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RECEIVED 10 July 2023

ACCEPTED 21 August 2023

PUBLISHED 07 September 2023

## CITATION

Alasadi GM and Al-Obaidi Z (2023),  
Synthesis of novel acylated and esterified  
ciprofloxacin derivatives as efficient  
anticancer and antimicrobial agents.  
*Front. Mater.* 10:1255955.  
doi: 10.3389/fmats.2023.1255955

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# Synthesis of novel acylated and esterified ciprofloxacin derivatives as efficient anticancer and antimicrobial agents

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Nowadays, various factors enhance the resistance of some microbes to antibiotics. Irrational antibiotic use is considered an example of such factors. Therefore, the synthesis and reporting of heterocyclic compounds with multiple biological properties are of considerable value. Ciprofloxacin is an antibiotic used to treat infections. New amidification and esterification derivatives of ciprofloxacin were synthesized. Their structure was identified and confirmed using both proton and carbon-13 nuclear magnetic resonance, mass spectrometry, and elemental analysis. With the employment of MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-terazoliumbromide) methods, *in vitro* anticancer activity was evaluated. The utilization of clinical and laboratory standards institute (CLSI) instructions was involved in the *in vitro* antimicrobial activity evaluation of the newly synthesized derivatives. In terms of anticancer activity, cell proliferation, viability, and IC<sub>50</sub>, as well as antimicrobial activities, IZD (Inhibition Zone Diameter), MIC (Minimum Inhibitory Concentrations), MBC (Minimum Bactericidal Concentrations), and MFC (Minimum Fungicidal Concentration) were measured and reported. The lowest cell proliferation and viability and IC<sub>50</sub> for 2,3-dihydroxypropyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate (compound 5) were observed to be 27.12% and 7.83 µg/mL. In antimicrobial activity, the lowest MIC for 2,3-dihydroxypropyl 7-(4-benzoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (compound 6c) was observed to be 2 µg/mL (against *Proteus mirabilis*) and the lowest MIC for 1-cyclopropyl-6-fluoro-7-(4-(4-(methylthio)benzoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (compound 3d) was observed to be 32 µg/mL (against *Candida albicans*, *Aspergillus fumigatus*). The effects were compared with commercially available drugs, and it was observed that some derivatives have the same efficacy as abemaciclib, which is used to treat breast cancer. Some derivatives were more effective than Cefazolin and Tolnaftate, well-known antibiotics and antifungals, respectively. Finally, a fairly clear relationship between the structure of the derivatives and their biological effectiveness was observed.

## KEYWORDS

ciprofloxacin, amidification, esterification, anticancer, antimicrobial

## 1 Introduction

A wide variety of antibiotics are used for various diseases every day worldwide. Unfortunately, the rate of improper utilization of antibiotics is increasing (Terreni et al., 2021) and this has led to the resistance of pathogenic microbial strains to many known antibiotics (Xiao et al., 2022). For this reason, there have been reports of modification to and changing the structure of antibiotics and the synthesis of new compounds (Das et al., 2020; Qu et al., 2023). For example, manganese ferrite-modified biochar from vinasse has been reported for enhanced adsorption of fluoroquinolone antibiotics. The fluoroquinolones kill bacteria by preventing DNA replication. Generally, the fluoroquinolone antibiotic inhibits the ligase activity of type II topoisomerases. With the ligase activity disrupted, DNA with single- and double-strand breaks lead to cell death (Aldred et al., 2014). In another proposed mechanism, fluoroquinolone antibiotics cause the oxidation of guanine nucleotides in the bacterial nucleotide pool, which contributes to their cytotoxicity (Foti et al., 2012). Delafloxacin, gemifloxacin, levofloxacin, moxifloxacin, and norfloxacin (Figure 1) are other examples of fluoroquinolone antibiotics (Hooper, 2001).

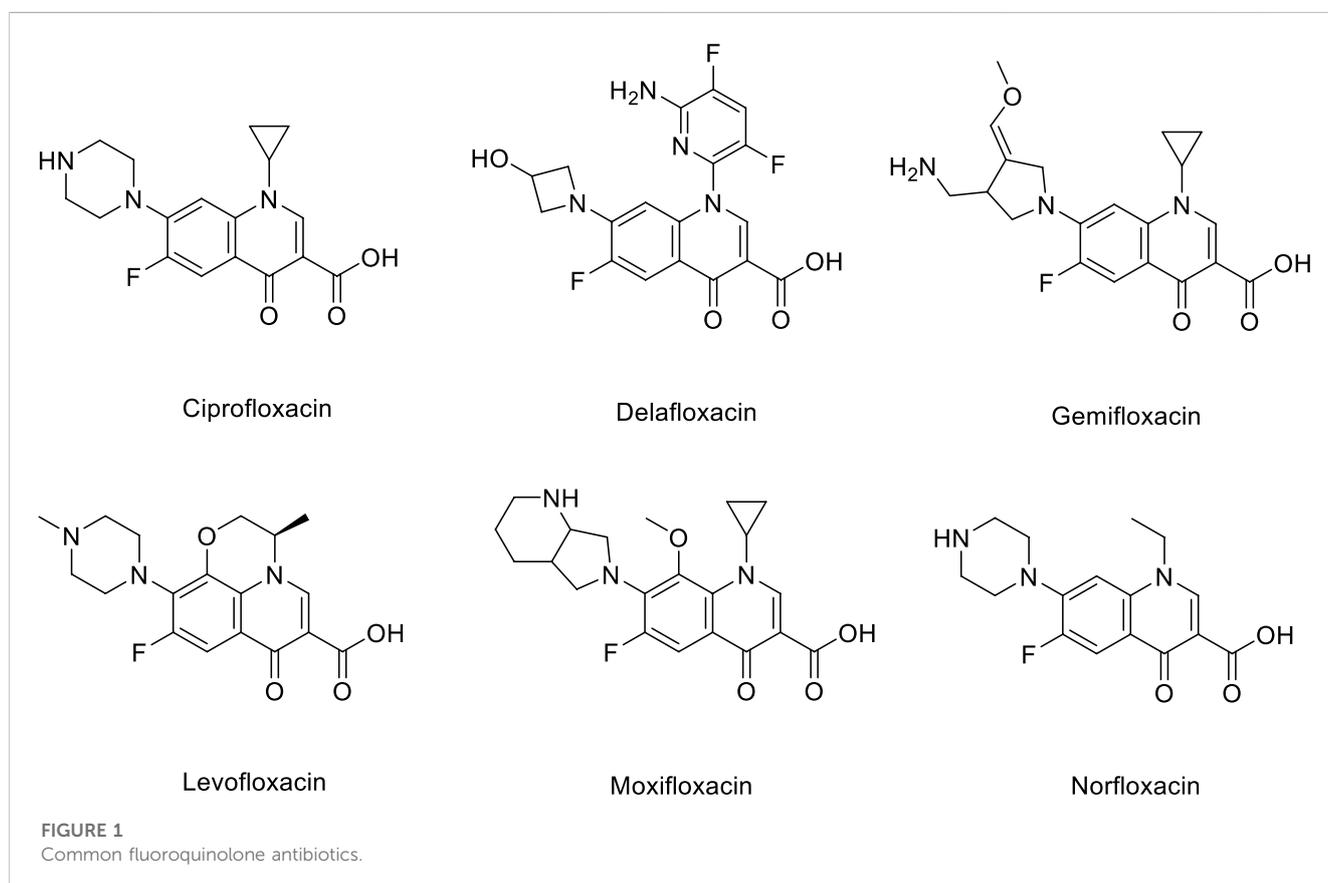
Ciprofloxacin is a fluoroquinolone antibiotic. Ciprofloxacin is an antibiotic used to treat infections, including joint and bone infections, intra-abdominal infections, certain types of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections. In the structure of some fluoroquinolone antibiotics such as ciprofloxacin, levofloxacin, and norfloxacin, two

