Ministry of Higher Education and Scientific Research

University Of Basrah - College of Pharmacy

Synthesis, characterization, of anti-breast cancer MCF-7 from new quinoline derivatives

A project

Submitted in partial fulfilment of the Requirements for the award of the degree of Bachelor of Pharmacy

BY

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الآية القرآنية:

"يَرْفَعِ اللَّـهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ"

صدق الله العلي العظيم

سورة المجادلةالآيه (١١)

Abstract

Due to the importance of quinolines and sulfa drug compounds as pharmacological and biological activities against many diseases, one of the objectives of this study was to prepare and study sulfa quinoline derivatives.

The first part included the preparation of a new series of sulfa-quinoline compounds by the condensation of quinoline derivatives and sulfa compounds. In the beginning quinoline derivative has been prepared that contains a bromide group, which was condensed with sulfa compound, the reaction was monitored by (TLC) technical and all synthesized compound were characterized by spectroscopic techniques such as FT-IR and ^{1}H-NMR

1. Introduction

Breast cancer is the most common malignancy among women worldwide. The incidence of breast cancer is increasing year by year by 3.1%, from about 641,000 cases in 1980 to more than 1.6 million in 2010. Interestingly, the global burden of this type of cancer is increasing in different countries regardless of the income of the population. This is primarily due to population growth and population aging. Therefore, the conclusions are not optimistic. Also, the exact number of patients with this disease is not known, because the official data mainly include prognosis and mortality but do not include relapses [1–3]. Breast cancer detected at an early stage of the disease, without metastases or cancer that has spread only to the axillary lymph nodes, is considered curable in about 70-80% of patients. In an advanced stage, with metastases to distant organs, it is considered incurable with currently available treatments. Advanced breast cancer is a disease for which current treatments aim to prolong life with acceptable side effects of treatment in order to maintain or improve quality of life. This is related to the genetic diversity of cells and the heterogeneity of their structures at the molecular level. This, in turn, is associated with activation of the human epidermal growth factor receptor HER2, encoded by ERBB2; hyperactivity of hormone receptors, including estrogen and progesterone receptors; and the BRCA mutation. The genetic variance of the tumor tissue determines, in this case, the optimal treatment selection, which in turn is related to the type of cell lines and the molecular subtype of the tumor. The treatment itself is multidisciplinary. They

include, among others, surgery along with radiotherapy against specific local tumors and systemic therapies. These include hormonal therapies for hormone receptor-positive tumors, and chemotherapy, or anti-HER2 therapy - for tumors with positive HER2 receptors present on the cell surface. In addition, bone-enhancing drugs and poly (ADP-ribose) polymerase inhibitors are used supportively in BRCA mutation carriers. In recent years, immunotherapy has also been introduced into the arsenal of anticancer agents [4–9]. The therapeutic concepts driving modern oncology aim to personalize treatment and therapy based on tumor biology and early response to treatment. Besides innovation, equal access to the latest therapies remains a challenge. The most abundant cell type in a tumor are tumor-associated fibroblasts. In addition, leukocytes, including lymphocytes, macrophages and stromal cells of myeloid origin, were found. Most of these cells are involved in the immune response [10]. Breast cancer tumor histology varies across molecular subtypes, which in turn are closely related to immunogenicity. The highest score is observed in tumors with TNBC and HER2(+) and lowest in luminal subtypes A and B [11,12]. In addition, response to neoadjuvant therapy and prognosis in breast cancer positively affects the number of tumor-infiltrating lymphocytes, which reflects the intensity of the immune response in the tumor [13, 14].

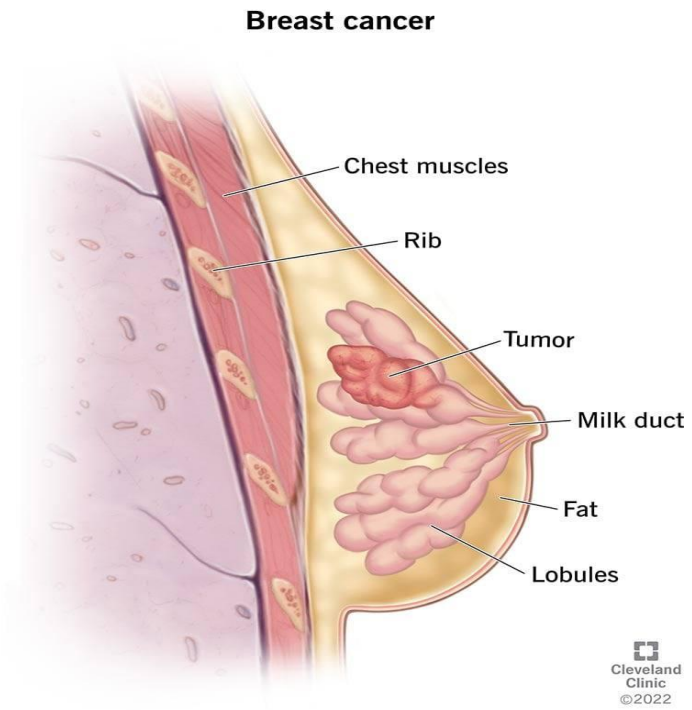


Figure (1)

2. Quinoline and sulfa drugs

2.1 Sulfonamide: Sulfonamide derivatives have been extensively studied due to their variety of biological activities such as antimicrobial, anticancer, and antiviral properties. The benzenesulfonamide derivative 1 (Figure 1) induced nuclear condensation, cell shrinkage, and nuclear fragmentation to induce apoptosis against the COLO-205Citation4 cell line. The sulfonamide derivative 2 could reduce the cell viability of the HT-29 cell line in a concentration-dependent manner with an IC value of 5.45 μM. Benzenesulfonamide 3 showed potent anti-proliferative activity against MCF-7 cells with an IC_{50} value of 3.96 μM as an apoptosis inducer. Quinoline sulfonamide 4 showed anticancer activity in vitro against T47D cells with an IC_{50} value of 0.27 μM. In addition, dithiocarbamates such as organic sulfur bonds have been successfully applied as anti-cancer agents. Disulfiram 5 can modulate ROS accumulation and overcome resistance to cisplatin synergistically against breast cancer cells. Dithiocarbamate 6 induced apoptosis via the mitogen-activated protein kinase signaling pathway. Dithiocarbamate 7 showed potent anticancer effects against SK-OV-3 and SK-BR-3 cells overexpressing HER2. Dithiocarbamate 8 has been developed as a candidate antitumor drug for the treatment of estrogen receptor positive breast cancer.

