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MEDICINAL CHEMISTRY RESEARCH

SYNTHESIS OF SOME NEW ANTIMICROBIAL THIADIAZOLYL AND OXADIAZOLYL QUINOLINE DERIVATIVES

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Abstract. Two series of substituted thiadiazolyl and oxadiazolylquinolines (**3a-h , 4a-h , 7a-f , 8a-f** and **9**) were synthesized and screened for their antimicrobial activity. Some of the tested compounds showed promising activity. Compound **4b** exhibited bactericidal activity against *S. aureus* at 31.25 μg/ml. While compound **8a** showed distinct antifungal activity against *C. albicans* (MIC at 31.25 μg/ml). The detailed synthesis, spectroscopic and biological data are reported.

The quinoline nucleus was reported to exhibit various biological activities such as antiamoebic [1], antimalarial [2,3], antiviral [4,5] as well as anti-inflammatory activity [6,7]. In addition, the discovery of nalidixic acid, a urinary tract antimicrobial drug [8], prompted the synthesis of many quinolone and quinoline derivatives and examination of their antimicrobial activity [9-11]. Norfloxacin, ofloxacin, and ciprofloxacin (nalidixic acid analogs) were marketed as active antimicrobial medications [12]. Besides, oxadiazole and thiadiazole rings are important examples of the heteroazoles that by themselves or in combination with other ring systems possess antimicrobial activity [13-15]. In view of these facts and as a continuation of a research program carried out in our laboratory [16-19] two series of substituted thiadiazolyl and oxadiazolylquinolines have been synthesized to investigate their antimicrobial activity.

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Chemistry

The synthetic pathway depicted to obtain the new compounds (3-9) is outlined in schemes 1-3. The starting materials, 2-substituted cinchoninic acids **1a-c** were prepared according to Pfitzinger reaction [20,21]. While, 2-amino-5-alkyl (or aralkyl) thio-1,3,4-thiadiazoles **2a-c,** were obtained by refluxing a mixture of thiosemi-carbazide, carbon disulfide and anhydrous sodium carbonate in absolute ethanol, followed by S-alkylation using either dialkylsulfates or aralkyl halides [22]. Condensation of the appropriate cinchoninic acid **1a-c** with the selected aminothiadiazole **2a-c** in the presence of DCC furnished the proposed 1,3,4-thiadiazol-2-yl-quinoline-4-carboxamides **3a-h** (Scheme 1, Table 1). Oxidation of the aforementioned compounds **3a-h** to the corresponding sulfonyl analogs **4a-h** was carried out using potassium permanganate in glacial acetic acid (Scheme 1, Table 1). On the other hand, the key intermediates 2-substituted cinchoninic acid hydrazides **5a,b** (Scheme 2) were obtained by following the reported procedure [23]. Treatment of the acid hydrazides **5a,b** with carbon disulfide in alkaline medium afforded the expected 5-thioxo-1,3,4-oxadiazolylquinolines **6a &b** [24] (Scheme 2, Table 2). These thiols **6a,b** were then alkylated to their corresponding alkyl or aralkyl thio analogs **7a-f** (Scheme 2, Table 2). Oxidation of **7b,d,e,f** was performed by potassium permanganate in glacial acetic acid at room temperature to afford the corresponding sulfonyl derivatives **8b,d,e,f** (Scheme 3, Table 2). While using the same conditions for compounds **7a,c**, both gave one and the same product, 2-methyl-4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)quinoline **9** (Scheme 3). Formation of this compound could be a result of further oxidation of the sulfones first formed. Thus, oxidation of **7a** and **7c** was repeated at 0-5°C to yield the expected sulfonyl products **8a,c** (Scheme 3, Table 2) as proved by interpreting their microanalytical and spectral data.