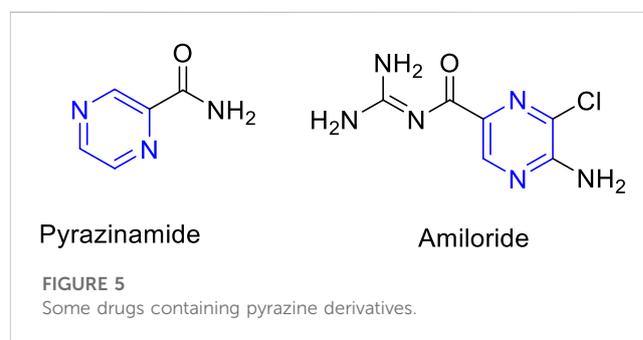
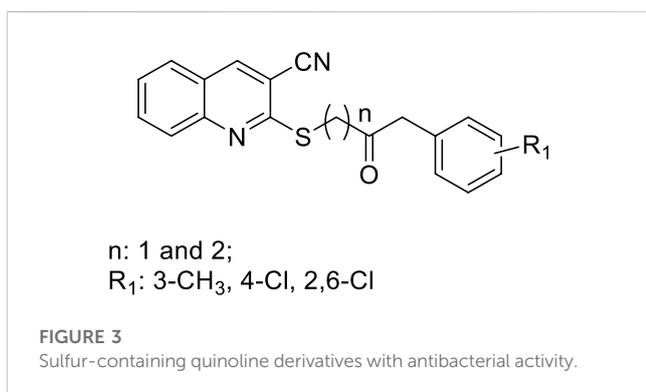
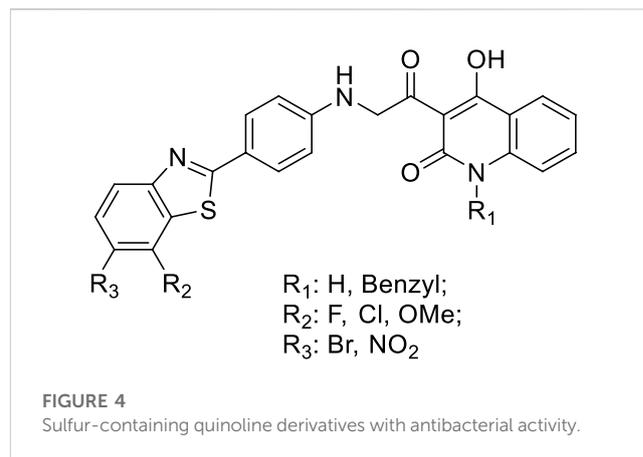
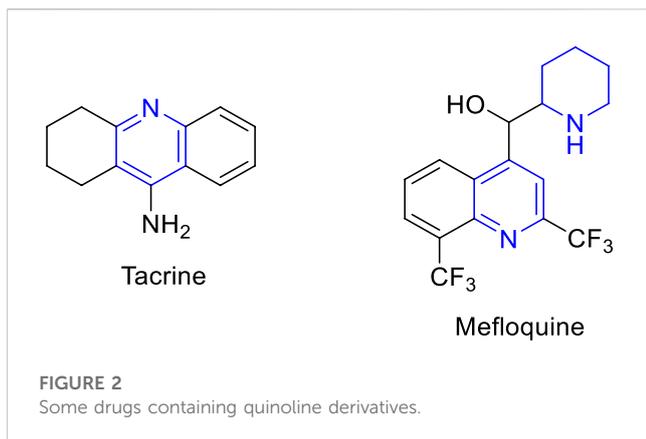
heterocyclic compounds of 1,4-dihydroquinoline and piperazine are present (Zhanet al., 2006; Uncu et al., 2019; Kudiyarova et al., 2021). The bicyclic heterocyclic compound of quinoline consists of a benzene ring connected with a pyridine ring. Derivatives of quinoline are abundantly found in nature, such as Galipea, Cinchona, Papaver, etc. In addition to Ciprofloxacin, commercial drugs such as Tacrine, Mefloquine, Bosutinib, etc., (Figure 2). Have quinoline derivatives in their structure.

Several other biological properties of quinoline-containing heterocyclic compounds have been reported (Matada et al., 2021). Some biological properties, such as anti-bacterial and anti-fungal activity (Dib et al., 2021; Jamshidi et al., 2022), anti-cancer activity (Gao et al., 2019), anti-plasmodial activities (Sharma et al., 2023), etc., have been reported from compounds containing this heteroatom. For example, in 2021, sulfur-containing quinoline derivatives (Figure 3) were reported that were effective against bacterial species such as *S. pneumonia*, *B. subtilis*, and *E. coli* (El-Shershaby et al., 2021).

Benzothiazolyl quinolone derivatives (Figure 4) are another polycyclic heterocyclic compounds that were reported in 2021 as newly synthesized heterocyclic structures effective against *E. coli* and *P. aeruginosa* bacterial species (Bolakatti et al., 2021).

Pyrazine is another bioactive compound that (Ferreira and Kaiser, 2012), in addition to Ciprofloxacin, is also present in the structure of Bosutinib. Medicines such as pyrazinamide (Figure 5), paritaprevir, bortezomib, amiloride, etc., have a pyrazine in their structure (Huigens et al., 2022; Tambat et al.,





2022). Other pharmacological activities, such as anti-inflammatory activity (Shashikant et al., 2022), antimicrobial activity (Schneider et al., 2022), anti-cancer activity (Tantawy et al., 2020), etc., have been reported from synthetic compounds containing pyrazine.

Considering the biological properties of Ciprofloxacin, as discussed, amidification and esterification of ciprofloxacin can be performed and compounds developed with biological properties. In this study, the derivatives mentioned above were synthesized and studied on various Gram-positive and Gram-negative pathogenic bacterial agents and common pathogenic bacterial strains between aquatic animals and humans. *In vitro* biological studies of the synthetic compounds were also evaluated on breast cancer cells and bacterial cultures. The results of these *in vitro* tests showed that the synthesized amidification and esterification derivatives of ciprofloxacin have high antimicrobial and anticancer activities. By performing other supplementary tests in subsequent studies, such as *in silico* studies, *in vivo* tests, cytotoxicity tests, etc., and if the results are acceptable, amidification and esterification derivatives of ciprofloxacin can be suitable candidates for developing effective antibiotic and anticancer drugs.

## 2 Experimental

### 2.1 Chemicals and reagents

All solvents, chemicals, and consumables utilized in this study were obtained from Merck Millipore and Sigma-Aldrich.

