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Design, practical synthesis and biological evaluation of novel 1,3,4-oxadiazole derivatives incorporated with quinolone moiety as microbial agents

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ABSTRACT

A series of novel 1,3,4-oxadiazole derivatives incorporated with quinolone moiety were designed and synthesized as biological compounds. In vitro antibacterial activity of the synthesized compounds ***(5a-g)*** *was performed against Gram +ve microorganisms Staphylococcus aureus (MTCC No. 96), Pseudomonas aeruginosa (MTCC No. 1688), Bacillus subtillis (MTCC No. 121) and Gram -ve microorganisms Escherichia coli (MTCC No. 521). In vitro* antifungal activity of compounds was screened against *Candida albicans.* The *structure of all new compounds was confirmed by IR, [1]H-NMR and Mass spectra.*

Key words: 1,3,4-oxadiazole, 4-oxoquinoline, antibacterial and antifungal activity.

INTRODUCTION

The major drawback of current treatment of infectious diseases are challenging due to resistance to antimicrobial agents and their side effects. In order to overcome this situation, it is necessary to continue the search for new antibacterial agents. In recent scenario heterocycles plays a major role in drug synthesis. In that respect oxadiazole and quinolone derivatives play a significant role among other heterocycles. From the literature survey oxadiazole was found to be having diverse activity such as anti-inflammatory, antimicrobial, antifungal, antiviral, analgesic, anti-mycobacterial, antidepressant and anticancer [1-3]. Quinolone derivatives exhibit anti HIV [4], antitubercular [5], antiplatelet [6, 7], antichlamydial [8] and antitumor activity [9-13] along with oxadiazole activities. The incorporation of 1,3,4-oxodiazole in 4-oxoquinoline frame work through N-CH_2 as bridging group was used as the target for chemical modification. Looking at the importance of these compounds, the present work aims to synthesise and screen the antibacterial and antifungal activity of 5-phenyl 1,3,4-oxadiazole-2-yl derivatives of different substituted 4-oxoquinoline nuclia.

MATERIALS AND METHODS

Melting points of the newly synthesized compounds was determined by open capillary method and are uncorrected. Purity of the compounds was determined by TLC on silica gel coated plates obtained from Merck as stationary phase and solvent mixture of n-hexane : ethyl acetate (3:7) was used as mobile phase at room temperature. IR spectra were recorded as KBr pellet on a Perkin Elmer - FTIR specrum-100. ^{1}H-NMR spectrum was recorded on

Bruker DRX 300 using $CDCl_3$ as a solvent and Mass spectra were recorded on Shimadzu GCMSQP5050A, Japan, DB-1 glass column 30, 0.25 mm, ionization energy 70 eV.

General procedure for the synthesis

Preparation of 8-chloro-1,4-dihydro-4-oxoquinoline-3-carbonitrile (2a)

1.99gm of ethyl-2-cyano-3-ethoxy acrylate (0.0117 mol) was added to 1gm of 2-chloro aniline (0.0078 moles) dissolved in diphenylether. The reaction mixture was maintained at reflux temperature for 12 hours. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled, poured on to ice water and then extracted with ethyl acetate. The organic layer was collected, dried over anhydrous sodiumsulphate and evaporated to get light brown color solid product. Yield: 80%, m.p. 2a:158-160, 2b:201-203, 2c:155-158, 2d:162-165, 2e:180-182, 2f:176-178 and 2g:161-163°C.

IR (KBr) cm^{-1}: 3090(Ar C-H str), 3310(N-H str), 2215(C≡N str), 1705(C=O str), 1627(C=C str).

Preparation of ethyl 2-(8-chloro-3-cyano-4-oxoquinolin-1(4H)-yl) acetate (3a)

0.0024 mol of triethylamine was added to 250 mg of 8-chloro-1,4-dihydro-4-oxoquinoline-3-carbonitrile (0.0012 mol) formed in the first step at 15^0C and maintained for 15 min at the same temperature. Then 0.18 ml of ethylchloroacetate (0.0024 moles) was added and refluxed for 2 hrs. The reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled, diluted with water and extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulphate and then evaporated. Yield: 75%, m.p. 3a:175-177, 3b:218-220, 3c:173-176, 3d:160-161, 3e:195-198, 3f:183-185 and 3g:168-170°C.

