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Research paper

Novel ciprofloxacin hybrids using biology oriented drug synthesis (BIODS) approach: Anticancer activity, effects on cell cycle profile, caspase-3 mediated apoptosis, topoisomerase II inhibition, and antibacterial activity

Asmaa E. Kassab*, Ehab M. Gedawy

Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, 33 Kasr El-Aini Street, Cairo 11562, Egypt

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ABSTRACT

As we are interested in synthetizing biologically active leads with dual anticancer and antibacterial activity, we adopted biology oriented drug synthesis (BIODS) strategy to synthesize a series of novel ciprofloxacin (CP) hybrids. The National Cancer Institute (USA) selected seventeen newly synthesized compounds for anticancer evaluation against 59 different human tumor cell lines. Five compounds **3e**, **3f**, **3h**, **3o** and **3p** were further studied through determination of IC_{50} values against the most sensitive cancer cell lines. *In vitro* results showed that the five compounds exhibited potent anticancer activity against test cell lines in nanomolar to micromolar range, with IC_{50} values between 0.72 and 4.92 μM, which was 9 to1.5 folds more potent than doxorubicin. In this study, two promising potent anticancer CP hybrids, **3f** and **3o,** were identified. The anti-proliferative activity of these compounds appears to correlate well with their ability to inhibit Topo II ($IC_{50} = 0.58$ and 0.86 μM). It is worth mentioning that compound **3f** was 6 folds more potent than doxorubicin, 5 folds more potent than amsacrine and 1.5 folds more potent than etoposide. At the same time, compound **3o** showed 4 folds more inhibitory activity against Topo II than doxorubicin, 3 folds more potent than amsacrine and almost equipotent activity to etoposide. Activation of damage response pathway of the DNA leads to cell cycle arrest at G2/M phase, accumulation of cells in pre-G1 phase and annexin-V and propidium iodide staining, indicating that cell death proceeds through an apoptotic mechanism. Moreover, compounds **3f** and **3o** showed potent pro-apoptotic effect through induction of the intrinsic mitochondrial pathway of apoptosis. This mechanistic pathway was confirmed by a significant increase in the level of active caspase-3 compared to control. This observation may indicate that both CP hybrids can chelate with zinc, a powerful inhibitor of procaspase-3 enzymatic activity, so procaspase-3 may process itself to the active form. The synthesized CP derivatives were tested for their *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* strains. The results proved that all of the test compounds have shown good to excellent antibacterial activity, as compared to its parent molecule ciprofloxacin. Compounds **2**, **3b**, **3k**, **3l**, **3m**, **3p, 5a**, **5b**, **5d** and **5e** exhibited equipotent or comparable activity to ciprofloxacin against the test strains. Compounds **3p** and **5a** were more potent than ciprofloxacin against *Pseudomonas aeruginosa,* a common organism causing infections in granulocytopenic cancer patients.

1. Introduction

Bacterial infections are a major cause of complications and death in cancer patients who become granulocytopenic because of intensive myelosuppressive chemotherapy [1,2]. In addition, infections are recognized of major obstacles impeding the successful management of patients with malignant diseases. The most common organisms causing these infections include *Pseudomonas aeruginosa* and *Staphylococcus aureus* [3]. There are hopes to find a much-needed antibiotic that may exert both antimicrobial and

* Corresponding author. Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt.
E-mail address: asmaa.kassab@pharma.cu.edu.eg (A.E. Kassab).

