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Physiochemical, antimicrobial and spectra profile of in-local synthesized Ciprofloxacin Dithiocarbamate

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Abstract: Ciprofloxacin dithiocarbamate was prepared by reaction of carbon disulfide (CS_2) and ciprofloxacin's secondary amine on the piperazine ring (C-7 substitute), the reaction was made in an alkaline medium (Ethanol 50%) and (Methanol 80%) at (0-4) ºC. The yield of reaction was over 90%.

The spectrums of the resulted compound (UV, IR, ^{13}C-NMR,^{1}H-NMR) were studied, and the physiochemical properties were determined. Ciprofloxacin dithiocarbamate showed more solubility than Ciprofloxacin and ciprofloxacin hydrochloride in distilled water, alkaline medium and moderate medium, and it was more lipophilicity at pH=7.4 and pH=6.8. The antimicrobial activity of Ciprofloxacin dithiocarbamate and ciprofloxacin was compared on 100 bacterial strains include gram negative and gram positive microorganisms, the results showed similar activity in 69% of strains, but the activity against ***Staphylococcus aurous***, and ***E. Coli*** increased. On the other hand, 57.14% of the gram positive strains were sensitive to Ciprofloxacin dithiocarbamate compared with 52.38% sensitive to ciprofloxacin, and 64.86% of the gram negative strains were sensitive to Ciprofloxacin dithiocarbamate compared with 56.76% sensitive to ciprofloxacin. In conclusion 60% of the strains were sensitive to Ciprofloxacin dithiocarbamate compared with 54% sensitive to ciprofloxacin.

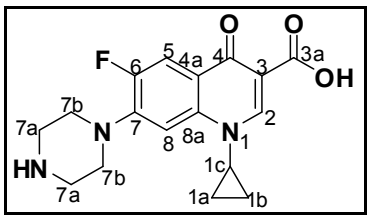
Key Words: ciprofloxacin, Ciprofloxacin dithiocarbamate, carbon disulfide, antimicrobial activity, pharmacokinetics.

Introduction:

Since their introduction in the 1960s with nalidixic acid, quinolone antimicrobial agents have undergone extensive synthetic and clinical development, resulting in improved antimicrobial activity, pharmacokinetic features and toxicity profiles.[1] .The antibacterial activity of fluoroquinolones depends not only on the bicyclic heteroaromatic pharmacophore but also on the nature of the peripheral substituents and their spatial relationship. These substituents exert their influence on antibacterial activity by providing additional affinity for the bacterial enzymes, enhancing the cell penetration, or altering the pharmacokinetics [2]. Flouroquinolones are an effective synthetic antibiotic and it is administrated as tablets in most cases because of low solubility in water and physiological mediums[3-5].

Administration of any antibiotic depends on its solubility and/or lipophilicity in physiological medium (pH=7.4 and 6.8) since these parameters influence the bioavailability and the growing number of microbial strains resistant to antimicrobial agent which becomes a serious problem when treating infections make it important to investigate new compounds with an improved pharmacokinetic profile [6-9].

CIP is the most popular Flouroquinolone, belongs to the second generation and has the formula described in figure 1:



Figure(1) the structure of ciprofloxacin

The C-7 position of the quinolone core has an important rule in modifying the physiochemical properties, subsequently the pharmacokenitics profile with/without minor effect on antimicrobial activity [6,10,11].

It is well known that the dithiocarbamate compounds (DTCs) have a remarkable effect in reducing inflammation severity and its activity against *E.Coli* and *Staphylococcus aureus* have been proved [12-14].

The aim of this study is to synthesis a structural analogue of CIP, depending on substitution on the secondary amine of pyridine ring on C-7 position. This modification was done using CS_2 in order to improve the bioavailability of CIP. The physiochemical properties and antimicrobial activity of the analogue were determined compared with CIP.

Experimental:

Materials:

All chemicals were of analytical grade and were obtained from Merck. Ciprofloxacin was obtained in pure form from a local pharmaceutical company (BAHRI) and was used without further purification.

Synthesis:

To a stirred solution of CIP.HCl.H_2O (0.01mol) in 150mL ethanol 50% (product C,D and F) or Methanol 80% (product B). Sodium Hydroxide (0.03mol) was added with constant stirring over ice bath, the solution became clear after few minutes then 1.2mL of CS_2 (0.2mol) was added dropwise. After 6 hours at (4°C) 100mL of ether was added. The precipitate was isolated and dried in desiccator over night.

