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SYNTHESIS AND ANTITUBERCULAR ACTIVITY OF OFLOXACIN DERIVATIVES

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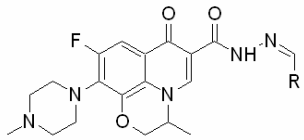
A series of novel ofloxacin derivatives were prepared and screened for their antimycobacterial activity. Among the synthesized compounds, 2, 3, 18, 19, 26 exhibited significant antimycobacterial activity. Additionally, all synthesized compounds were evaluated for their antibacterial and antifungal activity.



INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* and is still among the top ten causes of death worldwide.[1-3] According to the World Health Organization (WHO) Global Tuberculosis Report 2016, it was estimated that in 2015 there were 10.4 million new TB cases globally and 1.4 million deaths associated with TB.[2] The standard treatment for new TB cases is a 6-month regimen of first-line drugs, whereas the treatment for multidrug-resistant TB (MDR-TB) lasts for 20 months and it is treated with a second-line MDR-TB treatment regimen.[2,4-8] Second line treatment is less effective, more toxic with several side effects, poorly tolerated, longer and costs about US$ 2000–5000 per person.[2,4-8] Therefore, there is an urgent need to develop safe and more efficient antitubercular drugs that allow shorter therapies.

Fluoroquinolones with low price and toxicity are essential in the treatment of MDR tuberculosis and are being evaluated in shortened treatment duration. Especially, new generation fluoroquinolone moxifloxacin is used in the treatment of MDR-TB in accordance with the WHO recommendation.[2,4,9,10] However, a major problem with the fluoroquinolones is the development of resistant strains.[10-12] MDR strains that have developed resistance to the fluoroquinolones and also to any of the injectable second-line antitubercular drugs, such as amikacin, kanamycin, or capreomycin are extensively drug-resistant TB (XDR-TB) strains.[2,4,12]

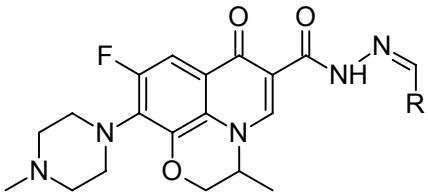


Fig. 1 – General structures of the synthesized compounds.

Ofloxacin, the second generation of fluoroquinolone, has good pharmacokinetic properties and intramacrophage penetration.[9,10,12] Besides, it is well known that the hydrazone moiety plays an important role for antimycobacterial activity and is used for prodrug design.[13-18] Thus, in the present study, a series of ofloxacin hydrazone derivatives were synthesized and tested for their ability to inhibit the

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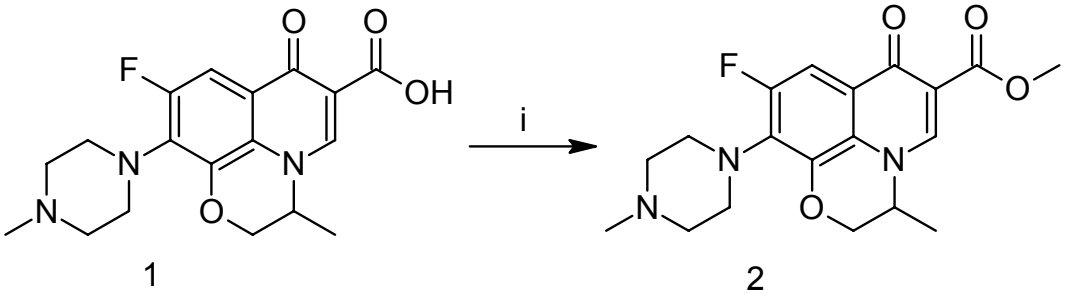
growth of selected mycobacterium, bacterial and fungal strains (Fig. 1).

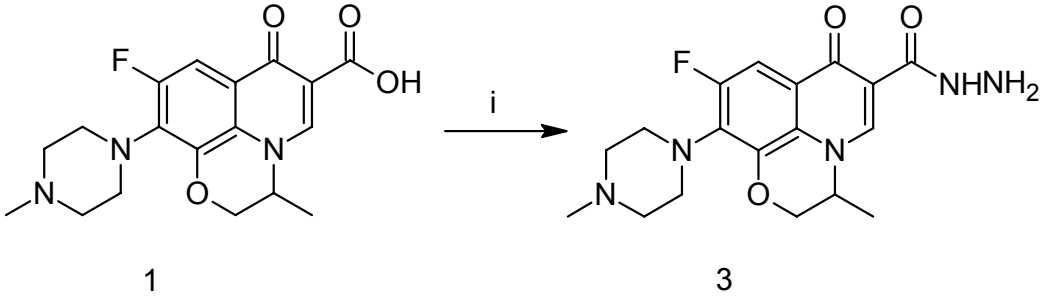
RESULTS AND DISCUSSION

The synthetic routes for the synthesized compounds are outlined in Schemes 1-3. Methyl ester derivative (2) was obtained by the reaction of ofloxacin (1) with methanol presence of sulfuric acid (Scheme 1). The treatment of compound 1 with hydrazine hydrate was given hydrazide derivative (3) (Scheme 2). Final compounds (4-26) were prepared by the reaction of compound 3 with appropriate aldehyde derivatives (Scheme 3). The structures of the synthesized compounds were elucidated by IR, ^{1}H-NMR and microanalyses.

