

Synthesis and Antimicrobial, Antiproliferative Evaluation of Novel Quinolone and Conazole Analogues via Conventional and Microwave Techniques

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Abstract

1,2,4-Triazole-3-one (**3**), acquired from cinnamaldehyde was converted to the corresponding carbox(thio)amides via several steps (**6a-c**). Their reaction with sodium hydroxide gave the 1,2,4-triazole derivatives (**7a-c**). Compound **3** treatment with 2-bromo-1-(4-chlorophenyl) ethanone or 2-chloro-1-(2,4-dichlorophenyl)ethanone afforded the compounds **8a,b** and by reducing these compounds reduction products were obtained (**9a,b**). The synthesis of (**10a-e**) was carried out by the reaction compounds **9a,b** with different benzyl chlorides. Then oxadiazol derivative (**12**) was obtained by ring closure from hydrazide compound **5**. Subsequently compounds **3**, **7a-c** and **12** were treated with various amines in the presence of formaldehyde to yield Mannich bases (**11a-e**, **14a-e**, **13a,b**). Microwave-assisted and conventional techniques were utilized for the syntheses. The structures of newly synthesized compounds were illuminated by spectroscopic methods. Their antimicrobial (MIC method), and anticancer activities (Abay's method) were examined. Results showed that most of the compounds exhibited good antimicrobial activities. Especially compounds **14a-e** which is a mannich base showed very good antitubercular activity against *Mycobacterium smegmatis* compared with Streptomycin standard drug. Also compounds **8a** and **9b** have been found to have strong antiproliferative effects on the HeLa cervical cancer cells and also these compounds did not have cytotoxic effect on normal cell.

1. Introduction

Cancer, is the result of uncontrolled growth of cells a major health concern and amongst the most important reasons of death worldwide [1–3]. The World Health Organization's cancer agency warns that there will be 22 million new cases of cancer every year within the next two decades [4]. Hence, cancer treatment is the primary task of today's medical research [5]. Despite the invention of several chemotherapeutic agents, still treatment of cancer is a major challenge, because of multi-drug resistance, toxicity or poor bioavailability. The effectiveness of many anticancer drugs is limited by acquired resistance to drugs, side effects due to their toxicity to normal cells because of their inability to differentiate between normal and cancerous cells. In this context, there is need to design and synthesize new, effective and less toxic anticancer agents throwing a challenge to medicinal chemists all over the World [6, 7].

Infectious diseases continue to be a leading threat to human health, and the rapid development of bacterial resistance to current antibiotic chemotherapies has rendered lots of therapy weapons less effective [8]. It is anticipated that antibiotic resistance is going to cause more than 10,000,000 deaths per year by the year 2050, posing a formidable challenge for disease treatment as pathogens become resistant to clinical drugs [9]. The World Health Organization has launched a global action plan calling on all countries to take measures towards drug-resistant microbes, and the discovery of efficacious and safer antimicrobials with new or multiple mechanisms of action has been an urgent need to combat resistant strains [10, 11].

Organic molecules bearing heterocyclic compounds have received a significant consideration in all fields of life comprising medicinal, pharmaceutical, combinatorial, natural resources, agriculture and dyes products [3, 12]. Hybrid compounds are molecules with two or more structural fields, which have diverse dual activities and biological features. These substances generally possess the capacity to get over medicine resistance, develop pharmacokinetic structures, and abate toxicity [13, 14].

Triazoles are an important class of aromatic five-membered heterocyclic compounds with a wide variety of biological activities. They consist of two structural isomers: 1,2,3- and 1,2,4-triazole. Compounds based on 1,2,4-triazole scaffold show diverse biological and pharmacological activities [15–17]. The synthesized compounds having 1,2,4-triazole-3-thione skeleton have been reported to possess biological and pharmacological activities such as anticonvulsant, urease inhibition, antioxidant, analgesic, anti-parasitic, antiulcer, anticancer, anti-HIV, anti-tuberculosis, antiamebic, anti-giardial, antiepileptic, anti-inflammatory, antidepressant and anxiolytic [18–24].

Quinolines are widespread pharmacophores in the anti-bacterial substances. Fluoroquinolones are noted as one of the most broadly used antibacterial agents for the cure of bacterial illness. The cytotoxic activity of quinolone derivatives has become the source of new anticancer agents, which might also help addressing side-toxicity and resistance [25]. Quinolines and related derivatives are useful compounds with diverse pharmaceutical applications, and some have even reached markets for treatment of various ailments [26]. Thus, the hybridization of quinoline/quinolone with 1,2,4-triazole is inclined to present hopeful anti-bacterial agents [27, 28].

Imidazoles and triazoles, as a group called conazoles, are currently used worldwide as fungicides for grain, vegetable, fruit, and flower production and as pharmaceuticals for treatment of human mycoses including vaginal mycoses in pregnant women and thrush in infants [29].

Recently, multicomponent reactions (MCRs) have received considerable attention by synthetic organic and medicinal chemists for the construction of complex molecules having biological activity. When compared with conventional organic reactions, MCRs have some superior properties including high conversion rate, minimal reaction time and structural complexity. Thus, MCRs are also considered as green chemical processes [30]. Among these, Mannich reaction, a one pot three-component condensation reaction, provide synthetically and biologically important β -aminoalkylated compounds, which are important intermediates for the construction of various nitrogen-containing natural products and pharmaceuticals [31].

Microwave-assisted organic synthesis of various heterocyclic moieties is an effective and environment-friendly synthetic approach and becoming an effective tool of green chemistry method. Microwave assisted techniques were reported to be more effective in perspective of environment, reaction time, high yields, ease of work-up and isolation of products. Moreover, solvents which are often expensive, toxic, difficult to remove in the case of aprotic dipolar solvents with high boiling point, and are environmentally polluting agents, are not necessary most of the microwave assisted synthesis [32, 33].

One of the main strategies for the discovery of new drugs is combining two or more pharmacophoric moieties in a single molecule to obtain the synergistic effect or to obtain antitumor agents that have a novel mode of action. For this purpose, various quinolone and conazole derivatives with biological activity were synthesized in this study.

2. Material And Methods

2.1 Experimental

2.1.1 General

The chemicals were obtained from Fluka Chemie AG Buchs (Switzerland) and utilized without further purification. Melting points of the synthesized molecules were found in open capillaries over a Büchi B-540 melting degree device and are uncorrected. Reactions were screened by thin-layer chromatography (TLC) over silicagel 60 F254 aluminium plates. Mobile stage was ethyl acetate:diethyl ether (1:1), and finding was performed utilizing UV lamp. FT-IR datas were founded utilizing a *Perkin Elmer* 1600 serial FTIR spectrometer. ^{13}C NMR and ^1H NMR spectra were recorded in $\text{DMSO-}d_6$ on a *BRUKER AVENE II* 400 MHz NMR Spectrometer (400.13 MHz for ^1H and 100.62 MHz for ^{13}C). The chemical ranges are dedicated at ppm interested to Me_4Si as an interior reference. J values are presented in Hz. Elemental assay was acquired over *aaCostech Elemental Combustion System* CHNS-O elemental analyzer. All the molecules present C, H and N assay within $\pm 0.4\%$ of the theoretical ranges. The mass spectra were provided on a *Quattro LC-MS* (70 eV) Instrument.

2.1.1.1 4-Amino-5-metil-2,4-dihidro-3H-1,2,4-triazol-3-on (2) [34]

Hydrazine hydrate (25 mmol) in 3% water solution was added to ethyl 2-(1-ethoxyethylidene) hydrazinecarboxylate (1) (10 mmol) and boiled under reflux for 8 hours. After the mixture was left in the freezer overnight, the white solid which had precipitated was filtered off and purified by crystallization from ethanol.

Yield: 70%, m.p: 210–212°C. FT-IR (ν_{max} , cm^{-1}): 3295 ve 3207 (NH_2), 3218 (NH), 1683 (C = O), 1588 (C = N). ^1H NMR ($\text{DMSO-}d_6$, δ ppm): 2.07 (3H, s, CH_3), 5.13 (2H, s, NH_2), 11.22 (1H, s, NH).

2.1.1.2 5-methyl-4-[(1Z,2Z)-3-phenylprop-2-en-1-ylidene]amino-2,4-dihidro-3H-1,2,4-triazol-3-one (3) [34]

In a round-bottom flask, a mixture of 1,2,4-triazol-3-one (2) (10 mmol) and cinnamaldehyde (10 mmol) was heated on oil bath at 110-120°C for 2 hours. The solid obtained by cooling the mixture to room temperature was crystallized from the ethanol: water (1: 3) mixture. The crystals obtained were purified

by crystallizing several more times from the same solvent and after drying in vacuo, it was identified as compound 3.

Yield: 78%, m.p: 190–192°C. FT-IR (ν_{\max} , cm^{-1}): 3167 (NH), 3041 (aromatic CH), 1687 (C = O), 1512 (C = N). ^1H NMR (DMSO- d_6 , δ ppm): 2.20 (3H, s, CH_3), 7.00 (1H, t, $J = 8.0$ Hz, arH), 7.04–7.40 (3H, m, arH), 7.65 (1H, d, $J = 8.0$ Hz, arH), 9.50 (3H, s, 3CH), 11.77 (1H, s, NH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.65 (CH_3), 125.41 (CH), arC: [128.02 (2CH), 129.32 (2CH), 129.98 (CH), 135.82 (C)], 143.69 (CH), 144.54 (triazole C-3), 151.71 (triazole C-5), 156.64 (CH). EI MS m/z (%): 135.28 (100), 113.14 (80), 251.32 ($[\text{M} + \text{Na}]^+$, 49), 103.33 (37), 114.21 (36), 215.22 (28), 182.20 (25), 152.26 (21).

2.1.1.3 Ethyl (3-methyl-5-oxo-4-[(1Z,2Z)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetate (4)

The solution of compound (3) (10 mmol) in absolute ethanol was refluxed with sodium (10 mmol) in absolute ethanol for 2 h. Then, ethyl bromoacetate (1.2 mL, 10 mmol) was added and refluxed for an additional 6 h. After evaporation of solvent under reduced pressure, a solid appeared. The crude product was recrystallized from ethanol-water (1: 1) to afford the desired compound.

Yield: 76%, m.p: 85–87°C. FT IR (ν_{\max} , cm^{-1}): 3037 (aromatic CH), 1734 (C = O), 1701 (C = O), 1170 (C-O), 1595 (C = N). ^1H NMR (DMSO- d_6 , δ ppm): 1.20–1.23 (3H, m, CH_3), 2.26 (3H, s, CH_3), 4.15–4.17 (2H, m, CH_2), 4.58 (2H, s, CH_2), 7.04–7.10 (1H, m, arH), 7.34–7.42 (4H, m, arH), 7.68–7.70 (2H, m, 2CH), 9.46 (1H, d, $J = 8.0$ Hz, CH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.51 (CH_3), 14.46 (CH_3), 46.77 (CH_2), 61.69 (CH_2), 125.08 (CH), 125.10 (CH), 128.48(CH), arC: [128.16 (CH), 129.35 (CH), 129.37 (CH), 130.14 (CH), 130.17 (CH), 144.00 (C)], 150.20 (triazole C-3), 157.38 (triazole C-5), 168.13 (C = O). EI MS m/z (%): 337.48 ($[\text{M} + \text{Na}]^+$, 100), 338.43 ($[\text{M} + \text{Na}]^+$, 38). Elemental analysis for: $\text{C}_{16}\text{H}_{18}\text{N}_4\text{O}_3$; Calculated (%): C, 61.13; H, 5.77; N, 17.82; Found (%): C, 61.45; H, 5.80; N, 17.90.

2.1.1.4 2-(3-methyl-5-oxo-4-[(1Z,2Z)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl)acetohydrazide (5)

A mixture of molecule 4 (10 mmol) in ethanol was refluxed with hydrazine hydrate (25 mmol) for 15 h. The solid acquired on keeping the reaction solution in a cold was filtered off, recrystallized from ethyl acetate:diethyl ether (1:3) to afford the desired product 5.

Yield: 78%, m.p: 177–179°C. FT IR (ν_{\max} , cm^{-1}): 3337 (NH), 3280 (NH_2), 3068 (aromatic CH), 1698 (C = O), 1655 (C = O), 1578 (C = N). ^1H NMR (DMSO- d_6 , δ ppm): 2.08 (3H, d, $J = 8.0$ Hz, CH_3), 4.18 (2H, s, CH_2), 5.25 (2H, s, NH_2), 6.08–7.03 (5H, m, arH), 8.55 (3H, s, 3CH), 9.17 (1H, s, NH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.07 (CH_3), 46.79 (CH_2), 77.90 (2CH), 84.80 (CH), arC: [100.80 (CH), 101.20 (2CH), 122.78 (2CH), 145.46

(C)], 153.69 (triazole C-3), 166.49 (triazole C-5), 170.68 (C = O). EI MS m/z (%): 323.21 ($[M + Na]^+$, 100), 355.22 (18), 363.27 (16). Elemental analysis for: $C_{14}H_{16}N_6O_2$; Calculated (%): C, 55.99; H, 5.37; N, 27.98; Found (%): C, 56.01; H, 5.40; N, 28.00.

