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Antimicrobial Activity of New Amide/thioamides Derivatives of Ciprofloxacin

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Abstract

A thioamide/ciprofloxacin hybrid **3a** has been designed and synthesized by acetylation/alkylation of ciprofloxacin then thioamidition on its corresponding amide **2a**. All the compounds have been characterized with spectral analysis. Both **2a** and **3a** compounds were undergone screening for antibacterial activities on some gram-positive and gram-negative bacteria. The antifungal activity of these compounds has also been made against *Candida albicans.* The amide derivative has shown enhanced antibacterial activity against both gram-positive and gram-negative bacteria than parent antibiotic only, on the other hand thioamide has shown potent antifungal activity and moderate antibacterial activity against gram negative and some stains of gram-positive bacteria than ciprofloxacin.

Keywords
ciprofloxacin, antibacterial, antifungal, thioamide

1. Introduction

Hydrogen sulfide (H_2S) is a noxious gas with the characteristic smell of rotten eggs, In the recent studies H_2S has been recognized as the third gaseous transmitter in addition to nitric oxide (NO) and carbon monoxide (CO), that influences various physiological processes. It plays a significant role in many pathophysiological processes such as regulation of inflammation, oxidation, ion channel regulation, cardiovascular protection, relaxation of vascular smooth muscles, mediate neurotransmission, endocrine regulation, elicit hibernation insulin signaling inhibition and tumor progression.[1,2] The importance of this molecule is being of its biological role in a concentration lower than100-160 µM unlike exposure to 300 ppm of H_2S leads to pulmonary edema while 1000 ppm causes immediate death, therefore, caution should be taken when working with H_2S.[3] On the other hand, endogenous concentrations of H_2S are generally low, making it difficult to perform precise biological functions so, we are in need to new H_2S releasing agent.[4]Hydrogen sulfide releasing agents (H_2S donors) have been widely used in many fields. These compounds are not only useful research tools, but they also have potential therapeutic use.[3] Hybrid based H2S donor is thiol-activated H_2S-releasing compounds where some of the first synthetic donors are reported, They can release H2S by reacting with endogenous thiol-containing molecules, such as *l*-cysteine and GSH which are relatively abundant in mammals.[2]

Fluoroquinolones were introduced into clinical practice for the treatment of various bacterial infections including upper and lower respiratory infections, and some bone, skin, soft tissue infections as well as community acquired pneumonia around three decades. they showed notable antimicrobial activity, excellent pharmacokinetic properties and few side effects. [5,6] Quinolones are synthetic antibacterial compounds based on a 4-quinolone skeleton, they target bacterial type II topoisomerases, generally DNA gyrase in Gram-negative bacteria and DNA topoisomerase IV in Gram-positive bacteria, cell death is caused by trapping the topoisomerase protein–DNA complex thus disrupting normal DNA replication, inducing oxidative damage, and triggering cell death signaling mechanisms.[5,7]Most of fluroquinolones which are currently at the market have only moderate activity against many Gram-positive cocci, including *Staphylococci* and *Streptococci*. This insufficient activity has not only limited their use in infections caused by these organisms, such as respiratory tract infections, but also has been believed to be one of the reasons for the rapidly developing quinolone resistance, in addition to the misuse and overuse of antibiotics. Bacteria have developed various mechanisms for resistance, such as changing membrane permeability via porin modification, decreasing intracellular drug concentration by efflux pump systems and enzymatically deactivating the antibiotic. [8,9] As a result, there is a critical need to create new antimicrobial agents with potent anti-drug-resistant microorganism activity.

Ciprofloxacin is one of the most potent second-generation fluoroquinolones. It offers potential anti-infective therapy for a wide range of bacteria, Gram-positive, and Gram-negative and for 12 medical treatments, including veterinary uses, the Food and Drug Administration (FDA) approved ciprofloxacin as exhibits antibacterial activity with minimal side effects and good pharmacokinetic properties. In addition, ciprofloxacin has a wide range of biological profiles and has been used to examine its antimalarial, anti-fungal, anti-tumor, and antibacterial properties in different areas of medical research.[6,10] Structure activity relationship (SAR) studies of fluoroquinolones have indicated that the only an area that substitution of bulky functional group is permitted as the basic group at the C-7 position that greatly influences their antibacterial potency, spectrum and safety. It is generally believed that the action of fluoroquinolones increases

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with the increase in lipophilicity consequently increase in bioavailability and antimicrobial activity.[7,11,12]

Based on the previous aspects, we aim to investigate the antibacterial and antifungal activity of thioamide/ ciprofloxacin hybrid as H_2S donor ciprofloxacin along with assaying the H_2S release. The compounds that will be discussed as H_2S donor in this research are thioamide hybrids-based fluroquinolones.