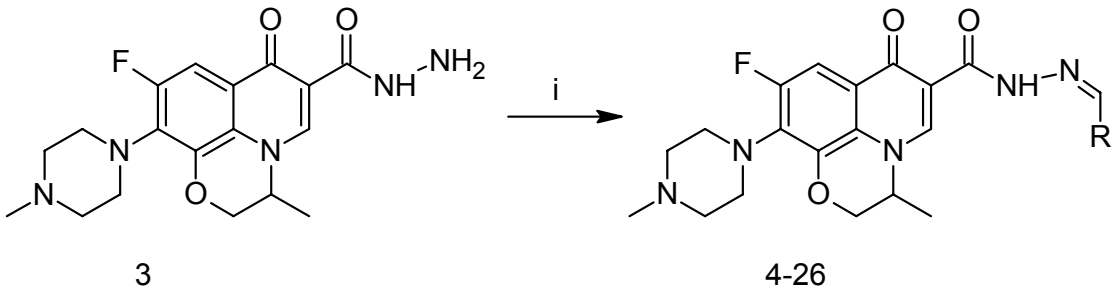
All the synthesized compounds were screened for their antibacterial activities against *Escherichia coli* ATCC 25922 (A), *E. coli* isolate (B), *Pseudomonas aeruginosa* ATCC 27853 (C), *P. aeruginosa* isolate (D), *Staphylococcus aureus* ATCC 29213 (E), *S. aureus* isolate (F), *Enterococcus faecalis* ATCC 29212 (G), *E. faecalis* isolate (H) and, for their antifungal activities against *Candida albicans* ATCC 10231 (I), *C. albicans* isolate (J) and, for their antimycobacterial activities against *Mycobacterium tuberculosis* H37Rv (K: 10^{-2} dilution of *M. tuberculosis* H37Rv, L: 10^{-4} dilution of *M. tuberculosis* H37Rv). The results were expressed as minimal inhibitory concentration (MIC, μg/mL). Antibacterial, antifungal and antimycobacterial activity of the synthesized compounds were given in Tables 1-3. Activity results of the standard drugs were given in Table 1.



Scheme 1 – Reagents: (i) methanol, H_2SO_4, reflux 20h.



Scheme 2 – Reagents: (i) NH_2NH_2, H_2O, reflux 16h.



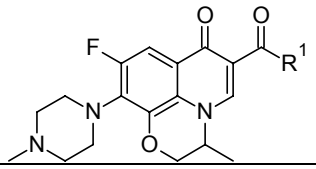
Scheme 3 – Reagents: (i) appropriate aldehyde derivatives, ethanol, and reflux 3h.

Among the synthesized compounds, 2, 3, 18, 19 and 26 were found the most active against 10^{-2} dilution of *M. tuberculosis* H37Rv with MIC value of 10 μg/mL. Compounds 9, 14, 15, and 16 exhibited moderate activity against 10^{-2} dilution of *M. tuberculosis* H37Rv with 20 μg/mL MIC value. In 10^{-4} dilution of *M. tuberculosis* H37Rv, the best MIC value was given by compound 19 having o-bromo substituent on the phenyl ring, which showed equal activity to ethambutol (MIC: 5 μg/mL). Compounds 2, 3, 5, 9, 14, 16, 18, and 26 also showed promising activity with MIC of 10 μg/mL against 10^{-4} dilution of *M. tuberculosis* H37Rv. However, ofloxacin (compound 1) and isoniazid showed more potent activity than the synthesized compounds against *M. tuberculosis* H37Rv as shown in Table 1. These results showed that ester and hydrazide moieties did not improve the activity against mycobacteria. In the hydrazone derivatives, except trifluoromethylphenyl and for methylphenyl derivatives, *ortho*-substituted phenyl derivatives showed a better antitubercular activity than *para*-substituted phenyl derivatives.

In the antibacterial activity test, most compounds showed weak activity with MICs values ranging between 64-256 μg/mL. Compound 15, 2-hydroxyphenyl derivative was found to be the most potent compound among the synthesized compounds and showed moderate antibacterial activity against *E. coli isolate* and *P. aeruginosa* ATCC 27853 (MIC: 32 μg/mL, 16 μg/mL, respectively). Surprisingly, compound 15 exhibited promising antifungal activity against *C. albicans* with MIC value of 16 μg/mL.

Table 1

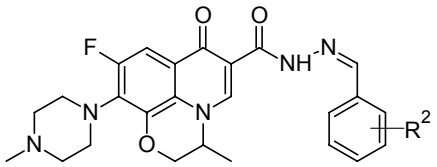
Antibacterial, antifungal and antimycobacterial activity of the synthesized compounds (1-3) and the standards



		Gram-negative bacteria				Gram-positive bacteria				Fungal sp.		Mycobacteria	
Comp.	R^1	A	B	C	D	E	F	G	H	I	J	K	L
1	OH	<0.25	<0.25	1	0.5	<0.25	64	1	32	128	128	<5	<5
2	OCH_3	128	64	64	64	32	256	32	128	64	64	10	10
3	$NHNH_2$	128	32	64	64	32	256	32	128	64	64	10	10
Ampicillin		4	16	-	-	1	16	1	>16	-	-	-	-
Ciprofloxacin		0.015	0.25	1	8	0.25	>16	1	>16	-	-	-	-
Meropenem		0.0625	0.0078	0.25	0.25	0.015	-	2	-	-	-	-	-
Cefotaxime		0.125	4	8	>16	2	>16	-	-	-	-	-	-
Fluconazole		-	-	-	-	-	-	-	-	1	1	-	-
Isoniazid		-	-	-	-	-	-	-	-	-	-	0.2	0.2
Ethambutol		-	-	-	-	-	-	-	-	-	-	5	5