General Method for the Synthesis of Compounds 6a-c

Phenyl isothiocyanate (for **6a**), benzyl isothiocyanate (for **6b**) or phenyl isocyanate (for **6c**) (20 mmol) was added to the mixture of molecule **5** (10 mmol) in a dried dichloro methane drop wise and the solution was stirred at room temperature for 24 h. The solid product was precipitated.

2.1.1.5 2-[(3-methyl-5-oxo-4-[(1E,2Z)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl]-N-phenylhydrazinecarbothioamide (**6a**)

Yield: 91%, m.p. 148–149°C. FT IR (ν_{max} , cm^{-1}): 3349 (NH), 3283 (2NH), 3061 (aromatic CH), 2924 (aliphatic CH), 1715 (C = O), 1543 (C = N), 1240 (C = S). 1H NMR (DMSO- d_6 , δ ppm): 1.84 (3H, s, CH_3), 4.48 (2H, d, $J = 4.0$ Hz, CH_2), 4.67 (2H, s, CH_2), 7.02–7.15 (8H, m, arH), 7.42 (2H, d, $J = 8.0$ Hz, arH), 8.28 (1H, s, CH), 9.16 (1H, s, CH), 9.25 (1H, s, CH), 9.91 (1H, s, NH), 11.28 (1H, s, NH), 11.38 (1H, s, NH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.53 (CH_3), 46.76 (CH_2), 90.76 (CH), 98.86 (CH), 100.73 (CH), arC: [125.15 (CH), 127.13 (CH), 127.40 (CH), 128.15 (2CH), 129.39 (CH), 130.16 (2CH), 135.75 (2CH), 140.84 (C), 143.81 (C)], 150.40 (triazole C-3), 157.14 (triazole C-5), 178.20 (C = O), 189.54 (C = S). EI MS m/z (%): 473.23 (40), 472.17 ($[M + Na]^+$, 100), 437.25 (35). Elemental analysis for: $C_{22}H_{23}N_7O_2S$; Calculated (%): C, 58.78; H, 5.16; N, 21.81; Found (%): C, 58.80; H, 5.20; N, 21.88.

2.1.1.6 N-benzyl-2-[(3-methyl-5-oxo-4-[(1E,2Z)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl]hydrazinecarbothioamide (**6b**)

Yield: 91%, m.p. 148–149°C. FT IR (ν_{max} , cm^{-1}): 3349 (NH), 3283 (2NH), 2924 (aliphatic CH), 3061 (aromatic CH), 1715 (C = O), 1543 (C = N), 1240 (C = S). 1H NMR (DMSO- d_6 , δ ppm): 1.84 (3H, s, CH_3), 4.48 (2H, d, $J = 4.0$ Hz, CH_2), 4.67 (2H, s, CH_2), 7.02–7.15 (8H, m, arH), 7.42 (2H, d, $J = 8.0$ Hz, arH), 8.28 (1H, s, CH), 9.16 (1H, s, CH), 9.25 (1H, s, CH), 9.91 (1H, s, NH), 11.28 (1H, s, NH), 11.38 (1H, s, NH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.53 (CH_3), 46.76 (CH_2), 47.11 (CH_2), 90.76 (CH), 98.86 (CH), 100.73 (CH), arC: [125.15 (CH), 127.13 (CH), 127.40 (CH), 128.15 (CH), 129.39 (CH), 130.16 (2CH), 135.75 (2CH), 140.84 (C), 143.81 (C)], 150.40 (triazole C-3), 157.14 (triazole C-5), 178.20 (C = O), 189.54 (C = S). EI MS m/z (%): 472.17 ($[M + Na]^+$, 100), 473.23 (40), 437.25 (35). Elemental analysis for: $C_{22}H_{23}N_7O_2S$; Calculated (%): C, 58.78; H, 5.16; N, 21.81; Found (%): C, 58.80; H, 5.20; N, 21.88.

2.1.1.7 2-[(3-methyl-5-oxo-4-[(1E,2Z)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl]acetyl]-N-

phenylhydrazinecarboxamide (6c)

Yield: 93%, m.p. 152–154°C. FT IR (ν_{\max} , cm^{-1}): 3360 (NH), 3198 (2NH), 3037 (aromatic CH), 1715 (C = O), 1626 (C = S), 1577 (C = N). ^1H NMR (DMSO- d_6 , δ ppm): 2.25 (3H, s, CH_3), 4.46 (2H, s, CH_2), 6.97–7.28 (4H, m, arH), 7.33–7.47 (4H, m, arH), 7.69 (2H, d, $J = 4.0$ Hz, arH), 8.26 (2H, s, 2CH), 8.78 (1H, s, CH), 9.50 (2H, s, NH), 10.04 (1H, s, NH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.56 (CH_3), 46.78 (CH_2), 91.75 (CH), 97.56 (CH), 101.43 (CH), arC: [118.93 (CH), 119.11 (CH), 122.51 (CH), 122.73 (CH), 125.18 (CH), 129.10 (CH), 129.16 (CH), 129.24 (CH), 129.38 (CH), 139.95 (C), 143.85 (C)], 155.54 (triazole C-3), 157.09 (triazole C-5), 166.90 (C = O), 173.56 (C = O). EI MS m/z (%): 442.31 ($[\text{M} + \text{Na}]^+$, 100), 134.98 (56), 447.25 (31), 301.28 (25), 420.35 ($[\text{M} + 1]^+$, 19). Elemental analysis for: $\text{C}_{22}\text{H}_{25}\text{N}_7\text{O}_3$; Calculated (%): C, 60.13; H, 5.05; N, 23.38; Found (%): C, 60.17; H, 5.01; N, 23.40.

General Method for the Synthesis of Compounds 7a-c

Method 1. A solution of the corresponding compound **6a-c** (10 mmol) in ethanol and water (1:1) was refluxed in the presence NaOH (2 N) for 6 h. Then the resulting solution was cooled to room temperature and acidified to pH 4 with 37% HCl. The precipitate formed was filtered off, washed with water, and recrystallized from ethyl acetate to afford the desired compound.

Method 2. The mixture of the corresponding compound **6a-c** (1 mmol) and NaOH (0.5 mmol) in ethanol (10 mL) was irradiated in monomode microwave reactor in closed vessel with the pressure control at 100°C for 16 min (hold time) at 150 W maximum power (the progress of the reaction was monitored by TLC). Then the resulting solution was cooled to room temperature and acidified to pH 5 with 37% HCl. The precipitate formed was filtered off, wash with water, and recrystallized from ethyl acetate (for **7a**), ethanol (for **7b** and **7c**) to give the target compounds.

2.1.1.8 2-[(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)methyl]-5-methyl-4-[(1E,2Z)-3-phenyl prop-2-en-1-ylidene]amino-2,4-dihydro-3H-1,2,4-triazol-3-one (7a)

Yield: 65% (Method 1), 80% (Method 2), m.p. 203–204°C. FT IR (ν_{\max} , cm^{-1}): 3029 (ar-CH), 2932 (SH), 1680 (C = O), 1585 (C = N). ^1H NMR (DMSO- d_6 , δ ppm): 2.02 (3H, s, CH_3), 5.08 (2H, d, $J = 8.0$ Hz, CH_2), 6.85 (2H, s, arH), 7.01–7.95 (8H, m, arH), 8.76 (1H, s, CH), 9.52 (2H, s, 2CH), 13.70 (1H, s, SH). ^{13}C NMR (DMSO- d_6 , δ ppm): 10.11 (CH_3), 48.67 (CH_2), 91.80 (CH), 95.98 (CH), 100.56 (CH), arC: [121.04 (2CH), 125.36 (CH), 126.10 (CH), 127.65 (CH), 128.93 (CH), 129.25 (CH), 130.67 (CH), 132.12 (2CH), 133.07 (C), 135.58 (C)], 152.64 (triazole C-3), 156.19 (triazole C-3), 166.40 (triazole C-5), 175.25 (triazole C-5). EI MS m/z (%): 454.26 ($[\text{M} + \text{Na}]^+$, 31), 428.23 (100), 406.27 (37), 287.27 (44), 261.24 (87), 187.16 (81). Elemental analysis for: $\text{C}_{22}\text{H}_{21}\text{N}_7\text{OS}$; Calculated (%): C, 61.23; H, 4.91; N, 22.72; Found (%): C, 61.27; H, 4.95; N, 22.75.

2.1.1.9 2-[(4-benzyl-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-5-methyl-4-[[[(1E,2Z)-3-phenyl prop-2-en-1-ylidene]amino]-2,4-dihydro-3H-1,2,4-triazol-3-one (7b)

Yield: 68% (Method 1), 86% (Method 2), m.p. 198–199°C. FT IR (ν_{\max} , cm^{-1}): 3032 (aromatic CH), 2929 (SH), 1699 (C = O), 1598 (C = N). ^1H NMR (DMSO- d_6 , δ ppm): 2.02 (3H, s, CH_3), 5.00 (2H, d, $J = 8.0$ Hz, CH_2), 5.21 (2H, d, $J = 12.0$ Hz, CH_2), 6.92 (2H, d, $J = 8.0$ Hz, arH), 7.10–7.80 (8H, m, arH), 8.73 (1H, s, CH), 9.34 (2H, s, 2CH), 14.02 (1H, s, SH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.12 (CH_3), 46.38 (CH_2), 50.20 (CH_2), 93.70 (CH), 98.58 (CH), 103.48 (CH), arC: [126.04 (2CH), 127.45 (CH), 128.20 (CH), 128.40 (CH), 128.83 (CH), 129.38 (CH), 129.52 (CH), 132.09 (2CH), 133.64 (C), 134.78 (C)], 155.54 (triazole C-3), 157.09 (triazole C-3), 167.20 (triazole C-5), 172.20 (triazole C-5). EI MS m/z (%): 428.23 (100), 261.24 (87), 187.16 (81), 287.27 (44), 406.27 (37), 454.26 ($[\text{M} + \text{Na}]^+$, 31). Elemental analysis for: $\text{C}_{23}\text{H}_{25}\text{N}_7\text{OS}$; Calculated (%): C, 61.23; H, 4.91; N, 22.72; Found (%): C, 61.27; H, 4.95; N, 22.75.

2.1.1.10 5-methyl-2-[(5-oxo-4-phenyl-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]-4-[[[(1E,2Z)-3-phenylprop-2-en-1-ylidene]amino]-2,4-dihydro-3H-1,2,4-triazol-3-one (7c)

Yield: 57% (Method 1), 76% (Method 2), m.p. 188–190°C. FT IR (ν_{\max} , cm^{-1}): 3370 (NH), 3027 (aromatic CH), 1683 (C = O), 1626 (C = O), 1594 (C = N). ^1H NMR (DMSO- d_6 , δ ppm): 2.20 (3H, s, CH_3), 4.53 (2H, s, CH_2), 7.29 (2H, d, $J = 8.0$ Hz, arH), 7.31–7.65 (8H, m, arH), 7.67 (1H, s, CH), 7.85 (1H, s, CH), 7.97 (1H, s, CH), 10.77 (1H, s, NH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.40 (CH_3), 47.25 (CH_2), 97.70 (CH), 99.74 (CH), 102.47 (CH), arC: [118.48 (CH), 119.70 (CH), 120.33 (CH), 122.93 (CH), 127.22 (CH), 127.47 (CH), 128.83 (CH), 128.90 (CH), 129.00 (CH), 134.85 (C), 139.53 (C)], 154.53 (triazole C-3), 158.10 (triazole C-3), 168.32 (triazole C-5), 169.78 (triazole C-5). EI MS m/z (%): 402.26 ($[\text{M} + 1]^+$, 100), 288.21 (95), 376.24 (87), 416.28 (76). Elemental analysis for: $\text{C}_{22}\text{H}_{23}\text{N}_7\text{O}_2$; Calculated (%): C, 62.83; H, 4.77; N, 24.42; Found (%): C, 62.85; H, 4.79; N, 24.50.

General Method for The Synthesis of Compounds 8a,b

Method 1. The solution of compound **3** (10 mmol) in ethanol was refluxed in the presence of sodium ethoxide (10 mmol) for 6 h. Then, 2-bromo-1-(4-chlorophenyl)ethanone (10 mmol), (**for 8a**), 2-chloro-1-(2,4-dichlorophenyl)ethanone (10 mmol) (**for 8b**), was added into it, and the mixture was refluxed for additional 18 h. After evaporation the solvent under reduced pressure, a solid appeared. This crude product was recrystallized from acetone:water (1:3) to give the target compound.

Method 2. The mixture of compound **3** (1 mmol) and sodium ethoxyde (1 mmol) in 10 mL of ethanol was irradiated in monomode microwave reactor in closed vessel with the pressure control at 120°C for 6 min (hold time) at 150 W maximum power. Then, 2-bromo-1-(4-chlorophenyl)ethanone (10 mmol), (**for**

8a), 2-chloro-1-(2,4-dichlorophenyl)ethanone (10 mmol) (**for 8b**), was added into it, and the mixture was irradiated for additional 10 min under the same conditions. After removing the solvent under reduced pressure, a solid appeared.