Results and Discussion

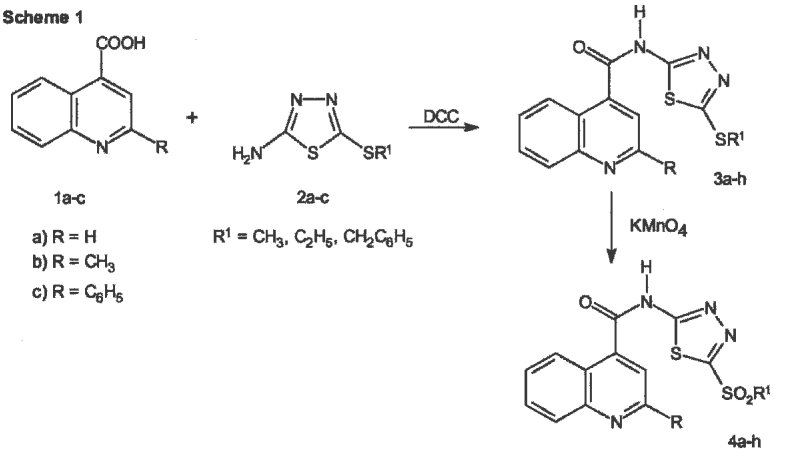
The newly prepared compounds were preliminarily evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* (ATCC 6538p) as an example of Gram-positive bacteria and *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (ATCC 9027) as representative examples of Gram-negative bacteria. They were evaluated for their *in vitro* antifungal activity against *Candida albicans* (ATCC

10231). Their inhibition zones using the cup diffusion technique were measured [25]. Compounds showing inhibition zones of 20 mm or more were considered to be active and were further evaluated for their minimal inhibitory concentration (**MIC**) and minimal bactericidal concentration (**MBC**) values using the two-fold serial dilution method [26].

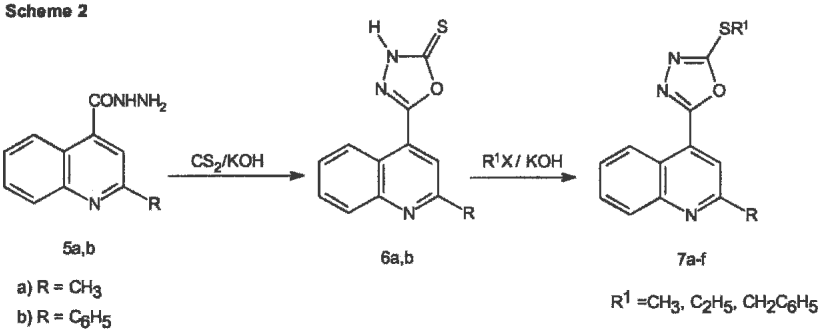
Ciprofloxacin was used a as standard antibacterial agent, while Nystatin was used as a standard antifungal agent. Dimethylformamide as a blank showed no antimicrobial activity.

The obtained data revealed that some of the tested compounds showed remarkable antimicrobial activity. Interestingly compounds **4b, e, f & g**. exhibited higher antimicrobial activity (IZ =30, 31, 27 & 28 mm, respectively) than ciprofloxacin (IZ = 25 mm) against *S. aureus*. On the other hand compounds **3b,e** and **4c,f** were found to be moderately active against Gram-negative bacteria, *P. aeruginosa* (IZ = 20 mm). Some compounds **(3g, 7a, 8a & 8c)** showed promising antifungal activity against *C. albcans* (IZ = 24-27 mm). However, none of the screened compounds showed significant activity against *E. coli*. It is worth mentioning that compound **4e** was proved to have bactericidal activity against *S. aureaus* (MBC = 125 μg/ml), while **4b**, the most active compound, caused cidal activity at 31.25 μg/ml. In general, the obtained results revealed that thiadiazole analogs are more active than the corresponding oxadiazoles. Sulfonylthiadiazoles, in particular are the most promising candidates for further structure modification study in order to increase their antimicrobial activity.

Scheme 1



Scheme 2



Scheme 3

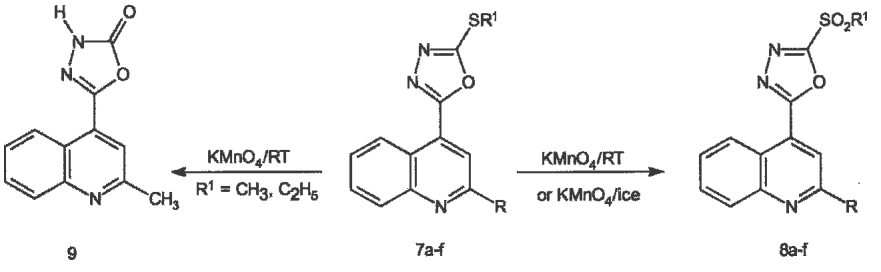


Table 1: Physical data, yields and crystallization solvents of the new compounds (3a-h and 4a-h)