Table 2

Antibacterial, antifungal and antimycobacterial activity of the synthesized compounds (4-20)



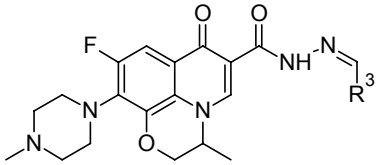
		Gram-negative bacteria				Gram-positive bacteria				Fungal sp.		Mycobacteria	
Comp.	R^2	A	B	C	D	E	F	G	H	I	J	K	L
4	H	128	128	64	64	128	256	32	64	64	64	40	20
5	4-CH_3	128	128	64	64	256	256	32	128	64	64	40	10
6	4-OCH_3	128	128	64	64	128	256	64	64	64	64	40	20
7	4-OH	128	128	64	64	64	256	128	64	64	64	80	40
8	4-F	128	128	64	64	128	256	64	64	64	64	40	20
9	4-CF_3	64	128	64	64	128	256	64	64	64	64	20	10
10	4-Cl	128	128	64	64	128	256	64	64	64	64	40	20
11	4-Br	128	128	64	64	128	256	64	64	64	64	40	20

Table 2 (continued)

12	4-NO_2	128	128	64	64	128	256	64	128	64	64	80	40
13	2-CH_3	128	128	64	64	128	256	64	128	64	64	40	20
14	2-OCH_3	128	128	64	64	128	256	64	128	64	64	20	10
15	2-OH	64	32	16	64	64	64	128	64	16	16	20	20
16	2-F	128	128	64	64	128	256	128	64	64	64	20	10
17	2-CF_3	128	128	64	64	128	256	128	64	64	64	40	20
18	2-Cl	128	64	64	64	128	128	128	64	64	64	10	10
19	2-Br	128	128	64	64	128	256	128	64	64	64	10	5
20	2-NO_2	128	128	64	64	64	256	128	64	64	64	40	20

Table 3

Antibacterial, antifungal and antimycobacterial activity of the synthesized compounds (21-26)



		Gram-negative bacteria				Gram-positive bacteria				Fungal sp.		Mycobacteria	
Comp.	R^3	A	B	C	D	E	F	G	H	I	J	K	L
21	4-pyridyl	64	128	64	64	128	256	128	64	64	64	80	40
22	2-pyridyl	64	128	64	64	128	256	128	64	64	64	80	40
23	2-furyl	64	128	64	64	128	256	128	64	64	64	80	40
24	2-thiophenyl	64	128	64	64	128	128	128	64	64	64	40	20
25	2,4-dichloro phenyl	128	128	64	64	128	256	128	64	64	64	40	20
26	biphenyl	64	128	64	64	128	128	128	64	64	64	10	10

EXPERIMENTAL PART

Chemistry

All chemicals and solvents were purchased locally from Merck AG and Aldrich Chemicals. Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectra were recorded on Perkin Elmer Spectrum 400 FT-IR and FT-NIR spectrometers with a Universal ATR sampler. ^{1}H-NMR spectra were recorded in DMSO-d_6 on a Varian Mercury 400, 400 MHz High Performance Digital FT-NMR spectrometer at the NMR facility of the Faculty of Pharmacy, Ankara University, Ankara, Turkey. All chemical shifts were recorded as δ (ppm). Microanalyses for C, H, and N were performed on a Leco-932 at the Faculty of Pharmacy, Ankara University, and they were within the range of ±0.4% of the theoretical value. The syntheses of 2,[19,20] 3,[21-23] 4,[22] 6,[22] 15,[22,23] 18[22] were previously reported.

Preparation of methyl 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylate (2)

1.8 g ofloxacin (0.005 mol) was dissolved in 30 mL of methanol, and added 0.6 mL of concentrated sulfuric acid. The mixture was heated to reflux and stirred for 20 hours. Then the precipitate was filtered by suction filtration. The remaining reaction medium was evaporated, and the residue was solved in water, and then neutralized with $NaHCO_3$. The formed precipitate was collected by suction filtration, washed with water and dried. Yield 23%; IR (ν, cm^{-1}): 1715, 1621 (C=O), 1240 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 8.62 (1H, s, H5), 7.41 (1H, d, H8), 4.71 (1H, dd, H2), 4.50 (1H, dd, H2), 4.30 (1H, dd, H3), 3.74 (3H, s, OCH_3), 3.23 (4H, m, piperazine), 2.42 (4H, t, piperazine), 2.22 (3H, s, N-CH_3), 1.39 (3H, d, CH_3). Anal. Calc. for $C_{19}H_{22}FN_3O_4.1/2H_2O$: C, 59.37; H, 6.03; N, 10.93. Found: C, 59.30; H, 5.86; N, 10.94%.