2.1.1.11 2-[2-(4-chlorophenyl)-2-oxoethyl]-5-methyl-4-[(1Z,2Z)-3-phenylprop-2-en-1-ylidene] amino}-2,4-dihydro-3H-1,2,4-triazol-3-one (**8a**)

Yield: 65% (Method 1), 82% (Method 2), m.p: 125–127°C. FT-IR (ν_{\max} , cm^{-1}): 3058 (aromatic CH), 1693 (C = O), 1625 (C = O). ^1H NMR (DMSO- d_6 , δ ppm): 1.03–1.16 (3H, m, CH_3), 5.38 (2H, s, CH_2), 7.27–7.38 (1H, m, arH), 7.38–7.40 (5H, m, arH), 7.64–7.67 (3H, m, arH), 9.47–9.53 (3H, m, 3CH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.55 (CH_3), 52.14 (CH_2), 125.15 (CH), arC: [125.45 (CH), 128.70 (CH), 128.95 (CH), 129.15 (CH), 129.25 (CH), 129.34 (CH), 129.36 (CH), 129.53 (CH), 129.99 (CH), 139.52 (C), 144.30 (C), 144.48 (C)], 144.02 (CH), 144.37 (CH), 150.63 (triazole C-3), 151.87 (triazole C-5), 192.56 (C = O). EI MS m/z (%): 403.39 ($[\text{M} + \text{Na}]^+$, 100), 405.40 (37), 251.35 (34), 449.38 (33), 341.39 (18), 381.37 ($[\text{M} + 1]^+$, 12).

2.1.1.12 2-[2-(2,4-dichlorophenyl)-2-oxoethyl]-5-methyl-4-[(1Z,2Z)-3-phenylprop-2-en-1-ylidene] amino}-2,4-dihydro-3H-1,2,4-triazol-3-one (**8b**)

Yield: 58% (Method 1), 77% (Method 2), m.p: 110–112°C. FT-IR (ν_{\max} , cm^{-1}): 3041 (aromatic CH), 1689 (C = O), 1625 (C = O). ^1H NMR (DMSO- d_6 , δ ppm): 2.21 (3H, s, CH_3), 5.25 (2H, s, CH_2), 7.29–7.33 (1H, m, arH), 7.38–7.42 (4H, m, arH), 7.50–7.67 (3H, m, arH), 9.53 (2H, d, $J = 12.0$ Hz, 2CH), 11.80 (1H, s, CH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.66 (CH_3), 48.05 (CH_2), 125.43 (CH), arC: [128.13 (CH), 129.33 (CH), 129.99 (CH), 130.47 (CH), 130.58 (CH), 130.93 (CH), 132.30 (CH), 132.36 (CH), 144.19 (C), 144.52 (C)], 143.72 (CH), 144.37 (CH), 150.46 (triazole C-3), 151.72 (triazole C-5), 194.43 (C = O). EI MS m/z (%): 437.25 ($[\text{M} + \text{Na}]^+$, 100), 251.30 (81), 439.26 (69), 447.58 (62), 360.54 (21), 341.40 (20).

General Method for The Synthesis of Compounds **9a,b**

Method 1: A solution of the corresponding compound **8a,b** (10 mmol) in absolute ethanol (40 mL) was refluxed in the presence of NaBH_4 (30 mmol) for 16 h. After evaporating the solvent under reduced pressure, an oily mass appeared. This was recrystallized from acetone:water (1:3) to afford the desired product.

Method 2: The mixture of the corresponding compound **8a,b** (1 mmol) and NaBH_4 (3 mmol) in ethanol was irradiated in monomode microwave reactor in closed vessel with the pressure control at 125°C for 8 min (hold time) at 150 W maximum power. (The progress of the reaction was monitored by TLC). Then solvent was removed under reduced pressure and a solid appeared. This crude product was washed with water and recrystallized from acetone:water (1:3).

2.1.1.13 2-[2-(4-chlorophenyl)-2-hydroxyethyl]-5-methyl-4-[(1E,2Z)-3-phenylprop-2-en-1-ylidene] amino}-2,4-dihydro-3H-1,2,4-triazol-3-one (**9a**)

Yield: 52% (Method 1), 85% (Method 2), m.p: 118–119°C. FT-IR (ν_{\max} , cm^{-1}): 3374 (OH), 3038 (aromatic CH), 1639 (C = O), 1630 (C = O). ^1H NMR (DMSO- d_6 , δ ppm): 2.21 (3H, s, CH_3), 4.84–4.88 (2H, m, CH_2), 5.41 (1H, s, OH), 6.25–6.32 (2H, m, arH), 6.39–6.43 (1H, m, arH), 7.01–7.07 (1H, m, arH), 7.23–7.43 (5H, m, arH), 7.68 (2H, d, $J = 8.0$ Hz, 2CH), 9.56 (1H, s, CH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.22 (CH_3), 51.74 (CH_2), 70.02 (CH), arC: [125.43 (CH), 126.21 (CH), 126.71 (CH), 128.11 (CH), 128.45 (CH), 128.47 (CH), 128.73 (CH), 128.76 (CH), 129.10 (CH), 132.21 (C), 136.89 (C), 142.20 (C)], 129.36 (CH), 133.46 (CH), 152.78 (triazole C-3), 156.72 (triazole C-5). EI MS m/z (%): 407.22 ($[\text{M} + \text{Na} + 2]^+$, 100), 409.29 (56).

2.1.1.14 2-[2-(2,4-dichlorophenyl)-2-hydroxyethyl]-5-methyl-4-[(1E,2Z)-3-phenylprop-2-en-1-ylidene]amino-2,4-dihydro-3H-1,2,4-triazol-3-one (9b)

Yield: 50% (Method 1), 82% (Method 2), m.p: 100–101°C. FT-IR (ν_{\max} , cm^{-1}): 3251 (OH), 3038 (aromatic CH), 1689 (C = O). ^1H NMR (DMSO- d_6 , δ ppm): 2.07 (3H, s, CH_3), 5.21 (2H, s, CH_2), 6.29 (1H, d, $J = 4.0$ Hz, CH), 6.44 (1H, d, $J = 8.0$ Hz, OH), 7.26–7.63 (8H, m, arH), 9.58 (3H, d, $J = 12.0$ Hz, 3CH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.67 (CH_3), 51.78 (CH_2), 70.07 (CH), 125.43 (CH), arC: [126.70 (CH), 126.70 (CH), 127.96 (CH), 128.04 (CH), 128.72 (CH), 129.07 (CH), 129.34 (CH), 130.00 (2CH), 139.76 (2C), 144.52 (C), 144.84 (C)], 135.85 (CH), 143.73 (CH), 151.72 (triazole C-3), 156.68 (triazole C-5). EI MS m/z (%): 117.14 (100), 360.48 (71), 221.01 (62), 155.06 (47), 227.14 (44).

General Method for The Synthesis of Compounds 9a-e

NaH (1 mmol) was added the solution of the corresponding compound **9a,b** (1 mmol) in THF and the mixture was irradiated in monomode microwave reactor in closed vessel with pressure control 100°C, for 5 min at 150 Watt. Then 4-chlorobenzyl chloride (for **10c and 10e**), 2,4-dichlorobenzyl chloride (for **10a and 10d**) or 2,6-dichlorobenzyl chloride (for **10b**) (3 mmol) was added and MW irradiation was maintained for additional 12 min under the same conditions. After evaporating the solvent under reduced pressure, an oily product appeared. Water was added into it and extracted with 15 mL of ethyl acetate three times in the presence of K_2CO_3 . The organic layer was dried on Na_2SO_4 and solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silicagel (n-hexane/ethyl acetate, 3:7).

2.1.1.15 2-{2-(4-chlorophenyl)-2-[(2,4-dichlorobenzyl)oxy]ethyl}-5-methyl-4-[(1 E,2Z) phenylprop-2-en-1-ylidene]amino-2,4-dihydro-3H-1,2,4-triazol-3-one (10a)

Yield: 57%. FT IR (ν_{\max} , cm^{-1}): 3061 (ar-CH), 1681 (C = O), 1557 (C = N). ^1H NMR (DMSO- d_6 , ppm): 2.07 (3H, s, CH_3), 4.90 (2H, s, CH_2), 5.69 (2H, d, $J = 4.0$ Hz, CH_2), 7.31–7.54 (12H, m, arH), 9.53 (1H, s, CH), 9.55 (1H, s, CH), 11.38 (1H, s, CH), 11.82 (1H, s, CH). ^{13}C NMR (DMSO- d_6 , ppm): 11.20 (CH_3), 51.77 (CH_2), 52.35 (CH_2), 128.03 (CH), 128.08 (CH), 128.42 (CH), 128.45 (CH), arC: [130.45 (CH), 131.87 (CH), 13.24 (CH), 133.30 (CH), 133.42 (CH), 133.47 (CH), 133.96 (CH), 135.61 (CH), 136.88 (CH), 142.18 (CH), 143.70

(CH), 144.54 (CH), 145.73 (C), 145.97 (2C), 151.73 (C), 152.81 (C), 153.44 (C)], 154.35 (triazole C-3), 158.47 (triazole C-5). EI MS m/z (%): 542.18 ($[M + 1]^+$, 53), 217.18 (100), 175.21 (71), 161.18 (65).

2.1.1.16 2-{2-(4-chlorophenyl)-2-[(2,6-dichlorobenzyl)oxy]ethyl}-5-methyl-4-[(1 E,2Z)-3-phenylprop-2-en-1-ylidene]amino}-2,4-dihydro-3H-1,2,4-triazol-3-one (10b)

Yield: 65%. FT IR (ν_{\max} , cm^{-1}): 3058 (aromatic CH), 1688 (C = O), 1581 (C = N). ^1H NMR (DMSO- d_6 , δ ppm): 2.07 (3H, s, CH₃), 4.90 (2H, s, CH₂), 5.69 (2H, d, J = 4.0 Hz, CH₂), 7.31–7.54 (12H, m, arH), 9.53 (1H, s, CH), 9.55 (1H, s, CH), 11.38 (1H, s, CH), 11.82 (1H, s, CH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.20 (CH₃), 51.77 (CH₂), 52.35 (CH₂), 128.03 (CH), 128.08 (CH), 128.42 (CH), 128.45 (CH), arC: [130.45 (CH), 131.87 (CH), 13.24 (CH), 133.30 (CH), 133.42 (CH), 133.47 (CH), 133.96 (CH), 135.61 (CH), 136.88 (CH), 142.18 (CH), 143.70 (CH), 144.54 (CH), 145.73 (C), 145.97 (C), 151.73 (C), 152.81 (C), 153.44 (C)], 154.35 (triazole C-3), 158.47 (triazole C-5). EI MS m/z (%): 300.55 (100), 287.91 (71), 421.83 (64), 564.87 ($[M + \text{Na}]^+$, 45).

2.1.1.17 2-[2-[(2,6-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-5-methyl-4-[(1 E,2Z)-3-phenylprop-2-en-1-ylidene]amino}-2,4-dihydro-3H-1,2,4-triazol-3-one (10c)

Yield: 58%. FT IR (ν_{\max} , cm^{-1}): 3057 (aromatic CH), 1691 (C = O), 1577 (C = N). ^1H NMR (DMSO- d_6 , ppm): 1.88 (3H, s, CH₃), 2.41 (2H, s, CH₂), 4.75 (2H, s, CH₂), 5.18 (1H, s, CH), 7.21 (3H, s, arH), 7.47 (4H, s, arH), 7.72–7.81 (4H, m, arH), 9.81 (1H, s, CH), 11.45 (1H, s, CH), 11.63 (1H, s, CH). ^{13}C NMR (DMSO- d_6 , ppm): 11.77 (CH₃), 50.43 (CH₂), 57.10 (CH₂), 121.18 (CH), 122.76 (CH), 123.54 (CH), 126.14 (CH), arC: [129.02 (CH), 130.04 (CH), 130.76 (CH), 131.13 (CH), 131.84 (CH), 131.90 (CH), 132.00 (CH), 132.14 (CH), 132.22 (CH), 132.41 (CH), 133.52 (CH), 133.76 (C), 134.15 (C), 134.78 (C), 135.17 (C), 135.88 (C), 135.96 (C), 141.03 (C)], 153.90 (triazole C-3), 157.83 (triazole C-5). EI MS m/z (%): 577.71 ($[M + 1]^+$, 83), 401.43 (52), 322.90 (47), 310.87 (100), 231.62 (34), 110.65 (65).