### 2.2 Amidification of ciprofloxacin

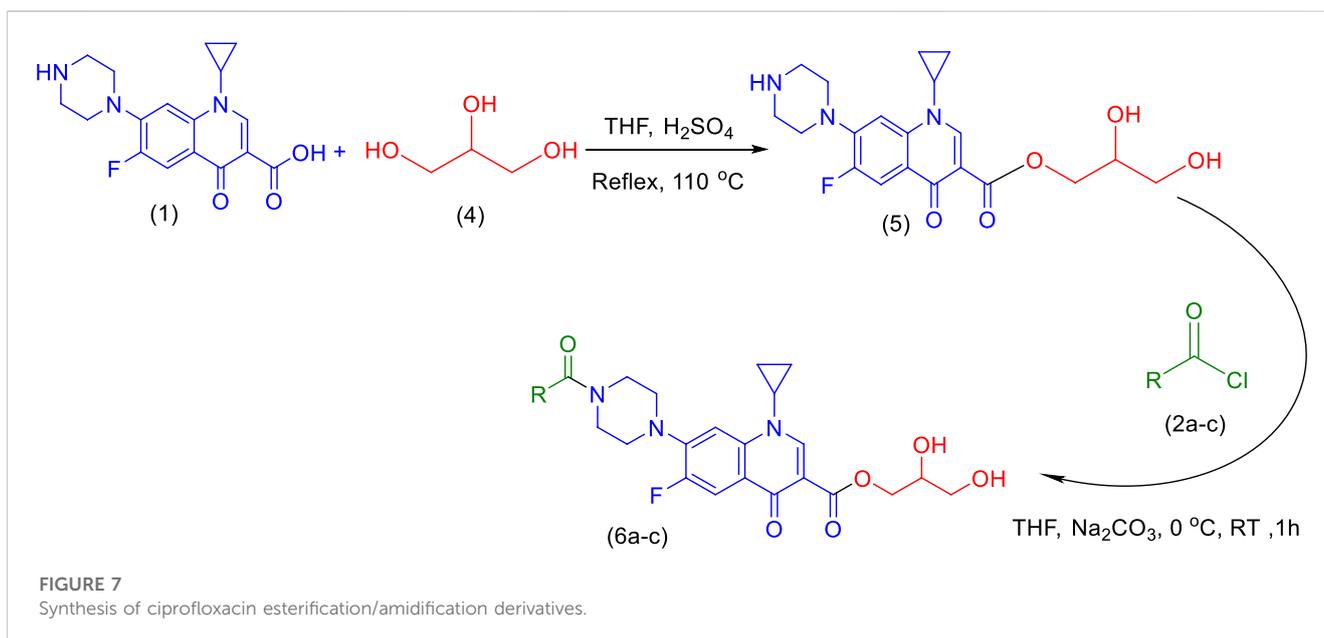
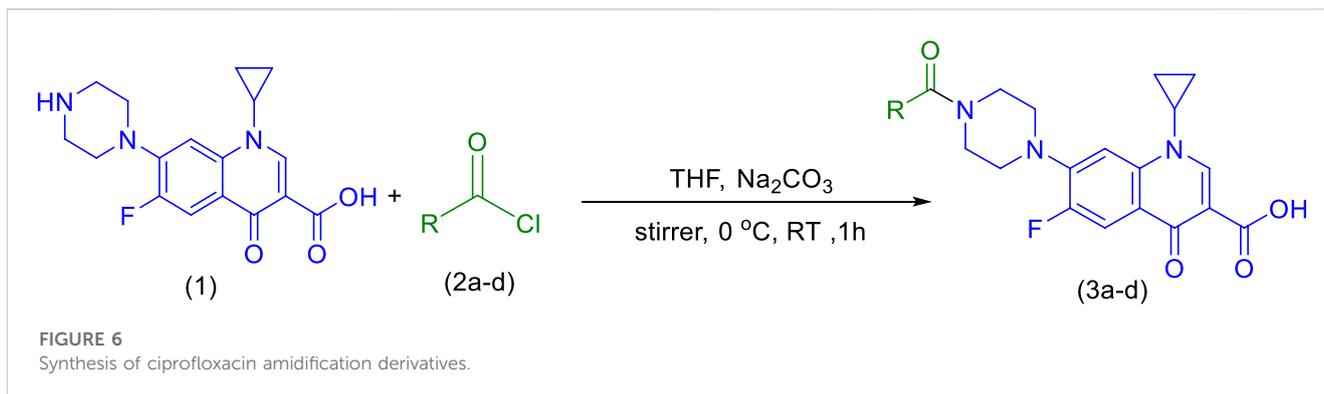
An accurately weighed amount of 10 mmol Acyl chloride derivatives was added to a stirring mixture of 1.84 g of ciprofloxacin hydrochloride (5 mmol) and 2.65 g of Na<sub>2</sub>CO<sub>3</sub> in 20 mL of tetrahydrofuran at 0°C (Table 1). The resultant mixture was continuously stirred for 1 h at 0°C. Thereafter, a yellow precipitate was observed. The column chromatography method (silica gel 60–120 mesh, a gradient solvent system of 0–20% methanol/DCM) was used for the purification of the synthesized derivatives.

7-(4-acetyl-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3a).

MS *m/z* 373 (M<sup>+</sup>). Element analysis. Calcd for C<sub>19</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>4</sub>: C, 61.12. H, 5.40. N, 11.25. O, 17.14. Found: C, 61.15. H, 5.38. N, 11.24. O, 17.17. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ = 1.05–4.07 (m, 2H), 1.38–1.42 (m, 2H), 2.18 (s, 3H), 3.36 (t, J = 8.0 Hz, 4H), 3.62 (t, J = 8.0 Hz, 4H), 4.21–4.25 (m, 1H), 6.17 (s, 1H), 7.86 (s, 1H), 8.25 (s, 1H), 12.37 (s, 1H). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ = 7.8, 21.9, 36.4, 44.7, 52.8, 103.2, 109.5, 111.3, 116.1, 135.8, 146.1, 147.8, 152.4, 165.4, 168.7, 177.3.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-pivaloylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (3b).

MS *m/z* 415 (M<sup>+</sup>). Element analysis. Calcd for C<sub>22</sub>H<sub>26</sub>FN<sub>3</sub>O<sub>4</sub>: C, 63.60. H, 6.31. N, 10.11. O, 15.40. Found: C, 63.62. H, 6.38. N, 10.10. O, 15.46. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ = 1.04, 1.08 (m, 2H), 1.25 (s, 9H), 1.35–1.39 (m, 2H), 3.31 (t, J = 7.5 Hz, 4H), 3.57 (t, J = 8.0 Hz, 4H), 4.15–4.120 (m, 1H), 6.09 (s, 1H), 7.82 (s, 1H), 8.17 (s, 1H), 12.32 (s, 1H). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ = 7.5, 28.3, 35.8, 38.3, 45.1, 53.2, 102.6, 109.7, 112.4, 115.8, 134.9, 147.3, 148.4, 152.7, 165.7, 176.3, 177.5.



7-(4-benzoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3c).

MS  $m/z$  435 ( $M^+$ ). Element analysis. Calcd for  $C_{24}H_{22}FN_3O_4$ : C, 66.20, H, 5.09, N, 9.65, O, 14.70. Found: C, 66.18, H, 5.07, N, 9.69, O, 14.69.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  = 1.01-1.05 (m, 2H), 1.32-1.40 (m, 2H), 3.28 (t,  $J$  = 8 Hz, 4H), 3.47 (t,  $J$  = 8.4 Hz, 4H), 4.12-4.17 (m, 1H), 6.04 (s, 1H), 7.52-7.58 (m, 3H), 7.96-8.05 (m, 3H), 8.24 (s, 1H), 12.42 (s, 1H).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  = 7.1, 35.4, 49.6, 52.9, 101.9, 109.3, 112.7, 115.3, 126.7, 128.1, 129.5, 134.3, 135.2, 146.9, 148.1, 152.3, 166.3, 168.6, 177.9.

1-cyclopropyl-6-fluoro-7-(4-(4-(methylthio)benzoyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3d).

