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Original Article

Synthesis, characterization and biological evaluation of new derivatives of ciprofloxacin and norfloxacin of interesting biological activities

Shakir Mahmood Alwan[1] and Awatef Abid Ebrahem[2]

[1]Pharmaceutical Chemistry Department, College of Pharmacy, University of Baghdad, Bab Al-Moadham, P.O. Box 14026, Baghdad, Iraq
[2]Pharmaceutical Chemistry Department, College of Pharmacy, University of Al-Mustansirya, Iraq

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ABSTRACT

Synthesis of new derivatives of ciprofloxacin **2** and norfloxacin **3** containing a tryptophan moiety linked to their NH of piperazine moiety forming amides was achieved by reaction of the carboxyl group of L-tryptophan. The chemical structures of these new compounds were confirmed by elemental (CHN) and spectral (IR, ^{1}H NMR) analyses. The minimum inhibitory concentrations (MICs) of these derivatives (**2** and **3)** were determined by a serial dilution method using *B. Subtilus* and *E. coli* and the results were comparable and promising, when compared with the parent compounds. The cell viability of these derivatives was also determined and the antitumor activity against three types of human cancer cell lines (Breast, Skin and Colorectal) was evaluated. Compound **2** has significant antiproliferative activity against breast and skin cancer cells, although slightly less active than ciprofloxacin. This result indicated that the new derivative of tryptophan-ciprofloxacin has selective antitumor activity against cancer cells and worth further investigation.

Keywords: ciprofloxacin, norfloxacin, breast cancer cells, colorectal cancer cell, human melanoma cells, tryptophan.

INTRODUCTION

Fluoroquinolones (FQ) is a family of synthetic potent and broad-spectrum bactericidal agents used against many clinically important pathogens, which are responsible for a variety of infections [**1**]. The FQ are widely used for the treatment of serious infections caused by G (-) organisms, including *Pseudomonas* species. The newer FQ have a wider clinical use and broader spectrum of antibacterial activities against G (+) and G (-), aerobic and anaerobic organisms. Most of the FQ are highly specific for prokaryotic type II topoisomerases and they are also very active against eukaryotic topoisomerases II [**1-3**]. Addition of various carboxylic acids at the NH- piperazine moiety forming amides showed good activity against G (+) and G (-) [**4**]. Linkage of bulky anthracene and phenanthrene moieties, as amides on the NH-piperazine reduced the antibacterial activities against G (+) and G (-) [**5**]. Quinolones represent a large number of antiproliferative agents exhibiting cytotoxicity through DNA interaction [**6-8**]. Ciprofloxacin has been shown to induce DNA strand breaks and to be clastogenic in mammalian cells [**9].** It is also known to induce G2M Cells and cycle arrest and apoptosis in a variety of cancer cell lines [**4, 10**].Ciprofloxacin was found to inhibit tumor cell growth of bladder transitional cell carcinoma and prostate cancer cell lines [**10-12**]. Ciprofloxacin acts as an anticancer drug against bladder cancer cells [**11]** and is distinguished by its strong inhibition of topoisomerase II. N-piprazinyl derivatives of ciprofloxacin showed significant cytotoxic activity against human breast tumor cell lines [**11].** Indole nucleus is continuously drawing attention for the development of newer drugs, due to its wide range of activities, such as anticancer, antibacterial, antifungal, anti-malarial, anticonvulsant and anti-inflammatory [**13-15**]. Indole-containing compounds also have appreciable antibacterial activities [**13, 16, 17**]. The objectives of synthesizing newer FQ should include; oral and parenteral dosing, a much broader spectrum of activity, good tissue distribution, improved pharmacokinetic profiles, good stability and a comparatively low incidence of adverse effects. In view of these desired objectives, an attempt was considered to design and synthesize new derivatives of ciprofloxacin and norfloxacin

***Corresponding Author Address: Dr. Shakir Mahmood Alwan,** Assist. Prof. in Medicinal Chemistry, College of pharmacy, University of Baghdad; E-mail: shakmawales@yahoo.co.uk*

containing tryptophan moiety linked through an amide at the NH-piperazine moiety of these drugs, in order to improve the antibacterial and/or the antitumor activity of these drugs.

