.7, 133.4, 127.0, 124.5, 120.8, 118.5, 116.1, 115.0, 61.5, 55.7. IR ν (cm^{-1}): 3408, 3010, 2924, 2827, 1607, 1517, 1495, 1457, 1457, 1433, 1398, 1338, 1292, 1242, 1192, 1151, 1099, 1038, 984, 924, 820, 774, 705, 667.

4.1.3.2. 5-chloro-7-(4-chlorophenylamino)-8-methoxyquinoline (5b)

Eluent hexane:EtOAc (8:2). Bright yellow solid. 50% overall yield: mp 151-157 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.79 (dd, 1H, J = 1.6, 4.0 Hz), 8.30 (dd, 1H, J = 1.6, 8.4 Hz), 7.50 (s, 1H), 7.22 (m, 3H), 7.04 (d, 2H, J = 8.8 Hz), 6.54 (s, 1H), 4.03 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 150.9, 143.4, 140.4, 139.8, 136.5, 133.5, 129.8, 128.2, 127.0, 121.8, 121.6, 119.3, 116.9, 61.9. IR ν (cm^{-1}): 3342, 3007, 2952, 1607, 1589, 1487, 1453, 1386, 1347, 1328, 1200, 1087, 985, 932, 836, 821, 804, 783, 642.

4.1.3.3. 5-chloro-7-(3,4-dichlorophenylamino)-8-methoxyquinoline (5c)

Eluent hexane:EtOAc (9:1). White solid. 55% overall yield: mp 167-169 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.88 (dd, 1H, J = 1.7, 4.3 Hz), 8.40 (dd, 1H, J = 1.7, 8.6 Hz), 7.68 (s, 1H), 7.37 (d, 1H, J = 8.8 Hz), 7.32 (dd, 1H, J = 4.3, 8.6 Hz), 7.27 (d, 1H, J = 2.6 Hz), 7.02 (dd, 1H, J = 2.6, 8.8 Hz), 6.59 (s, 1H), 4.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 151.0, 143.5, 141.2, 135.5, 133.6, 133.5, 131.3, 127.2, 126.0, 122.3, 121.2, 119.7, 119.0, 62.2. IR ν (cm^{-1}): 3396, 2947, 1596, 1492, 1477, 1460, 1388, 1337, 1304, 1104, 980, 930, 800, 779, 702, 647.

4.1.3.4. 5-chloro-7-(4-methylphenylamino)-8-methoxyquinoline (5d)

Eluent hexane:EtOAc (9:1). Yellow solid. 57% overall yield: mp 149-153 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.87 (dd, 1H, J = 1.7, 4.0 Hz), 8.37 (dd, 1H, J = 1.7, 8.5 Hz), 7.60 (s, 1H), 7.26 (dd, 1H, J = 4.0, 8.5 Hz), 7.18 (d, 2H, J = 8.4 Hz), 7.12 (d, 2H, J = 8.4 Hz), 6.57 (s, 1H), 4.12 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 150.8, 143.5, 139.5, 138.3, 137.7, 133.4, 133.4, 130.4, 127.0, 121.5, 121.2, 118.8, 116.7, 61.7, 21.02. IR ν (cm^{-1}): 3339, 3050, 3020, 2919, 1600, 1515, 1491, 1455, 1388, 1329, 1304, 1262, 1102, 988, 932, 876, 853, 782, 673.

4.1.3.5. 5-chloro-7-(pyrimidin-2-amino)-8-methoxyquinoline (5e)

Eluent hexane:EtOAc (9:1). Yellow solid. 66% overall yield: mp 159-161 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.13 (s, 1H), 8.89 (dd, 1H, J = 1.7, 4.0 Hz), 8.49 (d, 2H, J = 4.8), 8.45 (dd, 1H, J = 1.7, 8.5 Hz), 8.11 (s, 1H), 7.35 (dd, 1H, J = 4.0, 8.5 Hz), 6.80 (t, 1H, J = 4.8 Hz), 4.17 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.7, 158.2, 150.6, 142.6, 140.9, 133.3, 133.0, 126.4, 122.6, 120.0, 119.9, 113.7, 62.4. IR ν (cm^{-1}): 3400, 3010, 2943, 1612, 1585, 1564, 1519, 1495, 1444, 1422, 1379, 1253, 1092, 979, 927, 777.

4.2 Biological assay

The compounds were tested against several stains of ATCC bacteria, such as *Escherichia coli* (35218), *Pseudomonas aeruginosa* (27853), *Staphylococcus epidermidis* (35984), *Klebsiella pneumoniae* (70060), *Enterobacter aerogenes* (13048), *Shigella flexneri* (12022), *Enterococcus faecalis* (51299 and 29212), *Staphylococcus aureus* (33591, 29213, and 25923) by broth microdilution assay fallowing the document M100-S26 (CLSI, 2016) giving the MIC values in μg/mL [22].

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SUPPLEMENTARY INFORMATION

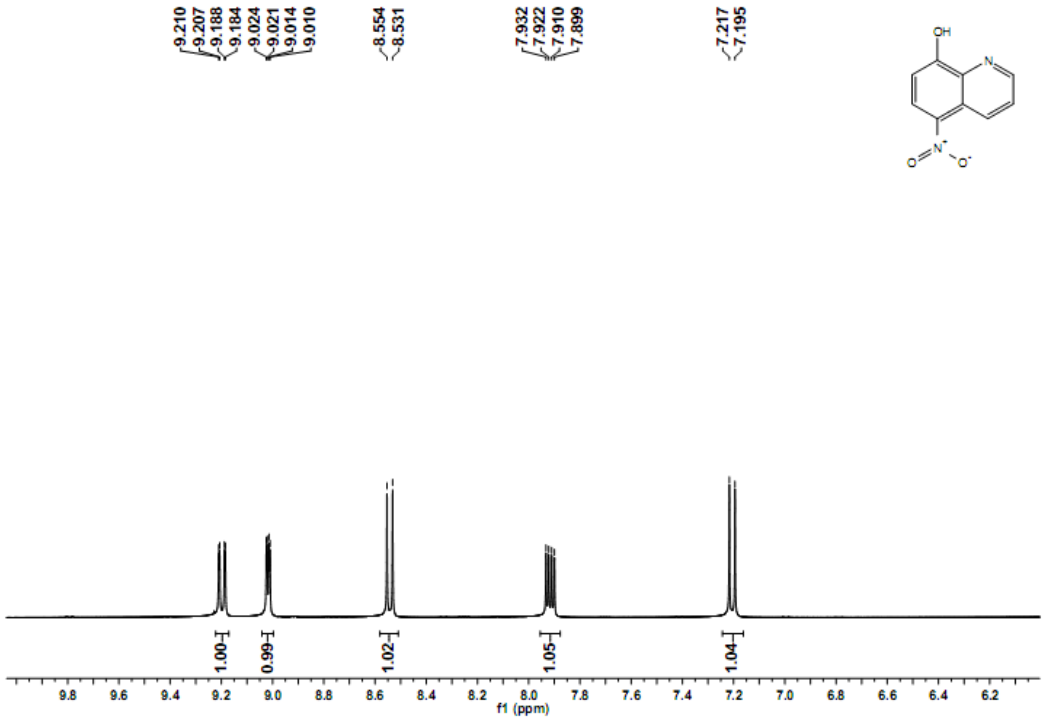

Figure 1 – NMR ^{1}H spectrum in DMSO-d_6 of **2**.