Comp. No.	R	R^1	Yield %	M.P.°C	Cryst. Solv.	Mol.Formula
3a	H	CH_3	53	272	(A)	$C_{13}H_{10}N_4OS_2$
3b	C_6H_5	CH_3	68.7	240-2	(A)	$C_{19}H_{14}N_4OS_2$
3c	H	C_2H_5	56.8	202-3	(A)	$C_{14}H_{12}N_4OS_2$
3d	CH_3	C_2H_5	66	170	(A)	$C_{15}H_{14}N_4OS_2$
3e	C_6H_5	C_2H_5	51	188	(A)	$C_{20}H_{16}N_4OS_2$
3f	H	$CH_2\ C_6H_5$	50.3	206-8	(A)	$C_{19}H_{14}N_4OS_2$
3g	CH_3	$CH_2\ C_6H_5$	76.5	200-2	(A)	$C_{20}H_{16}N_4OS_2$
3h	C_6H_5	$CH_2\ C_6H_5$	48	148-50	(A)	$C_{25}H_{18}N_4OS_2$
4a	H	CH_3	68.2	252	(A)	$C_{13}H_{10}N_4O_3S_2$
4b	C_6H_5	CH_3	82.5	268-70	(A)	$C_{19}H_{14}N_4O_3S_2$
4c	H	C_2H_5	69	240	(A)	$C_{14}H_{12}N_4O_3S_2$
4d	CH_3	C_2H_5	71.5	220	(A)	$C_{15}H_{14}N_4O_3S_2$
4e	C_6H_5	C_2H_5	83.3	260-2	(B)	$C_{20}H_{16}N_4O_3S_2$
4f	H	$CH_2C_6H_5$	68.3	214	(A)	$C_{19}H_{14}N_4O_3S_2$
4g	CH_3	$CH_2C_6H_5$	75.5	240	(A)	$C_{20}H_{16}N_4O_3S_2$
4h	C_6H_5	$CH_2C_6H_5$	84.1	250	(B)	$C_{25}H_{18}N_4O_3S_2$

Crystallization Solvent: A = Ethanol; B = Ethanol/Chloroform.

Table 2: Physical data, yields and crystallization solvents of the new compounds (7a-f and 8a-f)

Comp. No.	R	R^1	Yield %	M.P.°C	Cryst. Solv.	Mol.Formula
7a	CH_3	CH_3	75.7	164-6	(A)	$C_{13}H_{11}N_3OS$
7b	C_6H_5	CH_3	71.7	150-2	(B)	$C_{18}H_{13}N_3OS$
7c	CH_3	C_2H_5	71.8	80-2	(A)	$C_{14}H_{13}N_3OS$
7d	C_6H_5	C_2H_5	68.8	90-2	(C)	$C_{19}H_{15}N_3OS$
7e	CH_3	$CH_2C_6H_5$	73.2	148-9	(A)	$C_{19}H_{15}N_3OS$
7f	C_6H_5	$CH_2C_6H_5$	77.2	138-40	(A)	$C_{24}H_{17}N_3OS$
8a	CH_3	CH_3	77.4	158-9	(A)	$C_{13}H_{11}N_3O_3S$
8b	C_6H_5	CH_3	79.5	204-5	(B)	$C_{18}H_{13}N_3O_3S$
8c	CH_3	C_2H_5	73.3	146-8	(A)	$C_{14}H_{13}N_3O_3S$
8d	C_6H_5	C_2H_5	76.9	160-1	(A)	$C_{19}H_{15}N_3O_3S$
8e	CH_3	$CH_2C_6H_5$	80.5	128-9	(A)	$C_{19}H_{15}N_3O_3S$
8f	C_6H_5	$CH_2C_6H_5$	83.3	218-20	(B)	$C_{24}H_{17}N_3O_3S$

Crystallization Solvent: A=Ethanol; B=Ethanol/Chloroform; C=Aqueous ethanol.

Experimental

Melting points were determined in open glass capillaries on a Gallen Kamp melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded, for KBr discs, on a Perkin-Elmer 1430 Infrared spectrophotometer. ^{1}H-NMR spectra were determined on a JNM-LA 400 FTNMR system (400MHz), or Jeol (500MHz), or Brucker Avance spectrometer (300MHz), and are reported as values (ppm) relative to tetramethylsilane (TMS) as internal standard. ^{13}C-NMR spectra were recorded on Jeol spectrometer (500MHz). Elemental analyses were performed at the micro-

analytical unit, Faculty of Science, Cairo university, the microanalytical unit, Faculty of Science, Assuit University or National Research Center, Cairo.