2.Result and discussion

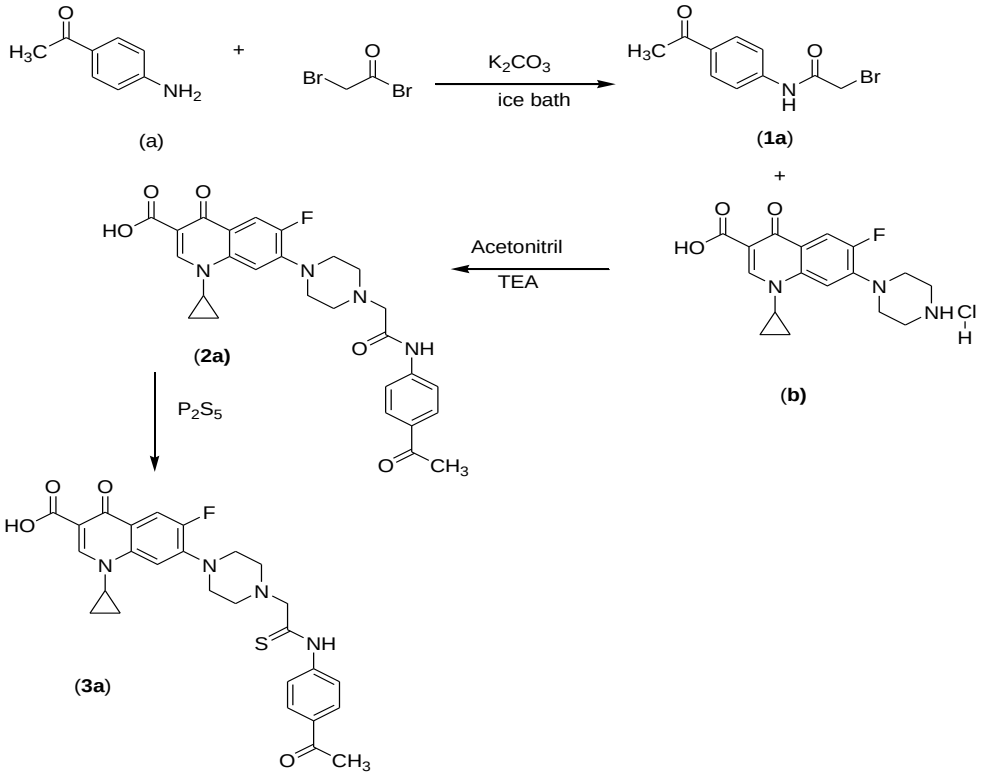
2.1. Chemistry

The target compounds were prepared as outlined in **scheme 1**. The acetylated amine**1a**, was synthesized using bromoacetyl bromide, via a biphasic reaction. Refluxing **1a** with ciprofloxacin (b) in basic medium afforded the amide **(2a)** in good yield. Thioamidation of **2a** using phosphorus pentasulphide in dioxin gave thioamide **3a**in moderate yield.

2.2. Antimicrobial activity

The target compound (**3a**) was tested as antibacterial in comparison with their amide (**2a**) to report the effect of H_2S release in such compounds using ciprofloxacin (**b**) as a reference compound, the method used was agar diffusion method, cultured with staphylococcus *aureus*, methicillin resistant *staphylococcus aureus*, *Escherichia coli* and *candida albicans* on nutrient agar plate incubated at 37C° for 24 h to show inhibition zone with Minimum Inhibiting Concentration (MIC) compared with reference compound.

as shown in the table 1 MIC of compound (**2a**) amide (0.35,0.41,0.42) shows lower MIC than reference (b) (0.58,0.56, 1.12) in gram positive bacteria and gram-negative bacteria respectively, but higher (0.61) than reference (0.43) in antifungal activity, *i.e.*, improved antibacterial activity than ciprofloxacin and decreased antifungal activity than ciprofloxacin (increase selectivity to antibacterial activity). The thioamide **3a** show (MIC) lower than reference and mild higher than its corresponding amide with *Staphylococcus aureus* (0.38) and *Escherichia coli* (0.45), and higher (MIC) than reference with Methicillin Resistant *Staphylococcus aureus* (0.75) i.e., lower antibacterial activity than ciprofloxacin, unlike its corresponding amide. compound **3a** show potent antifungal activity 10 folds higher than reference (0.06).