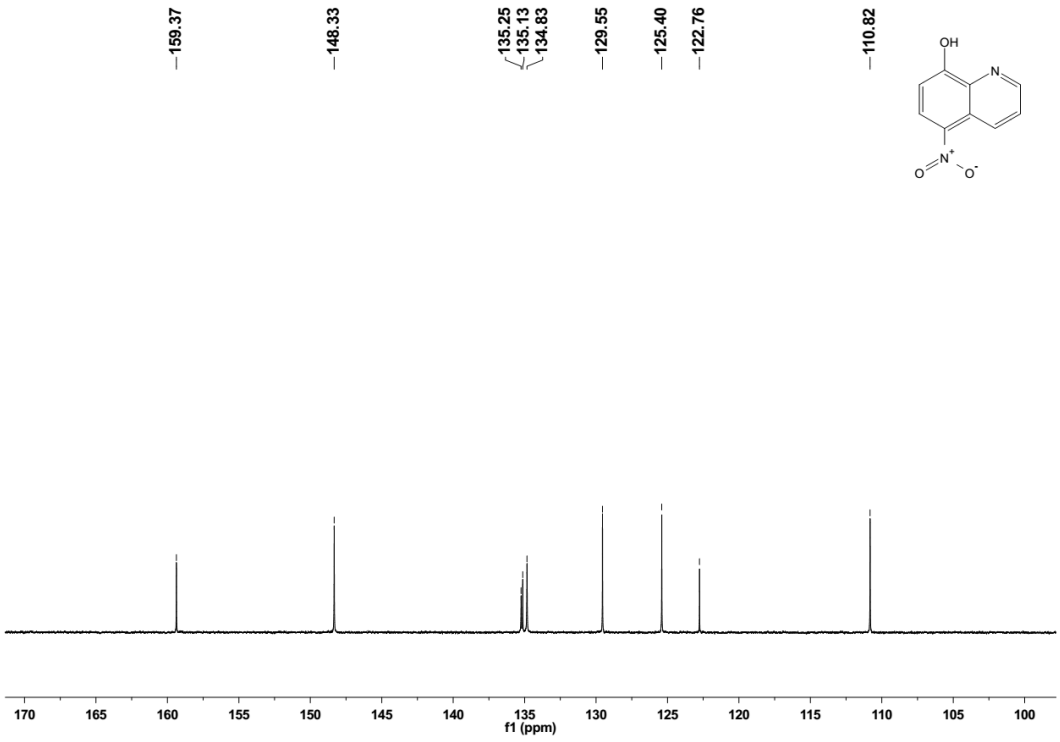

Figure 2 – NMR ^{13}C spectrum in DMSO-d_6 of **2**.

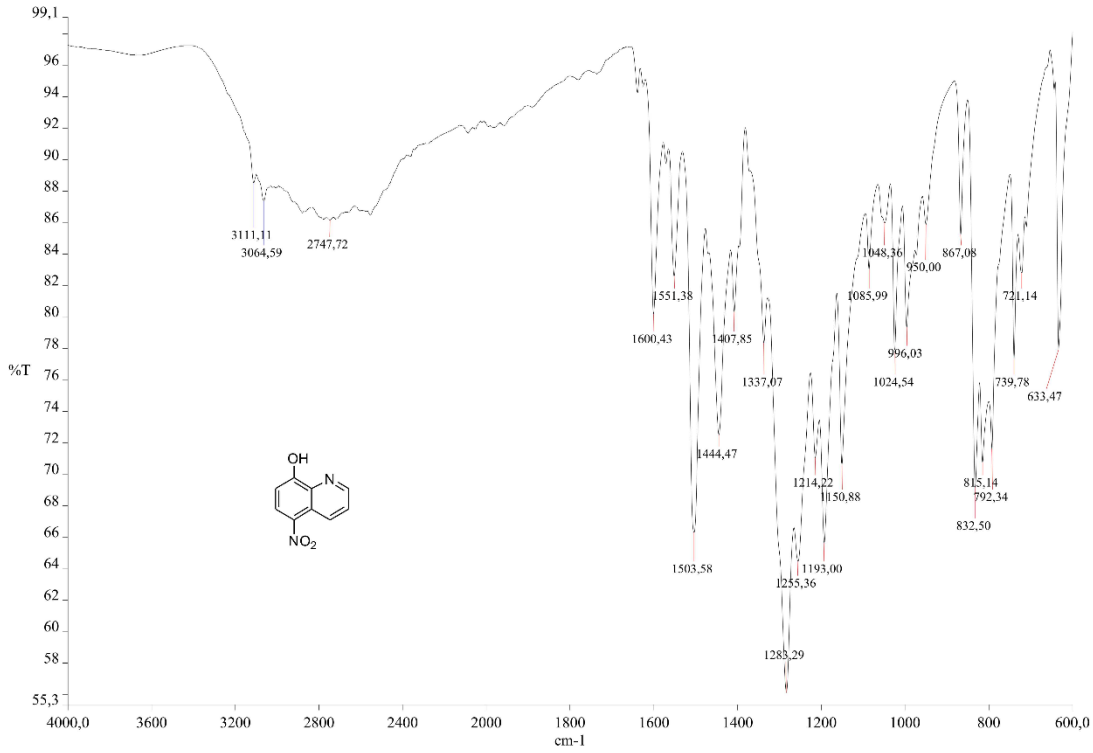

Figure 3 – FT-IR spectrum of **2**.

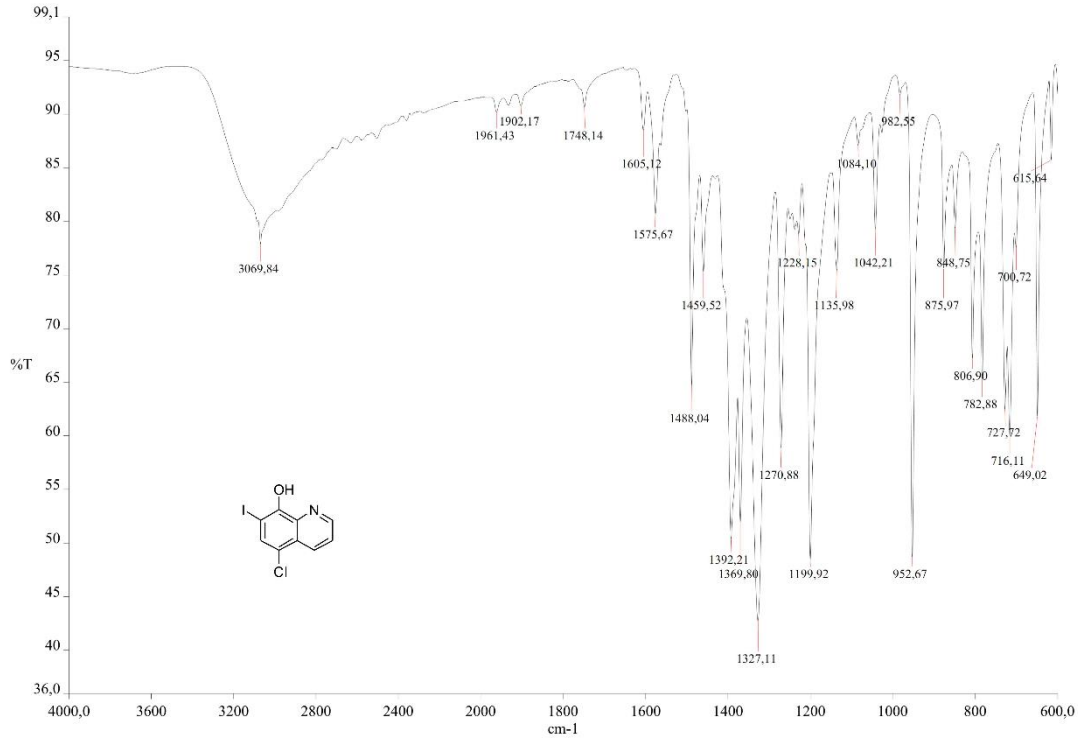

Figure 4 - FT-IR spectrum of **3**.