MATERIALS AND METHODS

General: Melting points were determined (uncorrected) by using electrical melting point apparatus, Electro-thermal 9300, USA. Infrared spectra were recorded in KBr disk using FT- IR spectrophotometer/ Shimadzu. Elemental micro-analyses were performed by Euro-vector EA 3000A. ^{1}H-NMR (400 MHz, DMSO-d6, δ / ppm), Bruker spectrometer, Switzerland. UV Spectra were recorded by UV Spectrophotometer type Analytikjena-Specord 40/ Germany. The products were detected for purity by passing through HPLC column (Waters Breeze, C18 with flow rate of 1.4mL/min.) using absolute methanol as a mobile phase. Ciprofloxacin and norfloxacin in methanol showed λ max at 287nm. Ciprofloxacin was obtained from Samara Drug Industries, Iraq. Norfloxacin was a kind gift from the JPM/Jordan. L-Tryptophan was obtained from Himedia. Ethyl chloroformate (ECF) was purchased from Sigma AG. MTT was purchased from Molecular Probes (Eugene, UR).

Chemical Synthesis: New derivatives of ciprofloxacin and norfloxacin were prepared by the formation of amides at NH-piperazine moiety with L-tryptophan carboxyl group. The α-carboxyl group of tryptophan was reacted with the NH-piperazine using ethylchloroformate (ECF) according to the mixed anhydride method [**18].**

Synthesis of amine-protected L-tryptophan 1a ***Ethyl-3-(2-(ethoxycarbonylamino)-3-methoxy-3-oxopropyl)-1H-indole-1-carboxylate:*** Protection of the amino groups of tryptophan was performed by ECF [**18**] and as follows; To a solution of L-tryptophan (10 mMol, 3.38gm) in NaOH (4N, 10mL) cooled in an ice bath at -5 to -10°C, a cold solution of ECF (10 mMol, 1.8 ml) in dioxane (10mL) was added drop wise with continuous stirring during 10min. A yellowish product was obtained, washed with distilled water, dried, triturated with petroleum ether (2 x 10mL) and then dried in an oven at 50°C. The crude yellowish oily residue was collected to afford compound **1a** (scheme). Yield: 73%, yellowish oily product, The IR spectra (υ, cm^{-1}); 3300-3550 (broad, NH indol and NH amide), 2980 (C-H str. vib. aromatic), 1705-1780 (broad, represent C=O of COOH and C=O of amide). This was freshly prepared and used directly for the next reaction step.

Synthesis of protected tryptophan-ciprofloxacin and tryptophan-norfloxacin 2a, 3a: Synthesis of these derivatives was performed by applying the mixed anhydride method [**18**] using ECF to afford compounds **2a** and **3a.** Compound **1a** (10 mMol) in dioxane (10mL) containing TEA (10 mMol) was reacted with ECF (10mMol) in an ice bath at -5 to -10°C, as previously described. Ciprofloxacin or norfloxacin sodium (10 mMol) was dissolved in distilled water (10mL) and was added at once with vigorous stirring to the above mixture. The reaction mixture was then stirred overnight at room temperature. A white precipitate was formed, filtered, washed with acetone and recrystallized with hot ethanol and dried in an oven at 50 ºC (scheme).

Synthesis of the amine-protected tryptophan-ciprofloxacin 2a ***1-cyclopropyl-7-(4-(3-(1-(ethoxy carbonyl)-1H-indol-3-yl)-2(ethoxycarbonylamino) -propanoyl)-piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid:*** Compound **1a** (10 mmol, 2.67g) in dioxane (10mL) containing TEA (10 mMol, 1.39 mL) was reacted with ECF (10mMol, 0.9 mL) in an ice bath at -5 to -10°C. Ciprofloxacin sodium (10 mMol, 3.4 g) in distilled water (20 mL) was added to the above solution and the mixture was treated as previously described (Scheme).Yield 70%, white powder, mp 230 ºC (decomposed). Anal. Calcd. for $C_{34}H_{35}FN_5O_8$ (660): C; 61.82, H; 5.30, N; 10.6. Found: C, 61.35, H; 5.18; N, 11.1. The IR spectra (υ, cm^{-1}) showed the following characteristic bands; 3402 (NH amide), 3097 (C=H str. aromatic), 1722 (C=O str. COOH), 1695 and 1629 (str. vib. of C=O amides). The ^{1}H-NMR spectra (δ, ppm); 1.22, 4.3 (CH and-CH_2, cyclopropyl), 1.3 (-CH_3), 3.3 (-CH_2-, tryptophan), 3.39-3.59 (-CH_2- piperazinyl protons), 4.06 (-CH-, tryptophan), 7.17 (-CH-, indol), 6.95-7.67 (CH, aromatic), 6.95, 8.1 and 8.66 (C_9-CH, C_6-CH and C_2-CH quinolone, respectively).

