ANTICANCER RESEARCH *40*: 5025-5033 (2020)
doi:10.21873/anticanres.14505

A New Ciprofloxacin-derivative Inhibits Proliferation and Suppresses the Migration Ability of HeLa Cells

MOUSTAFA FATHY[1,2], SIJIA SUN[3], QING-LI ZHAO[4], MOHAMED ABDEL-AZIZ[5], GAMAL EL-DIN A. ABUO-RAHMA[5], SURESH AWALE[3] and TOSHIO NIKAIDO[1]

[1]*Department of Regenerative Medicine, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan;*
[2]*Department of Biochemistry, Faculty of Pharmacy, Minia University, Minia, Egypt;*
[3]*Division of Natural Drug Discovery, Institute of Natural Medicine, University of Toyama, Toyama, Japan;*
[4]*Department of Radiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan;*
[5]*Department of Medicinal Chemistry, Faculty of Pharmacy, Minia University, Minia, Egypt*

Abstract. *Background/Aim: This study aimed to investigate the effect of a new 7-(4-(N-substituted carbamoylmethyl) piperazin-1-yl) ciprofloxacin-derivative on the proliferation and migration abilities of HeLa cells. Materials and Methods: Cell viability and morphological alterations were examined. Changes in migration were detected using wound healing and colony formation assays. Flow cytometry and western blotting were used to investigate the molecular mechanisms underlying this ciprofloxacin-derivative's action in HeLa cells. Results: The examined ciprofloxacin-derivative reduced viability of HeLa cells in a concentration-dependent manner and altered cellular morphology, indicating cell death. Furthermore, it significantly inhibited wound closure, even in a non-cytotoxic concentration, and reduced HeLa cell colony formation. In addition, apoptosis was increased probably through significant up-regulation of Bax protein expression and the generation of active cleaved caspase-3 protein. Conclusion: Our new derivative inhibits proliferation and induces apoptosis of HeLa cells. Furthermore, it suppressed the migration and colony formation abilities of HeLa cells. Therefore, it represents an attractive agent for drug development against cervical cancer based on its anti-metastatic effect.*

Correspondence to: Qing-Li Zhao, Department of Radiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. Tel: +81 764347267, Fax: +81 764345190, e-mail: zhao@med.u-toyama.ac.jp

Key Words: Apoptosis, ciprofloxacin, colony formation, HeLa cells, migration ability.

Cancer is a leading cause of death worldwide (1, 2). A total of 528,000 new cases of cervical carcinoma (CC) are diagnosed every year making it the second most common cancer in women (3, 4), and its prognosis is indigent when diagnosis occurs at the metastatic stage, which involves distant organs such as the brain, liver, lung and bone. Among women, it is the fourth leading cause of death related to cancer worldwide (5, 6). Due to the absence of an efficient treatment for patients with metastasis, CC has a five-year survival rate of 50% (7). Despite the great clinical surgical treatment achievements, recurrent, and metastatic cervical cancer remains one of the leading causes of deaths related to cancer (8). Therefore, novel therapeutic agents targeting metastases and migration of cancer cells are urgently needed.

Ciprofloxacin belongs to the category of 4-fluoroquinolone antibiotics that are remarkably employed in the treatment of many bacterial infections. It inhibits the bacterial DNA gyrase enzyme, which shows antimicrobial activity. However, this drug has additionally been shown to affect mammalian topoisomerase II (9). Recently, several studies have discussed in detail the role of fluoroquinolones, either alone or in combination with anti-cancer drugs, in inducing cell death in numerous cancer cell lines (10-12). It has been documented that the substituent insertion on the *N*-4-piperazinyl moiety of ciprofloxacin improved the physicochemical properties of the parent quinolone and produced major therapeutic changes (13-16).

According to these findings, our hypothesis was that the substituent insertion on the *N*-4-piperazinyl moiety of ciprofloxacin may potentially cause cancer cell death. Thus, the goal of this study was to investigate the anticancer effect of the new 7-(4-(*N*-substituted carbamoylmethyl) piperazin-1 yl)-derivative of ciprofloxacin. Therefore, we investigated the anti-proliferative effect of this new ciprofloxacin-

derivative on HeLa cells, human cervical carcinoma cells, and examined its ability to reduce their migration potential and induce apoptosis.

Materials and Methods

Chemistry. The examined compounds, compound 1 and 2, were prepared as reported previously through alkylation of ciprofloxacin with acetylated chalcone derivatives in acetonitrile using triethylamine as a base, as shown in Figure 1. They were identified by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry as previously reported (15).

Cell culture. HeLa cells, obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), were cultured in fresh Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich Co., St Louis, MO, USA) supplemented with 10% Fetal Bovine Serum (FBS, Biosolutions International, Melbourne, Australia), 1% L-glutamine (Sigma-Aldrich Co.), and 1% Penicillin-Streptomycin mixture (Invitrogen, Grand Island, NY, USA) and incubated in a humidified 5% CO_2 atmosphere at 37˚C.

Cell viability assay and morphological assessment. Cell viability assay was performed as previously described (17, 18) using the cell counting kit-8 (Dojindo Co., Kumamoto, Japan). Briefly, 5×10^3 cells/100 μl/well were seeded in triplicate in 96-well plates and allowed to proliferate for 24 h in fresh DMEM medium. Then the medium was replaced with DMEM containing serially diluted test compounds and cells were incubated for 72 h. The medium was then replaced with DMEM containing 10% WST-8 solution. Cells were incubated for 3 h and the absorbance was measured at 450 nm. Cell viability was expressed as a percentage relative to that of control untreated cells and the IC_{50} was calculated (*i.e.*, the compound concentration which kills 50% of the cells). Cell morphology was examined after 72 h incubation with the examined compound by capturing images using the EVOS FL cell imaging system (20× objective) in phase-contrast mode.