Scheme 1. Synthesis of the target compounds 2a and 3a.

Table 1. MIC of compounds 2a, 3a and the reference ciprofloxacin (b) against the tested strains

No.	Ciprofloxacin derivatives	MIC *(µg/ml)*			
		Staphylococcus aureus	*Methicillin Resistant Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
1	**2a**	0.35	0.41	0.42	0.61
2	**3a**	0.38	0.75	0.45	0.06
3	**b**	0.58	0.56	1.12	0.43

2.3. H_2S Release

The amount of H_2S released from the tested compound **3a** was measured using methylene blue assay method in phosphate buffer PH=7.4 in presence of *L*-cysteine as a source of thiol. The amount of H_2S released from the tested compound was measured relative to H_2S released from Na_2S standard solution.
The amount of H_2S released from the tested compound **3a** was measured was 58.64% of its theoretical release.H_2S release may implicate in increase antifungal activity of ciprofloxacin derivative (**3a**), but has low effect on its antibacterial activity.

3.Experimental

3.1. Chemistry

All chemicals and solvents used for the preparation of the intermediates and target compounds are of commercial grade and purchased from Aldrich and El-Nasr Pharmaceutical Chemical Companies. The reaction progress was monitored using and the spots were detected by exposure to UV lamp at λ 254 and 365 nm. Melting points were determined on Stuart electro-thermal melting point apparatus and were uncorrected.^{1}H NMR spectra were recorded on Bruker Advance III 400 MHz. Chemical shift (δ) in ppm relative to TMS (δ = 0 ppm) as internal standard and DMSO-d_6 or $CDCl_3$ as solvent. Coupling constant (*J*) in Hz and the signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.^{13}C NMR spectra were recorded on Bruker Advance III 100 MHz; faculty of Pharmacy, chemical shift (δ) in ppm relative to TMS (δ=0 ppm) as internal standard and DMSO-d_6 as solvent. Coupling constant (J) in Hz and the signals are designed as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

3.1.1. Synthesis of *N*-(-4-acetylphenyl)-2-bromoacetamide compound (1a)

To a stirred mixture of the appropriate amine (p-anisidine) (0.01 mmol) in dichloromethane (50 mL) and potassium carbonate (1.38gm, 0.01mmol) in 100 mL water in an ice bath, bromoacetyl bromide (3.03 g, 1.82 ml, 0.015 mmol) in 20 mL dichloromethane was added in a dropwise manner with stirring over 30 min. Stirring was continued for 2 h at 0ºC and at room temp. overnight. The reaction mixture was extracted with dichloromethane (2x60 mL) and the organic layer was washed with distilled water (2x40 mL), dried over anhydrous sodium sulfate, filtered, evaporated under vacuum and the residue was recrystallized from absolute ethanol.[13] product grayish powder , 80 % yield ,m.p:127-128℃ ;(reported 128-129℃).[14]

3.1.2. Synthesis of 7-(4-((4-formylphenylcarbamoyl) methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid compound (2a)

An equimolar mixture of compound 1a (0.8 mmol)and ciprofloxacin HCL (0.3g,0.8 mmol) in acetonitrile (50 mL) and TEA (0.17 g, 1.6 mmol) was heated at reflux for 8-10 h. Solvent was removed under reduced pressure.[13]the residue obtained was crystallized from acetonitrile to afford the target compound 2a. product :Pale yellow powder; 0.27 g 54.50 % yield; mp: 291-293ºC; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.20-1.21 (2H, m, cyclopropyl-*H*), 1.33-1.35 (2H, m, cyclopropyl-*H*), 3.21-3.34 (4H, m, piperazinyl-*H*), 3.51-3.55 (4H, m, piperazinyl-*H*), 3.86,4,26 (2H, s, N-C*H*$_2$-CO), 3.81-3.83 (1H, m, cyclopropyl-*H*), 3.74 (3H, s, OCH_3), 6.92-6.95 (2H, d, Ar-*H*), 7.54-7.56 (2H, d, Ar-*H*), 7.62-7.63 (1H, *J* = 8 Hz d, *H*-8), 7.92 (1H, d, *J* = 12 Hz, *H*-5), 8.67 (1H, s, *H*-2),10.69(1H, s, NH), 15.13 (1H, brs, COO*H*); ^{13}C-NMR (100 MHz, DMSO-d_6) δ7.63, 36.0, 46.30, 51.51, 55.21, 106.85, 106.94, 111.12, 111.35, 114.05, 119.42, 121.05, 131.03, 139.09,143.64,143.72, 148.23, 151.58,154.06, 155.82, 165.84, 176.37