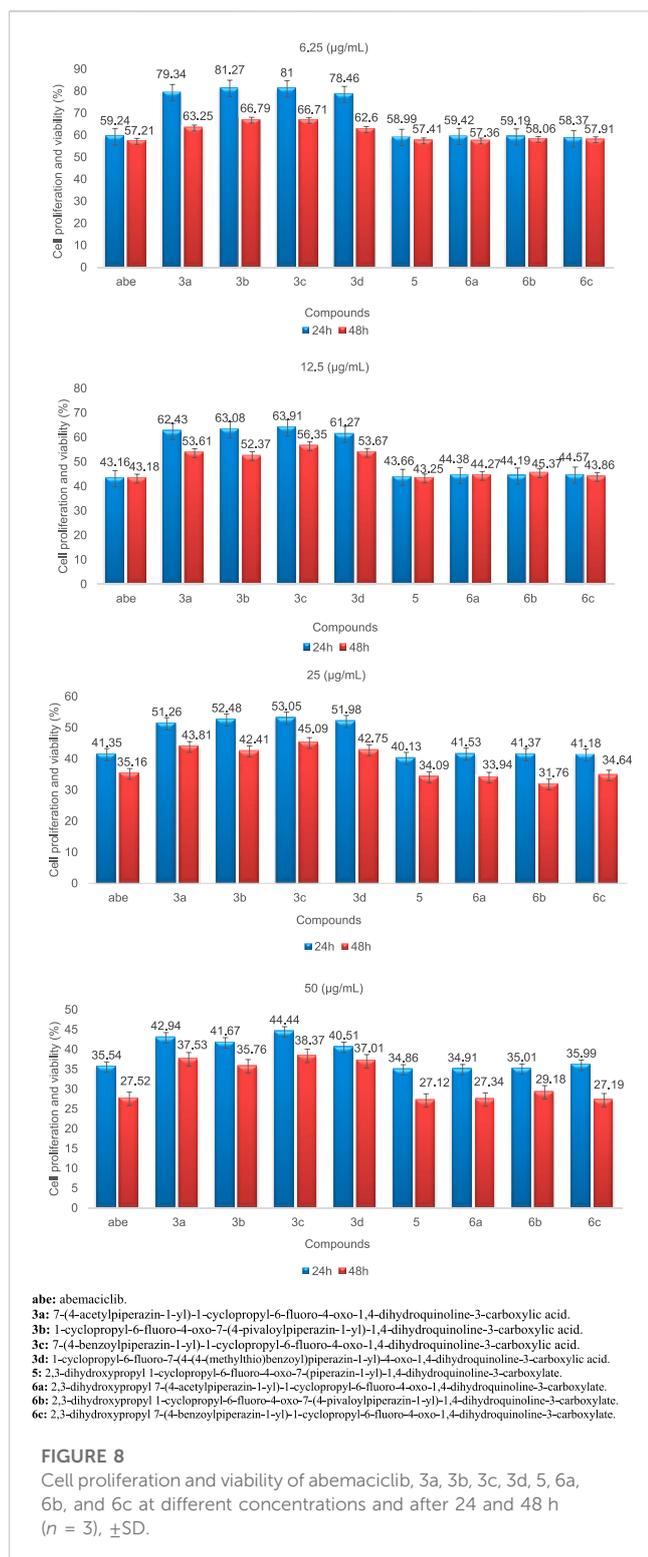
MS  $m/z$  481 ( $M^+$ ). Element analysis. Calcd for  $C_{25}H_{24}FN_3O_4S$ : C, 62.36, H, 5.02, N, 8.73, S, 6.66. Found: C, 62.35, H, 5.05, N, 8.70, S, 6.69.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  = 1.02-1.06 (m, 2H), 1.27-1.35 (m, 2H), 2.46 (s, 1H), 3.24 (t,  $J$  = 8.5 Hz, 4H), 3.41 (t,  $J$  = 8.5 Hz, 4H), 4.14-4.21 (m, 1H), 6.10 (s, 1H), 7.55 (d,  $J$  = 8.4 Hz, 2H), 7.91-7.99 (m, 3H), 8.41 (s, 1H), 12.36 (s, 1H).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  = 7.7, 14.9, 34.8, 50.1, 53.4, 101.5, 109.1, 112.4, 115.6, 126.1, 128.4, 132.9, 134.8, 140.2, 146.4, 149.9, 153.1, 165.9, 167.8, 178.1.

## 2.3 Esterification and amidification of ciprofloxacin

A total of 14.7 g of ciprofloxacin hydrochloride (40 mmol) was dissolved in 40 mL of tetrahydrofuran and then 11.1 g of glycerol (120 mmol) was added to the solution (Table 2). The mixture stirred and concentrated  $H_2SO_4$  (2 mL) was added dropwise. Thereafter, the mixture was refluxed for 4 h at 110°C. The column chromatography method (silica gel 60–120 mesh, a gradient solvent system of 0%–20% methanol/DCM) was used for the purification of the synthesized compounds.

2,3-dihydroxypropyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate (5).

MS  $m/z$  405 ( $M^+$ ). Element analysis. Calcd for  $C_{20}H_{24}FN_3O_5$ : C, 59.25, H, 5.97, N, 10.36, O, 19.73. Found: C, 21.18, H, 5.96, N, 10.33, O, 19.68.  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  = 1.03-1.09 (m, 3H), 1.29-1.34 (m, 2H), 2.75-2.79 (m, 4H), 3.44-3.52 (m, 6H), 3.98 (s, 1H), 4.12-4.31 (m, 4H), 5.73 (s, 1H), 6.08 (s, 1H), 7.99 (s, 1H), 8.57 (s, 1H).  $^{13}C$  NMR (DMSO- $d_6$ )  $\delta$  = 7.1, 35.5, 45.4, 51.2, 63.0, 67.6, 71.1, 102.7, 110.2, 119.9, 115.3, 134.6, 144, 147.5, 152.2, 165.8, 171.4.



An accurately weighed amount of 60 mmol Acyl chloride derivatives was added to a stirring mixture of 8.1g of compound 2,3-dihydroxypropyl 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate (20 mmol) and 10.5g of  $\text{Na}_2\text{CO}_3$  in 40 mL of tetrahydrofuran at  $0^\circ\text{C}$ . The resultant solution was continuously stirred for 1 h at  $0^\circ\text{C}$ . The column chromatography method (silica gel 60–120 mesh, isocratic

solvent system of 1:4 methanol/DCM) was used for the purification of the synthesized derivatives.

2,3-dihydroxypropyl 7-(4-acetylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (6a).

MS  $m/z$  447 ( $\text{M}^+$ ). Element analysis. Calcd for  $\text{C}_{22}\text{H}_{26}\text{FN}_3\text{O}_6$ : C, 59.05. H, 5.86. N, 9.39. O, 21.45. Found: C, 59.04. H, 5.85. N, 9.41. O, 21.44.  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  = 1.01-1.05 (m, 2H), 1.30-1.33 (m, 2H), 2.14 (s, 1H), 3.31 (t,  $J$  = 8Hz, 4H), 3.53-3.57 (m, 6H), 3.65 (s, 1H), 4.15-4.35 (m, 4H), 5.79 (s, 1H), 6.03 (s, 1H), 8.02 (s, 1H), 8.62 (s, 1H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  = 7.4, 21.9, 35.2, 46.7, 51.6, 63.5, 67.1, 71.9, 102.1, 110.3, 112.6, 115.0, 134.1, 144.6, 146.9, 152.5, 165.5, 167.4, 171.9.

2,3-dihydroxypropyl 1-cyclopropyl-6-fluoro-4-oxo-7-(4-pivaloylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylate (6b).

MS  $m/z$  489 ( $\text{M}^+$ ). Element analysis. Calcd for  $\text{C}_{25}\text{H}_{32}\text{FN}_3\text{O}_6$ : C, 61.34. H, 6.59. N, 8.58. O, 19.61. Found: C, 61.35. H, 6.62. N, 8.59. O, 19.62.  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  = 1.03-1.09 (m, 2H), 1.23-1.35 (m, 3H), 3.28 (t,  $J$  = 7.5 Hz, 4H), 3.52-3.59 (m, 6H), 3.77 (s, 1H), 4.12-4.36 (m, 4H), 5.75 (s, 1H), 6.10 (s, 1H), 8.12 (s, 1H), 8.76 (s, 1H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  = 7.1, 27.3, 35.7, 38.2, 46.9, 53.5, 63.2, 67.7, 71.9, 101.9, 110.8, 112.1, 115.6, 134.6, 145.2, 147.6, 152.1, 165.1, 169.8, 172.9.

2,3-dihydroxypropyl 7-(4-benzoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (6c).