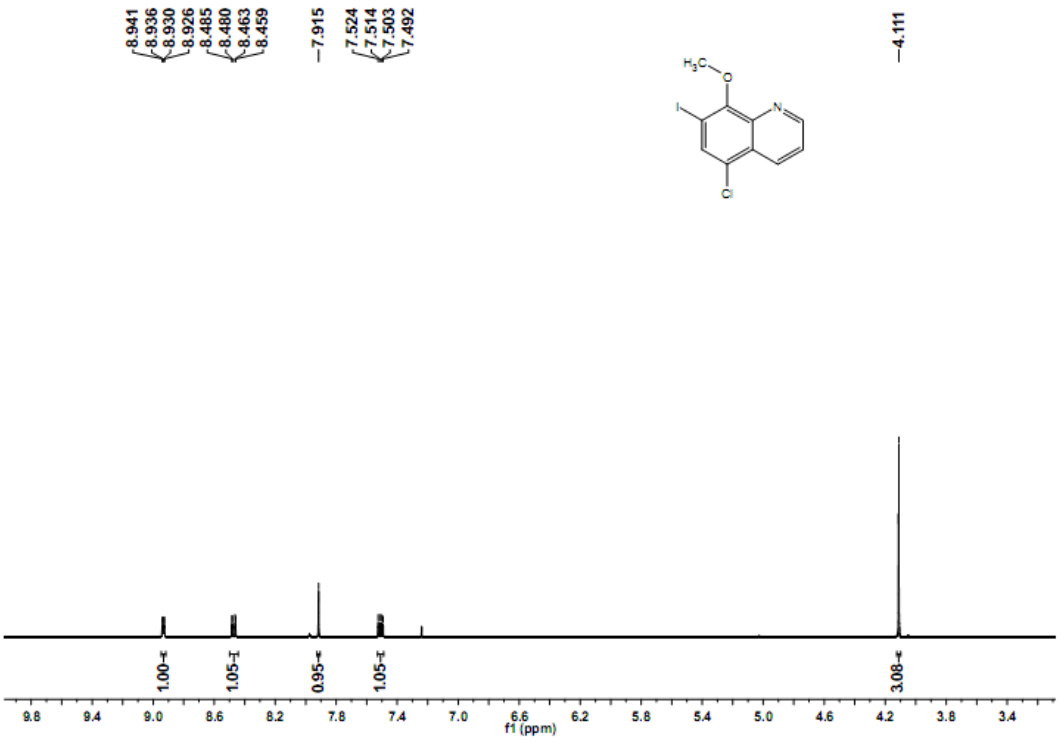

Figure 5 - NMR ^{1}H spectrum in $CDCl_3$ of **4**.

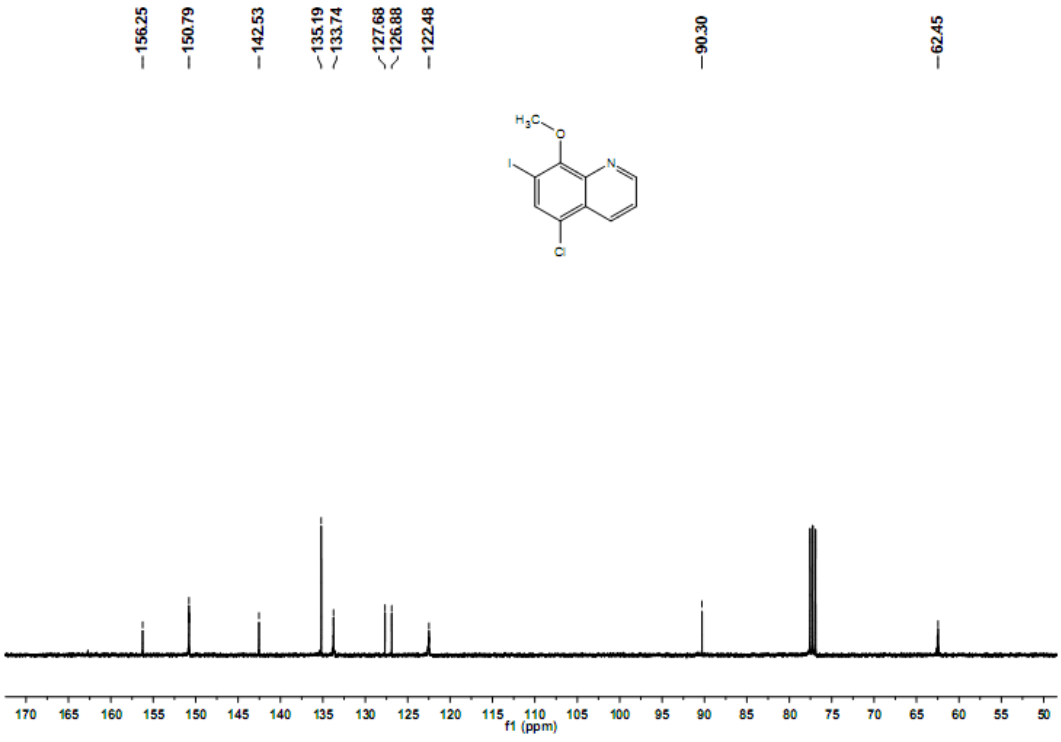

Figure 6 - NMR ^{13}C spectrum in $CDCl_3$ of **4**.

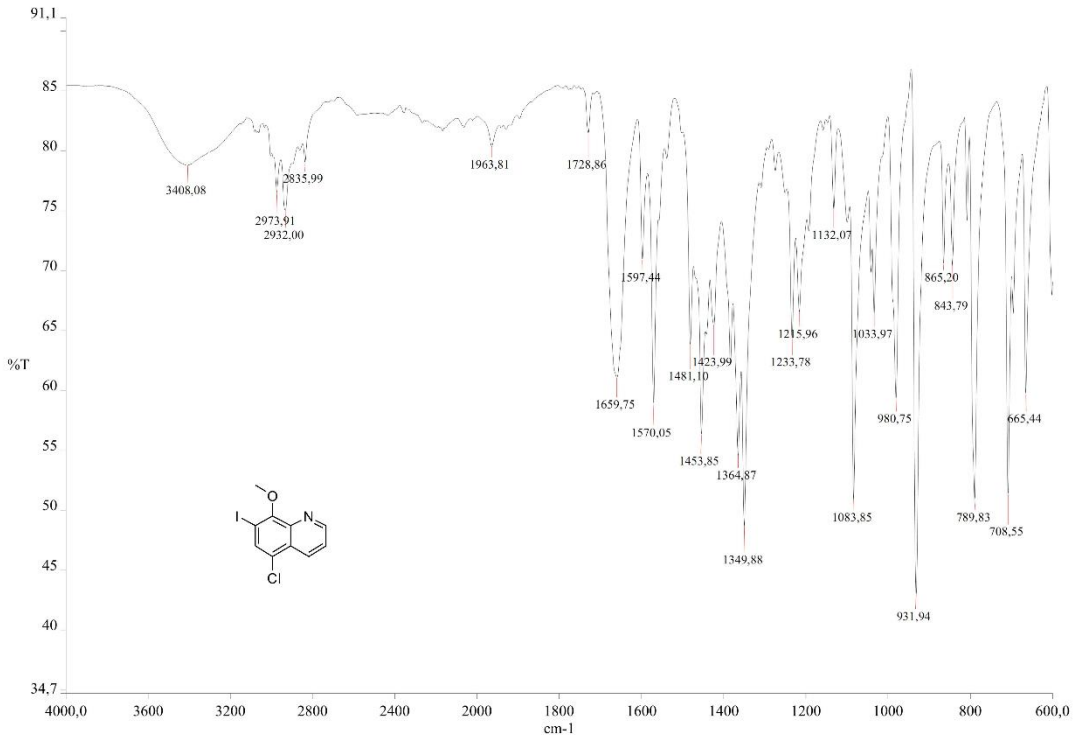

Figure 7 - FT-IR spectrum of **4**.