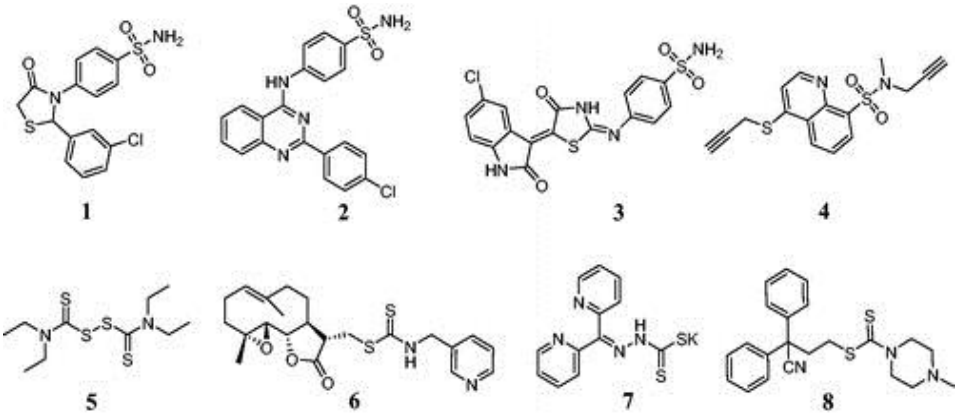


Figure 2. Antitumor sulfonamide and dithiocarbamate derivatives.

In addition, trimethoxyphenyl derivatives have enjoyed great success due to their strong chemical stability and pharmacokinetics for the treatment of cancer. These above interesting findings led to the implementation of a molecular hybridization strategy of the bioactive sulfonamide fragments, dithiocarbamate and dimethoxyphenyl to generate a novel scaffold with the aim of studying the effect of this modification on their antitumor activity. As shown in Figure 2, a molecular

hybridization strategy based on bioactive compounds 4 and 8 produced a new scaffold consisting of three parts: (1) a sulfonamide portion as the central backbone; (2) a dicarbonate bicarbonate fraction and (3) a trimethoxyphenyl fraction to increase antiproliferative activity. In this work, we synthesized and evaluated the in vitro and in vivo anticancer mechanisms of dicarbamate sulfonamide hybrids.

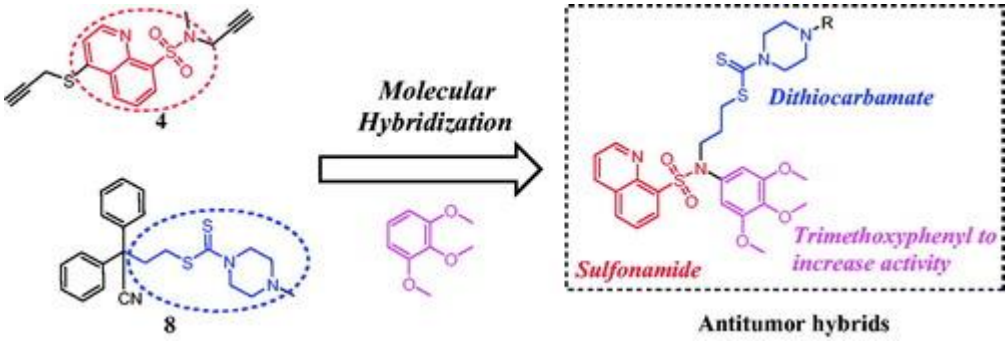


Figure 3. Illustration of the design strategy for sulfonamide hybrids.

2.2 Quinolones:

The quinoline-containing antimalarial drugs, chloroquine, quinine and mefloquine, are a vital part of our chemotherapeutic armoury against malaria. These drugs are thought to act by interfering with the digestion of haemoglobin in the blood stages of the malaria life cycle. Chloroquine is a dibasic drug which diffuses down the pH gradient to accumulate about a 1000-fold in the acidic vacuole of the parasite. The high intravacuolar concentration of chloroquine is proposed to inhibit the polymerisation of haem. As a result, the haem which is released during haemoglobin breakdown builds up to poisonous levels, thereby killing the parasite with its own toxic waste. The more lipophilic quinolinemethanol drugs, mefloquine and quinine, are not concentrated so extensively in the food vacuole and probably have alternative sites of action. The technique of photoaffinity labelling has been used to identify a series of proteins which interact specifically with mefloquine. These studies have led us to speculate that the quinolinemethanols bind to high density lipoproteins in the serum and are delivered to the erythrocytes where they interact with an erythrocyte membrane protein, known as stomatin, and are then transferred to the intracellular parasite via a pathway used for the uptake of exogenous phospholipid. The final target(s) of quinine and mefloquine action are not yet fully characterised, but may include parasite proteins with apparent molecular weights of 22 kDa and 36 kDa. As resistance to the

quinoline antimalarials rises inexorably, there is an urgent need to understand the molecular basis for decreased drug sensitivity. A parasite-encoded homologue of P-glycoprotein has been implicated in the development of drug resistance, possibly by controlling the level of accumulation of the quinoline-containing drugs. As our molecular understanding of these processes increases, it should be possible to design novel antimalarial strategies which circumvent the problem of drug resistance.

Various antibiotics are used in cancer treatment. Their antiproliferative and proapoptotic properties and influence on epithelial to mesenchymal transition are used for tumor growth inhibition. Also, quinolones, especially ciprofloxacin, were tested on many cell lines in vitro, indicating their potential usage for cancer patients. Induction of apoptosis, cell cycle arrest, and disruption of mitochondrial membrane potential are examples of quinolones' mechanism of action against cancer cells. Despite potential anticancer properties of different antibiotics, it should be noticed that these types of drugs can also negatively influence cancer development. Antibiotics, as well as chemotherapeutics, besides removing pathogenic bacteria, can also affect natural microbiota. Especially important is gut microbiota, whose disruption can lead to cancer generation by promotion of chronic inflammation, alteration of normal metabolism, genotoxicity, and weakening of the immune response. The microbiome is also present in the urinary tract, which for a long time was considered sterile.