MS  $m/z$  509 ( $\text{M}^+$ ). Element analysis. Calcd for  $\text{C}_{27}\text{H}_{28}\text{FN}_3\text{O}_6$ : C, 63.65. H, 5.54. N, 8.25. O, 18.84. Found: C, 63.70. H, 5.43. N, 8.28. O, 18.86.  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  = 1.0-1.10 (m, 2H), 1.21-1.29 (m, 3H), 3.33 (t,  $J$  = 8.4 Hz, 4H), 3.55-3.62 (m, 6H), 3.84 (s, 1H), 4.11-4.31 (m, 4H), 5.79 (s, 1H), 6.05 (s, 1H), 7.63-7.84 (m, 5H), 8.70 (s, 1H).  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  = 7.3, 34.9, 50.8, 53.1, 63.6, 67.1, 71.4, 102.3, 111.2, 116.4, 115.9, 127.2, 128.5, 129.9, 134.7, 135.1, 144.6, 147.8, 152.6, 165.4, 68.5, 171.3.

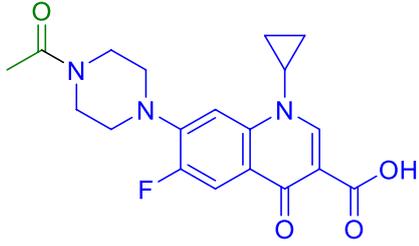
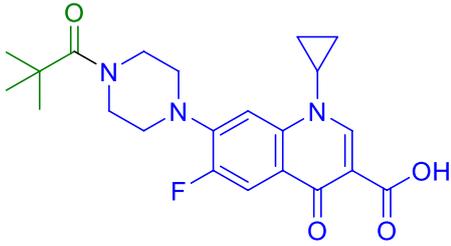
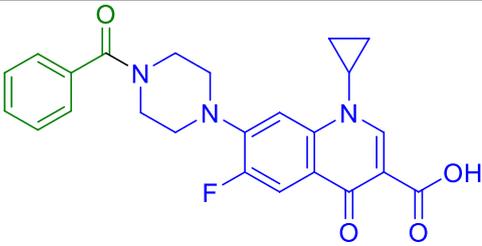
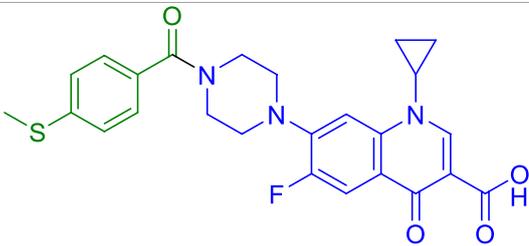
## 2.4 Anticancer activity tests

In the anticancer activity tests, the culture medium was Roswell Park Memorial Institute 1640 (RPMI), 10% Fetal Bovine Serum (FBS), and 200  $\mu\text{L}$  of antibiotic (G/streptomycin and penicillin). The test steps are as follows (Moghaddam-manesh et al., 2021; Moghaddam-Manesh and Hosseinzadegan, 2021):

First, breast cancer cells (MCF-7) were cultured in the culture medium. 200  $\mu\text{L}$  of MCF-7 with a density of  $1.2 \times 10^4$  cells per well was seeded in a microplate and incubated in suitable conditions. It should be noted that the suitable conditions were 5%  $\text{CO}_2$ ,  $37^\circ\text{C}$ , and 24 h. Then, concentrations of 6.25, 12.5, 25, and 50  $\mu\text{g}/\text{mL}$  of the derivatives were added to the wells and incubated for 24 and 48 h under the above conditions. After the tested times (24 and 48 h), the contents of the wells, including derivatives and culture medium, were emptied and 50  $\mu\text{L}$  of MTT solution and 150  $\mu\text{L}$  of culture medium were added to the wells and incubated in 5%  $\text{CO}_2$ ,  $37^\circ\text{C}$  for 4 h. Then, the culture medium and MTT were emptied and 200  $\mu\text{L}$  of dimethyl sulfoxide (DMSO) was added to the wells. Finally, the absorbance of the contents of the wells at 570 nm was read using an ELISA reader.

In the test stages, at the same time as studying the derivatives, a culture medium without derivatives was used as a control. The test steps were carried out on abemaciclib, a well-known commercial

TABLE 1 Synthesized ciprofloxacin amidification derivatives.

Derivative	R (2a-d)	Final structure	Efficiency (%)
3a	2a: acetyl chloride		88
3b	2b: pivaloyl chloride		89
3c	2c: benzoyl chloride		86
3d	2d: 4-(methylthio)benzoyl chloride		84

MCF-7 breast cancer cells drug, to compare the properties of the compounds. It should be noted that the MTT solution contained 2 µg/mL of MTT in phosphate-buffered saline (PBS).

## 2.5 Antimicrobial activity tests

In antimicrobial activity tests of the synthesized derivatives for antibacterial activity, IZD, MIC, and MBC were evaluated, whereas for the antifungal activity, IZD, MIC, and MFC were evaluated. The guidelines of CLSI (the Clinical and laboratory standards institute, guidelines M07-A9, M26-A, M27-A2) were used in all the tests (Etemadi et al., 2016; Beyzaei et al., 2017). Mueller Hinton Broth and Mueller Hinton Agar were used for the antibacterial tests and Dextrose Tryptone Broth and Dextrose Tryptone Agar were used as culture media for the antifungal tests. It should be noted that the final results are the average of the results of three repetitions of the

test. The test steps are as follows (Beyzaei et al., 2017; Moghaddam-Manesh et al., 2020):

First, using a spectrophotometer, a concentration of  $1 \times 10^5$  colony-forming unit/mL (CFU/mL) of bacterial and fungal species was prepared in a broth culture medium. Then, concentrations of 1–2,048 µg/mL (1, 2, 4, 8, 16, and 2,048 µg/mL) of the derivatives were prepared in DMSO.

To obtain MIC, 100 µL of the derivatives, 100 µL of the broth culture medium, and 10 µL of the studied bacterial or fungal species were added to a microplate (96 well plates). Then, it was placed in a shaker incubator for a suitable time and at a suitable temperature and was slowly stirred. After the necessary time, turbidity in the well indicated growth and transparency a lack of growth. Here, we define the MIC as the first concentration after which turbidity was observed. It should be noted that the proper time for the MIC test is 48 h and the proper temperature for bacterial species is 37°C and is 27°C for fungal species.

TABLE 2 Synthesized ciprofloxacin esterification/amidification derivatives.

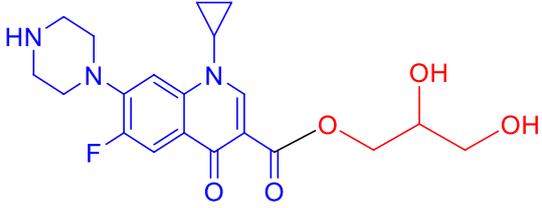
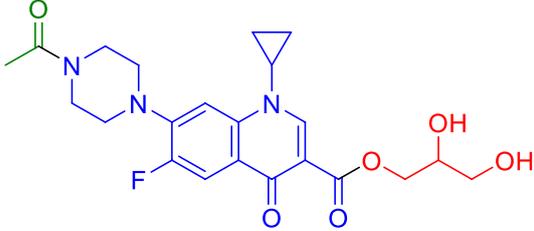
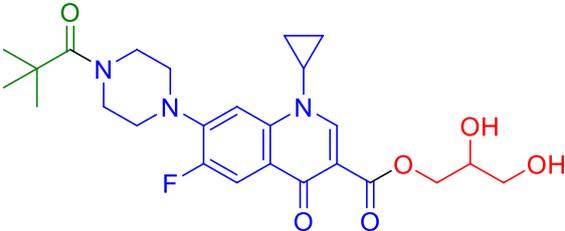
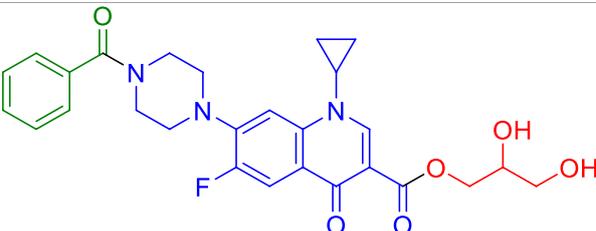
Derivative	R (2a-c)	Final structure	Efficiency (%)
5	-		86
6a	2a: acetyl chloride		89
6b	2b: pivaloyl chloride		85
6c	2c: benzoyl chloride		86

TABLE 3 Statistical studies of synthesized derivatives.