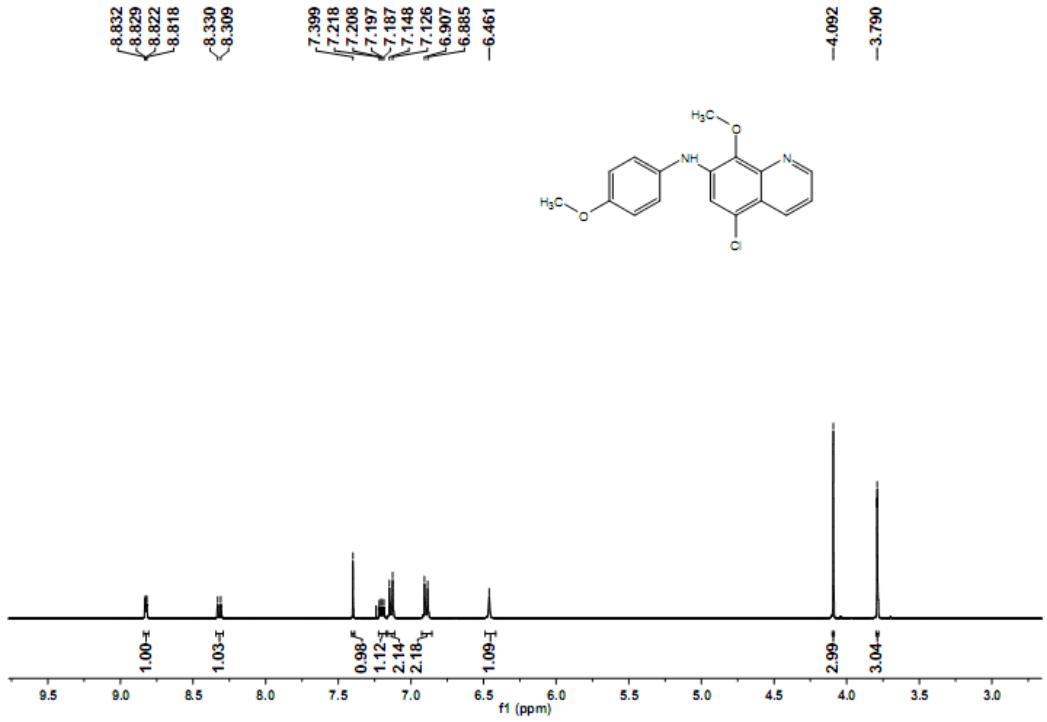

Figure 8 - NMR ^{1}H spectrum in $CDCl_3$ of **5a**.

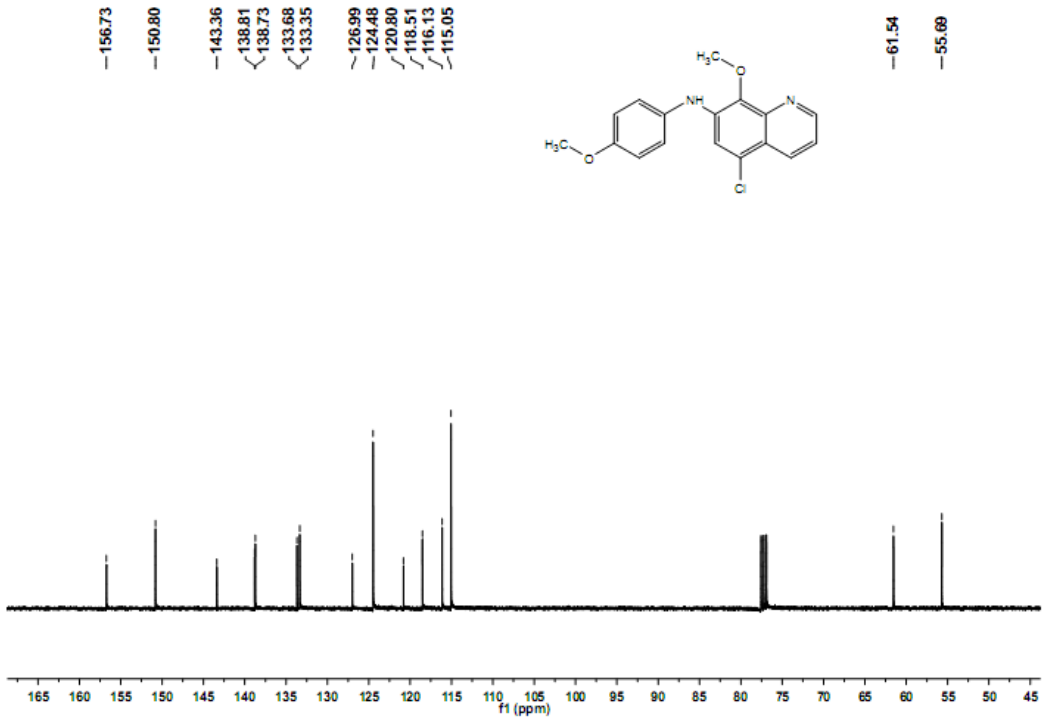

Figure 9 - NMR ^{13}C spectrum in $CDCl_3$ of **5a**.

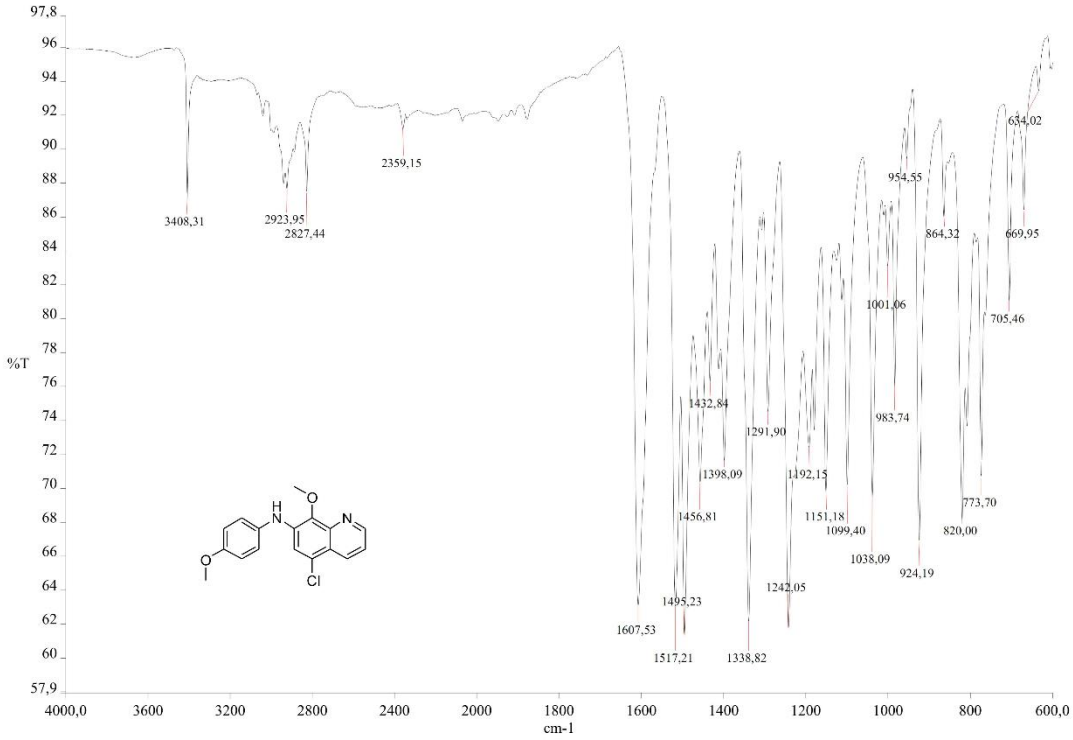

Figure 10 - FT-IR spectrum of **5a**.