Since the beginning of the COVID-19 pandemic, researchers have focused on repurposing of existing antibiotics, antivirals and anti-inflammatory drugs to find an effective therapy. Fluoroquinolones are broad spectrum synthetic antimicrobial agents, being chemical derivatives of quinoline, the prodrome of chloroquine. Interestingly, fluoroquinolones may exert antiviral actions against vaccinia virus, papovavirus, CMV, VZV, HSV-1, HSV-2, HCV and HIV. A recent in silico study has shown that the fluoroquinolones, ciprofloxacin and moxifloxacin, may inhibit SARS-CoV-2 replication by exhibiting stronger capacity for binding to its main protease than chloroquine and nelfinavir, a protease inhibitor antiretroviral drug. Remarkably, fluoroquinolones have shown multiple immunomodulatory actions leading to an attenuation of the inflammatory response through the inhibition of pro-inflammatory cytokines. Noteworthy, respiratory fluoroquinolones, levofloxacin and moxifloxacin, constitute fist line therapeutic agents for the management of severe community-

acquired pneumonia. They are characterized by advantageous pharmacokinetic properties; higher concentrations in the lungs; and an excellent safety profile comparable to other antibiotics used to treat respiratory infections, such as macrolides and b-lactams. Based on their potential antiviral activity and immunomodulatory properties, the favorable pharmacokinetics and safety profile, we propose the use of respiratory fluoroquinolones as adjuncts in the treatment of SARS-CoV-2 associated pneumonia.

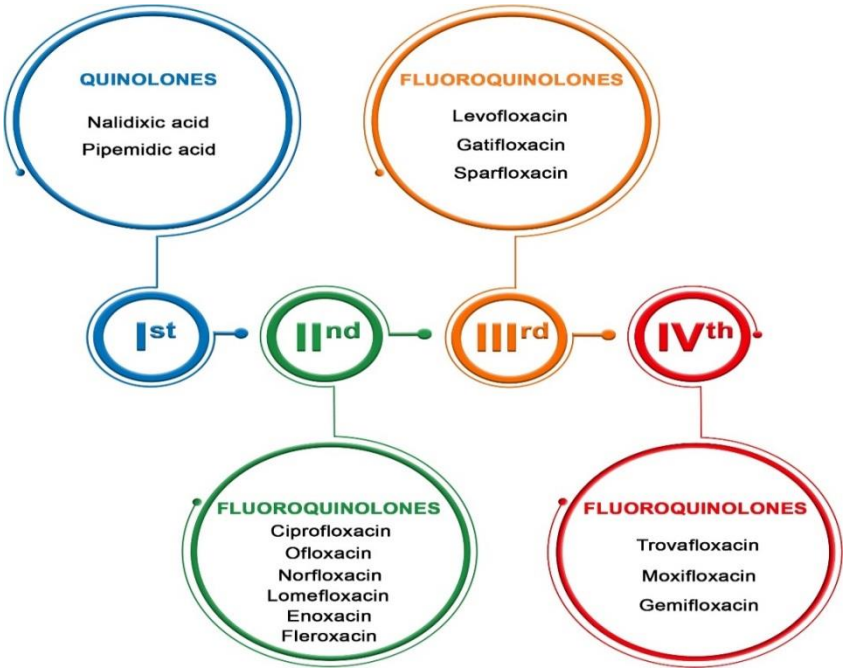


Figure 4 Generation of quinolones [24]

3. Materials and methods

The reaction was monitored by (TLC) technical and all synthesized compounds were characterized by spectroscopic techniques such as FT-IR, 1H-NMR

3.1 Instruments

3.1.1. Melting Point

The melting points of the studied compounds were measured using the electro-thermal apparatus, at Department of Chemistry/College of farmacy- University of Basrah.

3.1.2. Infrared Spectra

The infrared spectra for the studied compounds were measured using FT-IR spectrophotometer model FT-IR Affinity 1, as KBr disks at range (4000-400 cm-1) at

room temperature in the department of Chemistry- College of Education for pure sciences - University of Basrah. University of Basrah.

3.1.3. Nuclear Magnetic Resonance

1H-NMR spectra of the studied compounds were scanned on a BrukerAvance 500 MHz spectrometer internal standard was used as referenced to determined the 0.0 ppm. DMSO-d6 was used as a solvent

in the department of Chemistry- College of Education for pure sciences - University of Basrah.

3.2. General Synthesis of Acetanilide derivatives

3.2.1 Synthesis of N-(4-bromophenyl) acetamide

In a round bottom flask, 0.063 mol (6.5 gm) of acetic anhydride was added to (0.054 . 4- Bromoaniline -p-tulidine few drops of sulfuric acid were added to the mixture, the reaction mixture was heated in a water bath for three hours at 60 ^{o}C with stirring. The mixture cooled and poured in cold water (100 ml) with stirring. The obtained solid product filtrate was washed in cold water, dried, and recrystallized from Ethanol, the yield 55%, off white powder and obtain (N-(4-bromophenyl) acetamide m.p183-185.