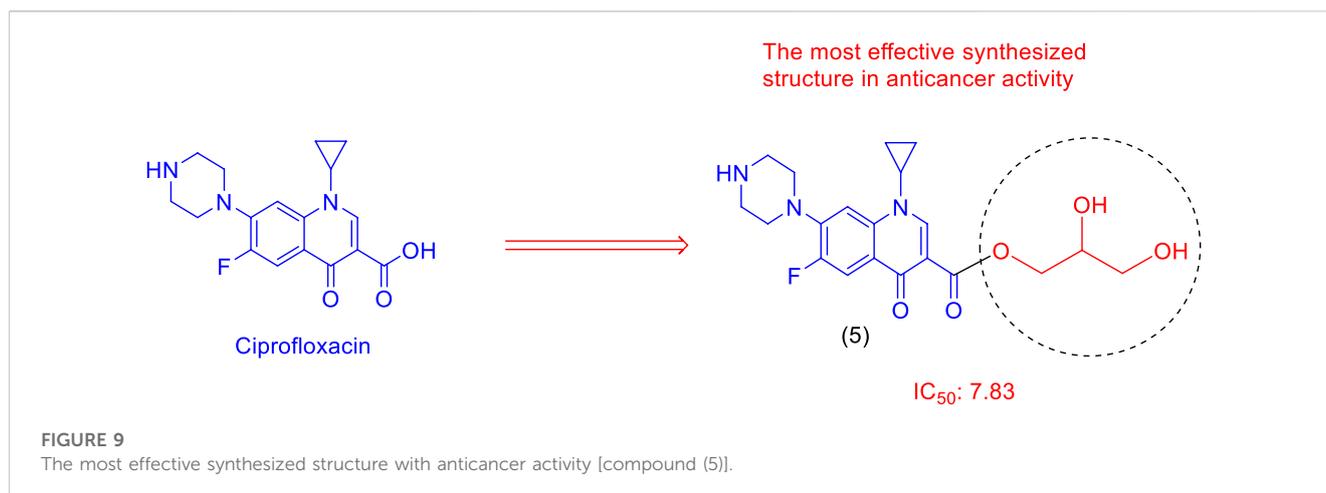
	Abemaciclib	3a	3b	3c	3d	5	6a	6b	6c	
<i>p</i> -values	24 (h)	0.001	0.001	0.000	0.004	0.003	0.001	0.000	0.003	0.001
	48 (h)	0.000	0.003	0.002	0.001	0.000	0.000	0.004	0.000	0.001

To obtain MBC and MFC, the contents of the MIC concentration and three more concentrated concentrations were cultured on the agar culture medium and placed inside the incubator at the proper time and temperature. Then, the concentration at which bacterial or fungal species did not grow was reported as MBC or MFC. It should be noted that the appropriate time for the MBC and MFC tests is 72 h and the proper temperature for bacterial species is 37°C and is 27°C for fungal species.

To obtain IZD, the studied bacterial or fungal species were first cultured on an agar medium. Then, a disc blank was placed on them. A total of 10 µL of the concentration obtained for the MIC of the

derivatives was injected on the disk blanks and was placed inside the incubator at the right time and temperature. Finally, the diameter of the halo created by the caliper was measured and reported. It should be noted that the appropriate time for the IZD test is 24 h and the proper temperature for bacterial species is 37°C and is 27°C for fungal species.

In the test stages, at the same time as studying the derivatives, the solvent (DMSO) without derivatives was used as a control. The test steps were carried out on several well-known commercial antibacterial and antifungal drugs to compare the properties of the compounds.



### 3 Results and discussion

#### 3.1 Amidification and esterification of ciprofloxacin

As mentioned in the introduction, in this study, considering the bioactivity of ciprofloxacin, its amidification derivatives and esterification/amidification derivatives were synthesized and their biological properties were tested. As shown in Figure 6, from the reaction of ciprofloxacin with acyl chloride derivatives, ciprofloxacin/ciprofloxacin/amidification derivatives were synthesized. The reaction was carried out in the presence of Na<sub>2</sub>CO<sub>3</sub> as a Brønsted–Lowry base. The structure of the derivatives was confirmed using proton nuclear magnetic resonance (<sup>1</sup>H NMR), carbon-13 nuclear magnetic resonance (<sup>13</sup>C NMR), mass spectrometry (MS), and elemental analysis (EA), the results of which are given in Sections 2, 3.

The advantages of the synthesis of the ciprofloxacin/amidification derivatives studied in this research include the synthesis of new derivatives, the use of Na<sub>2</sub>CO<sub>3</sub> as a green catalyst, and the high efficiency of the derivatives, as shown in Figure 6.

Esterification/amidification derivatives of ciprofloxacin were synthesized (Figure 7). For this purpose, first, an esterification reaction using ciprofloxacin and glycerol in the presence of sulfuric acid as Brønsted–Lowry acid was carried out. Then, the esterified ciprofloxacin was amidified under the aforementioned conditions and using Na<sub>2</sub>CO<sub>3</sub>.

The high efficiency of the derivatives and the greenness of Na<sub>2</sub>CO<sub>3</sub> can also be expressed here. By using <sup>1</sup>H NMR, <sup>13</sup>C NMR, MS, and EA, the synthesized esterification/amidification derivatives of ciprofloxacin were identified and confirmed.

#### 3.2 Examination of anticancer activities

The anticancer activity of the synthesized derivatives and abemaciclib was tested on breast cancer cells (MCF-7). Abemaciclib, a known breast cancer drug, was used to compare the anticancer properties of the compounds. As mentioned in

paragraphs 2–5, concentrations of 6.25, 12.5, 25, and 50 µg/mL of abemaciclib and compounds were investigated for 24 and 48 h. In the evaluations, the cell proliferation and viability were obtained at different concentrations for 24 and 48 h, and, finally, the IC<sub>50</sub> value was reported (Figure 8).

After 24 h and at a concentration of 50 µg/mL, the cell proliferation and viability for abemaciclib, 3a, 3b, 3c, 3d, 5, 6a, 6b, and 6c were determined to be 35.54%, 42.94%, 41.67%, 44.44%, 40.51%, 34.86%, 34.91%, 35.01%, and 35.99%, respectively, compared to the control. After 48 h and at a concentration of 50 µg/mL, the cell proliferation and viability for abemaciclib and 3a, 3b, 3c, 3d, 5, 6a, 6b, and 6c were determined to be 27.52%, 37.53%, 35.76%, 38.37%, 37.01%, 27.12%, 27.34%, 29.18%, and 27.19%, respectively, compared to the control. The results showed that in both times (24 and 48 h), in the abemaciclib and synthesized derivatives, the highest cell proliferation and viability is at the concentration of 50 µg/mL. Moreover, the effectiveness of abemaciclib and the derivatives, based on cell proliferation and viability, was higher after 48 h than 24 h.

The IC<sub>50</sub> values after 48 h for abemaciclib, 3a, 3b, 3c, 3d, 5, 6a, 6b, and 6c were determined to be 7.95 µg/mL, 22.61 µg/mL, 22.37 µg/mL, 26.15 µg/mL, 21.62 µg/mL, 7.83 µg/mL, 8.36 µg/mL, 8.49 µg/mL, and 8.96 µg/mL, respectively.