Preparation of 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carbohydrazide (3)

The mixture of ofloxacin (0.03 mol, 10g) and hydrazine hydrate (30 mL) in 50 mL water was heated to reflux and stirred for 16 hours. After the reaction mixture was cooled to the room temperature and the formed precipitate was filtered by suction filtration, it washed with water and dried. Yield 82%; IR (ν, cm^{-1}): 3504, 3317, 3234 (NH, NH_2), 1652, 1619 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 10.6 (1H, t, NH), 8.74 (1H, s, H5), 7.50 (1H, s, H8), 4.82 (1H, dd, H2), 4.55 (2H, d, NH_2), 4.50 (1H, dd, H2), 4.31 (1H, dd, H3), 3.23 (4H, m, piperazine), 2.41 (4H, t, piperazine), 2.20 (3H, s, N-CH_3), 1.39 (3H, d, CH_3). Anal. Calc. for $C_{18}H_{22}FN_5O_3.5/2H_2O$: C, 51.42; H, 6.47; N, 16.66. Found: C, 51.19; H, 6.28; N, 16.55%.

General procedure for the preparation of hydrazone derivatives (4-26)

0.002 mol appropriate aldehyde derivatives was added to the solution of 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carbohydrazide (0.001 mol, 0.375 g) in 10 mL ethanol and then heated to reflux and stirred for 3 hours. The formed precipitate was filtered by suction filtration, and crystallized using an appropriate solvent.

***9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-N'-[(E/Z)-phenylmethylidene]-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (4)**

Crystallized from isopropanol; yield 70%; IR (ν, cm^{-1}): 3048 (N-H), 1659, 1616 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.22 (1H, s, NH), 8.90 (1H, s, N=CH-), 8.41 (1H, s, H5), 7.77-7.75 (2H, m, phenyl), 7.55 (1H, d, H8), 7.48-7.43 (3H, m, phenyl), 4.90 (1H, d, H2), 4.55 (1H, d, H2), 4.36 (1H, d, H3), 3.26 (4H, t, piperazine), 2.43 (4H, t, piperazine), 2.23 (3H, s, N-CH_3), 1.44 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{26}FN_5O_3.1/2C_3H_8O$: C, 64.49; H, 6.12; N, 14.19. Found: C, 64.25; H, 6.15; N, 14.27%.

***9-Fluoro-3-methyl-N'-[(E/Z)-(4-methylphenyl)methylidene]-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (5)**

Crystallized from isopropanol; yield 58%; IR (ν, cm^{-1}): 3040 (N-H), 1656, 1605 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.15 (1H, s, NH), 8.87 (1H, s, N=CH-), 8.33 (1H, s, H5), 7.63 (2H, d, phenyl), 7.53 (1H, d, H8), 7.25 (2H, d, phenyl), 4.89 (1H, d, H2), 4.53 (1H, dd, H2), 4.34 (1H, dd, H3), 3.23 (4H, m, piperazine), 2.42 (4H, m, piperazine) 2.33 (3H, s, CH_3), 2.20 (3H, s, N-CH_3), 1.42 (3H, d, CH_3). Anal. Calc. for $C_{26}H_{28}FN_5O_3$: C, 65.39; H, 5.91; N, 14.67. Found: C, 65.11; H, 5.79; N, 14.55%.

***9-Fluoro-N'-[(E/Z)-(4-methoxyphenyl)methylidene]-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (6)**

Crystallized from ethanol; yield 66%; IR (ν, cm^{-1}): 3064 (N-H), 1662, 1602 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.11 (1H, s, NH), 8.86 (1H, s, N=CH-), 8.31 (1H, s, H5), 7.68 (2H, d, phenyl), 7.53 (1H, d, H8), 7.00 (2H, d, phenyl), 4.89 (1H, d, H2), 4.53 (1H, dd, H2), 4.34 (1H, dd, H3), 3.79 (3H, s, OCH_3), 3.24 (4H, m, piperazine), 2.41 (4H, m, piperazine), 2.21 (3H, s, N-CH_3), 1.42 (3H, d, CH_3). Anal. Calc. for $C_{26}H_{28}FN_5O_4.1/2H_2O$: C, 62.14; H, 5.82; N, 13.94. Found: C, 62.10; H, 5.89; N, 13.88%.

***9-Fluoro-N'-[(E/Z)-(4-hydroxyphenyl)methylidene]-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (7)**

Crystallized from ethanol; yield 74%; IR (ν, cm^{-1}): 3139-2505 (N-H, O-H), 1660, 1609 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.07 (1H, s, NH), 9.92 (1H, s, OH), 8.88 (1H, s, N=CH-), 8.27 (1H, s, H5), 7.59 (2H, d, phenyl), 7.55 (1H, d, H8), 6.84 (2H, d, phenyl), 4.90 (1H, d, H2), 4.55 (1H, d, H2), 4.36 (1H, d, H3), 3.26 (4H, m, piperazine), 2.43 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.44 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{26}FN_5O_4$: C, 62.62; H, 5.47; N, 14.61. Found: C, 62.49; H, 5.73; N, 14.64%.

***9-Fluoro-N'-[(E/Z)-(4-fluorophenyl)methylidene]-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (8)**

Crystallized from ethanol; yield 72%; IR (ν, cm^{-1}): 3045 (N-H), 1653, 1615 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.19 (1H, s, NH), 8.87 (1H, s, N=CH-), 8.40 (1H, s, H5), 7.81-7.77 (2H, m, phenyl), 7.52 (1H, d, H8), 7.30-7.25 (2H, m, phenyl), 4.89 (1H, d, H2), 4.53 (1H, dd, H2), 4.34 (1H, dd, H3), 3.24 (4H, m, piperazine), 2.43 (4H, m, piperazine), 2.21 (3H, s, N-CH_3), 1.42 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{25}F_2N_5O_3$: C, 62.36; H, 5.23; N, 14.55. Found: C, 62.19; H, 5.24; N, 14.55%.