The crude was purified either by recrystallization in Methanol 80% (product B,C and F) or by column chromatography (product D)(Silica gel 400-230 mesh) using 0.1% TFA in acetonitrile/0.1% TFA in water : 9/1 as mobile phase. The purity was checked on TLC.

Spectrum study:

Spectrometric measurements were performed on a Jasco UV-Vis V-530 instrument equipped with 10-mm quartz cuvettes, UV-Vis spectra were recorded in the range 200 to 400 nm at different pH mediums. Infrared spectra (IR) were recorded on Nicolt Impact 415 (4000–650cm $^{-1}$). NMR spectrum ^{13}C-NMR and ^{1}H-NMR were performed on Bruher Avance 500 MH_Z. The pH's of the solutions were determined using Hanna HI 9321 pH-meter. Melting point determination was done on Sanyo instrument.

Physiochemical study:

Solubility:

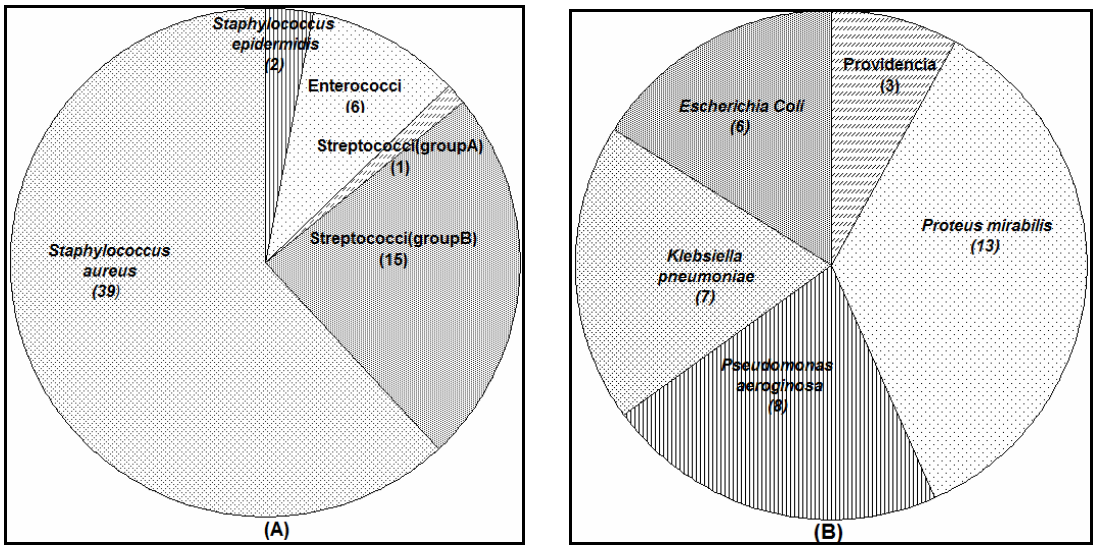
The solubility was studied at 25°C and 37°C in distilled water and at 37°C in phosphate buffer solutions (0.05M, pH=6.8 and 7.4). The concentrations were measured on spectrophotometer at wave length 273nm [15,16].

Partition coefficient determination (log $D_{Octanol/Buffer}$):

log $D_{O/B}$ was determined in n-octanol/Phosphate buffer (0.05M) system at pH=6.8 and 7.4 depending on Shack-Flask method. The concentrations were measured on spectrophotometer at wave length 287nm for n-octanol solutions[17,18].

Microbiological study:

This study was done on 63 strains of gram positive microorganisms (A) divided into 5 groups and 37 strains of gram negative microorganisms (B) divided into 5 groups. figure 2 :



Figure(2) the strains of gram positive microorganisms (A) and gram negative (B)

The microorganisms suspensions were prepared in a concentration of (10^5–10^6CFU/mL) and the study was done according to Break-Point method [19,20], in which two concentrations of CIP & CIP-CS_2 were used 2.5 and 0.625µg/ml corresponding to C_{max} and $\frac{1}{4}C_{max}$ of CIP respectively [7].

Petri dishes were prepared as follow: The amount of the antibiotic was dissolved in a small volume of double distilled water and then added to Mueller Hinton agar solution (34g/L). The final solution was autoclaved and poured in 11m diameter Petri dishes, after solidification 5µl of microbial suspension was added on the surface of growth medium, the dishes were then incubated at 35-37°C for 24 hours.The microorganisms were considered resistance (R) when growth happened in both concentrations, sensitive (S) when growth did not happen in any of concentrations and intermediate (I) when growth happened in $\frac{1}{4}C_{max}$ concentration only [19,21]. The blank dishes were prepared at the same manner without antibiotic substance.