3.2.2 Synthesis of QMBr

Dimethylformamide (9.13 gm, 0.125 mol) was chilled at 0 oC in the flask for 15 minutes before adding phosphoryl chloride (53.7 gm, 0.35 mol) dropwise with stirring. then the reaction mixture was treated with (N-(4-bromophenyl) acetamide. (0.214 mol) for 10 minutes before being heated reflex for 16 hours at 75 °C. The mixture was cooled, poured into ice water (100 ml), and agitated for 1 hour at 0-10 °C. Next, the mixture was filtered, washed, dried, brown powder was obtaind QMBr and recrystallized from ethyl acetate yield 23% ,m.p188-189 C and yellow pale powder

3.2.3 Synthesis Quinoline sulfa drugs :QSD, QST

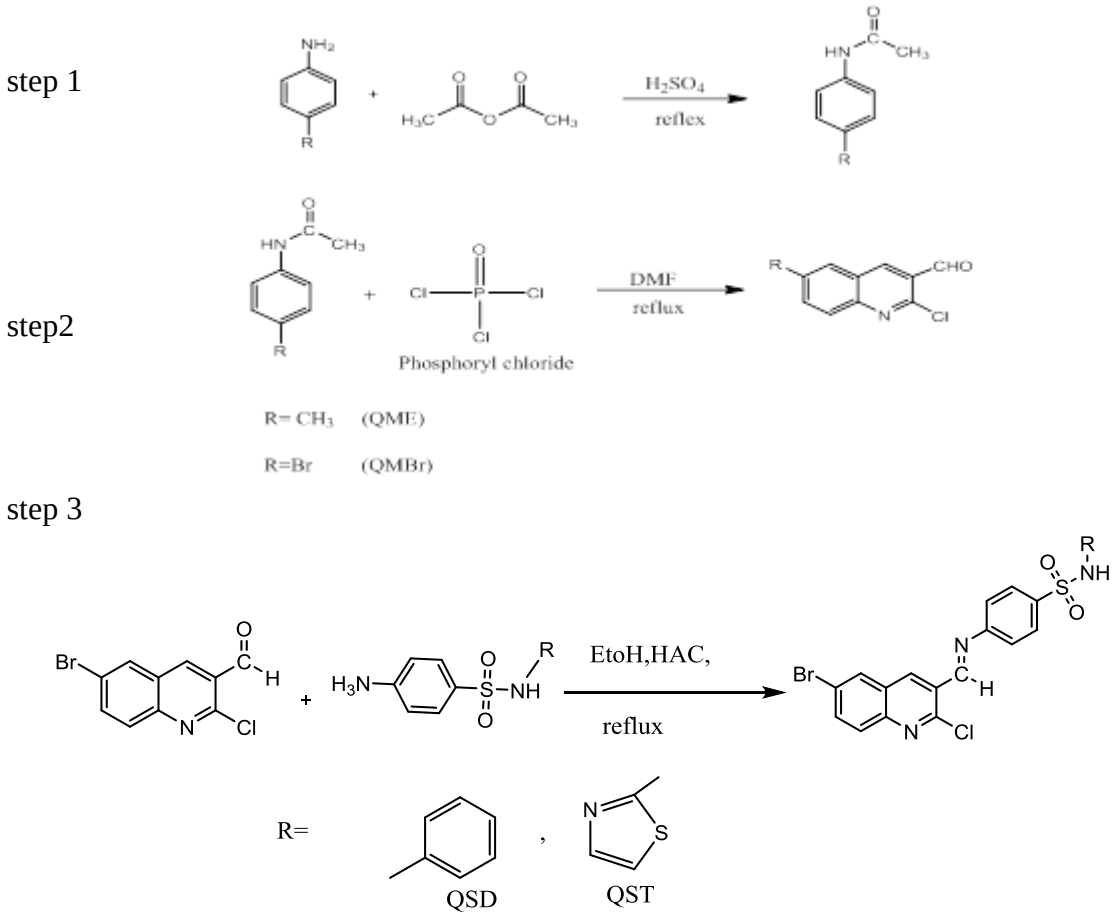
A mixture of Sulfa drug compound (1mmol) with (15 ml) Ethanol was added to quinoline-3-carbaldehyde derivatives (1mmol) in a round bottom flask with a few drops of glacial acetic acid. The mixture was stirred under reflux for 4 hrs, cooled, filtrated, and recrystallized in ethanol. The reaction mixture was monitored by TLC using Hexane ethylacetate (2:8 v/v) as eluent. We obtaind orange powder of compound QSD

yield 70% m.p 133-135 °C and yellow powder of compound QST yield 80% m.p 145-147° C.

4. Discussion

The present study involved the synthesis of new compounds from sulpha drugs with quinoline analogue. In the beginning were Prepared acetanilide from the reaction of aniline derivatives with acetic anhydride[62], then the quinoline analogue was synthesized according to the valsmier-Haack reaction. The target compounds were prepared from the condensation reaction of quinoline analogue and sulfa drugs compounds.

Acetanilide derivatives are prepared from the reaction of p-bromoaniline with acetic anhydride, the acetanilide product was treated with POCl3 and DMF under reflex to obtain the quinoline analogue (quinoline carbaldehyde) (scheme 3-1).



Scheme 3-1 synthesis pathway of acetanilide and quinolone carbaldehyde

FT-IR Spectra of Quinoline and their derivatives

The FT-IR spectra were recorded as KBr disk as shown in figures 5, 6, 7and the data are gathered below.

The IR spectra of compound QMBr, displayed a strong band at 1691 cm-1, attributed to the stretching vibration of the carbonyl of the formyl group. As well as the bands of stretching vibration of C=C at 1581 cm-1. The IR spectra of synthesized quinoline sulfonamide Schiff bases were displayed a strong stretching band at range (1622-1637) cm-1 attributed to the azomethine C=N, the spectra show disappearance of stretching band attributed to formyl of quinoline due to formation azomethine group. Also, the spectra display the stretching vibration band of the N-H group with in the range (3339- 3425) cm-1

The IR spectrum of all the synthesized Schiff base derivatives showed the bending vibration bands at 1298-1388 cm-1 were due to (S=O) asymmetric stretching vibration while the bands at (798-736) cm-1 were attributed to (C-Br) stretching vibration [65-66].