2.1.1.18 2-[2-[(2,4-dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-5-methyl-4-[(1 E,2Z)-3-phenylprop-2-en-1-ylidene]amino}-2,4-dihydro-3H-1,2,4-triazol-3-one (10d)

Yield: 93%. FT IR (ν_{\max} , cm^{-1}): 3087 (aromatic CH), 1698 (C = O), 1561 (C = N). ^1H NMR (DMSO- d_6 , ppm): 2.06 (3H, s, CH₃), 2.23 (2H, s, CH₂), 4.81 (2H, s, CH₂), 5.81 (1H, d, J = 4.0 Hz, CH), 7.45–7.48 (6H, m, arH), 7.63–7.69 (5H, m, arH), 9.53 (1H, d, J = 8.0 Hz, CH), 11.35 (1H, s, CH), 11.81 (1H, s, CH). ^{13}C NMR (DMSO- d_6 , ppm): 11.67 (CH₃), 51.77 (CH₂), 56.28 (CH₂), 126.17 (CH), 126.95 (CH), 127.96 (CH), 127.99 (CH), arC: [128.04 (CH), 128.07 (CH), 128.13 (CH), 128.33 (CH), 128.72 (CH), 129.07 (CH), 129.30 (CH), 129.34 (CH), 129.35 (CH), 129.44 (CH), 129.68 (CH), 133.02 (C), 133.33 (C), 134.68 (C), 134.73 (C), 135.83 (C), 136.89 (C), 139.73 (C)], 152.90 (triazole C-3), 156.57 (triazole C-5). EI MS m/z (%): 443.20 (35), 441.12 (48), 413.18 (59), 411.17 (100), 409.16 (60), 284.37 (28).

2.1.1.19 2-[2-[(4-chlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-5-methyl-4-[(1 E,2Z)-3-phenylprop-2-en-1-ylidene]amino}-2,4-dihydro-3H-1,2,4-triazol-3-one (10e)

Yield: 87%. FT IR (ν_{\max} , cm^{-1}): 3066 (aromatic CH), 1703 (C = O), 1597 (C = N). ^1H NMR (DMSO- d_6 , ppm): 2.09 (3H, s, CH_3), 3.36 (2H, s, CH_2), 3.65–3.68 (1H, m, CH), 4.77 (2H, s, CH_2), 7.25–7.49 (12H, m, arH), 9.48 (1H, s, CH), 9.71 (1H, s, CH), 11.50 (1H, s, CH). ^{13}C NMR (DMSO- d_6 , ppm): 11.21 (CH_3), 45.63 (CH_2), 62.55 (CH_2), 126.20 (CH), 126.70 (CH), arC: [128.09 (CH), 128.44 (CH), 128.64 (CH), 129.09 (CH), 129.11 (CH), 129.36 (CH), 130.05 (CH), 130.47 (CH), 131.18 (2C), 132.20 (CH), 133.45 (2C), 136.89 (2C), 137.19 (2C), 142.20 (3C)], 144.79 (triazole C-3), 152.78 (triazole C-5). EI MS m/z (%): 542.18 ($[\text{M} + 1]^+$, 21), 432.18 (51), 411.76 (65), 309.18 (75), 210.87 (100).

General Method for the Synthesis of Compounds 11a-e

The solution of norfloxacin (for **11a**) (10 mmol) or ciprofloxacin (for **11b**) (10 mmol), or morpholine (for **11c**) (10 mmol), or thiomorpholine (for **11d**) (10 mmol), or 4-phenylpiperazine (for **11e**) (10 mmol), formaldehyde (37%, 30 mmol), product **3** (10 mmol) and HCl (10% mol) was irradiated in monomode microwave reactor in a closed vessel with temperature control at 80 °C for 5 min at 100 W. After that resulting solution was poured into ice-water. The precipitated product was filtered off and recrystallized from DMSO/ H_2O (1:3) to give the target molecule.

2.1.1.20 1-ethyl-6-fluoro-7-{4-[(3-methyl-5-oxo-4-[(1E,2Z)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (11a)

Yield: 67%, m.p. 121–122 °C. FT IR (ν_{\max} , cm^{-1}): 3384 (OH), 3051 (aromatic CH), 1715 (C = O), 1624 (C = O), 1479 (C = N). ^1H NMR (DMSO- d_6 , ppm): 1.31 (3H, s, CH_3), 1.39 (3H, s, CH_3), 2.27 (2H, s, CH_2), 2.45 (2H, s, CH_2), 2.76 (2H, s, CH_2), 2.83 (2H, s, CH_2), 3.03 (2H, s, CH_2), 4.57 (2H, s, CH_2), 7.33–7.78 (7H, m, arH), 8.77 (2H, s, 2CH), 8.93 (2H, s, 2CH), 15.27 (1H, s, OH). ^{13}C NMR (DMSO- d_6 , ppm): 11.53 (CH_3), 18.19 (CH_3), 35.09 (CH_2), 36.71 (CH_2), 37.44 (CH_2), 39.81 (CH_2), 40.90 (CH_2), 47.88 (CH_2), 91.53 (CH), 93.49 (CH), 95.18 (CH), 96.72 (CH), arC: [120.60 (CH), 121.23 (CH), 122.62 (CH), 122.99 (CH), 123.51 (CH), 123.91 (CH), 124.59 (CH), 126.71 (C), 126.92 (C), 128.25 (C), 130.30 (C), 131.51 (C), 133.52 (C)], 156.10 (triazole C-3), 159.41 (triazole C-5), 173.87 (C = O), 177.81 (C = O). EI MS m/z (%): 582.27 ($[\text{M} + \text{Na}]^+$, 100), 560.24 ($[\text{M} + 1]^+$, 98), 485.17 (40), 332.20 (31), 354.28 (19). Elemental analysis for: $\text{C}_{29}\text{H}_{30}\text{FN}_7\text{O}_4$; Calculated (%): C, 62.24; H, 5.40; N, 17.52; Found (%): C, 62.31; H, 5.43; N, 17.60.

2.1.1.21 1-cyclopropyl-6-fluoro-7-{4-[(3-methyl-5-oxo-4-[(1E,2Z)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]piperazin-1-yl}-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (11b)

Yield: 63%, m.p. 126–128 °C. FT IR (ν_{\max} , cm^{-1}): 3416 (OH), 3079 (aromatic CH), 1718 (C = O), 1693 (C = O), 1595 (C = N). ^1H NMR (DMSO- d_6 , δ ppm): 1.15 (2H, s, CH_2), 1.31 (2H, d, $J = 8.0$ Hz, CH_2), 2.25 (3H, s, CH_3), 2.73 (2H, s, CH_2), 2.82 (2H, s, CH_2), 2.89 (2H, s, CH_2), 3.80 (2H, s, CH_2), 4.64 (2H, s, CH_2), 7.29–7.68 (6H, m, arH), 7.82 (1H, d, $J = 12.0$ Hz, arH), 8.61 (2H, s, 2CH), 9.49 (2H, d, $J = 8.0$ Hz, 2CH), 15.17 (1H, s, OH). ^{13}C NMR (DMSO- d_6 , ppm): 11.53 (CH_3), 34.10 (CH_2), 36.78 (CH_2), 39.98 (CH_2), 41.40 (CH_2), 42.23 (CH_2), 44.80 (CH_2), 46.71 (CH_2), 90.14 (CH), 91.90 (CH), 93.45 (CH), 94.17 (CH), 96.65 (CH), arC: [120.78

(CH), 121.65 (CH), 122.79 (CH), 124.61 (CH), 124.78 (CH), 126.34 (CH), 127.28 (CH), 129.87 (C), 130.01 (C), 130.77 (C), 131.98 (C), 133.50 (C), 136.90 (C)], 153.71 (triazole C-3), 156.02 (triazole C-5), 177.76 (C = O), 179.11 (C = O). EI MS m/z (%): 134.92 (100), 148.96 (59), 152.04 (50), 188.10 (44), 204.08 (41), 576.39 ($[M + 1]^+$, 36), 283.30 (31). Elemental analysis for: $C_{30}H_{30}FN_7O_4$; Calculated (%): C, 63.04; H, 5.29; N, 17.15; Found (%): C, 63.10; H, 5.33; N, 17.21.

2.1.1.22 5-methyl-2-(morpholin-4-ylmethyl)-4-[(1 E,ZZ)-3-phenylprop-2-en-1-ylidene]amino}-2,4-dihydro-3H-1,2,4-triazol-3-one (11c)

Yield: 4.45 m.p. 126–128°C. FT IR (ν_{max} , cm^{-1}): 3066 (aromatic CH), 1691 (C = O), 1490 (C = N). 1H NMR (DMSO- d_6 , δ ppm): 2.09 (3H, s, CH_3), 2.25 (2H, s, CH_2), 2.57 (2H, d, $J = 4.0$ Hz, CH_2), 3.34 (4H, s, $2CH_2$), 4.51 (2H, s, CH_2), 7.32–7.44 (3H, m, arH), 7.68 ve 7.69 (2H, d, $J = 4.0$ Hz, arH), 9.50 (3H, d, $J = 12.0$ Hz, 3CH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.57 (CH_3), 50.41 (CH_2), 52.06 (CH_2), 66.31 (CH_2), 66.49 ($2CH_2$), 125.24 (CH), 128.13 (CH), 129.37 (CH), arC: [130.12 (CH), 135.78 (C), 144.20 (2CH), 157.25 (2CH)], 140.38 (triazole C-3), 150.71 (triazole C-5). EI MS m/z (%): 145.93 (100), 114.20 (63), 328.31 ($[M + 1]^+$, 56), 213.00 (50), 382.06 (31), 350.21 ($[M + Na]^+$, 15). Elemental analysis for: $C_{17}H_{21}N_5O_2$; Calculated (%): C, 62.37; H, 6.47; N, 21.39; Found (%): C, 62.48; H, 6.57; N, 21.45.

2.1.1.23 5-methyl-4-[(1 E,ZZ)-3-phenylprop-2-en-1-ylidene]amino}-2-(thiomorpholin-4-ylmethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (11d)

Yield: 63%, m.p. 126–128°C. FT IR (ν_{max} , cm^{-1}): 3037 (aromatic CH), 1689 (C = O), 1578 (C = N). 1H NMR (DMSO- d_6 , δ ppm): 1.44 (3H, s, CH_3), 2.09 (2H, s, CH_2), 2.25 (2H, s, CH_2), 2.41 (2H, s, CH_2), 2.89 (2H, s, CH_2), 4.53 (2H, s, CH_2), 7.02–7.08 (3H, m, arH), 7.35 (2H, s, arH), 9.49 (2H, d, $J = 8.0$ Hz, 2CH), 11.74 (1H, s, CH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.57 (CH_3), 27.62 (CH_2), 51.25 (CH_2), 52.42 (CH_2), 53.39 (CH_2), 62.67 (CH_2), 125.23 (CH), 128.12 (CH), 129.36 (CH), arC: [130.11 (CH), 135.78 (C), 144.19 (2CH), 157.25 (2CH)], 143.34 (triazol C-3), 150.65 (triazol C-5). EI MS m/z (%): 344.78 ($[M + 1]^+$, 100), 478.14 (74), 510.51 (66), 123.76 (45). Elemental analysis for: $C_{17}H_{21}N_5OS$; Calculated (%): C, 59.45; H, 6.16; N, 20.39; Found (%): C, 59.80; H, 6.20; N, 20.45.

2.1.1.24 5-methyl-2-[(4-phenylpiperazin-1-yl)methyl]-4-[(1 E,ZZ)-3-phenylprop-2-en-1-ylidene] amino}-2,4-dihydro-3H-1,2,4-triazol-3-one (11e)

Yield: 63%, m.p. 126–128°C. FT IR (ν_{max} , cm^{-1}): 3035 (aromatic CH), 1697 (C = O), 1577 (C = N). 1H NMR (DMSO- d_6 , δ ppm): 2.09 (3H, s, CH_3), 2.25 (2H, s, CH_2), 2.59 (2H, s, CH_2), 2.73 (2H, s, CH_2), 3.12 (4H, s, $2CH_2$), 4.60 (2H, s, CH_2), 6.77 (1H, d, $J = 4.0$ Hz, arH), 6.91 (2H, d, $J = 8.0$ Hz, arH), 7.08–7.21 (2H, m, arH), 7.33–7.44 (3H, m, arH), 7.69 (2H, d, $J = 8.0$ Hz, arH), 9.51 (3H, d, $J = 8.0$ Hz, 3CH). ^{13}C NMR (DMSO- d_6 , δ ppm): 11.58 (CH_3), 48.73 (CH_2), 50.03 (CH_2), 51.50 (CH_2), 66.12 ($2CH_2$), 115.86 (CH), 116.09 (CH), 119.39 (CH), arC: [125.25 (2CH), 128.14 (2CH), 129.35 (2CH), 130.13 (2CH), 135.79 (C), 140.37 (C), 144.21 (CH), 157.27 (CH)], 150.71 (triazole C-3), 151.52 (triazole C-5). EI MS m/z (%): 403.18 ($[M + 1]^+$, 100), 256.78

(84), 189.90 (78), 357.81 (65), 288.90 (48). Elemental analysis for: C₂₃H₂₆N₆O; Calculated (%): C, 68.63; H, 6.51; N, 20.89; Found (%): C, 68.71; H, 6.57; N, 20.94.