Colony formation assay. Cells were seeded in triplicate in 12-well plates (5×10^3 cells/well) and allowed to attach for 24 h in fresh DMEM medium. Then the medium was changed to DMEM containing the examined compound (at concentrations of 0, 2.5, 5, or 10 μM), and cells were incubated for 24 h. Then cells were washed with phosphate-buffered saline (PBS) twice and allowed to grow in fresh DMEM medium without the examined compound for 12 days at 37˚C in humidified 5% CO_2 atmosphere. Then, cells were fixed in 4% formaldehyde, washed with PBS, and stained for 15 min with crystal violet. The colony area was estimated as previously described (19).

Wound healing assay. Cells (1×10^6 cells) were seeded in triplicate in 35 mm cell culture dishes in DMEM medium and incubated at 37˚C in a humidified 5% CO_2 atmosphere for 24 h to attach. Then a wound was created in the formed monolayers, using 200 μl tips. Cells were washed with PBS, treated with a non-cytotoxic concentration of the examined compound (2.5 μM) in DMEM, and incubated for 96 h. Photos were captured every 10 min using the CytoSMART live-cell imaging system (Lonza Walkersville, Inc., Walkersville, MD, USA) in the phase-contrast mode for 96 h. The rate of the closure of the scratch at different time points was calculated using ImageJ, and the data were analyzed using GraphPad Prism 6 software.

Apoptosis determination using Annexin V-FITC/PI staining. Apoptosis was detected by flow cytometry (FCM) using the Apoptosis Detection Kit, according to the manufacturer's instructions (Immunotech, Marseille, France). Briefly, Cells (5×10^5 cells) were seeded in triplicate in 35 mm cell culture dishes in DMEM medium and incubated for attachment at 37˚C in humidified 5% CO_2 atmosphere for 24 h. Then the medium was changed to DMEM containing the examined compound (at concentrations of 0, 2.5, 5, 10, or 20 μM), and cells were incubated for 72 h before they were collected, washed with cold PBS and suspended in the binding buffer. Staining, in the dark, with the labeled Annexin V and PI was performed for 30 min at 4˚C. Cells were analyzed using the FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA), counting at least 10^4 cells. Dot plots were created, and the fraction of total apoptosis was estimated as previously described (20, 21).

Western blot analysis. Cells (5×10^5 cells) were seeded in triplicate in 35 mm cell culture dishes in DMEM medium and incubated for attachment at 37˚C in humidified 5% CO_2 atmosphere for 24 h. Then the medium was changed to DMEM containing the examined compound (at concentrations of 0, 2.5, 5, 10 or 20 μM) and cells were further incubated for 24 or 48 h before they were collected for protein extraction in RIPA lysis buffer, containing 1 M Tris–Cl; 0.05% SDS, 5 M NaCl, 1% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, and 1% Nonidet P-40, supplemented with the complete protease inhibitor cocktail (Roche, Mannheim, Germany) for 30 min. The lysates, after sonication, were centrifuged and the protein concentration in the supernatants was determined using a Bio-Rad assay kit (Bio-Rad, Hercules, CA, USA). After denaturation, the lysates were separated by electrophoresis in SDS-polyacrylamide gel (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) and transferred to nitrocellulose membranes (Amersham Biosciences, Bucks, UK) using the Bio-Rad Trans-Blot SD Cell apparatus (Bio-Rad, Hercules, CA, USA). The membranes were incubated for 1 h at room temperature in Blocking Solution before incubation at room temperature with anti-Bax, anti-caspase-3, or anti-β-actin antibodies (New England Biolabs, Ipswich, MA, USA). After washing, membranes were incubated at room temperature for 1 h with the HRP-conjugated secondary antibody (New England Biolabs). According to the manufacturer's instructions, immunoreactive proteins were detected using an enhanced chemiluminescence kit (Amersham Biosciences) and a luminescent image analyzer (LAS-4000, Fujifilm Co., Tokyo, Japan) (21, 22). Bands corresponding to protein expression were analyzed densitometrically, using the ImageJ program, relative to that of cells cultured in DMEM containing the examined compound at a concentration of 0 μM, after normalization to β-actin levels.

Statistical analysis. Results were obtained from at least three independent experiments. Data were expressed as mean±standard deviation. The statistical significance of the differences was analyzed by post hoc Tukey's test after one-way analysis of variance (ANOVA) using GraphPad Prism 6 statistical software (GraphPad Software Inc., San Diego, USA). Differences were considered significant when the probability values (p) were less than 0.05.