Results:

synthesis:

The analogue was synthesized in high yield exceeded 90% in ethanol 50% and about 80% in Methanol 80%. The purity was more than 97% as shown on HPLC-C18, figure presents the HPLC profile for CIP-CS_2 and CIP.HCl.H_2O:

Spectrum analysis:

UV spectrum:

UV spectroscopy was used to get UV absorbance profile and to investigate the pH dependence absorbance. The spectrums of CIP.HCl.H_2O (A) and CIP-CS_2 (B,C,D,F) in double distilled water, figure 3:

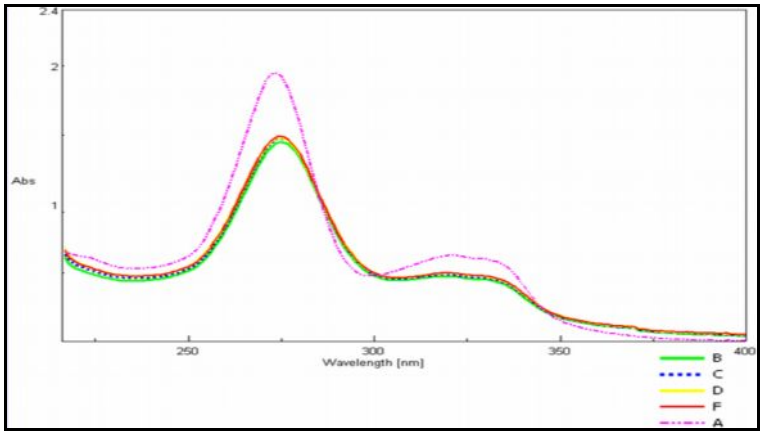


Figure 3: UV spectrums of CIP.HCl.H_2O (A) and CIP-CS_2 (F,D,C,B)

All the spectrum showed the same pattern of quinolones spectrum which could be divided into two major bands at 240-300nm and 300-375nm, which can be assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition respectively.

The spectrums of CIP.HCl.H_2O & CIP-CS_2 at 220-400nm in buffered mediums (pH=2-11) presented in figure 4 and 5 respectively:

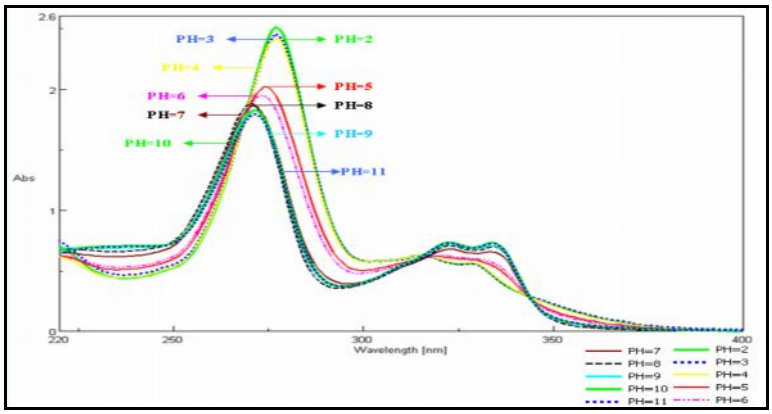


Figure 4: UV spectrums CIP.HCl.H_2O at 220-400 nm in buffered mediums (pH=2-11)

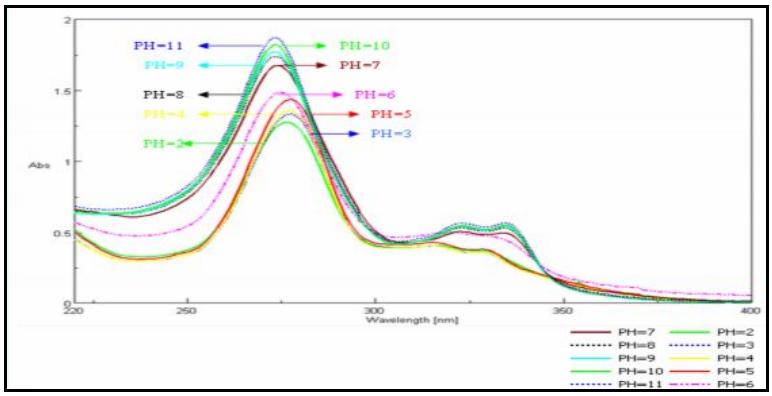


Figure 5: UV spectrums CIP-CS_2 at 220-400 nm in buffered mediums (pH=2-11)

The absorption maxima for an aqueous solution of CIP.HCl.H_2O and CIP-CS_2 at pH=2 appeared at 277, 315 and 328nm. On increasing the pH, the maximum at 277nm shifted to lower values 271nm (blue shift), and the maxima at 315 and 328nm shifted to higher values 323 and 334nm (red shift) at pH=11. These changes can be attributed to the extent of ionization of the carboxylic group as a consequence of the removal of a proton [15,22,23]. pH increasing led to absorbance increasing for both bands of CIP-CS_2 and pH increasing led to absorbance increasing for second bands and decreasing of absorbance for first band of CIP.HCl.H_2O.