2.1.1.25 5-methyl-4-([(1Z,2Z)-3-phenylprop-2-en-1-ylidene]amino)-2-[(5-sulfanyl-1,3,4-oxadiazol-2-yl)methyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (12)

CS₂ (10 mmol) was added to the solution of compound **5** (10 mmol) in 50 mL of ethanol and 50 mL of H₂O, and the mixture was refluxed in the presence of KOH (10 mmol) for 10 h. After cooling to room temperature, the mixture was neutralized with HCl. The solid precipitated was collected by filtration and recrystallized from ethanol to give the pure compound.

Yield 63%, m.p: 121–123°C. FT IR (ν_{\max} , cm⁻¹): 3044 (aromatic CH), 2749 (SH), 1667 (C = O), 1592 (C = N). ¹H NMR (DMSO-*d*₆, δ ppm): 2.12 (3H, s, CH₃), 5.01 (2H, s, CH₂), 5.32 (3H, s, 3CH), 7.06–7.12 (1H, m, arH), 7.38–7.46 (4H, m, arH), 14.66 (1H, brs, SH). ¹³C NMR (DMSO-*d*₆, δ ppm): 11.58 (CH₃), 56.51 (CH₂), 90.10 (CH), 97.45 (CH), 101.30 (CH), arC: [102.54 (CH), 107.76 (CH), 108.43 (CH), 110.78 (CH), 121.67 (CH), 125.87 (C)], 150.43 (oxadiazole C-2), 156.89 (oxadiazole C-5), 161.78 (triazole C-3), 167.65 (triazole C-5). EI MS *m/z* (%): 251.04 (100), 266.93 (44), 360.41 ([M + H₂O]⁺, 38), 127.03 (19), 381.43 ([M + K]⁺, 13).

General Method for the Synthesis of Compounds 13a,b

The solution of norfloxacin (for **13a**) (10 mmol) or ciprofloxacin (for **13b**) (10 mmol), formaldehyde (37%, 30 mmol), product **12** (10 mmol) and HCl (10% mol) was irradiated in a microwave reactor in a closed vessel with temperature control at 80 °C for 5 min at 100 W. After that resulting solution was poured into ice-water. The precipitated product was filtered off and recrystallized from DMSO/H₂O (1:3) to give the target molecule.

2.1.1.26 1-ethyl-6-fluoro-7-(4-[(5-[(3-methyl-5-oxo-4-[(1E,2E)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methyl]piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (13a)

Yield: 78%. FT IR (ν_{\max} , cm⁻¹): 3320 (OH), 3065 (aromatic CH), 1720 (C = O), 1698 (C = O), 1597 (C = N). ¹H NMR (DMSO-*d*₆, δ ppm): 2.25 (3H, s, CH₃), 2.40 (3H, s, CH₃), 2.65 (2H, s, CH₂), 2.70 (2H, s, CH₂), 2.95 (2H, s, CH₂), 3.78 (2H, s, CH₂), 4.10 (2H, s, CH₂), 4.68 (2H, s, CH₂), 4.80 (2H, s, CH₂), 6.98–7.00 (5H, m, arH), 7.12–7.40 (2H, m, arH), 8.32 (2H, s, 2CH), 8.88 (1H, s, CH), 9.12 (1H, s, CH), 15.40 (1H, s, OH). ¹³C NMR (DMSO-*d*₆, ppm): 11.53 (CH₃), 27.45 (CH₃), 36.09 (CH₂), 38.43 (CH₂), 47.32 (CH₂), 47.90 (CH₂), 48.10 (CH₂), 49.01 (CH₂), 50.43 (CH₂), 91.32 (CH), 91.98 (CH), 92.18 (CH), 96.09 (CH), arC: [112.32 (CH), 114.56 (CH), 116.87 (CH), 117.43 (CH), 118.18 (CH), 120.41 (CH), 125.87 (CH), 126.65 (C), 130.09 (C), 133.31 (C), 134.58 (C), 136.76 (C), 137.61 (C)], 150.09 (oxadiazole C-2), 151.78 (oxadiazole C-5), 156.45 (triazole C-3), 158.98 (triazole C-5), 178.09 (C = O), 180.32 (C = O). EI MS *m/z* (%): 300.98 (100), 478.23 (89), 674.98

([M + 1]⁺, 78), 509.41 (65), 600.87 (51), 267.47 (38). Elemental analysis for: C₃₂H₃₂FN₉O₅S; Calculated (%): C, 57.05; H, 4.79; N, 18.71; Found (%): C, 57.10; H, 4.84; N, 18.77.

2.1.1.27 1-cyclopropyl-6-fluoro-7-(4-[[5-[(3-methyl-5-oxo-4-[(1 E,2E)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]-2-thioxo-1,3,4-oxadiazol-3(2H)-yl]methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (13b)

Yield: 75%. FT IR (ν_{\max} , cm⁻¹): 3412 (OH), 3067 (aromatic CH), 1722 (C = O), 1696 (C = O), 1587 (C = N). ¹H NMR (DMSO-*d*₆, δ ppm): 1.30 (2H, s, CH₂), 1.45 (2H, s, CH₂), 2.41 (3H, s, CH₃), 2.65 (2H, s, CH₂), 2.71 (2H, s, CH₂), 2.88 (2H, s, CH₂), 3.76 (2H, s, CH₂), 4.90 (2H, s, CH₂), 5.11 (2H, s, CH₂), 6.98–7.12 (6H, m, arH), 7.15–7.20 (1H, m, arH), 8.10 (1H, s, CH), 8.56 (1H, s, CH), 9.27 (1H, s, CH), 9.77 (2H, s, 2CH), 15.56 (1H, s, OH). ¹³C NMR (DMSO-*d*₆, ppm): 12.67 (CH₃), 33.98 (CH₂), 35.61 (CH₂), 37.09 (CH₂), 46.32 (CH₂), 47.90 (CH₂), 48.65 (CH₂), 49.31 (CH₂), 50.12 (CH₂), 91.08 (CH), 92.13 (CH), 93.51 (CH), 94.65 (CH), 95.87 (CH), arC: [112.05 (CH), 113.56 (CH), 117.71 (CH), 118.54 (CH), 120.41 (CH), 123.76 (CH), 125.61 (CH), 130.78 (C), 131.90 (C), 132.46 (C), 133.51 (C), 135.09 (C), 136.87 (C)], 148.89 (oxadiazole C-2), 150.76 (oxadiazole C-5), 153.90 (triazole C-3), 158.41 (triazole C-5), 178.80 (C = O), 180.65 (C = O). EI MS *m/z* (%): 389.76 (100), 177.47 (89), 686.65 ([M + 1]⁺, 80), 471.98 (73), 512.67 (56), 300.54 (43). Elemental analysis for: C₃₃H₃₂FN₉O₅S; Calculated (%): C, 57.80; H, 4.70; N, 18.38; Found (%): C, 57.86; H, 4.76; N, 18.43.

General Method for The Synthesis of Compounds 14a-e

The mixture of norfloxacin (for **14a** and **14b**) or ciprofloxacin (for **14c**, **14d**, **14e**) (1 mmol) formaldehyde (37%, 3 mmol), compound **7a-c** (1 mmol) and HCl (10% mol) was irradiated in monomode microwave reactor in closed vessel with pressure control at 80 °C for 5 min at 100 W (the progress of the reaction was monitored by TLC). Then the resulting solution was poured into ice-water. The product precipitated was filtered off and recrystallized from DMSO/H₂O (1:3) to give the, desired compound.

2.1.1.28 1-ethyl-6-fluoro-7-[4-({3-[(3-methyl-5-oxo-4-[(1 E,2Z)-3-phenylprop-2-en-1-ylidene] amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]-4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl)piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid

(14a)

Yield: 67%, m.p. 121–122 °C. FT IR (ν_{\max} , cm⁻¹): 3059 (aromatic CH), 1710, 1669 and 1626 (3C = O), 1498 (C = N). ¹H NMR (DMSO-*d*₆, ppm): 1.42 (3H, s, CH₃), 2.12 (3H, s, CH₃), 2.73 (2H, s, CH₂), 2.89 (2H, s, CH₂), 2.98 (2H, s, CH₂), 4.59 (4H, s, 2CH₂), 4.97 (2H, s, CH₂), 5.22 (2H, s, CH₂), 7.19–7.78 (9H, m, arH), 7.89–7.95 (3H, m, arH), 8.94 (2H, d, *J* = 12.0 Hz, 2CH), 9.09 (1H, d, *J* = 8.0 Hz, CH), 9.31 (1H, s, CH), 15.36 (1H, s, OH). ¹³C NMR (DMSO-*d*₆, ppm): 11.31 (CH₃), 14.81 (CH₃), 36.24 (CH₂), 49.54 (2CH₂), 50.06 (2CH₂), 66.23 (2CH₂), 106.51 (CH), 107.55 (C), 111.74 (2CH), arC: [119.84 (C), 127.54 (2CH), 128.10 (2CH), 129.31 (2CH), 129.47 (3CH), 129.70 (3CH), 133.81 (C), 137.64 (2C), 146.89 (3C)], 157.12 (triazole 2C-3), 166.58 (triazole 2C-5), 172.15 (C = O), 176.64 (C = O). EI MS *m/z* (%): 749.61 ([M + 1]⁺, 63), 507.83 (100), 498.15

(50), 387.76 (69), 311.45 (77), 228.71 (37), 178.90 (41). Elemental analysis for: C₃₈H₃₇FN₁₀O₄S; Calculated (%): C, 60.95; H, 4.98; N, 18.70; Found (%): C, 70.00; H, 5.05; N, 18.78.

2.1.1.29 1-ethyl-6-fluoro-7-[4-({3-[(3-methyl-5-oxo-4-[(1E,2E)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]-4-benzyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (14b)

Yield: 75%, m.p.131–133°C. FT IR (ν_{\max} , cm⁻¹): 3210 (OH), 3057 (aromatic CH), 1695 (C = O), 1681 (C = O), 1507 (C = N). ¹H NMR (DMSO-*d*₆, δ ppm): 1,40 (3H, s, CH₃), 2,14 (3H, s, CH₃), 2,19 (2H, s, CH₂), 2,73 (2H, s, CH₂), 2,89 (2H, s, CH₂), 2,98 (2H, s, CH₂), 4,60 (4H, s, 2CH₂), 4,95 (2H, s, CH₂), 5,20 (2H, s, CH₂), 7,23–7,47 (6H, m, arH), 7,63 (6H, m, arH), 7,89 (1H, s, arH), 8,93 (1H, s, arH), 8,96 (2H, s, 2CH), 9,31 (1H, s, CH), 15,36 (1H, s, OH). ¹³C NMR (DMSO-*d*₆, δ ppm): 12,20 (CH₃), 25,65 (CH₃), 30,65 (CH₂), 31,84 (CH₂), 35,41 (CH₂), 38,74 (CH₂), 39,43 (CH₂), 40,65 (CH₂), 41,52 (CH₂), 48,20 (CH₂), 98,01 (CH), 98,86 (CH), 99,12 (CH), 100,06 (CH), arC: [111.87 and 111.98 (CH, d, *J* = 11.0 Hz), 112.20 (CH), 112.95 117.20 (CH), 119.30 (CH), 132.52 (CH), 133.57 (CH), 135.18 and 134.34 (CH, d, *J* = 16.0 Hz), 136.10 (CH), 137.32 (CH), 138.10 (CH), 139.12 (CH), 139.69 (CH), 140.02 (C), 140.49 (C), 141.05 (C), 141.94 (C), 142.01 (C), 142.10 and 142.65 (C, d, *J* = 55.0 Hz)], 153.54 (triazole C-3), 154.01 (triazole C-3), 160.71 (triazole C-5), 166.09 (triazole C-5), 171.10 (C = O), 177.73 (C = O). EI MS *m/z* (%): 763.85 ([*M* + 1]⁺, 100), 507.83 (63), 498. 90 (50), 118.80 (37). Elemental analysis for: C₃₉H₃₉FN₁₀O₄S; Calculated (%), C, 61.40; H, 5.15; N, 18.36; Found (%): C, 61.38; H, 5.14; N, 18.35.