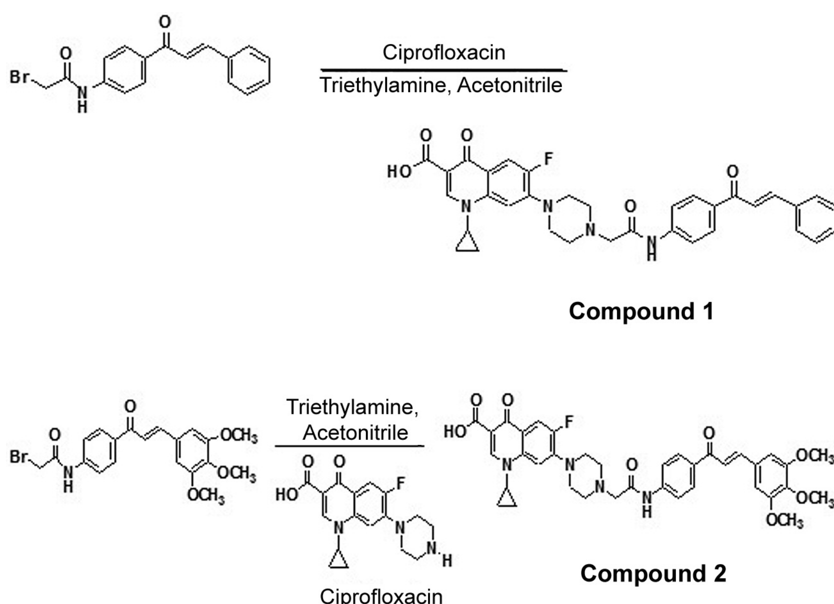

Figure 1. *Synthesis of the examined compounds (Compounds 1 and 2).*

Results

The effect on viability and morphology of HeLa cells. The effect of various concentrations of the examined compounds on the survival of HeLa cells following incubation for 72 h is shown in Figure 2A. Compound 1 showed a weak inhibitory effect on the proliferation of HeLa cells displaying an IC_{50} value greater than 100 μM. Compound 2 strongly inhibited the proliferation of HeLa cells in a concentration-dependent manner, displaying an IC_{50} value of 4.4 μM. So, compound 2 was used for all subsequent experiments to investigate its potential effect on HeLa cells.

As shown in Figure 2B, compared to the untreated cells, HeLa cells treated with different concentrations of the examined compound showed apparent abnormal alterations of cell morphology in a concentration-dependent manner.

The effect on colony formation ability. The effect of different concentrations of the examined compound on the colony formation ability of HeLa cells was investigated and the results are shown in Figure 3. Notably, after the initial treatment with 2.5, 5, and 10 μM of the examined compound for 24 h, the cells were further incubated for 12 days in culture and their colony formation ability was measured. Compared to the untreated cells, the compound significantly ($p<0.0001$) decreased colony formation in a concentration-dependent manner. Furthermore, treatment with 10 μM concentration significantly decreased the colony formation ability when compared to the treatment with 2.5 μM ($p<0.01$) or 5 μM ($p<0.05$).

The effect on HeLa cell migration. The ability of the examined compound to inhibit cell migration was investigated using a wound healing assay (Movie 1: https://www.researchgate.net/publication/340620331_Movie_1_Supporting_Movie, a time-lapse video showing the detailed events upon exposure of HeLa cells to 2.5 μM of the examined compound which is available as a video file in the HTML version of the paper). Figure 4 demonstrates that the control cells showed migration rate of 23%, 35%, 60%, and 96% at 24, 48, 72 and 96 h, respectively. However, treatment with the non-cytotoxic concentration 2.5 μM of the examined compound significantly inhibited migration to 5%, 19%, 28%, and 32% at 24, 48, 72 and 96 h, respectively.

The effect on cell apoptosis. Cell apoptosis was determined using FCM with Annexin V-FITC and PI staining. The treatment of HeLa cells with different concentrations of the examined compound induced apoptosis in a concentration-dependent manner, as shown in Figure 5. Compared to the untreated cells, the percentage of apoptosis was significantly ($p<0.0001$) increased to 22.51±1.5%, 32.73±1.4%, 43.53±2.9% or 48.4±2.08% when the cells were treated, for 72 h, with 2.5, 5, 10 or 20 μM of the examined compound, respectively.

The effect on the expression of apoptosis-associated proteins. Western blotting was performed to investigate the effect of the examined compound on the expression of the apoptosis-related proteins Bax and caspase-3 in HeLa cells. Figure 6 shows that the expression of Bax protein, after normalization to β-actin and compared to untreated cells, was significantly