Synthesis – Method A

2-Substituted N-(5-substituted thio-1,3,4-thiadiazol-2-yl) quinoline-4-carboxamides (3a-h). A solution of the appropriate 2-amino-5-substituted thio-1,3,4-thiadiazoles **2a-c** (5 mmole) in THF (6 ml) was added to a stirred mixture of an equimolar amounts of the appropriate cinchoninic acid **1a-c** and DCC (1.13 g; 5.5 mmole) in THF (6 ml). The stirred reaction mixture was heated under reflux for 20 hours and then left overnight. The precipitate formed was filtered off and washed with THF (3x5 ml). The combined filtrates were concentrated and the separated product was filtered, dried and crystallized from the proper solvent (Table 1). ^{1}H-NMR (DMSO-d_6), **3a**: δ 2.74 (s, 3H, S – CH_3); 7.69 (dd, 1H, J_1= 6.85 Hz, J_2= 1.55 Hz, quinolyl – C_6 – H); 7.82 - 7.85 (m, 2H, quinolyl – $C_{5\ \&\ 7}$ – H); 8.1 - 8.14 (m, 2H, quinolyl– $C_{3\ \&\ 8}$ – H), 9.05 (d, 1H, J= 4.6 Hz, quinolyl – C_2– H); 13.53 (s, 1H, NH, D_2O-exchangeable). ^{1}H-NMR (DMSO-d_6), **3e**: δ 1.35 (t, 3H, J = 7.65 Hz, CH_2-$\underline{CH_3}$); 3.26 (q, 2H, J = 7.65 Hz, $\underline{CH_2}$-CH_3); 7.49 – 7.58 (m, 3H, Ar – $C_{3,4\&5}$–H); 7.65 (t, 1H, J = 7.65 Hz, quinolyl–C_6–H); 7.83 (t, 1H, J = 7.95 Hz, quinolyl–C_7–H); 8.15 (d, 1H, J = 8.4 Hz, quinolyl–C_5–H); 8.21 (d, 1H, J = 8.4 Hz, quinolyl–C_8–H); 8.33 (d, 2H, J =7.65 Hz, Ar –$C_{2\&6}$–H); 8.48 (s, 1H, quinolyl– C_3–H); 13.6 (s, 1H, NH, D_2O exchangeable). ^{13}C -NMR (DMSO – d_6), **3a:** δ 16.56 (S – CH_3); 120.93, 124.24, 125.56, 128.56, 130.11, 130.71, 138.88, 148.42, 150.72 (quinoline C– 3, 6, 5, 4a, 7, 8, 4, 8a, 2 respectively); 158.88, 162.02 (thiadiazole C– 5,2 respectively) and 165.87 (C=O).

2-Substituted N-(5-substituted sulfonyl–1,3,4-thiadiazol-2-yl)quinoline-4-carboxamides (4a-h). An aqueous solution of 4% potassium permanganate was added dropwise to a stirred solution of the appropriate **3a-h** (0.5 mmole) in glacial acetic acid (10 ml). The addition of potassium permanganate was continued till the purple color persisted and stirring was maintained for further two hours. The reaction mixture was cooled to 5 °C and saturated sodium sulfite solution was added until the brown color disappeared. The white precipitate formed was filtered, washed with