IR spectrum:

The infrared spectrums of CIP.HCl.H_2O and CIP-CS_2 in a KBr pellet for wave number range of 4000–650cm^{-1} are presented in figure 6 and 7 respectively. The peak at 1702cm^{-1} shows the carbonyl stretching (C=O) of carboxylic acid, disappearing this peak in CIP-CS_2 spectrum might be a result of ionization state of CIP-CS_2 [15,22,24], especially with disappearing of carboxylic acid -OH band which will be in the region of 3300–2500cm^{-1} generally. The new peak at 1006cm^{-1} shows the thiocarbamate stretching (C=S) of dithiocarbamate[25]. The strong peak at 1204cm^{-1} might be corresponding to (N-CS_2) [26].

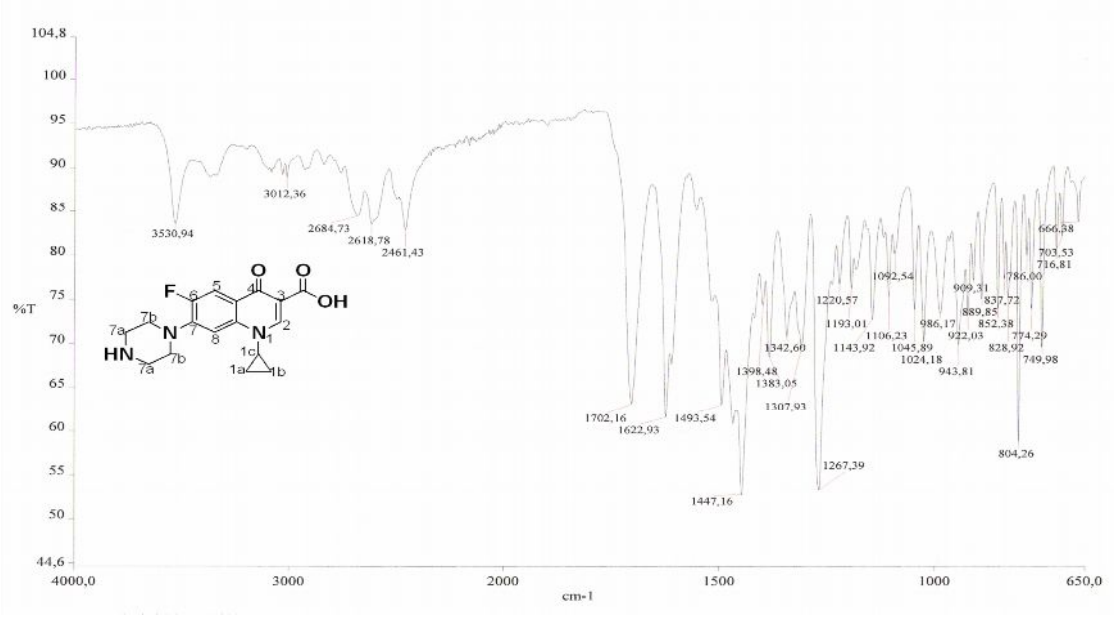


Figure 6 :IR spectrum of CIP.HCl.H_2O

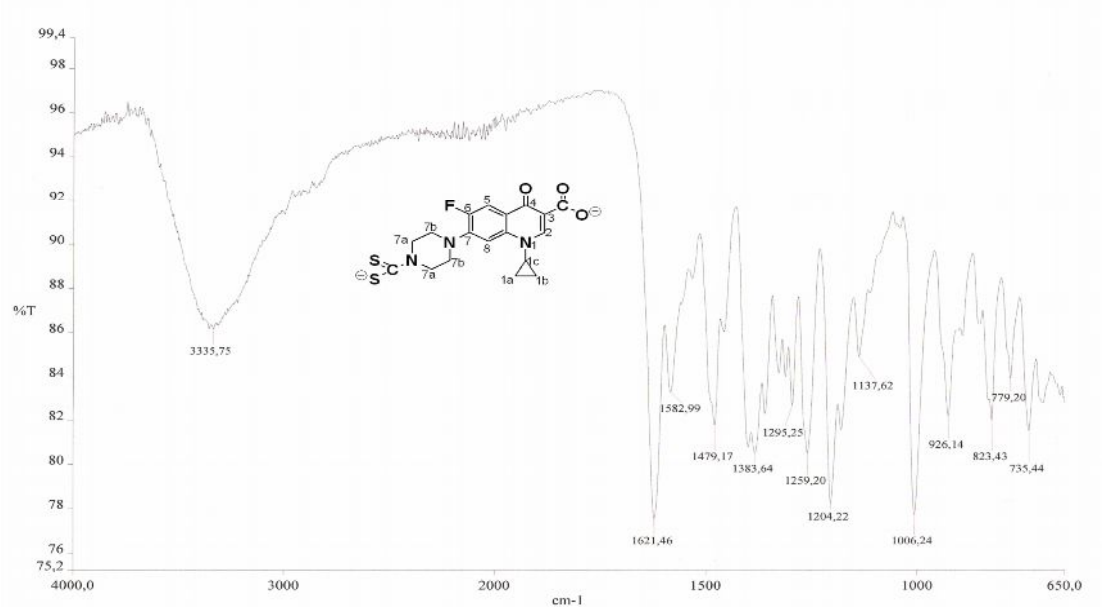


Figure 7 :IR spectrum of CIP-CS_2

NMR spectrums:

^{1}H–NMR spectrums:

The ^{1}H–NMR spectrums in D_2O of CIP.HCl.H_2O and CIP-CS_2 are presented in figure 8 and 9 respectively. These spectrums could be divided to three regions[26-30]: two chemical shifts at 0.5-1.5ppm assigned to 1a and 1b protons of cyclopropyl, three chemical shifts at 6.5-8.5ppm assigned to 8 protons of aromatic ring and two chemical shifts at 3.0-4.5ppm assigned to 7a and 7b protons of piperazine ring. The chemical shift at 3.230ppm in figure 9 assigned to 1c proton of cyclopropyl which is overlapped by the peaks of aromatic ring's protons in figure 8.