Synthesis of the amine-protected tryptophan-norfloxacin 3a ***7-(4-(3-(1-(ethoxycarbonyl)-1H-indol-3-yl)-2-(ethoxycarbonylamino)-propanoyl)-piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid:*** Compound **1a** (10 mMol, 2.67g) in dioxane (10mL) containing TEA (10 mMol, 1.39 mL) was reacted with ECF (10mMol, 0.9mL) in an ice bath at -5 to -10°C. Norfloxacin sodium (10 mMol, 3.4 g) in distilled water (20 mL) was added to the above solution and the mixture was treated as previously described (scheme).Yield 72%, white powder, mp 227°C (decomposed). Anal. Calcd. for $C_{33}H_{35}FN_5O_8$ (648): C; 61.11, H; 5.40, N; 10.8. Found: C, 60.86, H; 5.10, N; 10.46. The IR spectra (υ, cm^{-1}); 3475 (OH str. of COOH), 3075 (C-H str. of aromatic), 2983 (C-H of methyl), 1707 (C=O str. of COOH), 1627, 1676 (str. of amides) and 1143 (C-F). The ^{1}H-NMR spectra (δ, ppm); 1.22, 1.39 (CH_3 and-

CH_2, carbamoyl, respectively), 7.92 and 8.66 (C_2-CH, C_8-CH, quinolone, respectively), 3.32-3.59 (-CH_2-, piperazinyl protons), 7.21-7.92 (CH, aromatic), 3.3 (-CH_2-, tryptophan), 3.28 (-CH-, tryptophan).

***Chemical synthesis of tryptophan-ciprofloxacin and tryptophan-norfloxacin (2 and 3)*:** These compounds were prepared by deprotection of the ethylformate protecting group in compounds **2a** and **3a** (10mMol) using trifluoroacetic acid, TFAA (10mMol) [**19**]. Compounds **2a** or **3a** in dichloromethane (DCM) was reacted with TFAA and the mixture was stirred at room temperature for 2h. Cold distilled water (20 ml) was added and the pH was adjusted to 7 with sodium bicarbonate solution (5%). The mixture was filtered and the precipitate was collected, washed several times with distilled water and dried in an oven at 50°C. The precipitate was triturated with petroleum ether to afford compounds **2** or **3.**

Synthesis of tryptophan-ciprofloxacin 2 7-(4-(2-amino-3-(1H-indol-3-yl)-propanoyl)-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro quinoline-3-carboxylic acid: Compound **2a** (1.66mMol, 0.98g) in DCM (10mL) was reacted with TFAA (1.66mMol, 2mL) with continuous stirring for 2 h in an ice bath at 0°C and the mixture was treated as previously described to afford tryptophan-ciprofloxacin **2** (scheme). Yield 74.5%, white powder, mp 262-264^{o}C. The IR spectra (υ, cm^{-1}); 3431 (NH of amide), 3059 (C-H str. aromatic), 2860 (C-H str. methyl), 1710 (C=O COOH), 1674, 1624 (str. C=O amide) and 1112 (C-F). Anal. Calcd. for $C_{28}H_{28}FN_5O_4$ (517.55): C; 64.98, H: 5.45, N: 13.53; Found; C: 63.545, H: 4.94, N: 14.526. The ^{1}H-NMR spectra (δ, ppm); 1.22, 4.3 (CH and -CH_2, cyclopropyl), 6.95, 7.92 and 8.66 (C_9-CH, C_8-CH and C_2- CH quinolone, respectively), 3.39-3.59 (-CH_2-, piperazinyl protons), 6.95-7.67 (CH, aromatic), 3.3 (-CH_2-, tryptophan), 4.06 (-CH-, tryptophan), 7.17 (-CH-, indol), 5.3 (NH_2 of tryptophan).