water, dried and crystallized from the proper solvent (Table 1). ^{1}H-NMR (DMSO-d_6), **4c**: δ 1.26 (t, 3H, J = 7.65Hz, CH_2-$\underline{CH_3}$); 3.68 (q, 2H, J =7.65 Hz, $\underline{CH_2}$-CH_3); 7.71 (t, 1H, J = 8.4 Hz, quinolyl–C_6–H); 7.84 – 7.88 (m, 2H, quinolyl–$C_{5\&7}$–H); 8.13 (d, 1H, J = 8.45 Hz, quinolyl–C_3–H); 8.19 (d, 1H, J = 8.4 Hz, quinolyl–C_8–H); 9.08 (d, 1H, J = 3.8 Hz, quinolyl–C_2–H); 14.19 (s, 1H, NH, D_2O-exchangeable). ^{1}H-NMR (DMSO-d_6), **4h**: δ 5.11 (s, 2H, SO_2 – CH_2); 7.32 - 7.37 (m, 5H, benzyl – H); 7.52 - 7.60 (m, 3H, Ar – $C_{3,4\&5}$ – H); 7.68 (t, 1H, J = 7.65 Hz, quinolyl – C_6 –H); 7.85 (t, 1H, J = 7.65 Hz, quinolyl – C_7 –H); 8.17 and 8.22 (two d, each 1H, J = 7.65 Hz, quinolyl-$C_{2\&6}$-H); 8.34 (d, 2H, J = 7.65 Hz, Ar-$C_{2\&6}$-H); 8.55 (s, 1H, quinolyl-C_3-H); 14.19 (s, 1H, NH, D_2O - exchangeable).

2-Methyl (phenyl) 4-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)quinolines (6a,b)

To a cold stirred solution of the selected cinchoninic acid hydrazide **5a,b** (4.02 g, 20 mmole) in ethanol (20 ml) containing potassium hydroxide (1.12 g, 20 mmol), carbon disulfide (6 ml, 10 mmole) was gradually added. The reaction mixture was heated under reflux until hydrogen sulfide evolution ceased. Ethanol was removed under vacuum , the residue was stirred with water (40 ml), filtered and the filtrate was acidified with 10% HCl to pH 6. The precipitate was collected by filtration, washed with water and crystallized from ethanol. For compound **6a**, yield 4.15 g (82.3%); m.p. 304-6°C. Analysis ($C_{12}H_9N_3OS$. $^1/_2$ H_2O) : C,H,N. ^{1}H-NMR (DMSO-d_6), **6a**: δ 2.77 (s, 3H, CH_3); 3.42 (s, 1H, NH, under DMSO-d_6); 7.75 (t, 1H, J = 6.9 Hz, quinolyl – C_6 – H); 7.87 (t, 1H, J= 6.9Hz, quinolyl – C_7 – H); 7.98 (s, 1H, quinolyl – C_3 – H); 8.1 (d, 1H, J= 8.4 Hz, quinolyl – C_5 – H); 8.8 (d, 1H, J= 8.4 Hz, quinolyl – C_8 – H). For compound **6b**, yield 4.9 g (80%); m.p. 236.8°C (reported 233-5) [24].

2-Substituted 4-(5-substituted thio-1,3,4-oxadiazol-2-yl) quinolines (7a-f).

To a stirred solution containing the appropriate oxadiazolylquinoline **6a,b** (0.6 mmole) and potassium hydroxide (0.03g, 0.6 mmole) in 50% aqueous ethanol (10 ml), a solution of the proper alkyl or aralkyl halide (0.6 mmole) in ethanol (3ml) was added drop wise. The reaction mixture was stirred for additional two hours at room temperature. The precipitate formed was filtered, washed with water and crystallized from the suitable solvent (Table 2). ^{1}H-NMR (DMSO-d_6), **7a**: δ 2.71 (s, 3H, CH_3);