3.1.3. Synthesis of 7-(4-((4-acetylphenylthiocarbamoyl) methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid compound (3a)

To a stirred mixture of compound 2a (0.1 mmol) in 2ml of dioxin (0.2mmol) of P_2S_5 were added, the mixture is heated at 60-70℃ for 6 hours, ice was added to the reaction mixture to precipitate the product, filtration to afford the target compound 3a, product :Pale yellow powder;
0.2 g40.50 % yield; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.19-1.23 (2H, m, cyclopropyl-*H*), 1.33-1.35 (2H, m, cyclopropyl-*H*), 2.92 (4H, s, piperazinyl-*H*), 3.42-3.47 (4H, m, piperazinyl-*H*), 3.76 (3H, s, OCH_3),3.80-3.83 (1H, m, cyclopropyl-*H*),3.80,4.58 (2H, s, N-C*H*$_2$-CO), 6.89-6.91 (2H, d, Ar-*H*), 7.54-7.55 (2H, d, Ar-*H*), 7.59 (1H, *J* = 8 Hz d, *H*-8), 7.92 (1H, d, *J* = 12 Hz, *H*-5), 8.67 (1H, s, *H*-2),9.8(1H,s,NH), 15.13 (1H, brs, COO*H*); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 7.61, 35.92, 48.64 , 51.67, 52.16, 55.19, 60.29, 106.45, 106.77, 110.92, 111.15, 113.97, 118.73, 121.14, 131.54, 139.18, 144.77, 144.90, 148.04, 151.73, 154.21, 155.49, 161.52, 163.03, 165.92, 166.34, 176.38

3.2. H_2S Release measurement

A 5mM solution of Na_2S in sodium phosphate buffer (20 mM, pH7.4) was prepared ($Na_2S \cdot 9H_2O$, 120.20 mg in 100 mL volumetric flask) and used as the stock solution. Aliquots of 50, 100, 200, 400, 600, 800,1000, 1500 µL of the Na2S stock solution were added into a 50 mL volumetric flask and dissolved in sodium phosphate buffer to obtain the standard solutions in 5, 10, 20, 40, 60, 80, 100, 150 µM, respectively.
1 mL aliquot of the respective solution was reacted with the methylene blue (MB+) cocktail: 30mM FeCl3 (200 µL) in 1.2M HCl, 20mM of N, N-dimethyl-1,4- phenylenediamine sulfate (200 µL) in 7.2M HCl, 1%w/v of Zn $(OAc)_2$ (100 µL) in H_2O at room temperature for at least 15 min (each reaction was performed in triplicate). The absorbance of methylene blue was measured at λ_{max}=670 nm in UV–Vis spectrophotometer (Lambda25). The Na_2S calibration curve was obtained.
The reaction was initiated by adding 15 µL of stock solution of the compounds (60 µM) into pH7.4 phosphate buffer (30 mL) containing accelerator for L-cysteine (1.0 mM). Then 2.0 mL of reaction aliquots were periodically taken and transferred to colorimetric cuvette containing zinc acetate (1%w/v, 200 µL) and N,N-dimethyl-1,4-phenylenediamine sulfate (20 mM, 400 µL) in 7.2M HCl and ferric chloride (30 mM, 400 µL) in 1.2M HCl. The absorbance (670 nm) of the resulted solution was determined 15 min thereafter using an UV–Vis spectrometer (Lambda25). The H2S concentration of each sample was calculated against a calibration curve of Na_2S.

3.3. Antimicrobial activity

Agar diffusion method was carried by spreading the microbial inoculum of *S. aureus***,** MRSA**,** *E. coli* and *C. albicans* across the entire agar surface. The tested compounds are then added to the well in a volume (20-100 uL) at the different concentration **(**1,0.5,0.25,0.125mg**)** after an aseptic hole of 6 to 8 mm in diameter is punched with a sterile tip. The test microorganism is then placed on an appropriate agar plate, and the incubation

process is continued to 24hours, Inhibition zones were detected and MIC were obtained from concentration-inhibition zone curve.

Conclusion

The substitution of C-7 position in ciprofloxacin with bulky functional group greatly influences their antibacterial potency, spectrum and safety, Amidic group mainly enhance antibacterial activity only, however, thioamide group mainly enhances antifungal activity with notable change on the antibacterial activity of some bacterial strains. Therefore, it is obviously reveled that H_2S release is implicated in enhancing antifungal effect greater than the antibacterial effect that further studies may be needed in future.

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