The results show that the highest anticancer property was found in 5, and that the anticancer properties of ciprofloxacin esterification/amidification derivatives (6a, 6b, and 6c) are higher than ciprofloxacin amidation derivatives (3a, 3b, 3c, and 3d). A review of the literature shows that glycerin can increase anticancer properties (Pruchnik et al., 2016; Wang et al., 2018). Therefore, based on the structure of 5, ciprofloxacin esterification/amidification derivatives, and ciprofloxacin amidation derivatives, it can be concluded that the esterification of ciprofloxacin due to the addition of glycerol increases the anticancer property. Regarding the obtained results, it was found that the anticancer properties of compound 5 and ciprofloxacin derivatization (esterification/amidation) were close to each other. Moreover, the anticancer property of ciprofloxacin amidation is almost the same. Thus, it can be concluded that the groups binding to the pyrazole ring do not play an essential role in the anticancer properties of the products.

**TABLE 4 MIC value ( $\mu\text{g/mL}$ ) results in investigating the antimicrobial properties of the synthesized derivatives.**

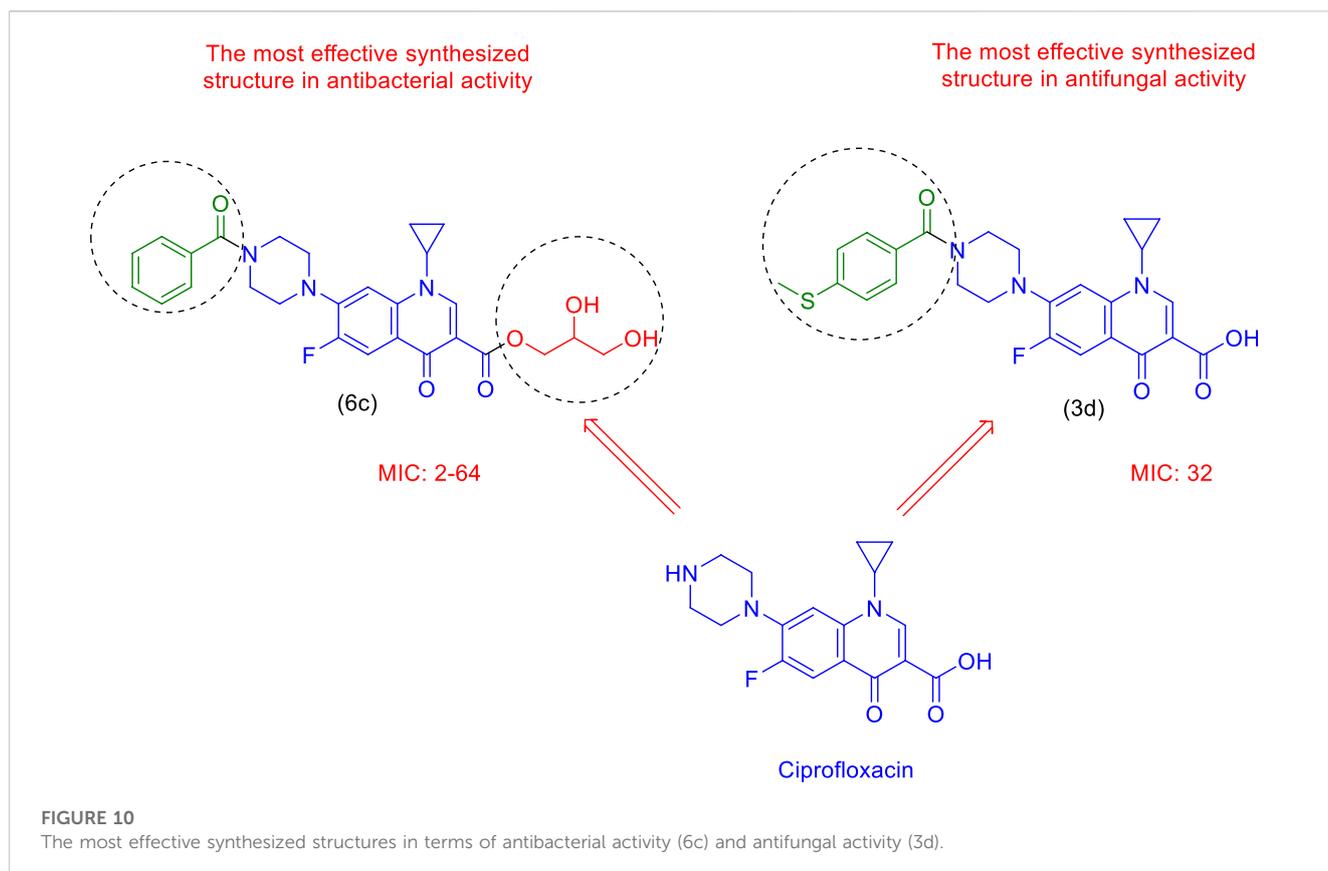
Compound	Gram-positive pathogenic bacterial				Gram-negative pathogenic bacterial				Fungi strain	
	ATCC 11778 <i>Bacillus cereus</i>	ATCC 15352 <i>Streptococcus equinus</i>	ATCC 25729 <i>Rhodococcus equi</i>	ATCC 29213 <i>Staphylococcus aureus</i>	ATCC 7002 <i>Proteus mirabilis</i>	ATCC 19606 <i>Acinetobacter baumannii</i>	ATCC 9610 <i>Yersinia enterocolitica</i>	ATCC 25922 <i>Escherichia coli</i>	ATCC 10231 <i>Candida albicans</i>	ATCC 1022 <i>Aspergillus fumigatus</i> Fresenius
3a	512	512	-	-	1024	-	2048	1024	-	512
3b	512	256	-	-	512	-	1024	512	-	512
3c	256	128	-	-	128	-	1024	256	-	256
3d	128	64	-	-	64	-	512	256	32	32
5	64	64	-	64	16	512	128	64	256	128
6a	32	16	-	32	4	128	64	32	128	64
6b	32	8	-	16	4	128	32	32	128	32
6c	16	8	-	4	2	64	32	16	64	32
Drug 1	2	4	2	2	1	32	8	8	32	64
Drug 2	-	32	-	8	2	-	16	8	-	-

**TABLE 5** MBC ( $\mu\text{g/mL}$ ) value for bacterial strains and MFC ( $\mu\text{g/mL}$ ) value for fungi strains results in investigating the antimicrobial properties of the synthesized derivatives.

Compound	Gram-positive pathogenic bacterial				Gram-negative pathogenic bacterial				Fungi strain	
	ATCC 11778 <i>Bacillus cereus</i>	ATCC 15352 <i>Streptococcus equinus</i>	ATCC 25729 <i>Rhodococcus equi</i>	ATCC 29213 <i>Staphylococcus aureus</i>	ATCC 7002 <i>Proteus mirabilis</i>	ATCC 19606 <i>Acinetobacter baumannii</i>	ATCC 9610 <i>Yersinia enterocolitica</i>	ATCC 25922 <i>Escherichia coli</i>	ATCC 10231 <i>Candida albicans</i>	ATCC 1022 <i>Aspergillus fumigatus Fresenius</i>
3a	1024	1024	-	-	2048	-	2048	2048	-	2048
3b	512	512	-	-	1024	-	2048	1024	-	1024
3c	256	256	-	-	256	-	1024	512	-	512
3d	256	128	-	-	256	-	1024	512	64	64
5	128	64	-	256	64	512	254	128	512	256
6a	64	32	-	64	16	512	128	128	512	128
6b	32	16	-	32	8	256	64	64	256	128
6c	32	16	-	8	4	128	32	32	128	64
Drug 1	4	8	4	4	2	64	8	16	64	128
Drug 2	-	64	-	16	4	-	32	32	-	-