2.81 (s, 3H, SCH_3); 7.69 (t, 1H, J= 7.6Hz, quinolyl – C_6 – H); 7.81 (t, 1H, J= 7.6Hz, quinolyl – C_7 – H); 7.93 (s, 1H,quinolyl – C_3 – H); 8.01 (d, 1H, J= 8.4 Hz, quinolyl – C_5 – H); 8.95 (d, 1H, J= 8.4 Hz, quinolyl – C_8 – H). ^{1}H-NMR (DMSO-d_6), **7d**: δ 1.45 (t, 3H, J = 6.9 Hz, CH_2–$\underline{CH_3}$); 3.38 (q, 2H, J= 6.9 Hz, $\underline{CH_2}$–CH_3); 7.51 - 7.56 (m, 3H, Ar – $C_{3,4\&5}$–H); 7.73 (t, 1H, J= 7.65 Hz, quinolyl – C_6–H); 7.86 (t, 1H, J= 8.4 Hz, quinolyl – C_7–H); 8.14 (d, 1H, J= 8.4 Hz, quinolyl – C_5–H); 8.27 (d, 2H, J= 6.85Hz, Ar–$C_{2\&6}$–H); 8.46 (s, 1H, quinolyl – C_3–H); 8.97 (d, 1H, J = 8.4 Hz, quinolyl – C_8–H). ^{1}H-NMR (DMSO-d_6), **7f**: δ 4.69 (s, 2H, S–$\underline{CH_2}$-C_6H_5); 7.27 - 7.37 (m, 3H, Ar – $C_{3,4\&5}$–H); 7.54 - 7.62 (m, 5H, CH_2-$\underline{C_6H_5}$); 7.77 (t, 1H, J = 7.6 Hz, quinolyl – C_6–H); 7.90 (t, 1H, J = 7.6 Hz, quinolyl –C_7–H); 8.2 (d, 1H, J= 8.56 Hz, quinolyl – C_5–H); 8.3 (d, 2H, J= 7.32 Hz, Ar – $C_{2\&6}$ –H); 8.5 (s, 1H, quinolyl – C_3 –H); 9.0 (d, 1H, J = 8.32 Hz, quinolyl – C_8 –H). ^{13}C-NMR (DMSO – d_6), **7d:** δ 15.38 (CH_3); 27.25 (S–CH_2); 118.70, 122.45, 126.1, 129.52, 130.55, 130.67, 138.17, 148.82, 156.33 (quinoline C–3, 6, 5, 4a, 7, 8, 4, 8a, 2 respectively); 127.83, 128.73, 128.90, 131.24 (phenyl C–2/6, 3/5, 4, 1 respectively); 164.07, 165.65 (oxadiazole C–5, 2 respectively).

2-Substituted 4-(5-substituted sulfonyl-1,3,4-oxadiazol-2-yl) quinolines (8b,d,e,f).

Method A: The title compounds were prepared following the same procedure for preparation of compounds **4a-h** (Table 2). ^{1}H-NMR (DMSO-d_6), **8d**: δ 1.39 (t, 3H, J = 7.32 Hz, CH_2 –$\underline{CH_3}$); 3.92 (q, 2H, J= 7.32 Hz, $\underline{CH_2}$ –CH_3); 7.55 - 7.63 (m, 3H, Ar – $C_{3,4\&5}$ –H); 7.83 (t, 1H, J = 8.28 Hz, quinolyl – C_6 –H); 7.95 (t, 1H, J = 8.28Hz, quinolyl – C_7 –H); 8.24 (d, 1H, J = 8.56 Hz, quinolyl – C_5 –H); 8.33 (d, 2H, J = 7.84 Hz, Ar – $C_{2\&6}$ –H); 8.65 (s, 1H, quinolyl – C_3 –H); 8.96 (d, 1H, J = 8.56Hz, quinolyl – C_8 –H). ^{1}H-NMR (DMSO-d_6), **8f** : δ 5.35 (s, 2H, $\underline{CH_2}$-C_6H_5); 7.40 - 7.45 (m, 5H, CH_2-$\underline{C_6H_5}$); 7.56 - 7.65 (m, 3H, Ar –$C_{3,4\&5}$–H); 7.83 (t, 1H, J = 8 Hz, quinolyl – C_6 –H); 7.96 (t, 1H, J= 8Hz, quinolyl – C_7 –H); 8.25 (d, 1H, J = 8.5Hz, quinolyl – C_5 – H); 8.3 (d, 2H, J = 7.1 Hz, Ar – $C_{2\&6}$–H); 8.51 (s, 1H, quinolyl – C_3 –H); 8.86 (d, 1H, J = 8.5 Hz, quinolyl – C_8 –H).

Method B: (For compounds 8a,c) an aqueous solution of 2% potassium permanganate was added dropwise to an ice cold stirred solution of **7a** or **7c** (0.5 mmole) in 5ml glacial acetic acid. Addition of potassium permanganate was carried out over a period of 4 hours till the purple color persisted. Cooling was maintained during this period, and then continued as mentioned for preparation of compounds **4a-h** (Table 2). ^{1}H-NMR (DMSO-d_6), **8c**: δ 1.04 (t, 3H, J = 7.65Hz, CH_2 – $\underline{CH_3}$), 2.4 (q, 2H, J = 7.65Hz, $\underline{CH_2}$-CH_3); 2.8 (s, 3H, CH_3); 7.79 (t, 1H, J = 7.65Hz, quinolyl– C_6– H); 7.93 (t, 1H, J = 7.65Hz, quinolyl– C_7– H); 7.98 (s, 1H, quinolyl– C_3– H); 8.09 (d, 1H, J = 8.4Hz, quinolyl– C_5– H); 8.89 (d, 1H, J = 8.4Hz, quinolyl– C_8– H).