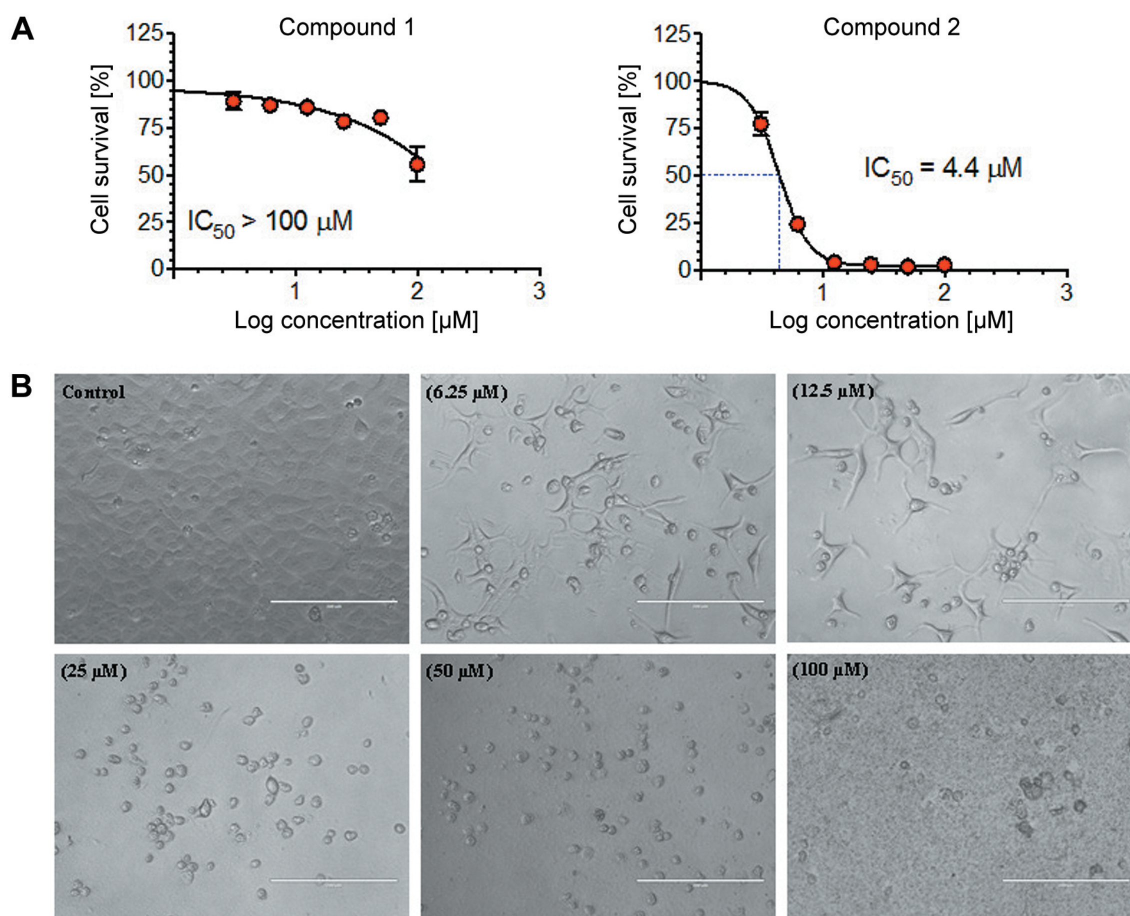

Figure 2. *Survival and morphological alterations of HeLa cells. (A) Survival of HeLa cells after treatment with different concentrations of the examined compounds for 72 h. Survival is expressed as a percentage relative to that of the untreated cells. Data represent mean±SD. (B) Morphological changes of HeLa cells after treatment with different concentrations of the examined compound (2) for 72 h (Scale bars represent 200 µm).*

increased in a concentration-dependent manner after treatment with the examined compound for 48 h ($p<0.01$ for 2.5, 5 and 10 µM and $p<0.001$ for 20 µM). There was a non-significant increase when the cells were treated for 24 h.

Furthermore, the expression of cleaved caspase-3 protein, which is a pro-apoptotic protein, was significantly ($p<0.0001$) increased in a concentration-dependent manner when the cells were treated for 48 h with 2.5, 5, 10 and 20 µM of the examined compound. There was also a significant ($p<0.05$) increase in its expression when the cells were treated for 24 h with 20 µM of the examined compound.

Discussion

In general, up to 90% of cancer-associated mortality is attributed to metastasis of highly invasive cancer cells from the primary site of cancer to distant tissues. Metastasis is a multistep phenomenon in which metastatic cancer cells separate away from the primary cancer tissue and move to another organ where they form secondary tumours (23, 24). In patients with malignant tumours, cancer metastasis, which is a malignancy hallmark, is the leading cause of mortality. Regarding cervical cancer, when patients are diagnosed at late stages with local invasion or metastasis, prognosis tumbles dramatically. Therefore, metastasis is the main reason of cervical cancer-related mortality (25, 26). Therefore, discovering new candidates affecting metastasis of cervical cancer is essential for improving effective treatment and increasing the survival rate.

Developing new anticancer drugs, which affect migration and reduce metastases, remains a challenging and a critical goal and thus the development of novel anticancer agents is very important (27). Fluoroquinolones are broad-spectrum synthetic antibiotics that are widely used as therapeutic

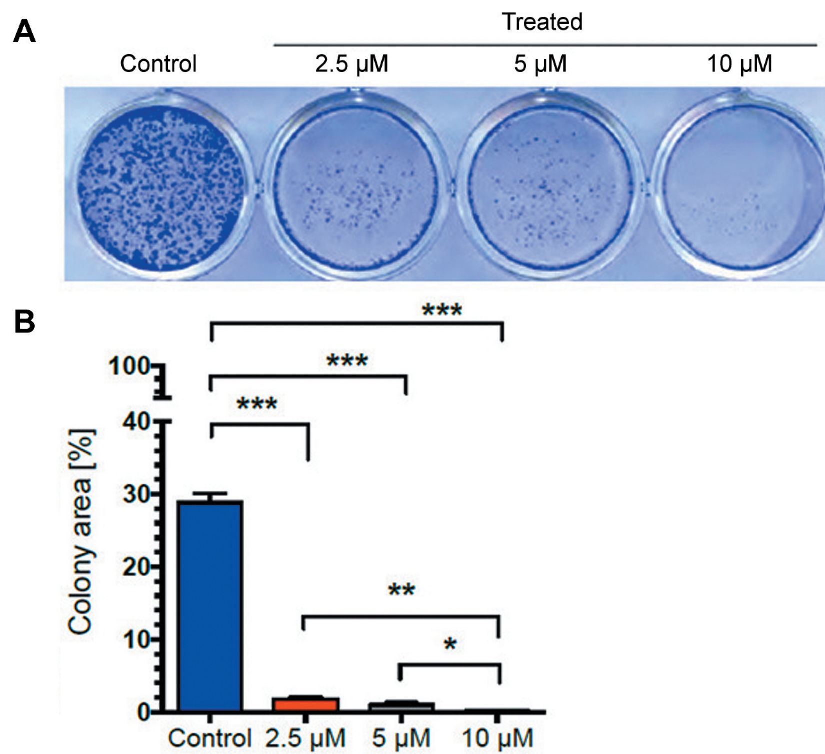

Figure 3. *The effect of the examined compound on colony formation ability of HeLa cells. (A) Colonies formed by HeLa cells after treatment with different concentrations of the examined compound. (B) Graph showing the area occupied by colonies formed by HeLa cells. Bars represent mean±SD. ***p<0.0001; **p<0.01; *p<0.05.*