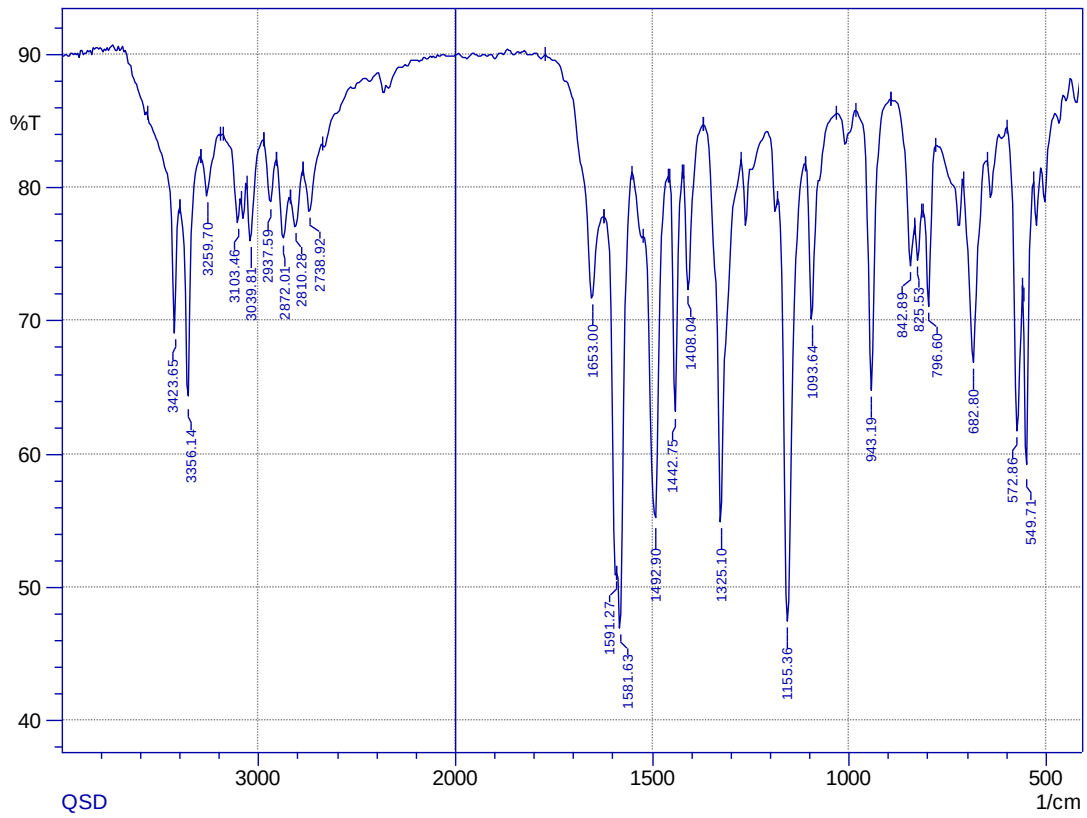


Figure (5) QSD

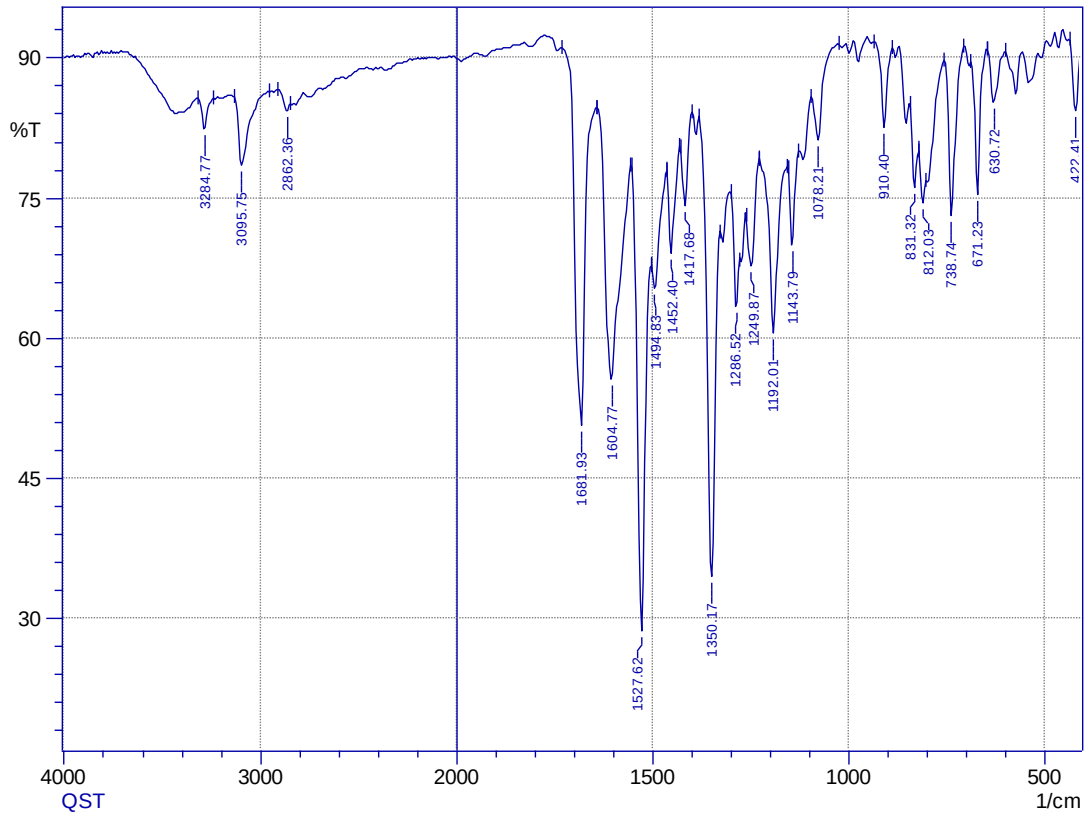


Figure (6) QST

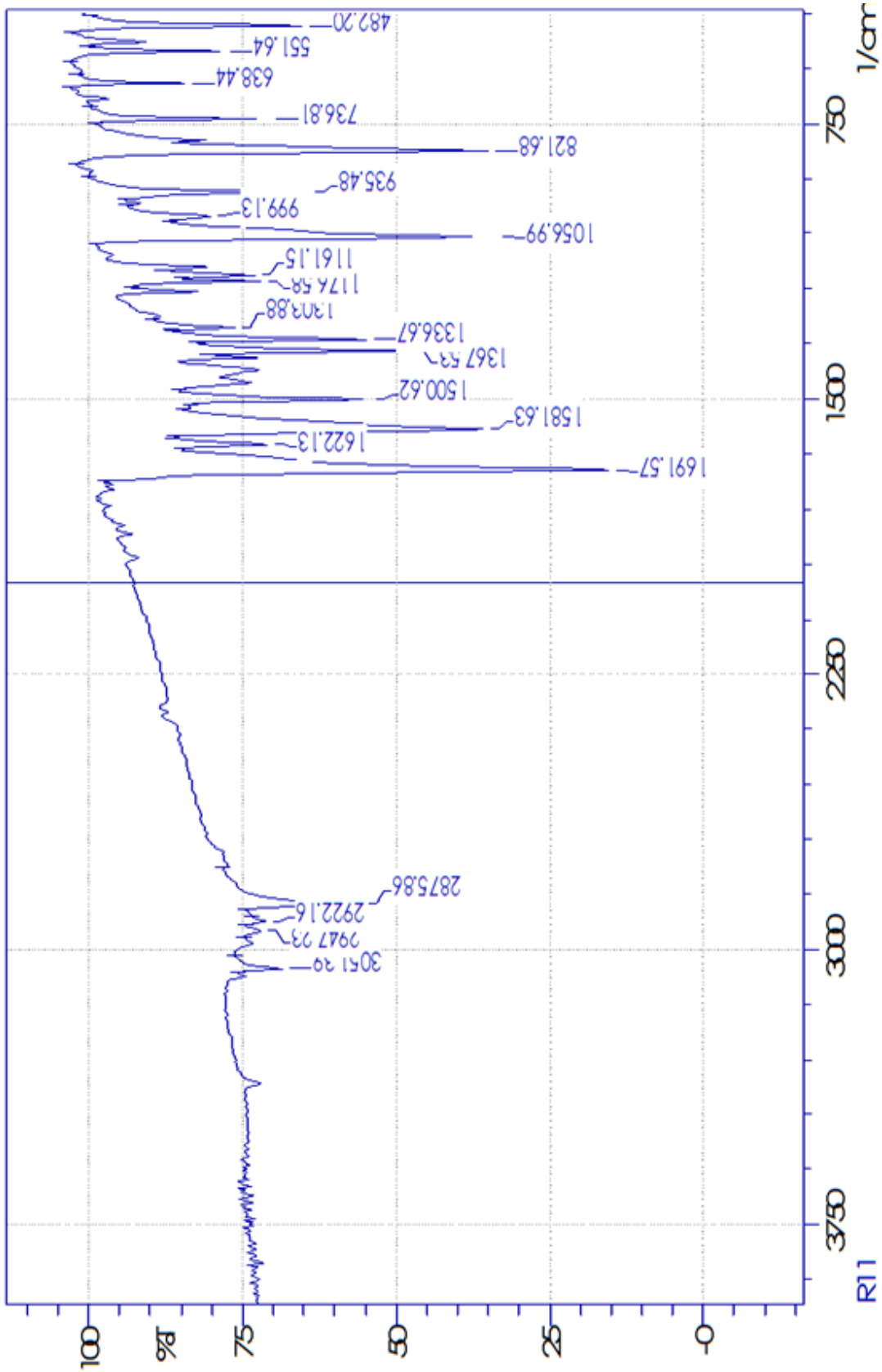


figure (7) I.R of QMBr

^{1}H-NMR Spectra of the Synthesized Compounds

The ^{1}H-NMR spectra of the synthesized compounds recorded on 500 MHz techniques used to confirm the proposed structure of the Quinoline derivatives in DMSO-d^{6}, with internal standard tetramethylsaline (TMS).

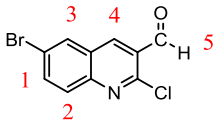
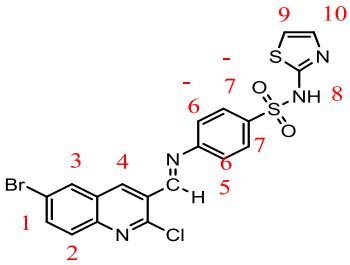
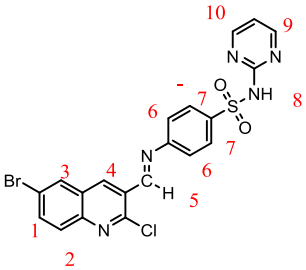
The ^{1}H-NMR spectra data are included in table (3-1) and the representive spectra are show in figure (8-10). In all case the peaks at 2.5 ppm and 3.3 ppm attributed to DMSO and DMSO water solution respectively .