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antitumor activity, to be used for prophylaxis as well as for treatment of bacterial superinfections in cancer patients, while being effective in preventing growth of cancer cells. Recently, a great deal of work has been devoted toward anticancer activity of fluoroquinolones and several studies prove them as potent cytotoxic agents [4–9]. Ciprofloxacin (CP), a commonly used broad-spectrum fluoroquinolone antibiotic, exhibit anti-proliferative activities against different human tumor cell lines. It is reported that CP accumulates in high concentration in urine, lung tissues and prostate gland, so it seems to be a good candidate for the treatment of bladder, lung and prostate cancers [10,11]. Moreover, several studies show efficient anti-proliferative activity of CP against colorectal, osteosarcoma and leukemic human cancer cell lines [7,12,13]. CP antitumor effect emanates mainly from its ability to inhibit significantly topoisomerase II enzyme (Topo II), which is a common cellular target for quinolone antibacterial agents and several clinically potent anticancer drugs, such as doxorubicin and mitoxantrone [14–16]. Furthermore, CP exerts its anticancer activity *via* other mechanisms, such as induction of intrinsic apoptotic pathway [17,18] by creating double strand breaks in nucleic acid or cell cycle arrest at the S/G2 stage [6]. Additionally, fluoroquinolones appear to have immunopotentiating properties [19,20], thus, CP is a privileged scaffold for designing new anticancer agents. The SAR studies reveal that fluorine atom, the 1-alkyl and 1,4-dihydro-4-oxo-quinoline-3-carboxylic acid skeleton are the essential pharmacophoric features for fluoroquinolones potency, as required for hydrogen-bonding interactions with DNA bases and Topo II inhibition [21,22]. It has become clear that novel fluoroquinolone derivatives may be designed through introduction of different modifications at N-4 position of piperazine, which improves the lipophilicity and the antibacterial efficacy [23,24]. The same strategy has been explored to develop improved Topo inhibitors as anticancer agents. Gimatecan is a modified lipophilic camptothecin developed to provide rapid uptake and accumulation in cells and a stable Topo DNA drug ternary complex [25]. Recently, *N*-acylarylhydrazone scaffold, played an important role as a building unit for several anticancer agents as PAC-1 (Fig. 1) due to the presence of hydrogen bond donors and acceptors, as well as to its flexible skeleton [26–28]. SAR studies prove that the anticancer activity of PAC-1 is dependent on the presence of the *o*-hydroxy *N*-acyl hydrazone motif that can chelate zinc, which is a powerful inhibitor of procaspase-3 enzymatic activity. Such mechanism allows procaspase-3 to process itself to the active form [29–31]. The 1, 3, 4-oxadiazole heterocycle, a good bioisostere of amides and esters, is widely used in the design and discovery of novel bioactive molecules and drugs. When combined with other heterocycles, it contributes in improving the pharmacological activity, as it can readily binds with various enzymes and receptors through hydrogen bonds [32,33]. Moreover, oxadiazole is a common structural motif of several marketed drugs, such as Zibotentan ® (Fig. 1) [34], a potent anticancer drug, and Furamizole antibiotic (Fig. 1) [35]. One of the most exciting new approaches frequently used in modern medicinal chemistry for the exploration of novel and highly potent molecules that triggers two or more cytocidal pharmacological mechanisms of action is biology oriented drug synthesis (BIODS). This strategy aims to the design and synthesis of libraries of compounds through structural modification on the skeleton of marketed drugs, to explore further biological potential [36–38]. Therefore, given our interest in the synthesis of biologically active leads with dual anticancer and antibacterial activity, we have constructed a 'biology oriented drug synthesis (BIODS)' model (Fig. 1), with Topo II inhibitor structural features, using CP core substituted at N-4 position of piperazine with biologically active building blocks, such as *N*-acylarylhydrazone (an apoptosis inducer), oxadiazole or its bioisostere triazole scaffolds. Various aryl or heteroaryl groups were introduced to the *N*-acylarylhydrazone moiety and diverse substitutions on the oxadiazole ring were established with the purpose of exploring the influence of substituents on anticancer and antibacterial activity. The anticancer activity of seventeen new compounds was evaluated against a panel of 59 human tumor cell lines provided by National Cancer Institute (USA). Five potent compounds were selected to be further studied through determination of their half maximal inhibitory concentration (IC_{50}) values against the most sensitive cancer cell lines. We anticipated that the designed molecules may be active against different biological targets, in order to explore the mechanistic pathways of the anticancer activity of the synthesized compounds, we chose the most potent compounds, **3f** and **3o**, to perform extra investigations, such as cell cycle analysis, Topo II assay and apoptosis marker (caspase-3). All the synthesized compounds were tested for their *in vitro* antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* strains.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds is outlined in Schemes 1 and 2. The primary starting **1** [39], was prepared by reacting ciprofloxacin with ethyl chloroacetate in dimethylformamide in the presence of triethylamine. The ^{1}H NMR spectrum of **1** displayed the presence of triplet and quartet signals at δ 1.27 and 4.14–4.19 ppm, respectively corresponding to CH_3CH_2 group. Compound **1** was reacted with hydrazine hydrate to afford **2**. The ^{1}H NMR spectrum of this compound displayed the presence of exchangeable singlet signals at δ 4.38 and 8.99 ppm, sequentially corresponding to NH_2 and NH protons. The hydrazones **3a-p** were obtained through reacting compound **2** with the appropriate aldehyde in ethanol. The ^{1}H NMR spectra of **3a-p** displayed the disappearance of the signal corresponding to NH_2 of the parent compound **2** in addition, they showed the presence of signals corresponding to different Ar-CH=N groups which were not present in **2**. Refluxing **2** with carbon disulphide in ethanolic potassium hydroxide gave the oxadiazole derivative **4**. The ^{1}H NMR spectrum of **4** showed the NH/SH exchangeable singlet signals at δ 14.63 and 14.67 ppm. The substitutedthio derivatives **5a-f** were achieved through the reaction of the appropriate halogen compound with **4** in DMF in the presence of potassium hydroxide. The ^{1}H NMR spectra of these compounds revealed the disappearance of the signal of NH/SH proton and appearance of the expected signals corresponding to the S-substituted groups which are indicative for the success of alkylation. Finally, compound **4** was reacted with hydrazine hydrate to give **6**. The ^{1}H NMR spectrum of **6** displayed the presence of exchangeable singlet signal at δ 5.58 ppm, corresponding to NH_2 protons.

2.2. Growth inhibition against a panel of 59 human tumor cell lines

In this study, 17 newly synthesized compounds were selected by National Cancer Institute (USA) for anticancer evaluation. The selected compounds were evaluated at a single dose (10^{-5} M) against 59 different human tumor cell lines, representing leukemia, melanoma and cancers of lung, colon, central nervous system (CNS), ovary, kidney, prostate as well as breast. The growth inhibition percentages (GI%) obtained from the single dose test (when GI% > 20%) for compounds **2**, **3b**, **3e**, **3g**, **3n, 3o**, **3p**, **4**, **5a**, **5d** and **5f** are shown in Table 1 and Table 2. Regarding the activity toward leukemia; compounds **2**, **3e**, **3p** and **4** showed GI% values in the range of 20.51–54.38 against this cancer type. Compound **3p** was