2-Methyl-4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)quinoline (9).

The title compound was obtained by oxidation of **7a** or **7c** (0.5 mmole) following the same procedure applied for preparation of compounds **4a-h**. The product was crystallized from ethanol. Yield 0.09g (69.2%); m.p. 278-80°C. Analysis ($C_{12}H_9N_3O_2$): C, H, N. ^{1}H-NMR (DMSO-d_6), δ 2.68 (s, 3H, CH_3); 3.35 (s, 1H, NH, under DMSO-d_6); 7.64 (t, 1H, J = 7.65 Hz, quinolyl – C_6 – H); 7.73 (s, 1H, quinolyl – C_3 –H); 7.77 (t, 1H, J = 7.65Hz, quinolyl –C_7 –H); 7.98 (d, 1H, J = 8.4Hz, quinolyl – C_5 –H); 8.75 (d, 1H, J = 8.4Hz, quinolyl –C_8 –H).

Antimicrobial

1. Inhibition zone measurement:

The compounds were dissolved in DMF in a concentration of 1 mg/ml. Sterile nutrient agar was inoculated with the test organisms. Each 100 ml of the medium received 1 ml of 24 hours broth culture. Then the seeded agar was poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar. Each cup received 0.1 ml of the test compound solution (1 mg/ml). The plates were then incubated at 37°C for 24 hours. The resulting inhibition zones are recorded (Table 3).

Table 3: The inhibition zones (IZ) in mm diameter

Compound No.	S. aureus	E. coli	P. aeruginosa	C. albicans	
3a	17	18	15	19	(-): No
3b	15	16	20	18	inhibition
3c	15	17	14	17	zones
3d	17	18	14	18	
3e	15	19	20	18	
3f	15	18	17	19	
3g	15	18	17	24	
3h	13	16	15	18	
4a	13	18	13	19	
4b	30	18	15	19	
4c	20	19	20	19	
4d	13	17	13	18	
4e	31	19	15	19	
4f	27	18	20	19	
4g	28	19	13	19	
4h	20	19	14	18	
6a	20	18	12	18	
7a	17	17	13	26	
7b	12	18	13	18	
7c	15	19	-	19	
7d	-	18	15	19	
7e	13	18	-	19	
7f	13	18	-	18	
8a	19	18	13	27	
8b	20	18	15	18	
8c	16	19	16	24	
8d	18	17	13	18	
8e	16	18	-	19	
8f	12	18	-	19	
Cipfrofloxacin	25	30	40	-	
Nystatin	-	-	-	37	

2. Minimal inhibitory concentration (MIC) measurement [25]:

The test organisms were grown in their suitable broth for 24 hours for bacteria and 48 hours for fungi at 37°C. Two fold serial dilutions of the test compounds solution were prepared using the suitable broth to obtain concentrations 500, 250, 125, 62.5, 31.25 and 15.62 µg/ml with the concentration of dimethylformamide not exceeding 2.5 %. The tubes were then inoculated with the test organisms; each 5 ml received 0.1 ml of the above inoculum and were incubated at 37°C for 48 hours. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in Table 4.

Table 4: MIC and MBC in µg/ml of the most active compounds:

Compound	**S.aureus**		**P.aeruginosa**		**C.albicans**	
No.	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
3b			250	>500		
3e			62.5	500		
3g					250	250
4b	31.25	31.25				
4c	125	>500	125	125		
4e	125	125				
4f	125	500	62.5	>500		
4g	62.5	>500				
4h	500	500				
6a	62.5	>500				
7a					125	125
8a					31.25	125
8c					125	125
Ciprofloxacin	2	-	4	-	-	-
Nystatin	-	-	-	-	25	-

3. Minimum bactericidal concentration (MBC) measurement [26]:

MIC tests were always extended to measure the MBC as follows:

A loopful from the tube not showing visible growth (MIC) was spread over a quarter of Muller-Hinton agar plate. After an overnight incubation (18 h), the plates were examined for growth. Again the tube containing the lowest concentration of the test compound that failed to yield growth on subculture plates was judged to contain the MBC of that compound for the respective test organism (Table 4).

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