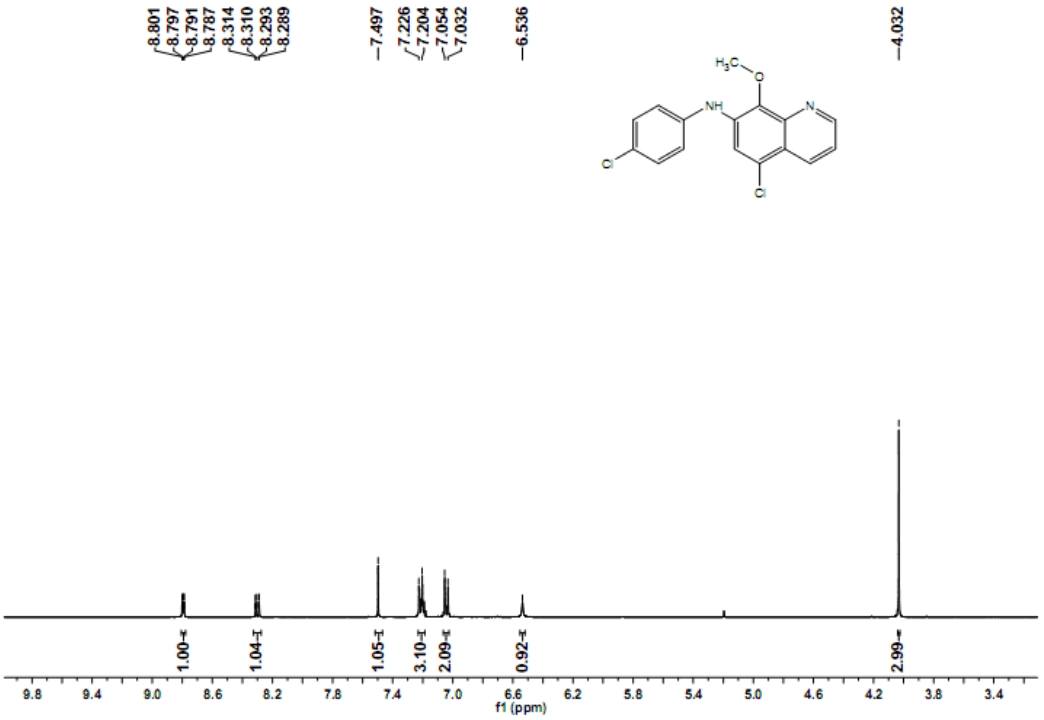

Figure 11 - NMR ^{1}H spectrum in $CDCl_3$ of **5b**.

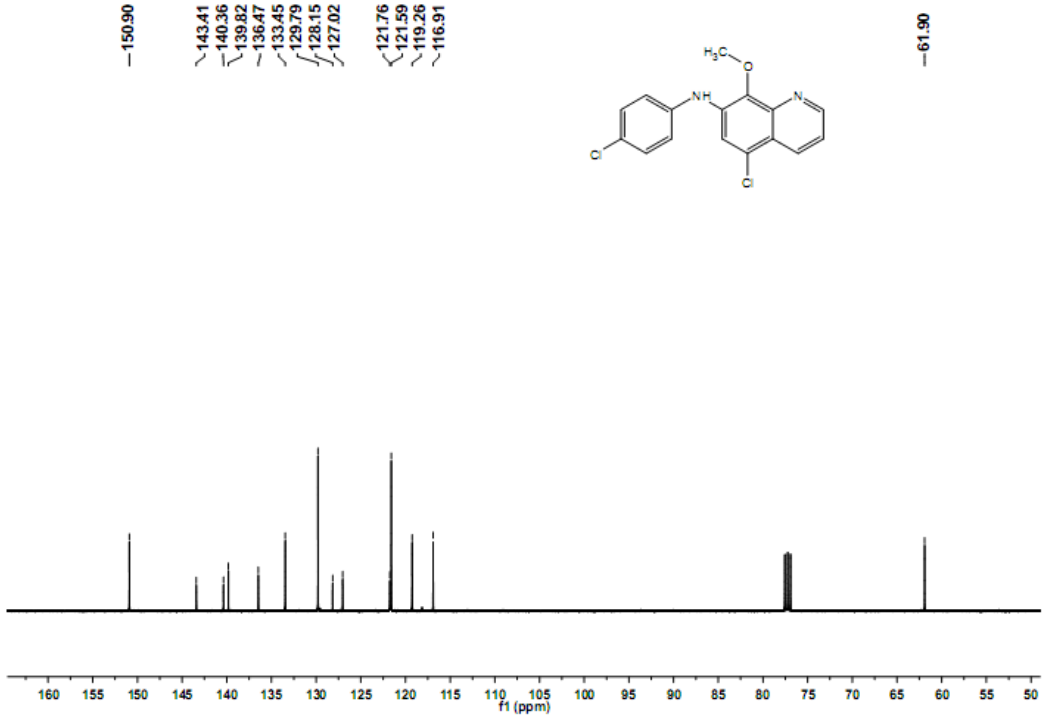

Figure 12 - NMR ^{13}C spectrum in $CDCl_3$ of **5b**.

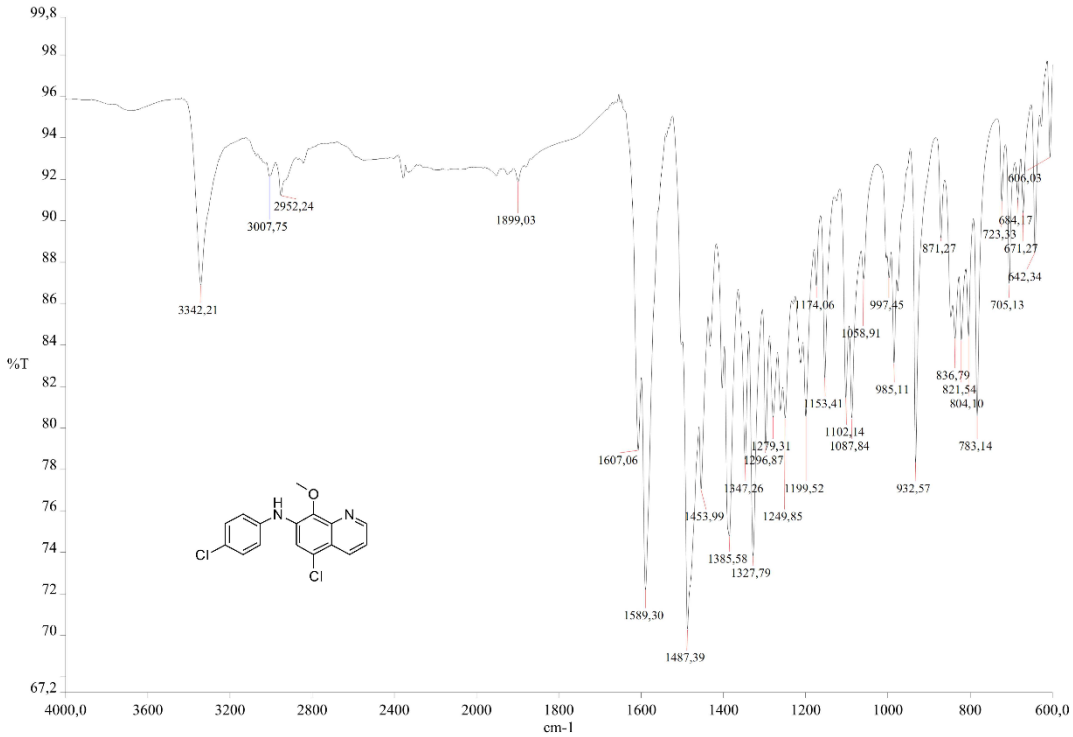

Figure 13 - FT-IR spectrum of **5b**.