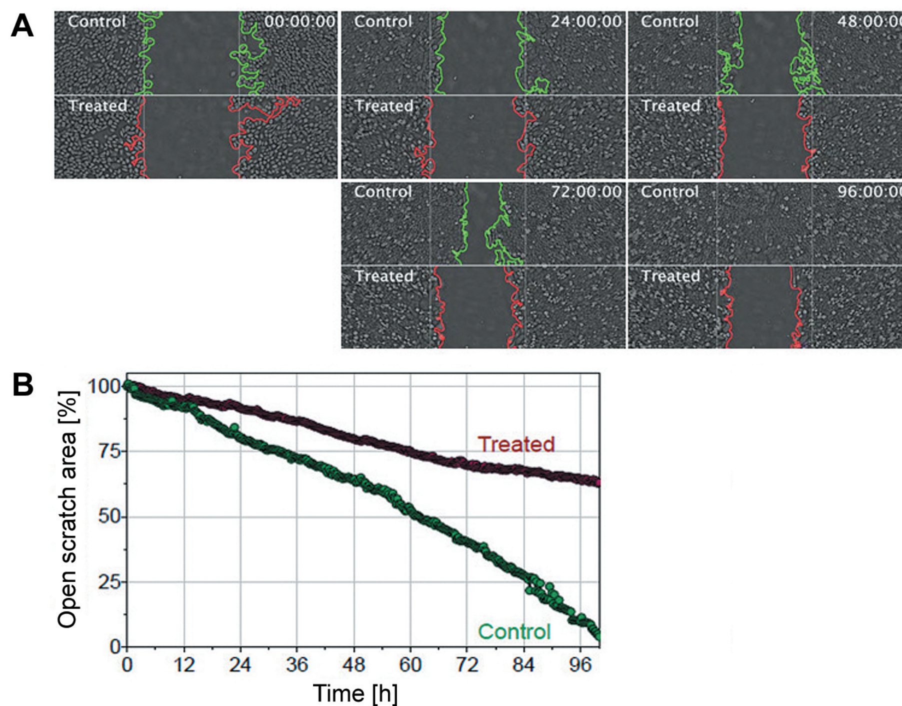

Figure 4. *The effect of the examined compound on the migration ability of HeLa cells. (A) Representative photos were taken at 0, 24, 48, 72 and 96 h for untreated cells (control) and cells treated with 2.5 μM of the examined compound after making the scratch area. (B) Quantification of the wound healing by measuring the open scratch area every 10 min for 96 h.*

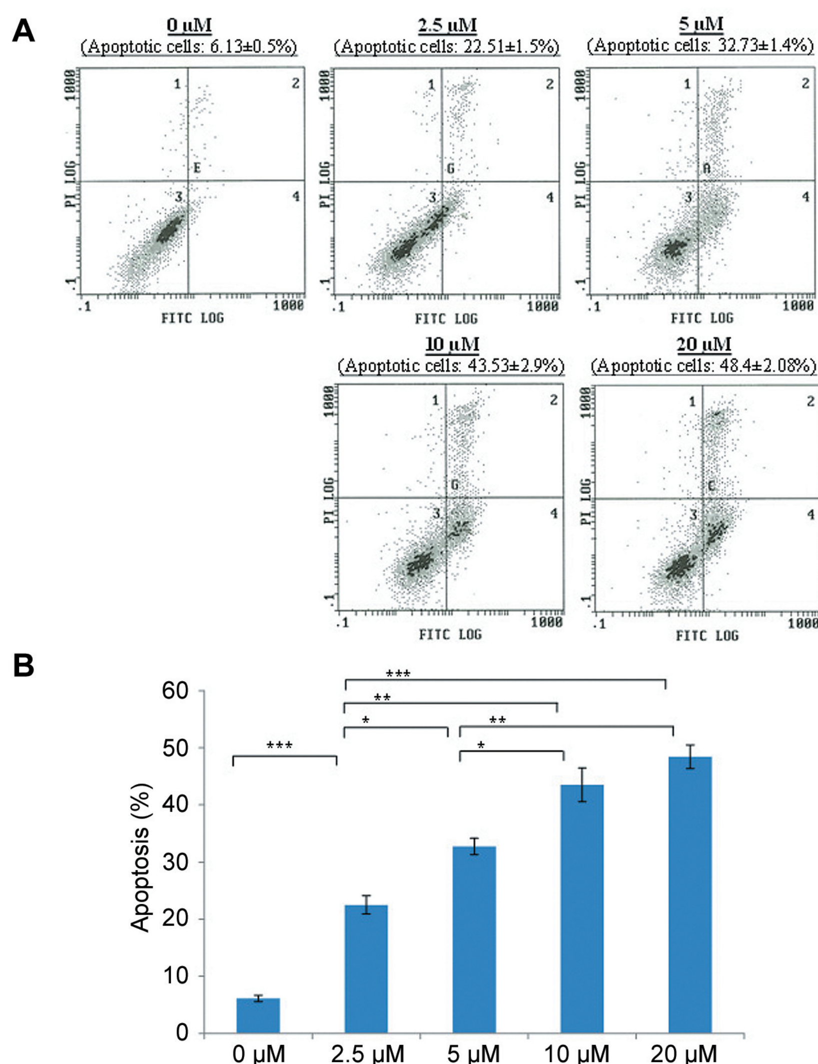

Figure 5. *The effect of the examined compound on HeLa cells apoptosis by flow cytometry analysis. (A) Representative dot plots for HeLa cells treated with different concentrations of the examined compound for 72 h after Annexin V-FITC / PI staining. (B) Graph showing the percentage of apoptosis occurred in HeLa cells after treatment with different concentrations of the examined compound. Bars represent mean±SD. ***p<0.0001; **p<0.001; *p<0.01.*

agents for various infections. Certain members of these antibiotics exhibit antitumor activity *in vitro* in several different cancer cell lines and also *in vivo* (11, 28), making them unique among other classes of antibiotics. This antitumor activity might be linked to the inhibition of the eukaryotic analog of DNA gyrase, topoisomerase IIα activity (29, 30).