2.1.1.30 1-cyclopropyl-6-fluoro-7-[4-({3-[(3-methyl-5-oxo-4-[(1E,2Z)-3-phenylprop-2-en-1-ylidene]amino)-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]-5-oxo-4-phenyl-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (14c)

Yield: 63%, m.p.126–128°C. FT IR (ν_{\max} , cm⁻¹): 3290 (OH), 3063 (aromatic CH), 1689 (C = O), 1626 (C = O), 1534 (C = N). ¹H NMR (DMSO-*d*₆, δ ppm): 1.18 (2H, s, CH₂), 1.31 (2H, s, CH₂), 2.25 (3H, s, CH₃), 2.73–2.89 (4H, s, 2CH₂), 3.82 (2H, s, CH₂), 4.14 (2H, s, CH₂), 4.45 (2H, s, CH₂), 4.50 (2H, s, CH₂), 7.04 (2H, s, arH), 7.29–7.55 (8H, m, arH), 7.69 (1H, s, arH), 7.89 (1H, s, arH), 8.66 (2H, s, 2CH), 9.19 (1H, s, CH), 9.48 (1H, s, CH), 10.04 (1H, s, CH), 15.23 (1H, s, OH). ¹³C NMR (DMSO-*d*₆, δ ppm): 11.34 (CH₃), 30.19 (CH₂), 31.21 (CH₂), 33.10 (CH₂), 33.95 (CH₂), 34.41 (CH₂), 36.89 (CH₂), 39.71 (CH₂), 46.53 (CH₂), 96.12 (CH), 97.61 (CH), 98.54 (CH), 99.18 (CH), 99.89 (CH), arC: [110.87 and 110.98 (CH, d, *J* = 11.0 Hz), 111.90 (CH), 112.45 (CH), 113.76 (CH), 121.17 (CH), 124.90 (CH), 126.78 (CH), 129.67 (CH), 130.43 (CH), 131.81 (CH), 132.64 (CH), 134.18 and 134.34 (CH, d, *J* = 16.0 Hz), 135.87 (C), 136.71 (C), 137.63 (C), 137.90 (C), 138.01 (C), 139.95 (C), 140.10 and 140.65 (C, d, *J* = 55.0 Hz)], 153.33 (triazole C-3), 154.10 (triazole C-3), 160.78 (triazole C-5), 166.94 (triazole C-5), 170.19 (C = O), 178.76 (C = O). EI MS *m/z* (%): 745.78 ([*M* + 1]⁺, 67), 543.65 (73), 498. 90 (70), 300.89 (100), 110.90 (43). Elemental analysis for: C₃₉H₃₇FN₁₀O₅; Calculated (%), C, 62.89; H, 5.01; N, 18.81; Found (%): C, 62.93; H, 5.09; N, 18.90.

2.1.1.31 1-cyclopropyl-6-fluoro-7-[4-({3-[(3-methyl-5-oxo-4-[(1E,2Z)-3-phenylprop-2-en-1-ylidene]amino}-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]-4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (14d)

Yield: 63%, m.p. 126–128°C. FT IR (ν_{\max} , cm^{-1}): 3059 (aromatic CH), 1711, 1670 and 1626 ($\text{C}=\text{O}$), 1546 ($\text{C}=\text{N}$). ^1H NMR ($\text{DMSO-}d_6$, ppm): 1.06 (3H, s, CH_3), 1.16 (2H, s, CH_2), 1.32 (2H, s, CH_2), 2.11 (2H, d, $J = 8.0$ Hz, CH_2), 2.73 (2H, s, CH_2), 2.89 (2H, s, CH_2), 2.98 (2H, s, CH_2), 4.97 (2H, s, CH_2), 5.22 (2H, s, CH_2), 7.26–7.47 (10H, m, arH), 7.62 (2H, m, arH), 7.95 (1H, s, CH), 8.63 (2H, d, $J = 16.0$ Hz, 2CH), 9.08 (1H, d, $J = 12.0$ Hz, CH), 9.30 (1H, s, CH), 15.20 (1H, s, OH). ^{13}C NMR ($\text{DMSO-}d_6$, ppm): 8.05 (CH_3), 36.24 (2 CH_2), 49.88 (2 CH_2), 50.01 (2 CH_2), 69.08 (2 CH_2), 106.97 (CH), 111.26 (CH), 111.49 (CH), 119.07 (CH), 119.14 (CH), arC: [124.87 (CH), 127.96 (CH), 128.07 (CH), 128.19 (CH), 129.30 (CH), 129.36 (CH), 129.46 (CH), 129.71 (CH), 132.13 (CH), 133.50 (C), 133.80 (C), 135.61 (C), 136.57 (C), 139.75 (C), 144.28 and 144.36 (C, d, $J = 8.0$ Hz), 146.90 (C), 149.01 (CH), 152.23 (CH)], 148.35 (quinolon CH), 154.62 (triazole C-3), 154.70 (triazole C-3), 157.09 (triazole C-5), 162.78 (triazole C-5), 169.98 ($\text{C}=\text{O}$), 176.78 ($\text{C}=\text{O}$). EI MS m/z (%): 761.81 ($[\text{M} + 1]^+$, 53), 507.21 (89), 451.76 (100), 443.43 (71), 410.65 (39), 127.78 (22). Elemental analysis for: $\text{C}_{39}\text{H}_{37}\text{FN}_{10}\text{O}_4\text{S}$ için; Calculated (%): C, 61.57; H, 4.90; N, 18.41; Found (%): C, 61.61; H, 4.96; N, 18.50.

2.1.1.32 1-cyclopropyl-6-fluoro-7-[4-({3-[(3-methyl-5-oxo-4-[(1E,2E)-3-phenylprop-2-en-1-ylidene]amino}-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]-4-benzyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)methyl]piperazin-1-yl]-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (14e)

Yield: 71%, m.p. 129–131°C. FT IR (ν_{\max} , cm^{-1}): 3217 (OH), 3061 (aromatic CH), 1678 ($\text{C}=\text{O}$), 1703 ($\text{C}=\text{O}$), 1523 ($\text{C}=\text{N}$). ^1H NMR ($\text{DMSO-}d_6$, δ ppm): 1.75 (3H, s, CH_3), 2.12 (2H, s, CH_2), 2.65 (2H, s, CH_2), 2.85 (2H, s, CH_2), 2.97 (2H, s, CH_2), 4.33 (2H, s, CH_2), 4.78 (4H, s, 2 CH_2), 5.05 (2H, s, CH_2), 5.29 (2H, s, CH_2), 7.10–7.39 (8H, m, arH), 7.45–7.69 (4H, m, arH), 8.87 (1H, s, CH), 8.94 (1H, s, CH), 9.12 (1H, s, CH), 9.38 (1H, s, CH), 15.40 (1H, s, OH). ^{13}C NMR ($\text{DMSO-}d_6$, δ ppm): 11.70 (CH_3), 30.18 (CH_2), 31.87 (CH_2), 36.41 (2 CH_2), 38.54 (CH_2), 39.17 (CH_2), 47.10 (CH_2), 48.19 (CH_2), 49.13 (CH_2), 98.15 (CH), 98.40 (CH), 99.06 (CH), 99.84 (CH), 100.01 (CH), arC: [110.87 and 110.98 (CH, d, $J = 11.0$ Hz), 111.85 (CH), 112.78 113.05 (CH), 114.52 (CH), 115.47 (CH), 116.73 (CH), 123.10 and 123.45 (CH, d, $J = 35.0$ Hz), 127.52 (CH), 129.41 (CH), 130.59 (CH), 131.84 (CH), 132.43 (CH), 139.20 (C), 140.45 (C), 141.37 (C), 142.73 (C), 143.11 (C), 143.79 (C), 144.10 and 144.65 (C, d, $J = 55.0$ Hz)], 155.41 (triazole C-3), 158.43 (triazole C-3), 161.08 (triazole C-5), 165.49 (triazole C-5), 170.21 ($\text{C}=\text{O}$), 173.23 ($\text{C}=\text{O}$). EI MS m/z (%): 797.29 ($[\text{M} + \text{Na}]^+$, 67), 774.29 ($[\text{M}]^+$, 90), 543.63 (53), 490.90 (40). Elemental analysis: for $\text{C}_{40}\text{H}_{39}\text{FN}_{10}\text{O}_4\text{S}$; Calculated (%), C, 62.00; H, 5.07; N, 18.08; Found (%): C, 62.01; H, 5.09; N, 18.06.

2.2. The Determination of Antimicrobial Activity

All bacterial and yeasts were acquired from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: “*Escherichia coli* ATCC35218, *Yersinia pseudotuberculosis* ATCC911, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212,

Bacillus cereus 709 ROMA, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193, *Saccharomyces cerevisiae* RSKK 251". Novel obtained hybrid molecules were weighed and dissolved in hexane to prepare extract stock solution of 20.000 microgram/milliliter ($\mu\text{g}/\text{mL}$).

Antibacterial impacts of the products were tested quantitatively in respective broth media by utilizing double microdilution and the minimal inhibition concentration (MIC) values ($\mu\text{g}/\text{mL}$) were obtained. The antimicrobial and antifungal processes were afforded in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH.7.3, respectively. The micro dilution trial plaques were incubated for 18–24 h at 35°C. Brain Heart Infusion broth (BHI) (Difco, Detroit, MI) was utilized for *M. smegmatis*, and waited for 48–72 h at 35°C [35]. Ampicillin (10 μg), Streptomisin (10 μg), and fluconazole (5 μg) were utilized as standard antimicrobial and antifungal medicines, respectively. Dimethylsulphoxide with dilution of 1:10 was utilized as solvent check.

2.3 Anticancer Activity

2.3.1 Cell Culture and Cell Proliferation Tests

2.3.1.1 Cell Culture

All the antiproliferative activity studies were performed in the sterile cabinet. The used medium (DMEM) in sterile culture flasks was transferred to the waste container. 10 mL of trypsin-EDTA was added to the culture flask containing the cells. The flask's lid was closed and incubated for 1–2 minutes at 37°C in a CO₂ incubator (5% CO₂). Thus, the cells adhering to the surface were removed from the surface. After incubation, the medium was neutralized by adding 10 mL of media (DMEM) to the container. The cell suspension was transferred in equal amounts to two separate 15-mL falcon tubes. These tubes were centrifuged at 600 rpm for 5 minutes to allow the cells to settle to the bottom of the tube. At the end of the centrifugation, the media were transferred from the falcon tube to the waste container and 3 mL of medium was added onto the cell sediment. Cells collected in the bottom of the tube were suspended with a sterile pipette. The Cedex Hires Analyzer (Roche) device was used for cell counting. Dead cells were labelled with trypan blue solution. The obtained data were used to determine the number of cells to be added to the wells of the E-Plate 96 plate.

2.3.1.2 Antiproliferatif Activity Tests

Antiproliferative activity tests were performed using real-time cell analyzer (xCelligence RTCA SP, ACEABIO, Inc.). RTCA SP is based on the principle of monitoring cell biology by microelectronic technique. The RTCA SP (Single Plate) unit consists of RTCA control unit, RTCA analyzer, RTCA SP station and E-Plate 96 components. One of the most important parts of the RTCA SP is the plate with 96 wells (E-Plate 96). At the bottom of the plate is a microelectronic cell sensor array suitable for cells adhesion. Biological alterations in the cells cause resistance changes on the RTCA SP sensors.

This resistance change is measured automatically and converted to digital signals so that the reader can read and analyze it. Cell viability, cell number, cell morphology and the attraction of molecules to each

other (adhesion) affect electrode resistance. If there is a cell on the electrode, the cell attached to the electrode surface behaves as insulators and cause an increase in the resistance of the medium. This means that the more cells there are on the electrode, the greater the change in resistance on the electrode. The cell index (CI, Cell Index) parameter is derived from the relative position of the cell state. The CI value is approximately zero when cells are not present or not well attached to the electrodes. If more cells are attached on the electrode in the same physiological conditions, the CI value is higher [36]. CI (Cell Index) is a unitless value. As the value of CI increases, it is understood that the cells hold onto the surface, they develop, the morphology does not show any change, and they divide and multiply. In other words, an increase in the CI value indicates that the cells are not deformed, that the cells do not enter the stress, that the conditions for the cells are appropriate and that there are no problems about proliferation.

Cells are added to the wells of E-Plate 96, which has previously been pre-filled with a certain amount of medium for each well. Within a short time, the cells touch and adhere to the surface of the sensor-electrode located at the bottom of the wells. The electronic properties of the sensor surfaces are monitored via the station located within a CO₂ incubator. Simultaneous quantitative information about the biological state of the cell such as cell viability, morphology and cytotoxicity is determined by the RTCA software in the RTCA analyzer and the control unit.

Antiproliferative activity studies were carried out according to the method used by Abay [37]. Samples were dissolved in sterile DMSO (as 20 mg/mL) and diluted in sterile tubes with DMEM (1:20). 50 µl culture medium (DMEM) was added in each well of E-plate 96. The plate was incubated in sterile cabinet for 15 minutes and then in the CO₂ incubator for 15 min (for the thermal balance). Subsequently, the plate was placed in the RTCA station and a background reading was performed. This reading lasted 1 minute and the status of E-plate 96 was evaluated. The counting of the cells to be added to the wells and the preparation of the suspension was carried out using with the cell counting device. 100 µl of this cell suspension was added to each well of the plate (25.000 cells/well) except last three wells. These wells were left without cells to check if there would be a CI change or not due to DMEM. The plate was reinserted in the RTCA station and the second phase (80 min.) was started. During this time, the cells were allowed to adhere to the bottom of the well and enter the growth process. At the end of this period, the e-plate was taken back to the sterile cabinet and the culture medium solution containing the molecule samples was added to the wells at different concentrations. The samples solutions were added to the wells at three different concentrations as 100, 50, and 10 µg/mL. The final volume of each well was adjusted to 200 µl with DMEM. Each dose was studied in triplicate. Then, the plate was placed on the RTCA for the last time. The life/death states of the cells were recorded for 48 hours. The standard deviation of triplicates of wells were analyzed by the RTCA Software.