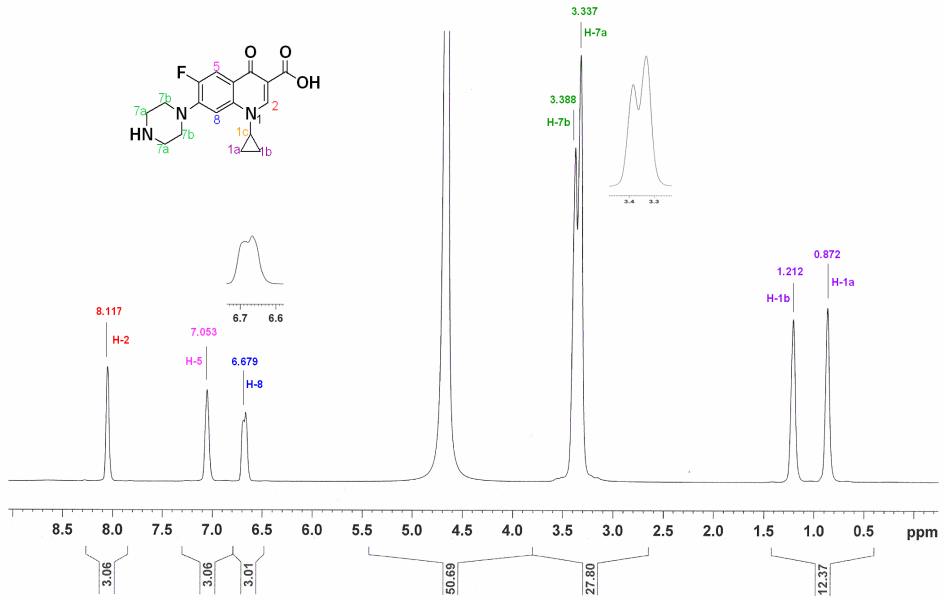


Figure 8: ^{1}H-NMR spectrum of CIP.HCl.H_2O in D_2O

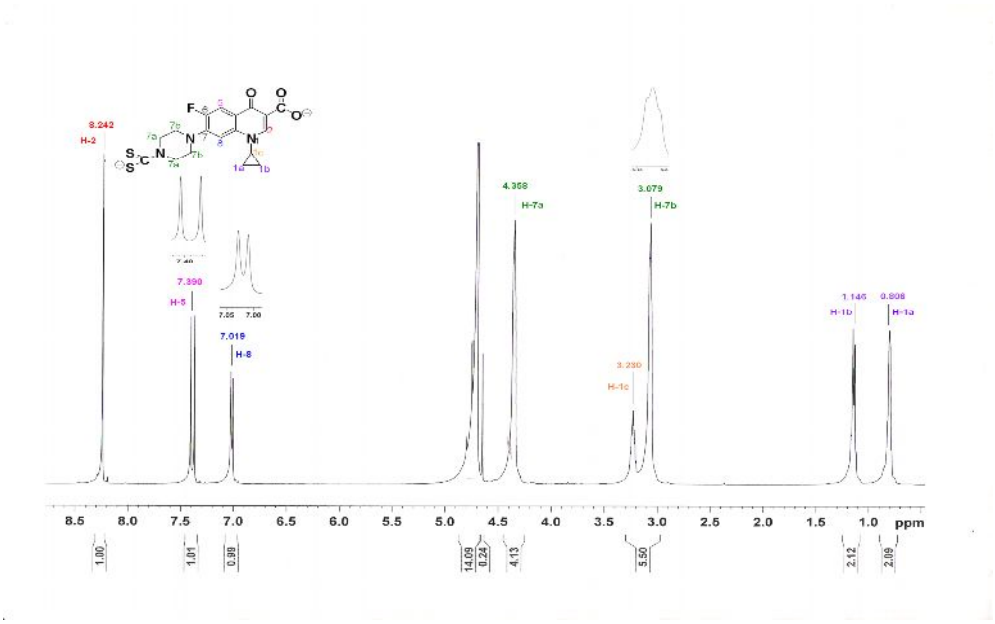


Figure 9: ^{1}H-NMR spectrum of CIP-CS_2in D_2O

^{13}C–NMR spectrums: [26-30]

The ^{13}C–NMR spectrums in D_2O of CIP.HCl.H_2O and CIP-CS_2 are presented in figure 10 and 11 respectively. In Comparison of spectrums a few points could be noticed: No changing in C-1b, C-1a, C-4 signals, some signals showed a shift up to 7ppm, but the biggest change happened to C-7a about 7.27ppm and C-7b about 7ppm. The new signal at 209.25ppm assigned to (-N-CS_2) [31].