This study investigated the effect of our new ciprofloxacin derivative (Compound 2) on the viability and cell morphology of HeLa cells. Treatment with the examined compound led to a concentration-dependent decrease in cell viability and significantly altered cellular morphology, indicating cell death.

Cervical cancer cells are characterized by high metastatic ability. During metastasis, metastatic cancer cells migrate into distant sites, invade healthy tissues and form tumor colonies. As a result, compounds inhibiting cancer cell migration could provide therapeutic benefits against cancer metastasis. To assess the efficacy of our compound against HeLa cell migration, a wound-healing assay was performed. The exposure of HeLa cells even to a non-cytotoxic concentration of our compound significantly inhibited migration.

During cancer invasion and metastasis, micrometastases, which are small colonies of cancer cells formed after adaptation of the invading cancer cells into the foreign tissue microenvironment, grow into large tumors. This process is

Figure 6. *Effect of the examined compound on the expression of apoptosis-related proteins. (A) Representative western blots of Bax and caspase-3 protein levels in HeLa cells treated with different concentrations of the examined compound for 24 and 48 h. β-actin was used as an internal control. (B) Expression of Bax and cleaved caspase-3 proteins in HeLa cells after treatment with different concentrations of the examined compound for 24 and 48 h relative to untreated cells after normalization to the corresponding β-actin protein expression. Bars represent mean±SD. ****p<0.0001; ***p<0.001; **p<0.01; *p<0.05 when compared to the untreated cells.*

known as "colonization" (31). In the present study, the effect of this new ciprofloxacin-derivative (Compound 2) against HeLa cell colony formation was thus further investigated. More specifically, treatment with our compound significantly and drastically reduced the colony formation ability of HeLa cells in a concentration-dependent manner. These results suggest that our new compound has anti-metastatic potential.

To understand the mechanism of the effects of this compound on proliferation, migration and colony formation abilities of HeLa cells, FCM and western blot analysis were performed. One of the most common pathways of programmed cell death is apoptosis which is one of the main anticancer therapy mechanisms (32). Accordingly, the ability of the examined compound to induce apoptosis was investigated by FCM following Annexin V/PI staining. Our results demonstrated that the percentage of apoptosis was increased in a concentration-dependent manner after treatment of HeLa cells with the examined compound. Therefore, it may have reduced the viability, migration and colony formation ability of HeLa cells through the induction of apoptosis. To affirm this conclusion, western blot analysis was carried out. Mechanistically, one of the noteworthy features of apoptosis is Bax up-regulation and cleavage of caspase-3 into active cleaved caspase-3, which are well-known pro-apoptotic proteins. The results of this study indicated that the treatment of HeLa cells with the examined compound significantly up-regulated the expression of Bax protein and the levels of active cleaved caspase-3 protein in a concentration-dependent manner. These results indicated that this derivative induces apoptosis of HeLa cells. Similar results were obtained in different cancer cell lines including MDA-MB-231 breast cancer, colorectal carcinoma, and prostate cancer cells (29, 33, 34).

It has been reported that fluoroquinolones inhibit bacterial type II topoisomerase (DNA gyrase); however, there is also evidence to suggest that they may affect the viability of cells,

including cancer cells (35). The antitumor activity of topoisomerase inhibitors may potentially occur *via* the inhibition of mitochondrial DNA synthesis, which subsequently induces mitochondrial injury, disorders in the respiratory chain, and depletion of ATP stores. Energy depletion favors apoptosis, as it may cause cell cycle arrest in the G_2/M and/or S-phases (11, 18, 36). Furthermore, it has been shown that induction of apoptosis in pancreatic cancer cells is involved in the anti-proliferative effect of moxifloxacin and ciprofloxacin (11).

The present study is the first to indicate that this newly synthesized ciprofloxacin-derivative (Compound 2) decreased HeLa cell viability, migration and colony formation in a concentration-dependent manner. Therefore, this compound has a pronounced anti-metastatic activity. Mechanistically, it strongly induced apoptosis through the up-regulation of Bax and cleaved caspase-3 protein expression. Together, these results provide convincing experimental evidence and a novel perspective on the therapeutic properties of this new ciprofloxacin-derivative to treat cervical cancer. Still, further studies using animal models are required for estimating the *in vivo* application and further understanding the molecular mechanism that underlies the effects of this new derivative.

Conflicts of Interest

The Authors declare no potential conflicts of interest regarding this study.

Authors' Contributions

M.F., S.A. and T.N. designed and supervised the research, evaluated the data and wrote the manuscript. S.S. and Q.Z. were involved in data curation, formal analysis and writing–original draft preparation. G.A. assisted with experimental design and writing–original draft preparation. M.A. conceived the study and was involved in project administration. All Authors read and approved the final article.

Acknowledgements

This work was supported in part by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number JP18K07708.