Synthesis of tryptophan-norfloxacin 3 ***7-(4-(2-amino-3-(1H-indol-3-yl) propanoyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid:*** Compound **3a** (1.66 mMol, 0.97g) in DCM (10 mL) was reacted with TFAA (1.66mMol, 2mL), as previously described. An off-white powder of tryptophan-norfloxacin **3** (scheme) was obtained. Yield 78%, mp 265-267°C. Anal. Calcd. for $C_{27}H_{28}FN_5O_4$ (515.54): C; 64.15, H; 6.45, N; 13.85. Found: C; 71.089, H; 6,712, N; 11.363. The IR spectra (υ, cm^{-1}); 3430-3440 (str. OH of carboxyl), 1685 (str. C=O amide), 1138 (C-F). The ^{1}H-NMR spectra (δ, ppm); 6.95, 7.92 and 8.66 (C_9-CH, C_8-CH and C_2- CH quinolone, respectively), 3.32-3.59 (-CH_2-, piperazinyl protons), 7.21-7.92 (CH, aromatic), 3.3 (-CH_2-, tryptophan), 4.9 (-CH-, tryptophan), 7.23 (-CH-, indol), 5.3 (NH_2 of tryptophan).

***Determination of the minimum inhibitory concentrations (MICs values)*:** The MIC values (µg/mL) were determined by the serial dilution method [**20]**.The new derivatives were dissolved in dimethylsulphoxide (DMSO). Ciprofloxacin (30 µg) was used as the standard antibacterial drug. DMSO was diluted with distilled water (1:30) and used as the solvent. The microtitre plates were incubated at 37 °C for 24 h and were examined for growth using automatic reader at 400 nm. The MICs values were the lowest concentrations in the medium that completely kill growth. All experiments were performed in triplicates. The breakpoints indicated in the last edition of the National Committee for Clinical Laboratory Standards, 2003 (NCCLS tables M100-S13 (M7) were used to determine the susceptibility and resistance.

Cell Viability Assay: **Cell Lines:** Human melanoma (MV3), breast cancer (MCF7), colorectal (HCT116) cell lines were used to evaluate the antitumor activity. The cytotoxic effect of the newly synthesized derivatives of ciprofloxacin and norfloxacin on human cancer cells was determined using MTT assay [**21**]. Briefly, cells were seeded at 5000/well onto flat-bottomed 96-well culture plates and allowed to grow for 24h before treatment with the compounds. Cells were then labeled with MTT from Vybrant MTT cell proliferation assay kit and the resulting formazan was solubilized with dimethylsulphoxide and read at 540nm.

RESULTS AND DISCUSSION

***Chemical synthesis*:** The chemical synthesis of **2** and **3** and their intermediates were successfully achieved with reasonable yields (scheme). Their chemical structures were confirmed by spectral and elemental microanalysis (CHN). The IR spectra of **2** and **3** showed the following characteristic absorption bands (υ, cm^{-1}); 3430-3440 (broad NH of amide of N-piperazine, NH indol, α-NH_2 of tryptophan and OH of carboxyl of quinolone nucleus. 1134 (C-F at C-6 position) and 1695-1629 for C=O stretching vibration of amides. The (CHN) analysis of the synthesized compounds confirmed the proposed chemical structures and the results comply with the acceptable range. The ^{1}H-NMR results displayed certain protons that indicated and confirmed the chemical structures and the detailed results were listed for each compound.

Minimum Inhibitory Concentrations (MICs): The MIC values (µg/mL) were determined by the serial dilution method [**20**]. The new derivatives (30 µg) showed less antimicrobial activities when compared with ciprofloxacin (30 µg). However, compound **2** has comparable and promising results, while, compound **3** showed poor results compared to those of ciprofloxacin and the results are shown on Table.

Antitumor Activity: The results of the cytotoxic effect of the newly synthesized derivatives **2** and **3** on human cancer cells are summarized on Table. Compound **2** retained the activity, although was slightly less potent than ciprofloxacin. The incorporation of an indole moiety was expected to add antitumor activity on top of that of ciprofloxacin. However, retaining the activity is a success for such complicated molecule. Compound **3 s**howed much less activity against microbes and cancer cell lines.

CONCLUSION

The linkage of the carboxyl moiety of tryptophan to the NH-piperazine of ciprofloxacin through an amide bond was successful and the antimicrobial and antitumor activities were retained and showed promising results when compared with ciprofloxacin and this may be considered as a successful and encouraging result for further investigations.