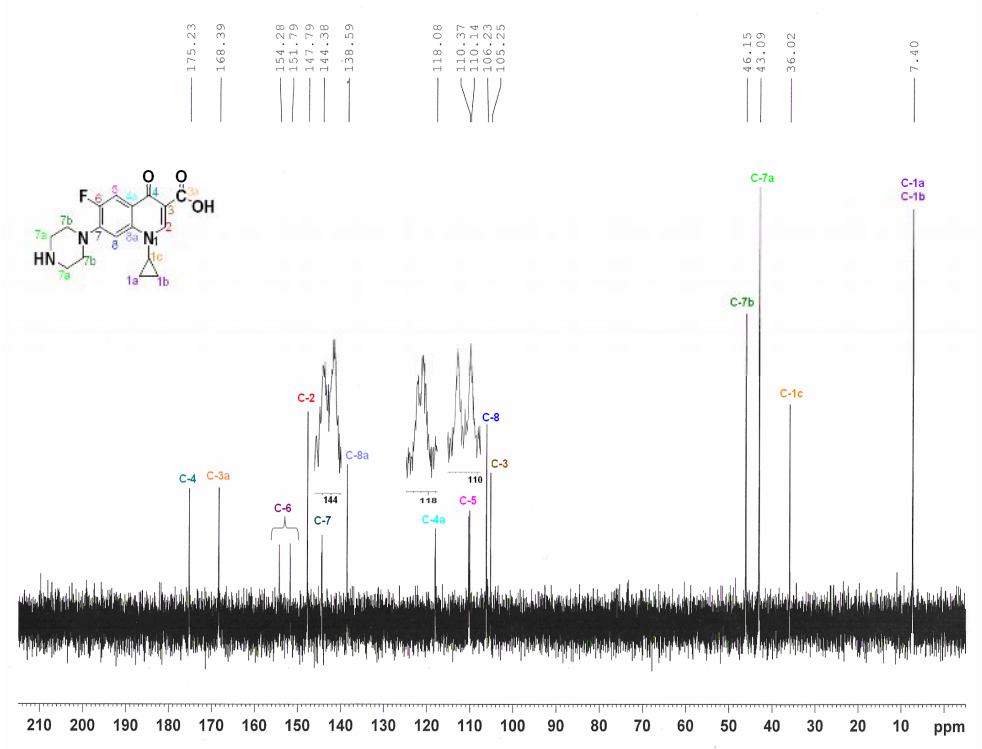


Figure 10: ^{3}C -NMR spectrum of CIP.HCl.H_2O in D_2O

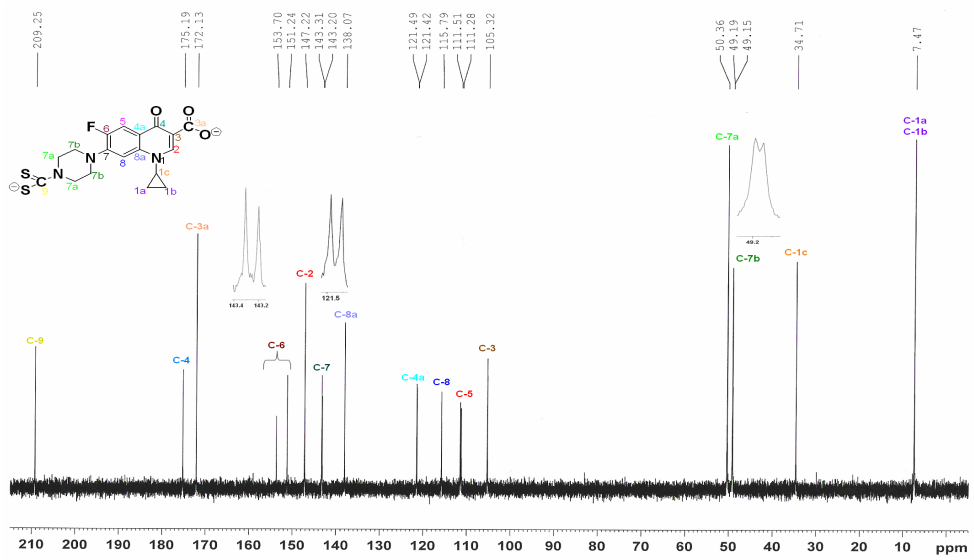


Figure 11: ^{13}C -NMR spectrum of CIP-CS_2in D_2O

Physiochemical properties:

Figure 12 presents the chemical formula of the new analogue: sodium 1-cyclopropyl-7-(4-dithiocarboxypiperazinyl)-6-fluoro-4-oxo-quinoline-3-carboxylate

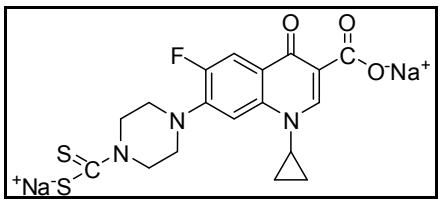


Figure 12: chemical formula of CIP-CS_2

CIP-CS_2 is a yellow crystalline powder, decompose at 257°C. Its 2.5% solution in double distilled water has a pH=8-9 compared to 3-3.5 for