References

1 Gado A, Ebeid B, Abdelmohsen A and Axon A: Colorectal cancer in egypt is commoner in young people: Is this cause for alarm? Alexandria Med J 50(3): 197-201, 2014. DOI: 10.1016/j.ajme.2013.03.003

2 Varmus H: The new era in cancer research. Science *312(5777)*: 1162-1165, 2006. PMID: 16728627. DOI: 312/5777/1162 [pii] 10.1126/science.1126758

3 Boom K, Lopez M, Daheri M, Gowen R, Milbourne A, Toscano P, Carey C, Guerra L, Carvajal JM, Marin E, Baker E, Fisher-Hoch S, Rodriguez AM, Burkalter N, Cavazos B, Gasca M, Cuellar MM, Robles E, Lopez E and Schmeler K: Perspectives on cervical cancer screening and prevention: Challenges faced by providers and patients along the texas-mexico border. Perspect Public Health *139(4)*: 199-205, 2018. PMID: 30117782. DOI: 10.1177/1757913918793443

4 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in globocan 2012. Int J Cancer *136(5)*: E359-386, 2015. PMID: 25220842. DOI: 10.1002/ijc.29210

5 Liu C, Wang J, Hu Y, Xie H, Liu M and Tang H: Upregulation of kazrin f by mir-186 suppresses apoptosis but promotes epithelial-mesenchymal transition to contribute to malignancy in human cervical cancer cells. Chin J Cancer Res *29(1)*: 45-56, 2017. PMID: 28373753. DOI: 10.21147/j.issn.1000-9604.2017.01.06

6 Zhang X, Wang Y, Cao Y and Zhao H: Increased ccl19 expression is associated with progression in cervical cancer. Oncotarget *8(43)*: 73817-73825, 2017. PMID: 29088748. DOI: 10.18632/oncotarget.17982

7 Huang BX and Fang F: Progress in the study of lymph node metastasis in early-stage cervical cancer. Curr Med Sci *38(4)*: 567-574, 2018. PMID: 30128863. DOI: 10.1007/s11596-018-1915-0

8 Li X: Emerging role of mutations in epigenetic regulators including mll2 derived from the cancer genome atlas for cervical cancer. BMC Cancer *17(1)*: 252, 2017. PMID: 28390392. DOI: 10.1186/s12885-017-3257-x

9 Castora FJ, Vissering FF and Simpson MV: The effect of bacterial DNA gyrase inhibitors on DNA synthesis in mammalian mitochondria. Biochim Biophys Acta *740(4)*: 417-427, 1983. PMID: 6309236. DOI: 10.1016/0167-4781(83)90090-8

10 Reuveni D, Halperin D, Shalit I, Priel E and Fabian I: Quinolones as enhancers of camptothecin-induced cytotoxic and anti-topoisomerase i effects. Biochem Pharmacol *75(6)*: 1272-1281, 2008. PMID: 18191106. DOI: 10.1016/j.bcp.2007.11.014

11 Yadav V, Varshney P, Sultana S, Yadav J and Saini N: Moxifloxacin and ciprofloxacin induces s-phase arrest and augments apoptotic effects of cisplatin in human pancreatic cancer cells *via* erk activation. BMC Cancer *15:* 581, 2015. PMID: 4531397. DOI: 10.1186/s12885-015-1560-y

12 Tsai WC, Hsu CC, Tang FT, Wong AM, Chen YC and Pang JH: Ciprofloxacin-mediated cell proliferation inhibition and g2/m cell cycle arrest in rat tendon cells. Arthritis Rheum *58(6)*: 1657-1663, 2008. PMID: 18512786. DOI: 10.1002/art.23518

13 Yogeeswari P, Sriram D, Kavya R and Tiwari S: Synthesis and *in vitro* cytotoxicity evaluation of gatifloxacin mannich bases. Biomed Pharmacother *59(9)*: 501-510, 2005. PMID: 16263236. DOI: 10.1016/j.biopha.2005.06.006

14 Nieto MJ, Alovero FL, Manzo RH and Mazzieri MR: Benzenesulfonamide analogs of fluoroquinolones. Antibacterial activity and qsar studies. Eur J Med Chem *40(4)*: 361-369, 2005. PMID: 15804535. DOI: 10.1016/j.ejmech.2004.11.008

15 Abdel-Aziz M, Park SE, Abuo-Rahma Gel D, Sayed MA and Kwon Y: Novel n-4-piperazinyl-ciprofloxacin-chalcone hybrids: Synthesis, physicochemical properties, anticancer and topoisomerase i and ii inhibitory activity. Eur J Med Chem *69:* 427-438, 2013. PMID: 24090914. DOI: 10.1016/j.ejmech.2013.08.040

16 Rajabalian S, Foroumadi A, Shafiee A and Emami S: Functionalized n(2-oxyiminoethyl) piperazinyl quinolones as new cytotoxic agents. J Pharm Pharm Sci *10(2)*: 153-158, 2007. PMID: 17706174.