3. Results

3.1 Chemistry

The primary target of this study was to develop antimicrobial hybrid substances covering various pharmacophore structures. Reactions of last and intermediate compounds were achieved as pictorial in Scheme 1, Scheme 2, Scheme 3, and Scheme 4. The synthesis was carried out by utilizing microwave-assisted and conventional methods. The finishing of the synthesis was observed via the thin-layer chromatography (TLC) process. All product structures were based on the foundation of spectral and physicochemical data.

3.2 Antimicrobial Activity

All newly products were tested for their antimicrobial properties utilizing the minimal inhibition concentration method (MIC) and the outcomes for active molecules are illustrated in Table 1. The antimicrobial activities of compounds (2–9) were tested against 4 bacteria and 3 yeasts.

Table 1. Antimicrobial activity of the compounds ($\mu\text{g}/\text{mL}$)

Comp. No.	Minimal Inhibition Concentration Values (µg/mL)									
	Ec	Yp	Pa		Sa	Ef	Bc	Ms	Ca	Sc
3	-	-	500	-	-	-	-	-	500	-
5	-	-	-	-	-	-	-	-	125	-
6c	-	-	-	-	250	-	-	-	-	-
7a	125	-	-	-	-	-	-	-	-	-
7b	-	-	-	-	-	-	-	62.5	-	-
7c	-	-	-	-	-	-	-	-	125	250
8b	62.5	-	-	125	-	125	1.95	-	-	-
9a	0.24	-	500	-	-	-	-	-	-	-
9b	250	-	-	-	-	-	-	-	-	-
10a	-	-	-	-	250	-	-	-	-	-
10b	-	-	-	-	-	-	-	-	625	-
10d	-	-	-	-	-	-	-	125	-	-
10e	-	-	-	-	-	-	-	-	625	-
11a	<1	1.9	3.9	-	3.9	7.8	3.9	-	-	-
11b	1.9	62.5	15.6	-	15.6	31.25	31.25	-	-	-
11c	-	62.5	-	-	-	-	62.5	312.5	312.5	312.5
11d	62.5	-	625	-	-	-	-	312.5	312.5	312.5
11e	15.6	-	>1000	-	-	-	-	312.5	156	156
12	-	-	500	-	-	500	-	250	-	-
13a	<1	7.8	-	156	19	39	3.9	312.5	78	78
13b	31.25	<1	-	9.7	9.7	9.7	<1	312.5	312.5	312.5
14a	<1	<1	<1	-	<1	1.9	<1	-	-	-
14b	<1	<1	<1	-	<1	<1	<1	-	-	-
14c	<1	<1	<1	-	<1	1.9	<1	-	-	-
14d	<1	<1	1.9	-	-	1.9	1.9	-	-	-
14e	<1	<1	3.9	-	<1	1.9	<1	-	-	-

Amp.	10	18	>128	35	10	15
Strep.						4
Flu.					<8	<8

Ec: *E. coli* ATCC 35218, Yp: *Y. pseudotuberculosis* ATCC 911, Pa: *P. aeruginosa* ATCC 10145, Sa: *S. aureus* ATCC 25923, Ef: *E. faecalis* ATCC 29212, Bc: *B. cereus* 709 Roma, Ms: *M. smegmatis* ATCC607, Ca: *C. albicans* ATCC 60193, *S. cerevisiae* RSKK 251, Amp.: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole, (–): no activity of test concentrations

Among the synthesized compounds, compounds 3–9 displayed moderate activity in relation to test microorganisms while showing better activities on the *M. smegmatis* and *E. coli*. That is compounds **7b** and **8b** have more effective for *M. smegmatis* than the other molecules with the MIC values of 62.5 and 1.95 µg/mL respectively. *M. smegmatis* is one of an acid-fast, aerobic-rapidly growing bacterial species in the genus *Mycobacterium* and constitutes one of a characteristic tuberculosis agent causing the death and morbidity. On the other hand, compound **9a**, an intermediate, showed excellent activity on *E. coli*, a gram-negative bacterium, at 0.24 µg/mL MIC value. Namely **9a** exhibited much better activity against to *E. coli* than ampicillin used as standard drug. *E. coli* which is rod-shaped, gram-negative enteric bacteria which lives in the enteric systems of humans and animals and are found in aqua and fecal substance.

Compounds **10a-e** which are conazole derivatives exhibited slight activity on the gram-positive and gram-negative bacteria and yeasts. But compound **10c** no antimicrobial effects were found towards to the test microorganisms.

Mannich bases **11a-e** displayed good antibacterial activity to test microorganisms. However these compounds presented not good antimicrobial effect against the yeast strains *C. albicans* and *S. cerevisiae*. Only compound **11e**, containing phenyl piperazine, showed moderate activity MIC value of 156 µg/mL on *S. cerevisiae*.

Oxadiazole compound, **12**, obtained as a result of ring closure, has low activity on all the microorganisms. But, mannich bases **13a,b** obtained from the reaction of the oxadiazole compound with the quinolone showed strong activities. Especially compound **13a** exhibited the best activity among all compounds synthesized against yeast strains with 78 µg/mL MIC value on *S. cerevisiae*. That is **13a** has a good antifungal activity.

Among the synthesized compounds, **11a-e** mannich bases demonstrated the best and excellent activity against gram-positive and gram-negative bacteria with MIC values between < 1 and 31.25 µg/mL, while showing no activity against yeast strains. At the same time, these compounds showed very good antitubercular activity against *M. smegmatis* compared with Streptomycin standard drug.

No antimicrobial effects were found against to the test microorganisms in the studied concentration ranges of the 4, 6a,b, 8a and 10c compounds. Therefore, these results are not included in the table.

3.3 Anticancer Activity

The antiproliferative activity potentials of the molecules were examined by Abay's [37] method against HeLa cells. Three different doses of the samples showed different antiproliferative activity against the cells (Fig. 1 and Fig. 2). High Cell Index (CI) values (red line) were obtained from the wells without the molecules samples. Lower CI values were obtained from the wells in which the molecules were added. Only the medium (DMEM) was added to the final three wells. These three wells were used to obtain a baseline. No impedance change was observed in these wells containing only DMEM. Therefore, the Cell Index (CI) values of these three wells did not increase at all and straight continued to the end of the experiment (green line) similarly.

CI values obtained from only DMEM and cell-added wells were shown as the red line. The HeLa cells that continue to develop without encountering any obstacles have caused the CI to rise rapidly. This situation shows that the cells are attached to gold-plated microelectrodes in the well ground during the experiment and there is no proliferative negativity. The more the cells attached to the electrodes on the plate floor, the greater the impedance change. This results in an increase in CI value. On the contrary, the decrease in CI value means that the proliferation of the cells is suppressed or inhibited.

The hard CI decline seen at the 2nd hour is due to the change in the temperature of the e-Plate 96 taken from the RTCA station in the incubator (95% CO₂, 37°C) to the sterile cabinet and the sample addition. After the addition of the molecule samples, the E-Plate 96 inserted into the station in the CO₂ incubator again. The cells in the wells with no added samples were rapidly increased the CI values (red line). However, the proliferation of the cells in the wells the samples were added was strongly suppressed by the samples' molecules. This situation resulted in low CI values.

80 minutes after the addition of the cells to the wells, the e-plate was removed from the station and taken to the sterile cabinet. The samples were added to the wells three different concentrations (100, 50 and 10 µg mL⁻¹). Each doses of the samples were evaluated in triplicate. The antiproliferative effect of the molecules against HeLa cells was monitored in real time every 10 minutes and followed for 48 hours. The mean CI values from the wells were calculated automatically with the xCelligence RTCA SP software and the standard deviations are shown as vertical bars.

Dose effect investigations are performed by considering terms such as a dose dependent effect, dose-dependent reverse effect, hormesis and inverse hormesis [38–40]. Antiproliferative effects of these molecules against HeLa cells are shown in Fig. 1 and Fig. 2. CI values obtained from wells with no cells increased rapidly (red line). Different doses of DMSO were added to the wells with HeLa cells and no DMSO-induced antiproliferative activity was observed (Fig. 3). The effect of all doses of 5FU used as a positive control was strong, and CI values were very close to each other. None of the lower doses of the molecules didn't showed antiproliferative effect (10 µg mL⁻¹, turquoise line). All of the low doses of the

molecules produced the same CI as the control group. Only the low dose of compound **8a** partially dissociated from the control group towards the end of the experiment (after 35 hours). However, this situation cannot be considered as a net antiproliferative effect. On the 5FU graph (Fig. 4) used as a positive control, it is seen that the low dose (turquoise line) has a clearly different CI value from the red colored CI values (negative control).

Middle doses of **8a** and **9b**, molecules ($50 \mu\text{g mL}^{-1}$, pink line) showed strong antiproliferative activity. They maintained their antiproliferative effects throughout the experiment. High doses of molecules have also been found to have high antiproliferative activities. High doses of the **9b** molecule ($100 \mu\text{g mL}^{-1}$, dark blue line) showed very strong antiproliferative activity. High doses of the **8a** molecule also showed a strong antiproliferative effect ($100 \mu\text{g mL}^{-1}$, dark blue line) on HeLa cells. The effects of high doses of the **9b** molecules were so potent that the resulting CI values were the same as the 0 CI values (green line) obtained from wells with no added cells. This strong effect continued throughout the experiment.

The dose effect differences of these molecules were most clearly seen in **8a** and **9b**. These effects of molecules with strong antiproliferative effects are due to their structural forms. The antiproliferative activity potentials of the molecules that didn't show any effect or have a weak effect against to HeLa cells should be examined against other cancer cell lines and their effects should be investigated.

4. Discussion

Schiff base (**3**) was obtained from the reaction of 3-alkyl-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-one (**2**) compound with cinnamaldehyde, which was obtained from the reaction of ester ethoxycarbonylhydrazone (**1**) and hydrazine hydrate [34]. The object was to combine the 1,2,4-triazole nucleus to the cinnamaldehyde since it is known that more efficacious antimicrobial substances can be discovered by adding two biologically effective components together into a single molecular structure [41, 42]. Thereafter product (**3**) was transformed to the corresponding hydrazide derivative (**5**) by the creation of ester (**4**). With introduction of hydrazide function in the molecule, two signals appeared at 4.23 and 0.88 ppm as D_2O exchangeable singlets at the ^1H NMR data of molecule (**5**). With introduction of hydrazide group in the compound, two signals emerged at 5.25 and 9.17 ppm as D_2O exchangeable singlets at the ^1H NMR data of molecule.

Molecules (**6a-c**) were performed by the treatment of molecule (**5**) with phenyl- (for **6a**), or benzyl isothiocyanate (for **6b**), or phenyl isocyanate (for **6c**) in dichloromethanolic solution in good yields and molecule constructions were confirmed via FT IR, ^1H NMR, ^{13}C NMR, mass data.

The intramolecular cyclization of products (**6a-c**) at basic media afforded in the transformation of carbox(thio)amide function alter to 5-oxo(mercapto)-1,2,4-triazole derivatives and so molecules **7a-c** were synthesized. These compounds were characterized by the presence of a signal at 13.70 and 14.02 ppm in the ^1H NMR data as a D_2O exchangeable singlet confirming the existence of a -SH function (**7a,b**) and

10.77 ppm –NH function (for **7c**). The stretching band derived from this groups appeared at 2929 and 2932 cm^{-1} , and C = O (for **7c**) observed at 1626 cm^{-1} at the FT-IR data of these molecules (Scheme 1).

Alkylation of products **3**, via 2-bromo-1-(4-chlorophenyl)-ethanone or 2-chloro-1-(2,4-dichlorophenyl)-ethanone in ethanol performed compound **8a,b**. These reactions, which took place in 1440 minute in the conventional method, took place in 6 minute in the microwave irradiated method. Studies related to microwave synthesis which support organic synthesis in a shorter time and higher efficiency are available in the literature [43–45]. NH proton attached to the triazole group disappeared for compound **3** at the ^1H NMR spectra. New aromatic peaks were resonated in the region 7.27–7.67 ppm. In ^{13}C NMR datas of molecules, the carbon atom (C = O) were observed between 192.56 and 194.43 ppm for the newly added carbonyl group.

Compounds **9a,b** was obtained with the reduction of the carbonyl structure of products **8a,b** with sodium borohydride utilizing both classical heating and MW irradiation. When we compare the traditional and microwave method MW irradiation reduced the reaction time from 960 min. to 8 min. and increased the yields. Looking at compound number **9a,b**, the carbonyl group peak has evanesced at the ^1H NMR and ^{13}C NMR datas and OH peak added between 5.41 and 6.44 ppm in the ^1H NMR. The spreading band obtained this group (OH) appeared between 3374 and 3251 cm^{-1} , in the FT-IR data of molecules.