CIP.HCl.H_2O . The solubility at 25°C in double distilled water is up to 8.55%(w/v) compared to 3.5%(w/v) for CIP.HCl.H_2O, but at 37°C (figure 13) the solubility increases dramatically in pH=7.35 and 8.5, although in pH=6.7, it is a little bit less than that of CIP.HCl.H_2O [32].

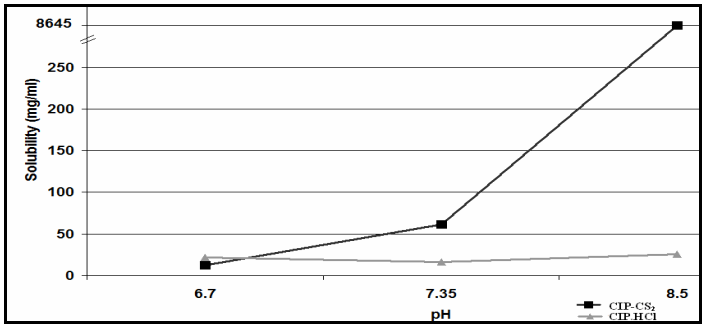


Figure 13: comparison between solubilities of CIP.HCl.H_2O and CIP-CS_2 in different pHs at 37°C

The figure 14 presents log D results for CIP.HCl.H_2O and CIP-CS_2 in pH=6.7 and 7.4. It is clear that CIP-CS_2 has a higher lipophilicity than CIP.HCl.H_2O in both pH points [18].