17 Fathy M, Fawzy MA, Hintzsche H, Nikaido T, Dandekar T and Othman EM: Eugenol exerts apoptotic effect and modulates the sensitivity of hela cells to cisplatin and radiation. Molecules *24(21)*: 3979, 2019. PMID: 31684176. DOI: 10.3390/molecules24213979
18 Fathy M, Awale S and Nikaido T: Phosphorylated akt protein at ser473 enables hela cells to tolerate nutrient-deprived conditions. Asian Pac J Cancer Prev *18(12)*: 3255-3260, 2017. PMID: 29286216. DOI: 10.22034/APJCP.2017.18.12.3255
19 Guzman C, Bagga M, Kaur A, Westermarck J and Abankwa D: Colonyarea: An imagej plugin to automatically quantify colony formation in clonogenic assays. PLoS One *9(3)*: e92444, 2014. PMID: 24647355. DOI: 10.1371/journal.pone.0092444
20 Reddy R: Antioxidant therapeutics for schizophrenia. Antioxid Redox Signal *15(7)*: 2047-2055, 2011. PMID: 20977337. DOI: 10.1089/ars.2010.3571
21 Zhao QL, Fujiwara Y and Kondo T: Synergistic induction of apoptosis and caspase-independent autophagic cell death by a combination of nitroxide tempo and heat shock in human leukemia u937 cells. Apoptosis *15(10)*: 1270-1283, 2010. PMID: 20577812. DOI: 10.1007/s10495-010-0522-8
22 Li P, Zhao QL, Jawaid P, Rehman MU, Ahmed K, Sakurai H and Kondo T: Enhancement of hyperthermia-induced apoptosis by 5z-7-oxozeaenol, a tak1 inhibitor, in molt-4 cells. Int J Hyperthermia *33(4)*: 411-418, 2017. PMID: 28111999. DOI: 10.1080/02656736.2017.1278629
23 Thiery JP, Acloque H, Huang RY and Nieto MA: Epithelial-mesenchymal transitions in development and disease. Cell *139(5)*: 871-890, 2009. PMID: 19945376. DOI: S0092-8674(09)01419-6
24 Zhao Z, Zhou W, Han Y, Peng F, Wang R, Yu R, Wang C, Liang H, Guo Z and Gu Y: Emt-regulome: A database for emt-related regulatory interactions, motifs and network. Cell Death Dis *8(6)*: e2872, 2017. PMID: 28617437. DOI: 10.1038/cddis.2017.267
25 Dong J, Wang Q, Li L and Xiao-Jin Z: Upregulation of long non-coding rna small nucleolar rna host gene 12 contributes to cell growth and invasion in cervical cancer by acting as a sponge for mir-424-5p. Cell Physiol Biochem *45(5)*: 2086-2094, 2018. PMID: 29533945. DOI: 10.1159/000488045
26 Shih CC, Chou HC, Chen YJ, Kuo WH, Chan CH, Lin YC, Liao EC, Chang SJ and Chan HL: Role of pgrmc1 in cell physiology of cervical cancer. Life Sci *231:* 116541, 2019. PMID: 31216441. DOI: 10.1016/j.lfs.2019.06.016
27 Eckhardt S: Recent progress in the development of anticancer agents. Curr Med Chem Anticancer Agents *2(3)*: 419-439, 2002. PMID: 12678741. DOI: 10.2174/1568011024606389
28 Kloskowski T, Gurtowska N, Olkowska J, Nowak JM, Adamowicz J, Tworkiewicz J, Debski R, Grzanka A and Drewa T: Ciprofloxacin is a potential topoisomerase ii inhibitor for the treatment of nsclc. Int J Oncol *41(6)*: 1943-1949, 2012. PMID: 3583647. DOI: 10.3892/ijo.2012.1653
29 Beberok A, Wrzesniok D, Rok J, Rzepka Z, Respondek M and Buszman E: Ciprofloxacin triggers the apoptosis of human triple-negative breast cancer mda-mb-231 cells *via* the p53/bax/bcl-2 signaling pathway. Int J Oncol, 2018. PMID: 29532860. DOI: 10.3892/ijo.2018.4310
30 Elmore S: Apoptosis: A review of programmed cell death. Toxicol Pathol *35(4)*: 495-516, 2007. PMID: 17562483. DOI: 10.1080/01926230701320337
31 Valastyan S and Weinberg RA: Tumor metastasis: Molecular insights and evolving paradigms. Cell *147(2)*: 275-292, 2011. PMID: 22000009. DOI: 10.1016/j.cell.2011.09.024
32 Zhang Y, Chen X, Gueydan C and Han J: Plasma membrane changes during programmed cell deaths. Cell Res *28(1)*: 9-21, 2018. PMID: 29076500. DOI: 10.1038/cr.2017.133
33 Herold C, Ocker M, Ganslmayer M, Gerauer H, Hahn EG and Schuppan D: Ciprofloxacin induces apoptosis and inhibits proliferation of human colorectal carcinoma cells. Br J Cancer *86(3)*: 443-448, 2002. PMID: 11875713. DOI: 10.1038/sj.bjc.6600079
34 Aranha O, Grignon R, Fernandes N, McDonnell TJ, Wood DP, Jr. and Sarkar FH: Suppression of human prostate cancer cell growth by ciprofloxacin is associated with cell cycle arrest and apoptosis. Int J Oncol *22(4)*: 787-794, 2003. PMID: 12632069. DOI: 10.3892/ijo.22.4.787
35 Yadav V, Sultana S, Yadav J and Saini N: Gatifloxacin induces s and g2-phase cell cycle arrest in pancreatic cancer cells *via* p21/p27/p53. PLoS One *7(10)*: e47796, 2012. PMID: 3485023. DOI: 10.1371/journal.pone.0047796
36 Beberok A, Wrzesniok D, Otreba M and Buszman E: Impact of sparfloxacin on melanogenesis and antioxidant defense system in normal human melanocytes hema-lp – an *in vitro* study. Pharmacol Rep *67(1)*: 38-43, 2015. PMID: 25560573. DOI: 10.1016/j.pharep.2014.07.015

Received March 17, 2020
Revised April 12, 2020
Accepted April 14, 2020