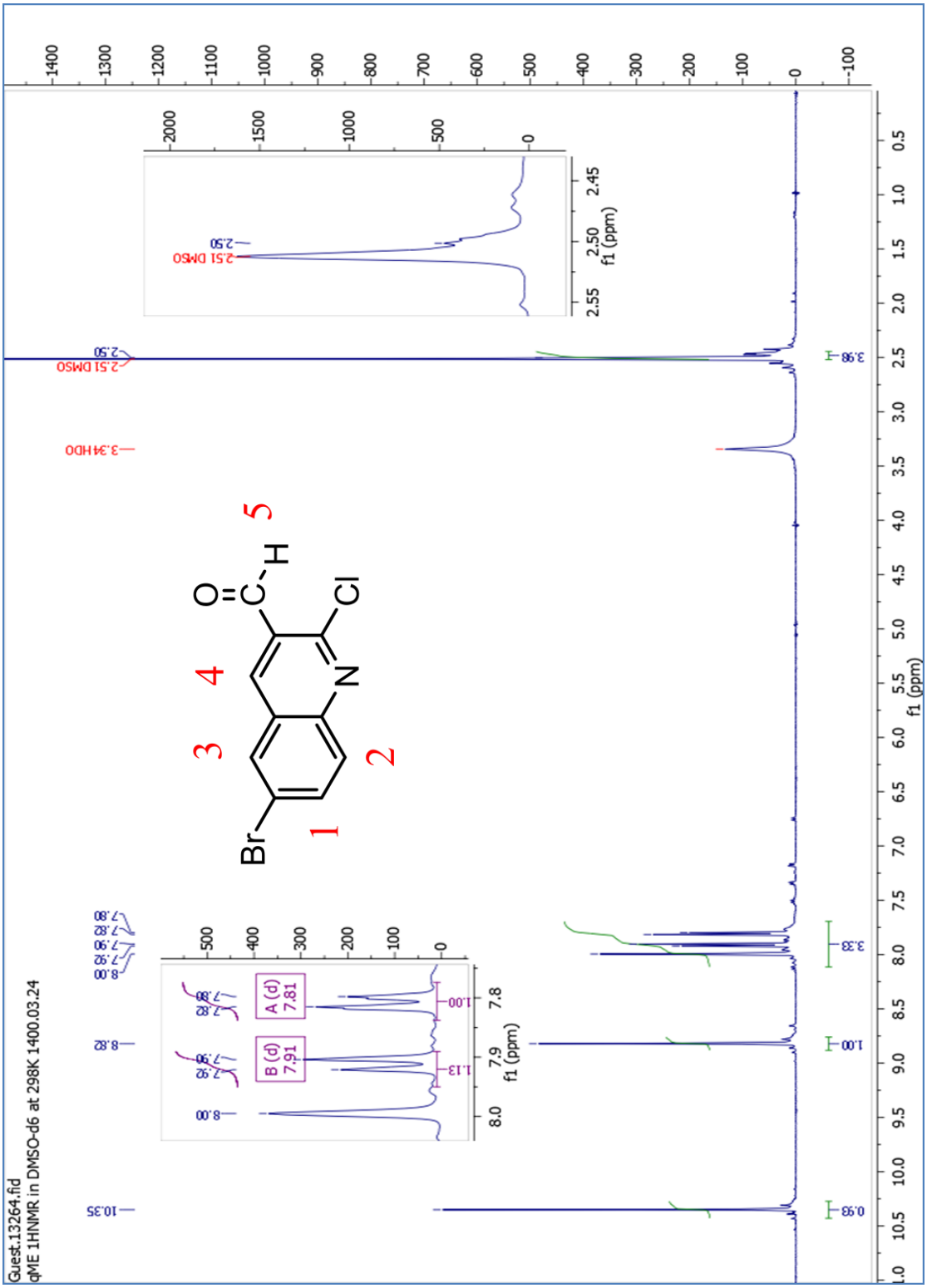
The spectrum of compound QMBr (Figure-8) display two groups of resonance, resonance which the signals of aromatic protone which appear various signals with the range (7.8-8.0) ppm as well as singlet signal of aromatic C-H nieghbours the formyl group display at 8.82 ppm, the formyl proton appear at 10.35 ppm. The ^{1}H-NMR spectra of quinoline sulfa evidence of prepared new compound from condensation sulfa drugs with quinoline. In addition, all the synthesized compounds (QMBr) demonstrated avarious signals within range at (6.8-8.4 ppm) due to the proton of the aromatic ring [68], the proton of the methyl groups which appear singlate signal with in the range at (1.05-2.5) ppm.

The N-H group proton resonance which appear at the downfield with in the rang at

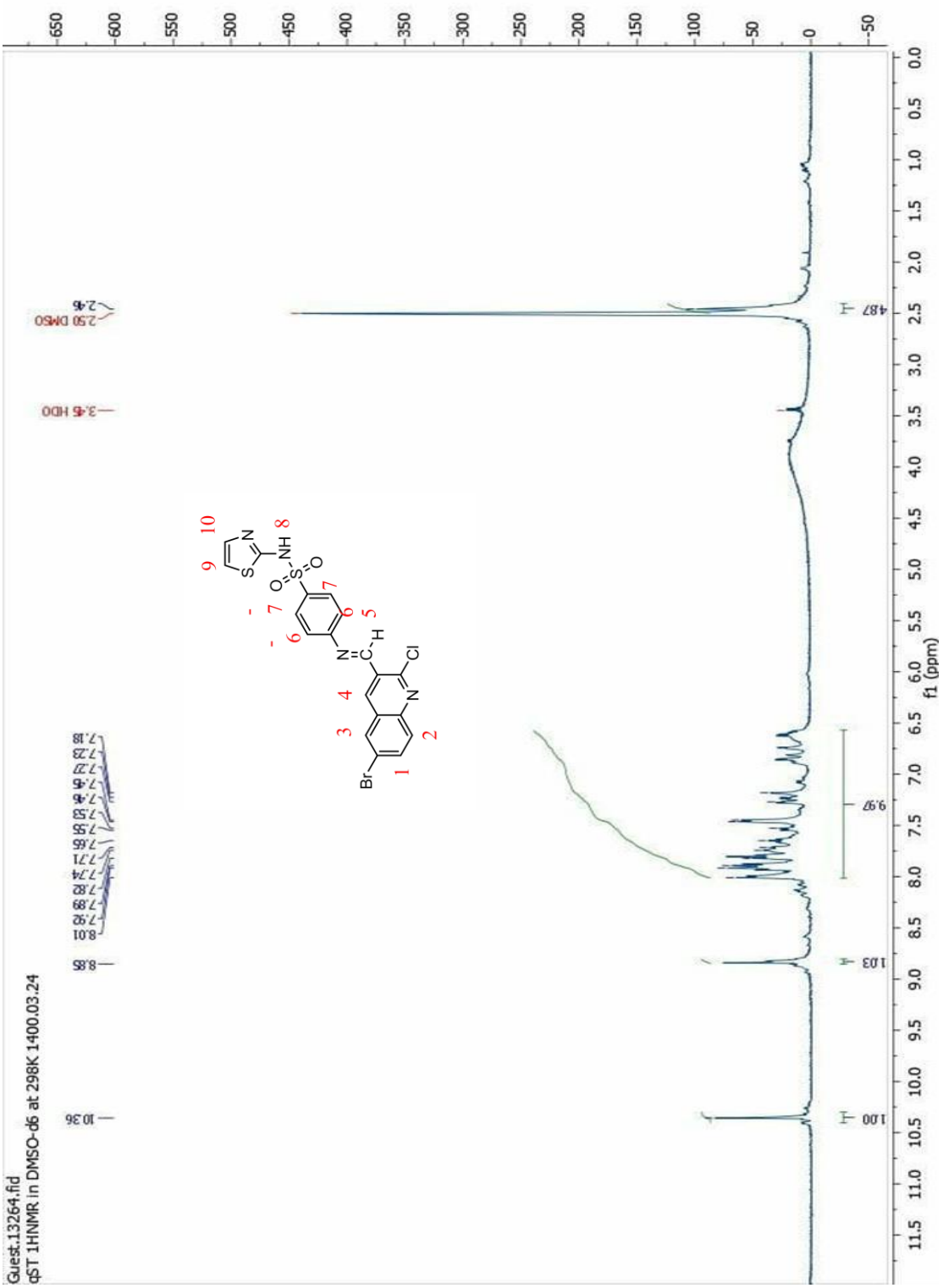
(10.35-10.96) ppm due to the deshelding effect of SO2 group.