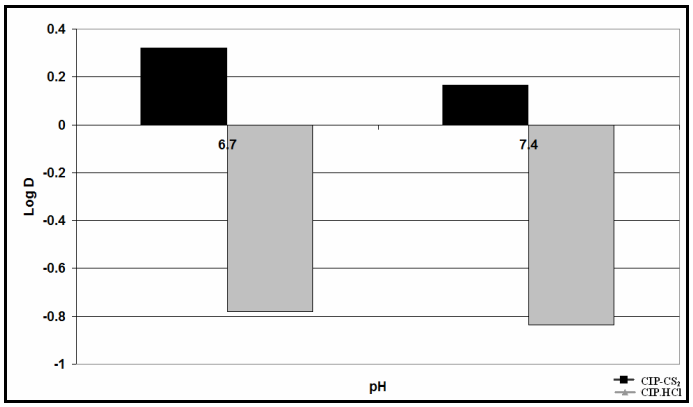


Figure 14: comparison between log D of CIP.HCl.H_2O and CIP-CS_2 in pH 6.7 and 7.4

The differences in physiochemical properties between CIP.HCl.H_2O and CIP-CS_2 might be because of bifunctional acidic group for CIP-CS_2 as shown in figure (15).

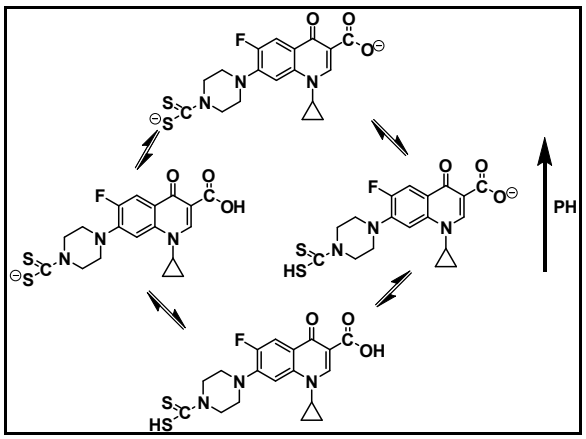


Figure 15: Protolytic equilibrium in CIP-CS_2 water solutions

Microbiological study:

Table 1 and 2 indicate the results of microbiological activity against gram positive and gram negative microorganisms respectively.

Table 1: The results of microbiological activity against gram positive microorganisms

	CIP			CIP-CS_2		
	S	I	R	S	I	R
Staphylococcus aureus	*17	6	16	20	2	17
Streptococcus B	11	0	4	11	2	2
Streptococcus A	1	0	0	1	0	0
Enterococci	3	2	1	3	2	1
Staphylococcus epidermidis	1	1	0	1	0	1

*indicate to the number of strains.

Table 2: The results of microbiological activity against gram negative microorganisms

	CIP			CIP-CS_2		
	S	I	R	S	I	R
proteus mirabilis	*7	3	3	7	2	4
Pseudomonas aeroginosa	5	3	0	5	2	1
Klebsiella pneumoniae	4	1	2	4	1	2
E. Coli	3	3	0	5	0	1
providencia	2	1	0	3	0	0

*indicate to the number of strains

Results and Discussion:

CIP-CS_2 was synthesized as a yellow crystalline powder in alcoholic medium with more than 90% yield. UV, IR, NMR spectrum were fully studied. NMR spectrums indicated that the major changes happened on piperazine ring as a result to CS_2 reaction on secondary amine, in addition to a new peak at 209ppm assigned to (-N-CS_2). Physiochemically, there are a considerable differences between CIP-CS_2 and CIP.HCl.H_2O. The solubility of CIP-CS_2 is two times more than that of CIP.HCl.H_2O in distilled water at 25°C and four times more at 37°C in pH=7.4 and has a slightly basic medium compared to acidic medium of CIP.HCl.H_2O.

The higher $logD_{pH=6.7,7.4}$ for CIP-CS_2 compared to CIP.HCl.H_2O influences the renal and hepatic elimination and consequently pharmacokinetic profile. High solubility of CIP-CS_2 slightly basic medium make it more compatible and easier for drug formulation and administration.

The antimicrobial activity of CIP-CS_2 and CIP.HCl.H_2O was compared on 100 bacterial strains include

gram negative and gram positive microorganisms, the results showed similar activity in 69% of strains, but the activity against *Staphylococcus aurous* and *E. Coli* increased which might be assigned to dithiocarbamate moiety . On the other hand, 57.14% of the gram positive strains were sensitive to CIP-CS_2 compared with 52.38% to CIP.HCl.H_2O and 64.86% of the gram negative strains were sensitive to CIP-CS_2 compared with 56.76% to CIP.HCl.H_2O. In conclusion, 60% of the strains were sensitive to CIP-CS_2 compared with 54% to CIP.HCl.H_2O.

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