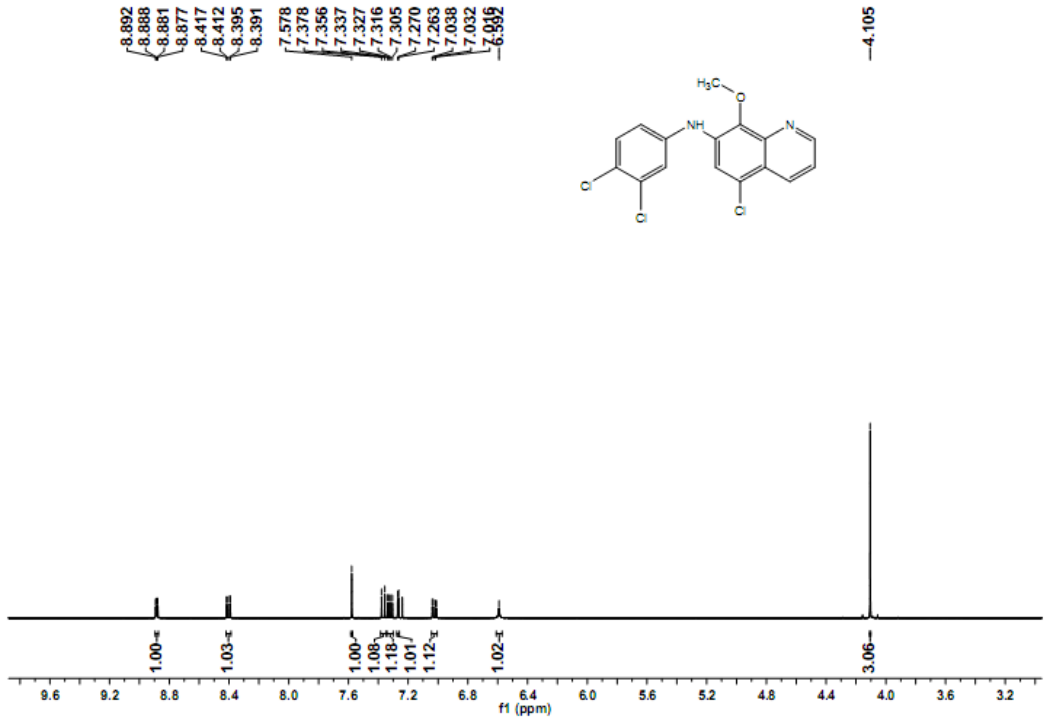

Figure 14 - NMR ^{1}H spectrum in $CDCl_3$ of **5c**.

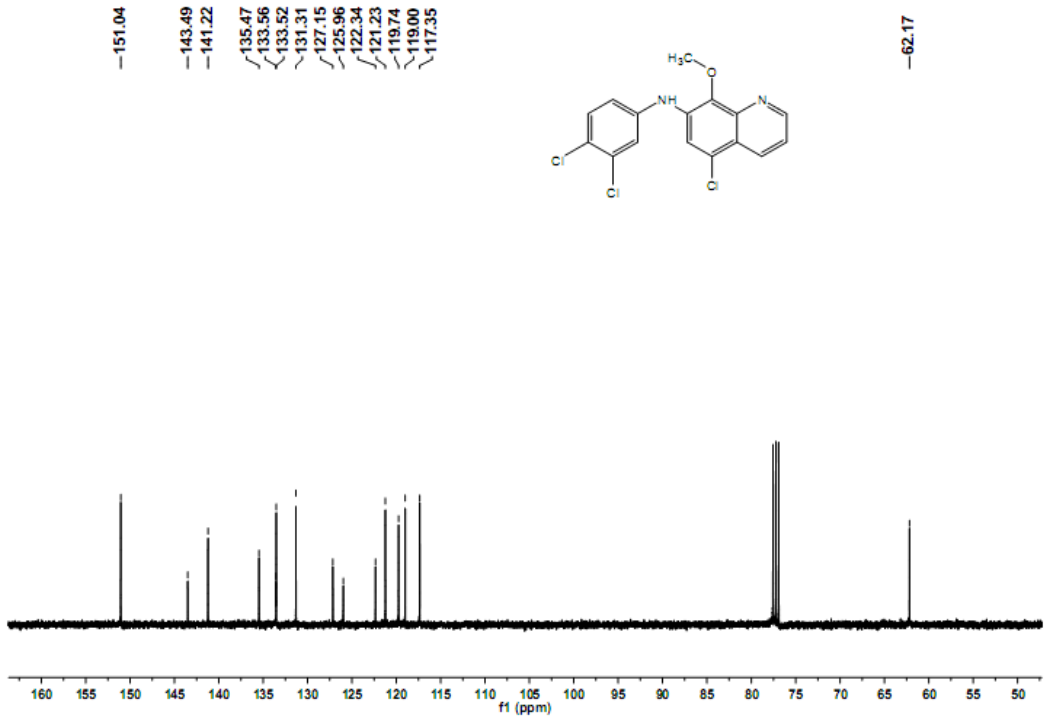

Figure 15 - NMR ^{13}C spectrum in $CDCl_3$ of **5c**.

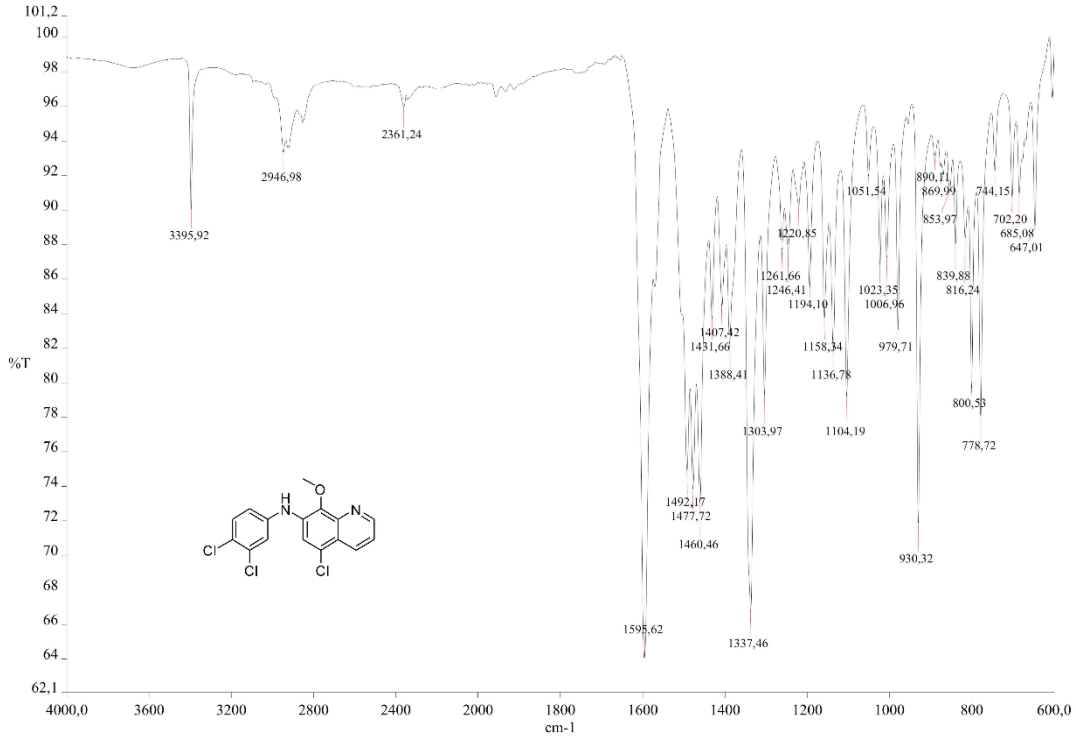

Figure 16 - FT-IR spectrum of **5c**.