Reactions of molecules **10a-e**, were afforded reaction of molecule **9a,b** and benzyl chlorides, such as 2,4-dichloro-, 2,6-dichloro- and 4-chlorobenzyl, chlorides in a ambience with of NaH via MW synthesis method at 80°C and 100 W for 5 min. In both FT-IR and ^1H NMR datas of the molecules, the peaks due to the –OH group have disappeared. Another peaks approving molecule structures were displayed at the concerned chemical ranges in the ^1H NMR and ^{13}C NMR spectra. Moreover, [M + 1] ion signals were appeared at the concerned m/z ranges auxiliaring the offered structures of molecules **10a-e** (Scheme 2).

Oxadiazole compound (**12**) was obtained as a result of ring closure reaction of hydrazide compound (**5**) with CS_2 in basic media. The NH and NH_2 peaks resulting from the hydrazide compound disappeared in both the FT-IR and ^1H NMR data of the oxadiazol derivative compound. Instead of those peaks, SH peaks were added at 14.66 ppm in ^1H NMR and 2749 cm^{-1} in the FT-IR. In addition synthesized molecules confirmed ^{13}C NMR and Mass spectral data and elemental analysis results consistent with the assigned sutructures (Scheme 3).

Mannich reaction is a three-component condensation reaction involving active hydrogen containing compound, formaldehyde and a secondary amine. The amino alkylation of aromatic substrates by Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds [46]. Mannich bases found numerous practical applications in the field of medicinal chemistry, it could be responsible for enhancing physicochemical properties [47]. Mannich bases linked 1,2,4-triazole derivatives as containing a significant biological activity that has been reported in the literature [48, 49]. Furthermore, several Mannich bases of triazole derivatives including piperazine, thiomorpholine, or morpholine moiety were synthesized as antimicrobial agents in our laboratory [50, 51].

Fluoroquinolones are known as the most broadly utilized synthetic antimicrobial substances; privileged with wide spectrum antibacterial property, relatively low occurrence of toxic and adverse effects along with an perfect safety profile [47].

Considering these facts in this research, the aminoalkylation of structures **3**, **7a–c**, and **12** with different amines, such as norfloxacin (for **11a**, **13a**, **14a**, **14b**), ciprofloxacin (for **11b**, **13b**, **14c**, **14d**, **14e**), morpholine (for **11c**), thiomorpholine (**11d**), and 4-phenylpiperazine (for **11e**) in an ambience with formaldehyde was performed using the MW-assisted Mannich synthesis reactions (Scheme 2, Scheme 3 and Scheme 4). No signal symbolizing the presence of the NH band exists on the ¹H NMR and FT-IR spectra of products (**11a–e**, **13a,b**, **14a–e**) and in the ¹H NMR and ¹³C NMR spectra of molecules extra signals originated from amine moieties were observed at the concerned chemical ranges. These molecules displayed mass spectral datum records reasonable with their constructions.

The use of microwave (MW) irradiation method consequences in very influential and clean results with notable developments compared to classical processes. The process via MW irradiation ensured the more helpful road with developed synthesis yields and shorter synthesis times [54]. Green Chemistry would like the high yield of synthetic processes, the use of less toxic solvents, and the decrease in phases of synthetic schemes [53].

5. Conclusions

This study statements the synthesis of novel compounds having various bioactive units via microwave irradiation and conventional techniques. Microwave method provided more efficient way the synthesis of desired compounds. Also, antibacterial, and antiproliferative activity of the synthesized molecules were determined. Among the synthesized compounds, the best antimicrobial activities were found to show compounds **14a–e** which are Mannich bases. Especially these compounds showed very good antitubercular activity against *M. smegmatis* compared with Streptomycin standard drug. And also they exhibited better activity against to *E. coli* than ampicillin used as standard drug. Middle and high doses of compound **8a** and **9b** were found to have strong anticancer activity on the HeLa cervical cancer cells.

Declarations

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Conflict of Interest

The authors declare no conflict of interest.

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Schemes

Schemes 1-4 are in the Supplemental Files section.

Figures

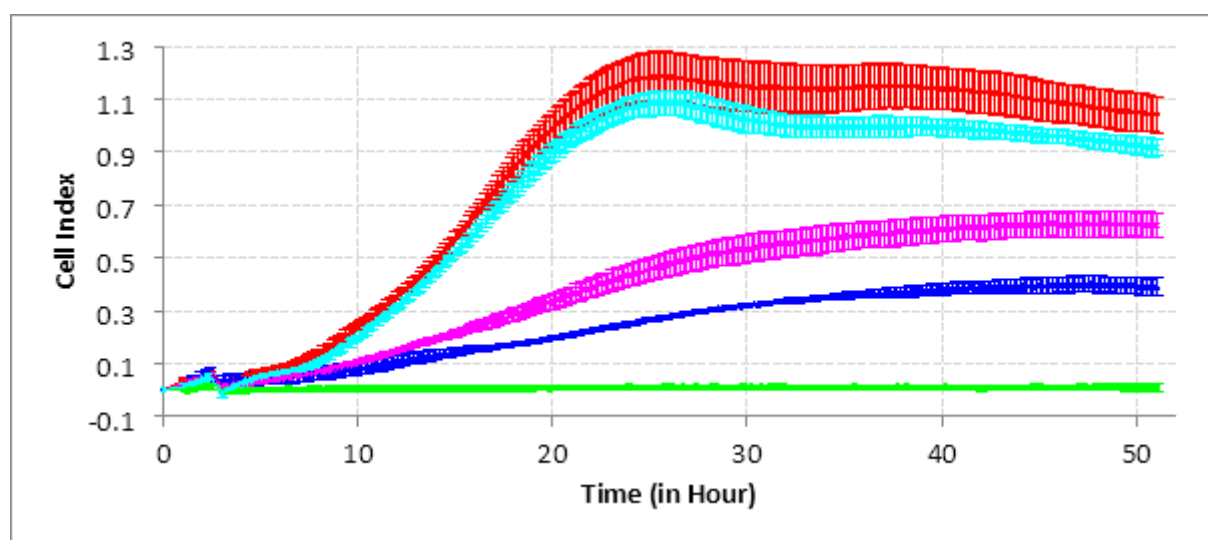


Figure 1

Time-CI (Cell Index) plot showing the antiproliferative activity test results of Compound 8a against HeLa cell line. (The concentration unit is $\mu\text{g}/\text{mL}$).

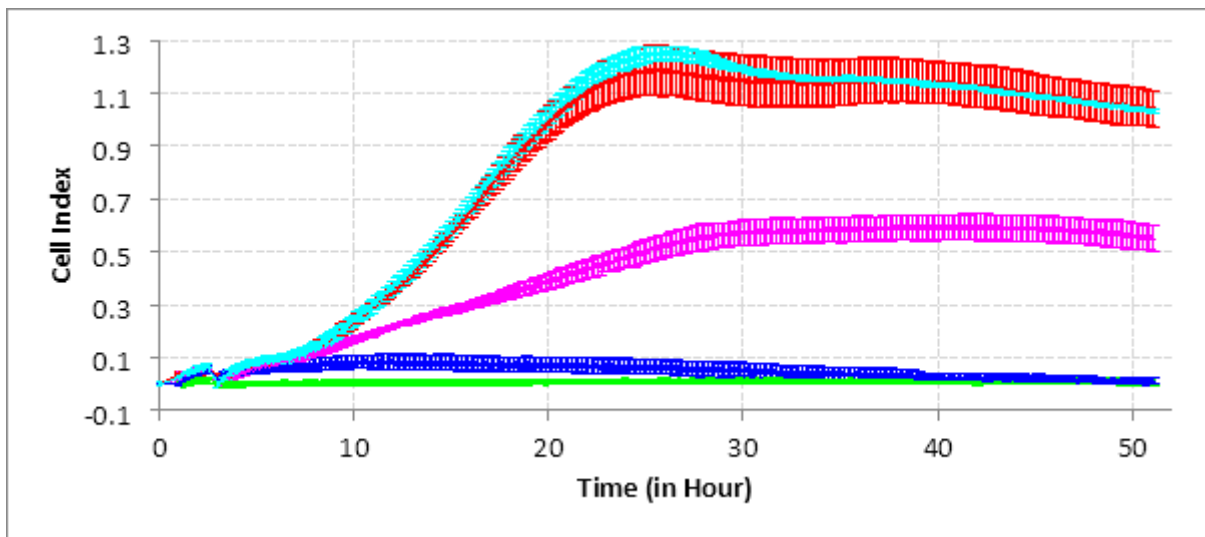


Figure 2

Time-CI (Cell Index) plot showing the antiproliferative activity test results of Compound 9b against HeLa cell line. (The concentration unit is $\mu\text{g/mL}$).

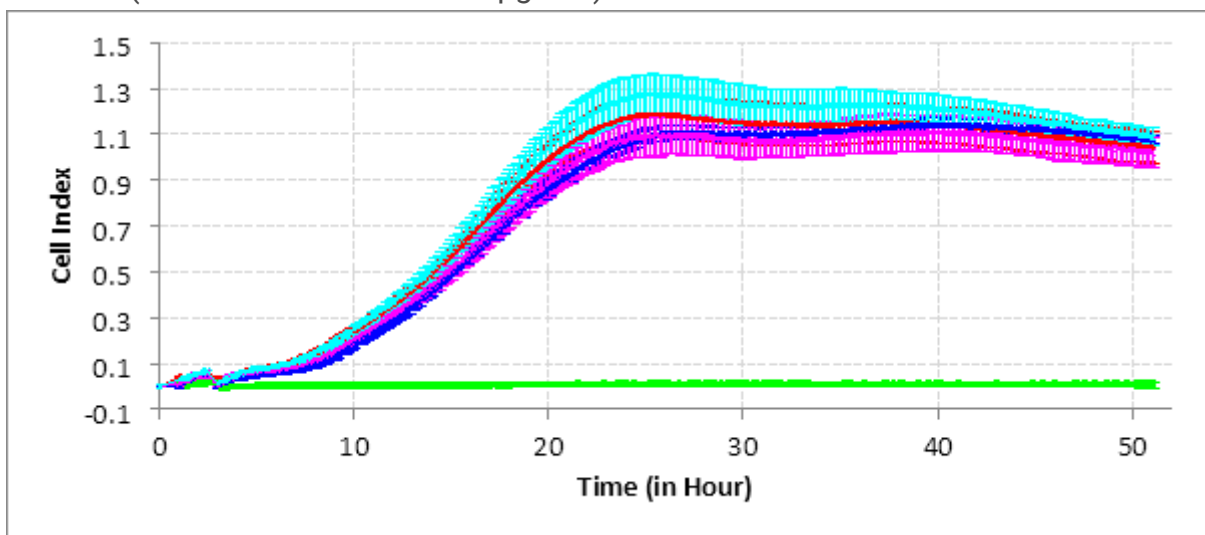


Figure 3

Adding different doses of DMSO to HeLa cell wells (The concentration unit is $\mu\text{g/mL}$).

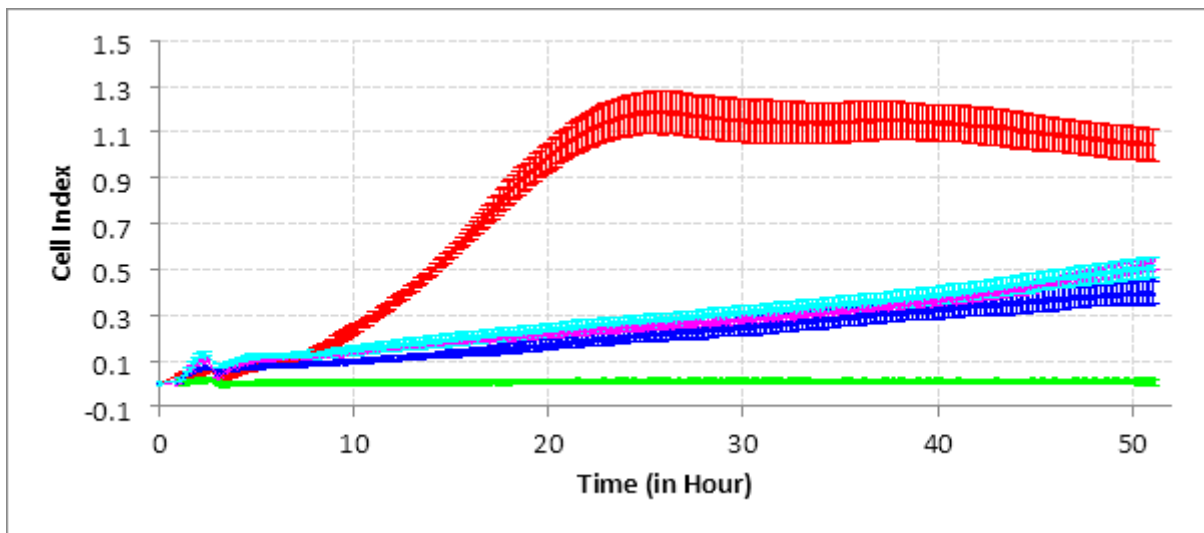


Figure 4

Effect of all 5FU doses used as positive control (The concentration unit is $\mu\text{g}/\text{mL}$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Scheme1.png](#)
- [Scheme2.png](#)
- [Scheme3.png](#)
- [Scheme4.png](#)