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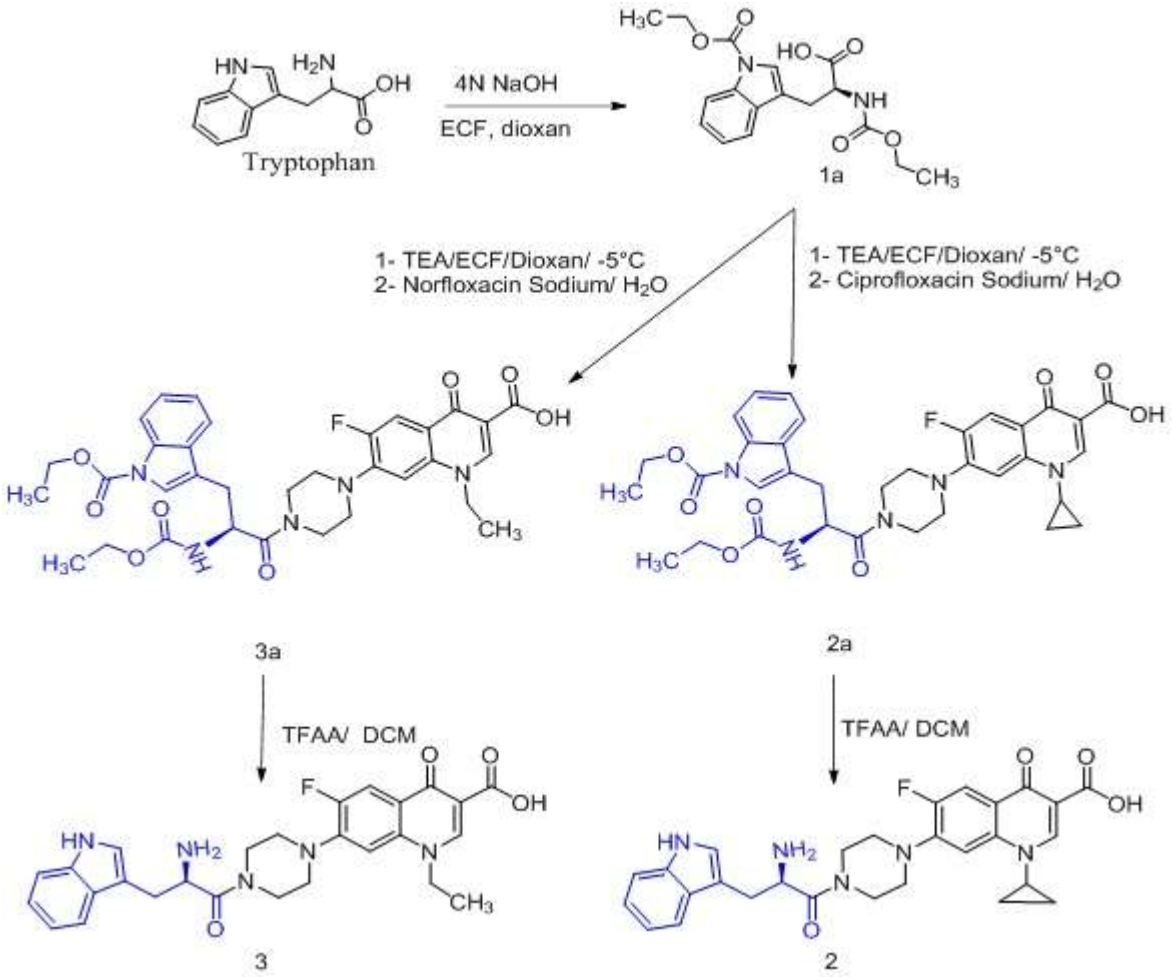

Scheme: Chemical synthesis of tryptophan-ciprofloxacin and tryptophan-norfloxacin derivatives.

Table: The biological activities of the new derivatives of ciprofloxacin 2 and norfloxacin 3.

Compound	MICs (µg/ml)		Antitumor activity IC50 (µg/ml)		
	B. Subtilus	***E. Coli***	**MCF-7**	**MV-3**	**HCT-116**
2	**< 25**	**25**	**< 50**	**< 100**	**> 200**
3	**< 100**	**> 200**	**> 200**	**> 200**	**> 200**
Ciprofloxacin	**< 25**	**12.5**	**< 20**	**< 50**	**< 20**

Key notes: MCF-7: breast cancer cell line; MV-3: skin cancer cell line; HCT-116: colorectal cancer cell lines. MIC (µg/ml): < 25 = highly sensitive, < 100 = Intermediate sensitive, > 200 = least sensitive.

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