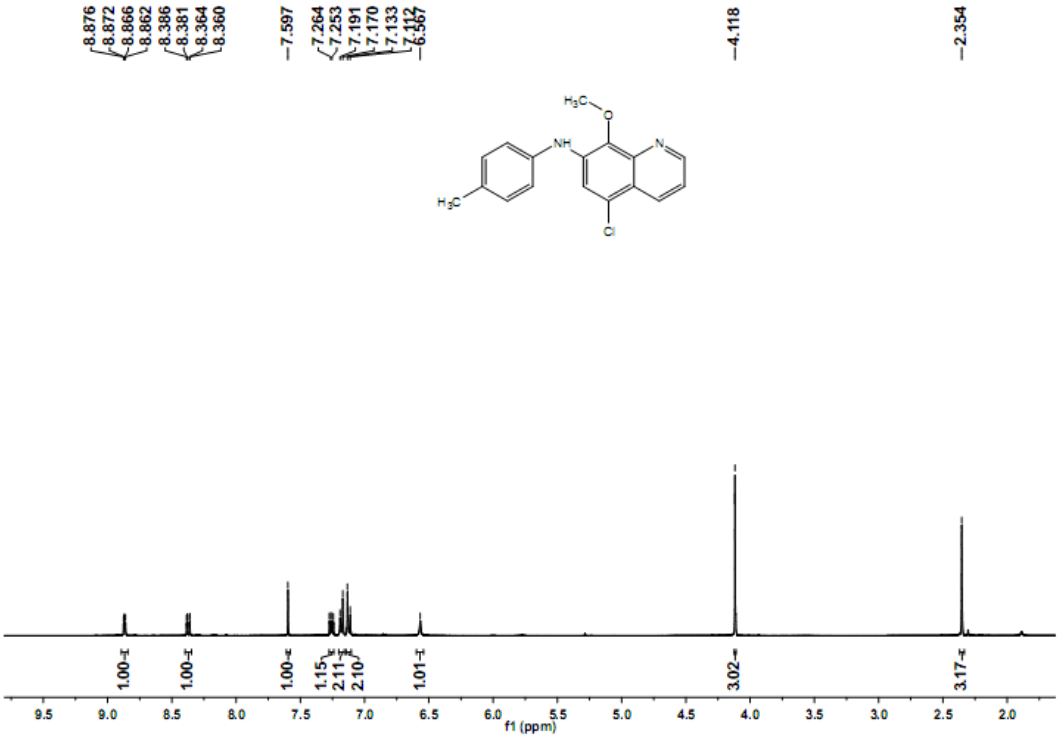

Figure 17 - NMR ^{1}H spectrum in $CDCl_3$ of **5d**.

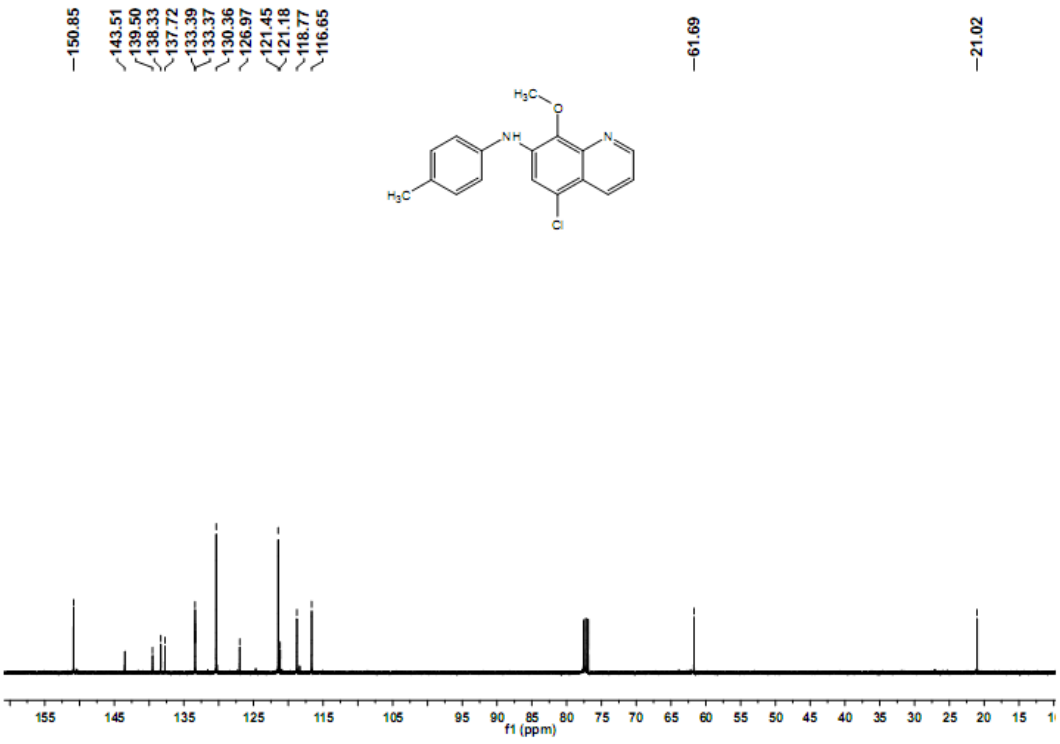

Figure 18 - NMR ^{13}C spectrum in $CDCl_3$ of **5d**.

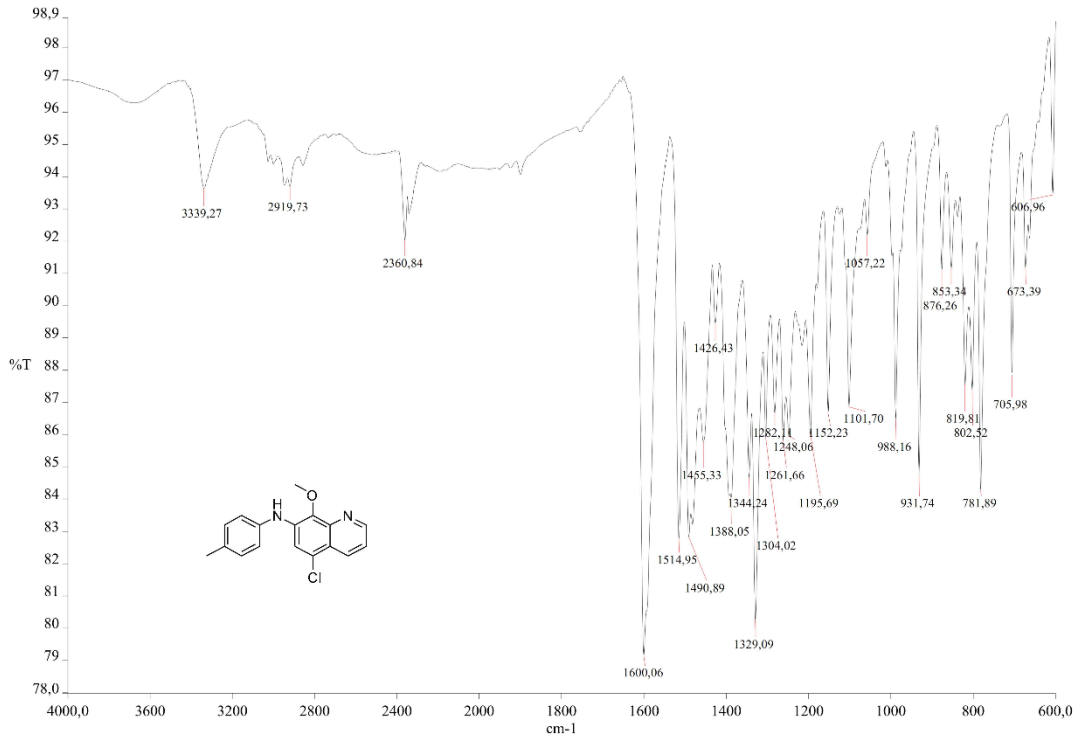

Figure 19 - FT-IR spectrum of **5d**.