Table (3-1) ^{1}H-NMR

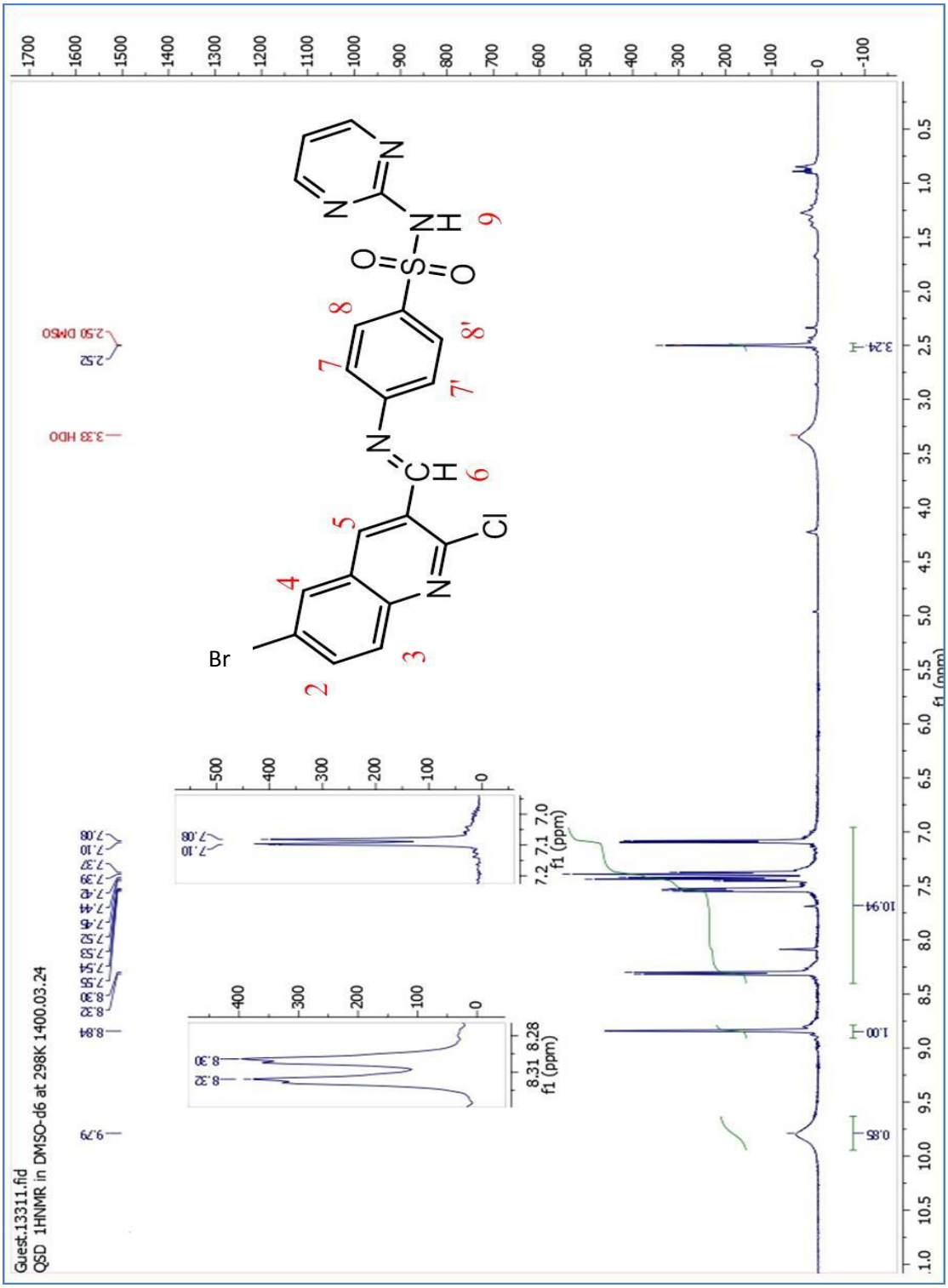
Code	Comp. Structure	Chemical shift (ppm)
QMBr Figure(8)		(1)7.80-7.82 (d, 1H (*J*=10), Ar-H) (2) 8.00 (s, 1H , Ar-H) (3) 7.90-7.92 (d,1H (*J*=10) ,Ar-H) (4) 8.82 (s, 1H, CH=CHO) (5)10.35 (s, 1H, CHO)
QST Figure(9)		6.7-7.9 (various bands, 10H, Ar-H (5)8.85 (s, 1H, CH=N) (8) 10.36 (s, 1H, N-H)
QSD Figure(10)		(10) 6.92 (s, 1H, AR-H) (7) 7.49 (d , 2H, J=10, Ar-H) (6) 7.63 (d, 2H, J=10, Ar-H) (1) 7.69 (d, 2H, J=10, Ar-H) (3) 8.31 (s, 1H, Ar-H) (9) 8.35 (s, 1H, Ar-H) (4) 8.58 (s, 1H, Ar-H) (5) 8.91 (s, 1H, CH=N) (8) 10.37 (s, 1H, N-H)



Figure(8)NMR QMBr



Figure(9) NMR QST



Figure(10) NMR QSD

5. Conclusion

1. The possibility of preparing and purifying quinoline derivatives easily and with high yield.

2. Synthesis of a series of new compounds resulting from the union of quinoline moiety with sulfa compounds.

3. Sulfa compounds with quinoline derivatives have activity against breast cancer cells (MCF-7).

6. References

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