***9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-N'-{(E/Z)-[4-(trifluoromethyl)phenyl]methylidene}-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (9)**

Crystallized from ethanol; yield 68%; IR (ν, cm^{-1}): 3043 (N-H), 1657, 1619 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.34 (1H, s, NH), 8.89 (1H, s, N=CH-), 8.49 (1H, s, H5), 7.95-7.92 (2H, d, phenyl), 7.81-7.79 (2H, d, phenyl), 7.53 (1H, d, H8), 4.90 (1H, d, H2), 4.55 (1H, dd, H2), 4.35 (1H, dd, H3), 3.24 (4H, m, piperazine), 2.42 (4H, m, piperazine), 2.20 (3H, s, N-CH_3), 1.43 (3H, d, CH_3). Anal. Calc. for $C_{26}H_{25}F_4N_5O_3$: C, 58.75; H, 4.74; N, 13.18. Found: C, 58.67; H, 4.85; N, 13.21%.

***N'-[(E/Z)-(4-chlorophenyl)methylidene]-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (10)**

Crystallized from ethanol; yield 80%; IR (ν, cm^{-1}): 3059 (N-H), 1662, 1616 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.22 (1H, s, NH), 8.86 (1H, s, N=CH-), 8.39 (1H, s, H5), 7.75-7.73 (2H, m, phenyl), 7.53-7.48 (3H, t, H8, phenyl), 4.87 (1H, d, H2), 4.53 (1H, d, H2), 4.32 (1H, d, H3), 3.23 (4H, m, piperazine), 2.40 (4H, m, piperazine), 2.19 (3H, s, N-CH_3), 1.41(3H, d, CH_3). Anal. Calc. for $C_{25}H_{25}ClFN_5O_3$: C, 60.30; H, 5.06; N, 14.06. Found: C, 60.25; H, 5.03; N, 14.10%.

***N'-[(E/Z)-(4-bromophenyl)methylidene]-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (11)**

Crystallized from ethanol; yield 55%; IR (ν, cm^{-1}): 3082 (N-H), 1663, 1617 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.27 (1H, s, NH), 8.90 (1H, s, N=CH-), 8.41 (1H, s, H5), 7.71-7.65 (4H, m, phenyl), 7.55 (1H, d, H8), 4.91 (1H, d, H2), 4.55 (1H, d, H2), 4.36 (1H, d, H3), 3.25 (4H, m, piperazine), 2.43 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.44 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{25}BrFN_5O_3$: C, 55.36; H, 4.65; N, 12.91. Found: C, 55.15; H, 4.65; N, 12.90%.

***9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-N'-[(E/Z)-(4-nitrophenyl)methylidene]-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (12)**

Crystallized from ethanol; yield 60%; IR (ν, cm^{-1}): 3074 (N-H), 1667, 1615 (C=O), 1517, 1336 (NO_2); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.43 (1H, s, NH), 8.92 (1H, s, N=CH-), 8.57 (1H, s, H5), 8.30 (2H, d, phenyl), 8.00 (2H, d, phenyl), 7.55 (1H, d, H8), 4.92 (1H, d, H2), 4.56 (1H, dd, H2), 4.37 (1H, dd, H3), 3.25 (4H, m, piperazine), 2.49 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.45 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{25}FN_6O_5.1/3H_2O$: C, 58.36; H, 5.03; N, 16.33. Found: C, 58.49; H, 5.14; N, 16.29%.

***9-Fluoro-3-methyl-N'-[(E/Z)-(2-methylphenyl)methylidene]-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (13)**

Crystallized from ethanol; yield 66%; IR (ν, cm^{-1}): 3046 (N-H), 1658, 1616 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.18 (1H, s, NH), 8.89 (1H, s, N=CH-), 8.59 (1H, s, H5), 7.85 (1H, d, phenyl), 7.55 (1H, d, H8), 7.32-7.24 (3H, m, phenyl), 4.91 (1H, d, H2), 4.56 (1H, dd, H2), 4.37 (1H, dd, H3), 3.26 (4H, m, piperazine), 2.49 (3H, s, CH_3), 2.44 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.45 (3H, d, CH_3). Anal. Calc. for $C_{26}H_{28}FN_5O_3$: C, 65.39; H, 5.91; N, 14.67. Found: C, 64.98; H, 6.29; N, 14.64%.

***9-Fluoro-N'-[(E/Z)-(2-methoxyphenyl)methylidene]-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (14)**

Crystallized from ethanol; yield 60%; IR (ν, cm^{-1}): 3046 (N-H), 1658, 1616 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.21 (1H, s, NH), 8.87 (1H, s, N=CH-), 8.53 (1H, s, H5), 7.85 (1H, d, phenyl), 7.53 (1H, d, H8), 7.41 (1H, t, phenyl), 7.09 (1H, d, phenyl), 7.01 (1H, t, phenyl), 4.89 (1H, d, H2), 4.53 (1H, dd, H2), 4.34 (1H, dd, H3), 3.86 (3H, s, OCH_3), 3.25 (4H, m, piperazine), 2.41 (4H, m, piperazine), 2.20 (3H, s, N-CH_3), 1.42 (3H, d, CH_3). Anal. Calc. for $C_{29}H_{28}FN_5O_4$: C, 63.27; H, 5.72; N, 14.19. Found: C, 63.05; H, 5.90; N, 14.19%.