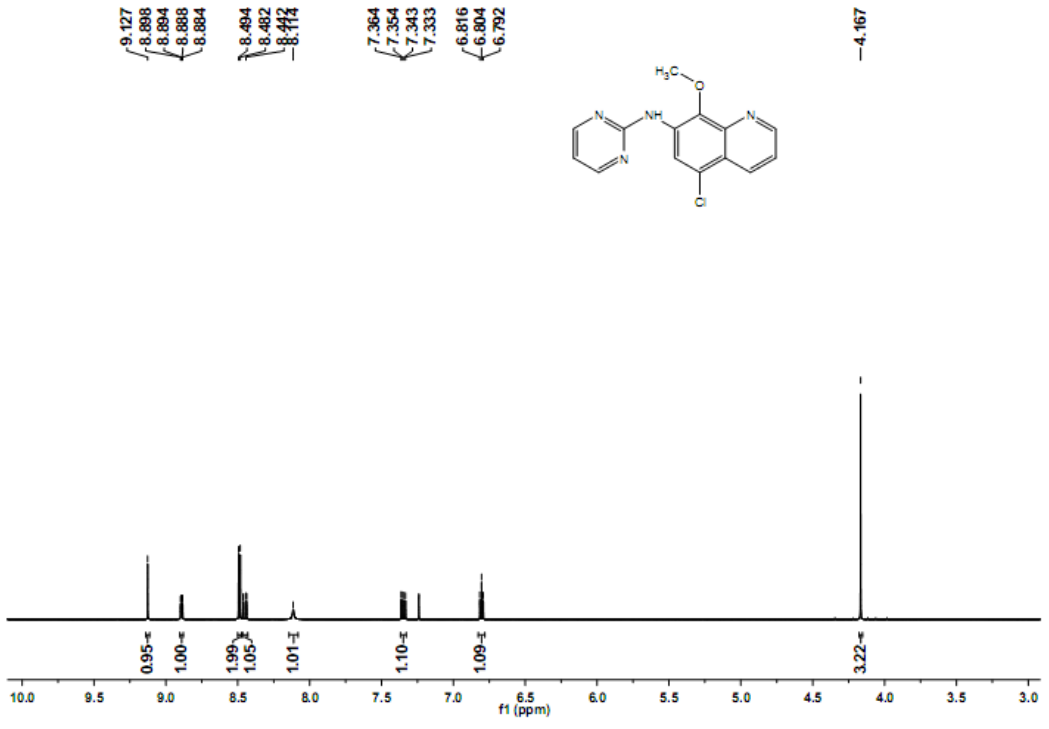

Figure 20 - NMR ^{1}C spectrum in $CDCl_3$ of **5e**.

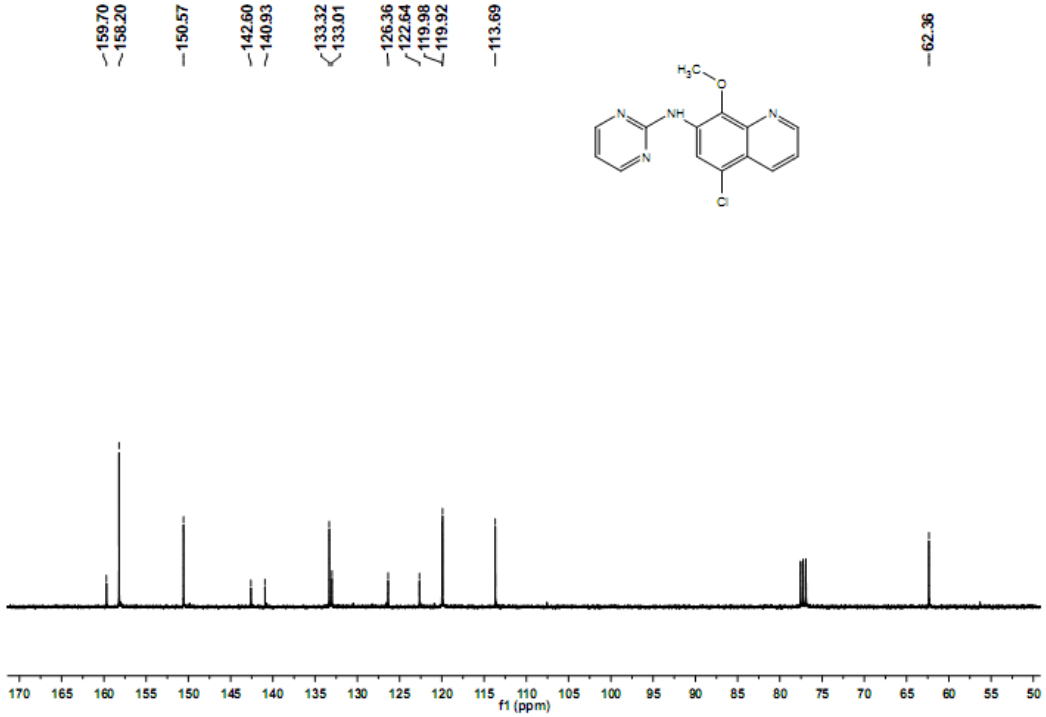

Figure 21 - NMR ^{13}C spectrum in $CDCl_3$ of **5e**.

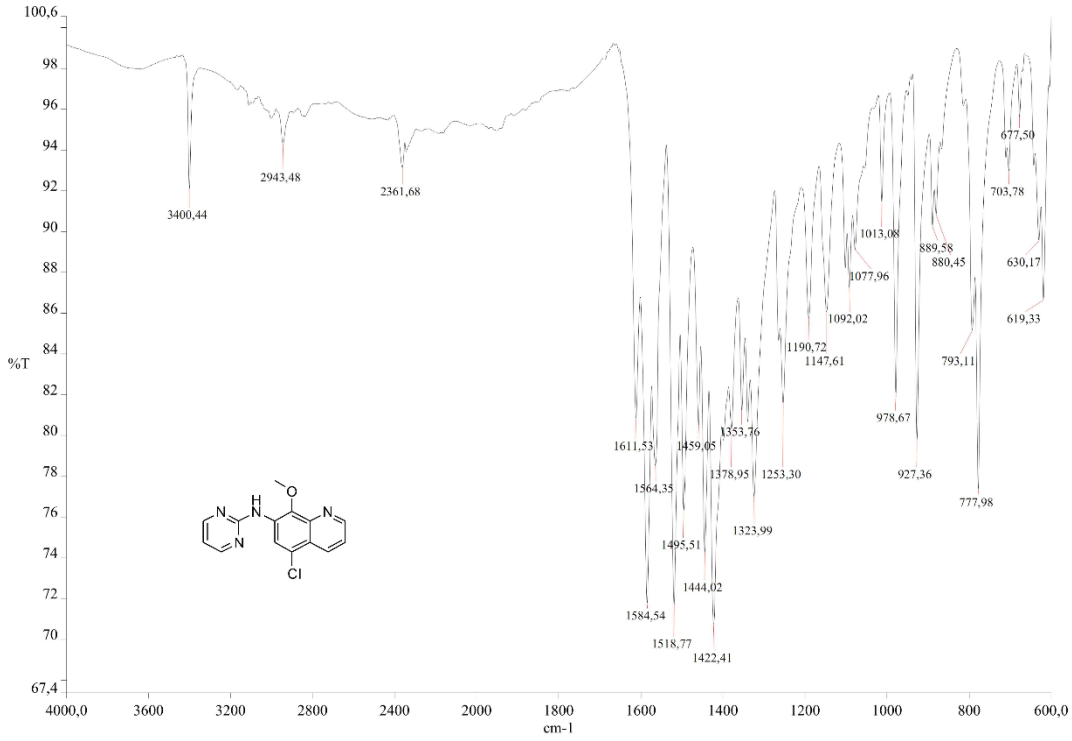

Figure 22 - FT-IR spectrum of **5e**.

ANEXO 1

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

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AUTHOR INFORMATION PACK

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