IR (KBr): 3100(Ar C-H str), 2995(Ali C-H str), 2221(C≡N str), 1705(C=O str), 1627(C=C str), 1210(C-O str).

Preparation of 2-(8-chloro-3-cyano-4-oxoquinolin-1(4H)-yl) acetohydrazide (4a)

Ethyl 2-(8-chloro-3-cyano-4-oxoquinolin-1(4H)-yl) acetate (0.0175 mole) and hydrazine hydrate (0.0525 mole) were dissolved in ethanol. The contents of the reaction was maintained at reflux temperature for 6 hrs. The completion of the reaction was monitored by TLC by using chloroform: methanol (9:1) as an eluent and observed under UV light. After completion of the reaction, the reaction mixture was cooled and the solid product obtained was collected by filtration. It was recrystallized from ethanol. Yield: 85%, m.p: 4a:182-185, 4b:206-208, 4c:165-168, 4d:176-178, 4e:208-210, 4f:205-207 and 4g:196-198°C.

IR(KBr): 3250(N-H str), 3090(Ar C-H str), 2230(C≡N str), 1700(C=O str), 1627(C=C str), 1514(N-N str).

Table-1: Physicochemical parameters of the synthesized compounds (5a-g)

compound	(R)	Mol.formula	[a]M.P (°C)	Mol.wt.	[b]R_f	Elemental analysis % calculated		
						C	H	N
5a	8-Cl	$C_{19}H_{11}ClN_4O_2$	255	362	0.60	62.80 (62.91)	2.98 (3.06)	15.32 (15.44)
5b	7-NO_2	$C_{19}H_{11}N_5O_4$	264	373	0.54	61.00 (61.13)	2.90 (2.97)	18.62 (18.76)
5c	7-CH_3	$C_{20}H_{14}N_4O_2$	231	342	0.60	70.04 (70.17)	4.23 (4.12)	16.24 (16.37)
5d	7-Br	$C_{19}H_{11}BrN_4O_2$	199	406	0.61	55.90 (56.04)	2.63 (2.72)	13.59 (13.76)
5e	8-OCH_3	$C_{20}H_{14}N_4O_3$	261	358	0.68	66.85 (67.03)	3.83 (3.94)	15.54 (15.63)
5f	7,8-Di chloro	$C_{19}H_{10}Cl_2N_4O_2$	230	396	0.74	57.37 (57.45)	2.43 (2.54)	13.92 (14.10)
5g	8-C_2H_5	$C_{21}H_{16}N_4O_2$	226	356	0.79	70.65 (70.77)	4.37 (4.53)	15.54 (15.72)

[a]Solvent of crysllization-Ethanol

[b]Solvent system- Ethyl acetate: n-Hexane (7:3)

Preparation of 8-chloro-1,4-dihydro-4-oxo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl) quinoline-3-carbonitrile (5a)

A mixture of 0.5 g of 2-(8-chloro-3-cyano-4-oxoquinolin-1(4H)-yl) acetohydrazide (0.0019 mol) formed in the third step and 0.35g (0.0029 mol) of benzoic acid was dissolved in 4 ml of phosphorous oxy chloride and refluxed for 20 h. The completion of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was

cooled to the room temperature and slowly poured on to crushed ice and kept overnight. The solid mass thus separated out was filtered and recrystallized from ethanol. Yield: 70%, m.p: 255-256°C.

All the compounds 2b-2g, 3b-3g, 4b-4g and 5b-5g were prepared by similar method. Compounds 5a-5g were recrystallized from alcohol. Physicochemical properties of all compounds are shown in **Table-1**.

Spectral data:

8-chloro-1,4-dihydro-4-oxo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)quinoline-3-carbonitrile (5a)
IR(KBr) υ_{max} cm^{-1}: 3010(Ar C-H str), 2120(C≡N str), 1719(C=O str), 1615(C=N str), 1140(C-O-C str), 735(C-Cl str).; **Mass m/z (M^+)** 362; **^{1}HNMR(300MHz,$CDCl_3$)δ:** 4.30(s, 2H, N-CH_2), 6.75, 7.56, 7.63(m, 4H, Quinoline-H), 7.20, 7.36, 7.43(m, 5H, Aromatic-H).