***9-Fluoro-N'-[(E/Z)-(2-hydroxyphenyl)methylidene]-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (15)**

Crystallized from ethanol; yield 85%; IR (ν, cm^{-1}): 3043 (N-H), 1659, 1605 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.27 (1H, s, NH), 11.30 (1H, s, OH), 8.90 (1H, s, N=CH-), 8.63 (1H, s, H5), 7.56-7.51 (2H, m, phenyl, H8), 7.30 (1H, t, phenyl), 6.94-6.91 (2H, m, phenyl), 4.90 (1H, d, H2), 4.55 (1H, d, H2), 4.37 (1H, d, H3), 3.26 (4H, m, piperazine), 2.43 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.45 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{26}FN_5O_4.1/4C_2H_5OH$: C, 62.37; H, 5.65; N, 14.26. Found: C, 62.70; H, 5.67; N, 14.13%.

***9-Fluoro-N'-[(E/Z)-(2-fluorophenyl)methylidene]-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (16)**

Crystallized from ethanol; yield 73%; IR (ν, cm^{-1}): 3045 (N-H), 1664, 1617 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.25 (1H, s, NH), 8.86 (1H, s, N=CH-), 8.50 (1H, s, H5), 7.81-7.90 (1H, t, phenyl), 7.52 (1H, d, H8), 7.49-7.43 (1H, m, phenyl), 7.27 (2H, t, phenyl), 4.88 (1H, d, H2), 4.53 (1H, d, H2), 4.34 (1H, d, H3), 3.23 (4H, m, piperazine), 2.41 (4H, m, piperazine), 2.19 (3H, s, N-CH_3), 1.41 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{25}F_2N_5O_3.1/4H_2O$: C, 61.78; H, 5.29; N, 14.41. Found: C, 61.92; H, 5.14; N, 14.38%.

***9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-N'-{(E/Z)-[2-(trifluoromethyl)phenyl]methylidene}-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (17)**

Crystallized from ethanol; yield 77%; IR (ν, cm^{-1}): 3045 (N-H), 1662, 1615 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.39 (1H, s, NH), 8.91 (1H, s, N=CH-), 8.66 (1H, s, H5), 8.21 (1H, d, phenyl), 7.83-7.76 (2H, m, phenyl), 7.65 (1H, t, phenyl), 7.56 (1H, d, H8), 4.91 (1H, d, H2), 4.55 (1H, d, H2), 4.37 (1H, d, H3), 3.25 (4H, m, piperazine), 2.43 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.44 (3H, d, CH_3). Anal. Calc. for $C_{26}H_{25}F_4N_5O_3$: C, 58.75; H, 4.74; N, 13.18. Found: C, 58.44; H, 4.93; N, 13.19%.

***N'-[(E/Z)-(2-chlorophenyl)methylidene]-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (18)**

Crystallized from ethanol; yield 75%; IR (ν, cm^{-1}): 3045 (N-H), 1660, 1617 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.35 (1H, s, NH), 8.91 (1H, s, N=CH-), 8.67 (1H, s, H5), 8.02-8.00 (1H, m, phenyl), 7.57-7.44 (4H, m, H8, phenyl), 4.91 (1H, d, H2), 4.55 (1H, d, H2), 4.37 (1H, d, H3), 3.26 (4H, m, piperazine), 2.43 (4H, t, piperazine), 2.23 (3H, s, N-CH_3), 1.44 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{25}ClFN_5O_3$: C, 60.30; H, 5.06; N, 14.06. Found: C, 60.34; H, 5.37; N, 14.05%.

***N'-[(E/Z)-(2-bromophenyl)methylidene]-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (19)**

Crystallized from ethanol; yield 78%; IR (ν, cm^{-1}): 3045 (N-H), 1660, 1616 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.36 (1H, s, NH), 8.91 (1H, s, N=CH-), 8.61 (1H, s, H5), 7.99 (1H, d, phenyl), 7.71 (1H, d, phenyl), 7.56 (1H, d, H8), 7.48 (1H, t, phenyl), 7.38 (1H, t, phenyl), 4.91 (1H, d, H2), 4.56 (1H, d, H2), 4.36 (1H, d, H3), 3.25 (4H, m, piperazine), 2.43 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.44 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{25}BrFN_5O_3$: C, 55.36; H, 4.65; N, 12.91. Found: C, 55.19; H, 4.86; N, 12.92%.

***9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-N'-[(E/Z)-(2-nitrophenyl)methylidene]-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (20)**

Crystallized from isopropanol; yield 36%, IR (ν, cm^{-1}): 3043 (N-H), 1664, 1616 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.41 (1H, s, NH), 8.92 (1H, s, N=CH-), 8.72 (1H, s, H5), 8.09 (2H, d, phenyl), 7.83 (1H, t, phenyl), 7.69 (1H, t, phenyl), 7.56 (1H, d, H8), 4.91 (1H, d, H2), 4.55 (1H, d, H2), 4.37 (1H, d, H3), 3.28 (4H, m, piperazine), 2.43 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.45 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{25}FN_6O_5$: C, 59.05; H, 4.96; N, 16.53. Found: C, 59.17; H, 5.01; N, 16.49%.