**TABLE 6** IZD (mm) value results in investigating the antimicrobial properties of the synthesized derivatives.

Compound	Gram-positive pathogenic bacterial				Gram-negative pathogenic bacterial				Fungi strain	
	ATCC 11778 <i>Bacillus cereus</i>	ATCC 15352 <i>Streptococcus equinus</i>	ATCC 25729 <i>Rhodococcus equi</i>	ATCC 29213 <i>Staphylococcus aureus</i>	ATCC 7002 <i>Proteus mirabilis</i>	ATCC 19606 <i>Acinetobacter baumannii</i>	ATCC 9610 <i>Yersinia enterocolitica</i>	ATCC 25922 <i>Escherichia coli</i>	ATCC 10231 <i>Candida albicans</i>	ATCC 1022 <i>Aspergillus fumigatus Fresenius</i>
3a	14.29	14.53	-	-	17.38	-	15.37	13.02	-	15.19
3b	14.01	14.45	-	-	17.50	-	15.16	12.86	-	18.73
3c	14.57	14.76	-	-	18.67	-	14.81	13.52	-	17.91
3d	14.35	14.38	-	-	18.94	-	15.37	13.44	25.12	27.53
5	14.58	16.12	-	16.94	20.89	11.99	17.08	16.41	18.23	19.56
6a	17.76	15.99	-	19.43	21.02	12.64	17.96	17.73	20.12	23.17
6b	17.29	17.68	-	19.83	20.84	13.16	17.92	17.35	19.46	25.32
6c	18.19	17.31	-	20.03	21.99	12.50	18.15	17.38	20.75	24.01
Drug 1	20.21	16.85	22.43	19.38	23.67	15.29	18.72	16.41	23.48	21.40
Drug 2	-	15.96	-	19.61	20.48	-	17.81	17.37	-	-



Based on the values of cell proliferation and viability, as well as  $IC_{50}$  related to 5 and abemaciclib, it was found that the anticancer property of 5 is slightly higher than abemaciclib, which is a known anti-breast cancer drug.

Statistical studies were performed for abemaciclib, 3a, 3b, 3c, 3d, 5, 6a, 6b, and 6c at 24 h and 48 h and  $IC_{50}$  values;  $p$ -values were obtained, and the results are shown in Table 3.

Based on the results shown in the Table, it can be concluded that concentration is a critical parameter at 24 and 48 h. Therefore, the  $IC_{50}$  values and the effectiveness of the compounds depend on the exposure time.

As a final result, it can be said that 5 has the ability to be used as an effective anticancer drug against breast cancer, and, after other supplementary tests, it can be developed as a potent drug (Figure 9).

### 3.3 Examination of antimicrobial activities

The antimicrobial properties of the synthesized derivatives along with drugs 1 and 2 were investigated on four Gram-positive strains (ATCC11778/*Bacillus cereus*, ATCC15352/*Streptococcus equinus*, ATCC25729/*Rhodococcus equi*, and ATCC29213/*Staphylococcus aureus*), four Gram-negative strains (ATCC7002/*Proteus mirabilis*, ATCC19606/*Acinetobacter baumannii*, ATCC9610/*Yersinia enterocolitica*, and ATCC25922/*Escherichia coli*) and two fungal species (ATCC10231/*Candida albicans* and ATCC1022/*Aspergillus fumigatus Fresenius*). The results of the antimicrobial activity of the synthesized compounds, Gentamicin, Cefazolin,

Terbinafine, and Tolnaftate, including the MIC value, MBC/MFC value, and IZD value, are shown in Tables 4–6.

Gentamicin, Cefazolin, Terbinafine, and Tolnaftate are known antibiotics, and, in this study, they were used to compare the antibacterial properties of the synthetic compounds. Gentamicin (antibacterial activity Tables, drug 1) and Cefazolin (antibacterial activity Tables, drug 2) are recognized potent antibacterial drugs, while Terbinafine (antifungal activity Tables, drug 1) and Tolnaftate (antifungal activity Tables, drug 2) are recognized potent antifungal drugs.

In terms of antimicrobial activity, the highest effectiveness was observed for 6c. The order of antimicrobial activity is described as  $6b > 6a > 5 > 3d > 3c > 3b > 3a$ . From the comparison of the structures, it can be concluded that the effectiveness of the compounds against bacterial species depends first on the hydroxyl and ester groups of the compounds, then on having an electron-rich benzene ring, and finally on substitutions with more methyl. The review of the literature showed that compounds containing oxygen and esters have high antibacterial properties (Shafiee et al., 1973; Dongamanti et al., 2015; Xie et al., 2015; Qin et al., 2020; Shi et al., 2021); here, it was also determined that the esterification of ciprofloxacin by glycerol led to the synthesis of compounds with significant antimicrobial properties.

In terms of antifungal activity, the highest effectiveness was observed for 3d. The MIC values for this combination were 32  $\mu\text{g/mL}$  for both *Candida albicans* and *Aspergillus fumigatus Fresenius*. The presence of sulfur and the electron-rich benzene ring in the structure of this compound is the reason for the effectiveness of this compound in terms of antifungal activity. The order of antifungal activity is described as  $6c > 6b > 6a > 5 > 3c > 3b > 3a$ . By examining the

structures of the derivatives, the order of effectiveness can be attributed to compounds with hydroxyl and ester groups, followed by the benzene ring, and, finally, substitutions with more methyl, just like the case for the antibacterial activity. Therefore, it was proved here that the presence of sulfur in the structure of 3d (methylthio) plays a significant role in antifungal activity (Pathania et al., 2019; Hua et al., 2020; Asif and Imran, 2022).

Comparing the results with known drugs such as Gentamicin, Cefazolin, Terbinafine, and Tolnaftate proved that the synthesized compounds are more active than them. For example, Cefazolin was ineffective against *Bacillus cereus* and *Acinetobacter baumannii*, but the MIC values for 6c were observed to be 16 µg/mL and 64 µg/mL, respectively. In terms of antifungal activity, Tolnaftate was not effective against *Candida albicans* and *Aspergillus fumigatus Fresenius*, but an MIC value of 32 µg/mL was observed for 3d.

As a final result, 6c and 3d can be reported as effective agents with antimicrobial and antifungal activities, respectively (Figure 10). After being proven to be safe and passing additional supplementary pharmacology tests, including *in vivo*, cytotoxicity, etc., they can be suggested as strong effective drugs.

## 4 Conclusion

In summary, in this research, ciprofloxacin was used as a starting material for synthesizing new amidification and esterification derivatives of ciprofloxacin. The synthetic compounds were confirmed by using proton nuclear magnetic resonance (<sup>1</sup>H NMR), carbon-13 nuclear magnetic resonance (<sup>13</sup>C NMR), mass spectrometry (MS), and elemental analysis (EA). IZD, MIC, MBC, and MFC were measured for antimicrobial properties, including Gram-positive antibacterial, Gram-negative antibacterial, and antifungal based on clinical and laboratory standards. In some derivatives, more effective antibacterial and antifungal properties were observed than for some commercial drugs. In continuation of the biological investigations, the *in vitro* anticancer activity of the derivatives against breast cancer cells was evaluated by the MTT method. In terms of anticancer properties, the effectiveness of some derivatives was similar to abemaciclib, a well-known anti-breast cancer drug. In biological evaluations, a clear relationship between the structure of the derivatives and biological properties such as antibacterial, antifungal, and anticancer was observed. As the results of biological activities have proven, some derivatives have higher antimicrobial and anticancer properties than

commercial drugs used in the market. Therefore, the synthesized compounds in this study are important in this regard; it is suggested that other supplementary pharmacological and biological assays, such as *in silico* studies on the mechanism of action, *in vivo* tests, cytotoxicity tests, etc., be performed and if positive findings can be seen, then these compounds can be reported as effective leads for further preclinical and clinical studies.

## Data availability statement

The original contributions presented in the study are available upon request, further inquiries can be directed to the corresponding author.

## Author contributions

GA: Writing—original draft. ZA-O: Conceptualization, Supervision, Writing—original draft, Writing—review and editing.

## Funding

The authors declare that no financial support was received for the research, authorship, and/or publication of this article.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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