***9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-N'-[(E/Z)-pyridin-4-ylmethylidene]-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (21)**

Crystallized from ethanol; yield 64%; IR (ν, cm^{-1}): 3042 (N-H), 1657, 1603 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.39 (1H, s, NH), 8.89 (1H, s, N=CH-), 8.64-8.62 (2H, m, pyridyl), 8.42 (1H, s, H5), 7.66-7.64 (2H, m, pyridyl), 7.53 (1H, d, H8), 4.90 (1H, d, H2), 4.55 (1H, dd, H2), 4.36 (1H, dd, H3), 3.24 (4H, m, piperazine), 2.42 (4H, m, piperazine), 2.20 (3H, s, N-CH_3), 1.43 (3H, d, CH_3). Anal. Calc. for $C_{24}H_{25}FN_6O_3$: C, 62.06; H, 5.42; N, 18.09. Found: C, 61.75; H, 5.75; N, 17.96%.

***9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-N'-[(E/Z)-pyridin-2-ylmethylidene]-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (22)**

Crystallized from ethanol; yield 64%; IR (ν, cm^{-1}): 3044 (N-H), 1655 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.31 (1H, s, NH), 8.91 (1H, s, N=CH-), 8.64-8.63 (1H, m, pyridyl), 8.36 (1H, s, H5), 7.97 (1H, d, pyridyl), 7.91-7.87 (1H, m, pyridyl), 7.57 (1H, d, H8), 7.44-7.41 (1H, m, pyridyl), 4.92 (1H, d, H2), 4.56 (1H, dd, H2), 4.37 (1H, dd, H3), 3.26 (4H, m, piperazine), 2.43 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.45 (3H, d, CH_3). Anal. Calc. for $C_{24}H_{25}FN_6O_3$: C, 62.06; H, 5.42; N, 18.09. Found: C, 61.90; H, 5.64; N, 17.98%.

***9-Fluoro-N'-[(E/Z)-furan-2-ylmethylidene]-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (23)**

Crystallized from ethanol; yield 36%; IR (ν, cm^{-1}): 3087 (N-H), 1660 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.11 (1H, s, NH), 8.89 (1H, s, N=CH-), 8.32 (1H, s, H5), 7.85 (1H, d, furyl), 7.54 (1H, d, H8), 6.89 (1H, d, furyl), 6.65-6.63 (1H, m, furyl), 4.90 (1H, d, H2), 4.55 (1H, dd, H2), 4.36 (1H, dd, H3), 3.26 (4H, m, piperazine), 2.45 (4H, m,

piperazine), 2.23 (3H, s, N-CH_3), 1.44 (3H, d, CH_3). Anal. Calc. for $C_{23}H_{24}FN_5O_4.1/2H_2O$: C, 59.73; H, 5.45; N, 15.14. Found: C, 59.71; H, 5.20; N, 15.08%.

***9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-N'-[(E/Z)-thiophen-2-ylmethylidene]-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (24)**

Crystallized from ethanol; yield 71%; IR (ν, cm^{-1}): 3044 (N-H), 1658 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.17 (1H, s, NH), 8.87 (1H, s, N=CH-), 8.65 (1H, s, H5), 7.68 (1H, d, thiophenyl), 7.54 (1H, d, H8), 7.45 (1H, d, thiophenyl), 7.16-7.14 (1H, dd, thiophenyl), 4.91 (1H, d, H2), 4.57 (1H, d, H2), 4.36 (1H, d, H3), 3.26 (4H, m, piperazine), 2.44 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.44 (3H, d, CH_3). Anal. Calc. for $C_{23}H_{24}FN_5O_3S.1/3C_2H_5OH$: C, 58.62; H, 5.40; N, 14.44. Found: C, 58.79; H, 5.45; N, 14.48%.

***N'-[(E/Z)-(2,4-dichlorophenyl)methylidene]-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (25)**

Crystallized from ethanol; yield 77%; IR (ν, cm^{-1}): 3042 (N-H), 1663, 1617 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.37 (1H, s, NH), 8.91 (1H, s, N=CH-), 8.64 (1H, s, H5), 8.02 (1H, d, phenyl), 7.73 (1H, d, phenyl), 7.57 (1H, d, H8), 7.55-7.52 (1H, dd, phenyl), 4.91 (1H, d, H2), 4.55 (1H, d, H2), 4.36 (1H, d, H3), 3.24 (4H, m, piperazine), 2.43 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.44 (3H, d, CH_3). Anal. Calc. for $C_{25}H_{24}Cl_2FN_5O_3$: C, 56.40; H, 4.54; N, 13.15. Found: C, 56.34; H, 4.56; N, 13.30%.

***N'-[(E/Z)-biphenyl-4-ylmethylidene]-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carbohydrazide* (26)**

Crystallized from ethanol; yield 54%; IR (ν, cm^{-1}): 3039 (N-H), 1654 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 13.27 (1H, s, NH), 8.91 (1H, s, N=CH-), 8.46 (1H, s, H5), 7.86-7.40 (10H, m, phenyl, H8), 4.92 (1H, d, H2), 4.56 (1H, d, H2), 4.37 (1H, d, H3), 3.30 (4H, m, piperazine), 2.44 (4H, m, piperazine), 2.23 (3H, s, N-CH_3), 1.45 (3H, d, CH_3). Anal. Calc. for $C_{31}H_{30}FN_5O_3.1/4H_2O$: C, 68.43; H, 5.65; N, 12.87. Found: C, 68.44; H, 5.68; N, 12.90%.

Biological Activity

Antibacterial and Antifungal Activity [24, 25]

Microorganisms

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231 standard strains and clinical isolates provided from Trakya University Health Center for Medical Research and Practice Microbiology Laboratory were used in the study.

Microdilution Method

Standard powders of ampicillin (Sigma), cefotaxim (Sigma), ciprofloxacin (Sigma), meropenem (Sigma) and fluconazole (Sigma) were used as standard antimicrobial agents. Stock solutions of the test compounds were prepared in DMSO (Merck). Ampicillin was prepared in phosphate buffer solution and other antibiotic solutions were prepared in sterile distilled water according to the guideline of CLSI M100-S25.

Antimicrobial susceptibility testing was performed through CLSI M100-S25 and CLSI M27-A3 directions. Mueller Hinton Agar (MHA) (Merck), Mueller Hinton Broth (MHB) (Merck), Sabouraud Dextrose Agar (SDA) (Merck), Sabouraud Liquid Medium (SLM) (Merck) and RPMI-1640 medium (Sigma) with L-glutamine buffered pH 7 with 3-[N-morpholino]-propansulfonic acid (MOPS) (Sigma) were used for microbial cultures. Bacterial isolates were subcultured in Mueller Hinton Agar (MHA) plates and incubated over night at 37^{o}C and *C. albicans* was subcultured in Sabouraud Dextrose Agar (SDA) plates at 35 ^{o}C for 24-48 h. Pure colonies were transferred to MHB and SLM for bacteria and fungi, respectively. They were incubated in the appropriate conditions overnight. After the incubation, the bacterial suspensions used for inoculation were prepared at $5x10^5$ CFU/mL by diluting fresh cultures at McFarland 0.5 density ($1\text{-}2x10^8$ CFU/mL).Yeast suspensions were also prepared according to McFarland 0.5 density and a working suspension was made by a 1:100 dilution followed by a 1:20 dilution of the stock suspension ($2.5x10^3$ CFU/mL).

Susceptibility testing was performed with MHB for bacteria and RPMI-1640 medium with L-glutamine buffered pH 7 with 3-[N-morpholino]-propansulfonic acid (MOPS) for fungi. The solutions of the newly synthesized compounds and standard drugs were prepared at 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 µg/mL and 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015, 0.0078 µg/mL concentrations, respectively by diluting the stock concentrations in a microdilution tray with a multichannel pipette.

After the dilution, a 10 µL bacterial or fungal inoculum was added to each well of the microdilution trays. The trays were incubated at 37 ^{o}C for bacteria and 35 ^{o}C for fungi, in a humid chamber and MIC endpoints were read after 24 h of incubation. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported.

All organisms were tested in triplicate in each run of the experiments. Solvents, pure microorganisms and pure media were used as control wells.

Antimycobacterial Activity

Agar proportion method

The minimum inhibitory concentration (MIC) values of each synthesized compound were tested by agar dilution in duplicate as recommended by the Clinical Laboratory Standards Institute (CLSI).[26,27] Positive and negative growth controls were run in each assay. Isoniazid (INH) (Sigma I3377) and ethambutol (EMB) (Sigma E4630) were used as control agents. *M. tuberculosis* H37Rv was used as the standard strain and was provided from Refik Saydam National Public Health Agency, National Tuberculosis Reference Laboratory, Ankara, Turkey. Stock solutions of the synthesized compounds and reference compounds were prepared in DMSO/H_2O (50%) at a concentration of 1000 µg/mL. These solutions were then filtered through a 0.22 µm membrane filter (Ministar, Sartorius Stedim Biotech GmbH, Goettingen, Germany). Middlebrook 7H10 agar medium (BBL, Becton Dickinson and Company, Sparks, MD, USA) was supplemented with oleic acid-albumin-dextrose-catalase (OADC, BBL, Becton Dickinson and Company, Sparks, MD, USA). The synthesized compounds and control agents were added to obtain an appropriate final concentration in the medium. The final concentrations of INH and EMB were 0.2 µg/mL and 5 µg/mL, respectively. Synthesized compounds were prepared at final concentrations of 5, 10, 20, 40 and 80 µg/mL. Agar without any references and the synthesized compounds were used as a positive growth

control, and 3 mL of the prepared medium was dispensed into sterile tubes. The DMSO concentration in the final solutions was not above 1% for antimycobacterial activity.

Inoculum preparation

H37Rv was maintained in Lowenstein-Jensen medium. A culture suspension was prepared by subculturing in Middlebrook 7H9 broth (BBL, Becton Dickinson and Company, Sparks, MD, USA) supplemented with 10% OADC at 37°C for 7-10 days, until a density corresponding to 10^{-2} to 10^{-4} dilutions were obtained from McFarland standard No. 1. Then 0.1 mL of the diluted suspension was inoculated onto the control and the other tubes with compounds in different concentrations. The tubes were incubated at 37°C in an atmosphere of 5% CO_2 for 3 weeks. The MIC values were defined as the lowest concentration that inhibited more than 90% of the bacterial growth and the results of INH and EMB were interpreted according to the CLSI. The MIC was considered the lowest concentration that showed no visible colonies in all dilutions.

CONCLUSIONS

In this study, new hydrazone derivatives of ofloxacin were synthesized and screened for their antimycobacterial, antibacterial and antifungal activities. Among the synthesized compounds, methyl ester (compound 2), hydrazide (compound 3), 2-chlorophenyl (compound 18), 2-bromophenyl (compound 19) and biphenyl (compound 26) derivatives exhibited good antimycobacterial activity. 2-Hydroxyphenyl derivative (compound 15) was found to have a promising antibacterial and antifungal activity in the synthesized compounds.

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