7-nitro-1,4-dihydro -4-oxo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)quinoline-3-carbonitrile(5b)
IR(KBr) υ_{max} cm^{-1}: 3090(Ar C-H str), 2120(C≡N str), 2060(NO_2 str), 1715(C=O str), 1621(C=N str), 1120(C-O-C str).; **Mass m/z (M^+)** 373; **^{1}HNMR(300MHz,$CDCl_3$)δ:** 4.30(s, 2H, N-CH_2), 7.10, 7.64, 7.76(m, 4H, Quinoline-H), 7.20, 7.35, 7.42(m, 5H, Aromatic-H).

7-methyl-1,4-dihydro -4-oxo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)quinoline-3-carbonitrile (5c)
IR(KBr) υ_{max} cm^{-1}: 3095(Ar C-H str), 2987(C-H str CH_3), 2115(C≡N str), 1715(C=O str), 1621 (C=N str), 1125(C-O-C str).; **Mass m/z (M^+)** 342; **^{1}HNMR(300MHz,$CDCl_3$)δ:** 4.35(s, 2H, N-CH_2), 2.32(s, 3H, CH_3), 6.54, 7.68, 7.76(m, 4H, Quinoline-H), 7.21, 7.34, 7.41(m, 5H, Aromatic-H).

7-bromo-1,4-dihydro-4-oxo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)quinoline-3-carbonitrile (5d)
IR(KBr) υ_{max} cm^{-1}: 3095(Ar C-H str), 2115(C≡N str), 1715(C=O str), 1621(C=N str), 1145(C-O-C str), 559(C-Br str); **Mass m/z (M^+)** 406; **^{1}HNMR(300MHz,$CDCl_3$)δ:** 4.33(s,2H, N-CH_2), 6.75, 7.52, 7.78(m, 4H, Quinoline-H), 7.20, 7.35, 7.42(m, 5H, Aromatic-H).

8-methoxy-1,4-dihydro-4-oxo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)quinoline-3-carbonitrile (5e)
IR(KBr) υ_{max} cm^{-1}: 3095(Ar C-H str), 2115(C≡N str), 1715(C=O str), 1621(C=N str), 1154(C-O-C str). ; **Mass m/z (M^+)** 358; **^{1}HNMR(300MHz,$CDCl_3$)δ:** 4.33(s, 2H, N-CH_2), 3.60(s, 3H, OCH_3), 6.60, 7.62, 7.72(m, 4H, Quinoline-H), 7.21, 7.32, 7.40(m, 5H, Aromatic-H).

7,8-dichloro-1,4-dihydro-4-oxo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)quinoline-3-carbonitrile (5f)
IR(KBr) υ_{max} cm^{-1}: 3010(Ar C-H str), 2124(C≡N str), 1719(C=O str), 1632(C=N str), 1140(C-O-C str), 772(C-Cl str).; **Mass m/z (M^+)** 396; **^{1}HNMR(300MHz,$CDCl_3$)δ:** 4.30(s,2H, N-CH_2), 6.60, 7.56, 7.68(m, 3H, Quinoline-H), 7.20, 7.36, 7.43(m, 5H, Aromatic-H).

8-ethyl-1,4-dihydro-4-oxo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)quinoline-3-carbonitrile (5g)
IR(KBr) υ_{max} cm^{-1}: 3095(Ar C-H str), 2987(C-H str CH_3), 2115(C≡N str), 1715(C=O str), 1621 (C=N str), 1135(C-O-C str).; **Mass m/z (M^+)** 356; **^{1}HNMR(300MHz,$CDCl_3$)δ:** 4.35(s,2H, N-CH_2), 1.26(t, 3H, CH_3), 2.64(q, 2H, CH_2), 6.54, 7.60, 7.70(m, 4H, Quinoline-H), 7.22, 7.35, 7.42(m, 5H, Aromatic-H).

Antibacterial Activity

The compounds 5a-g were screened for their antibacterial activity against four different strains, viz three gram positive bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtillis)* and one gram negative bacteria (*Escherichia coli*) at 250 & 500 µg/l concentrations by filter paper disc method [14,15]. Standard antibacterial drugs Ciprofloxacin, Ampicillin were also tested under the similar conditions for comparison. Antifungal activity was carried out against *C.albicans* and the results were compared with the standard drugs Ketoconazole, Fluconazole by the same method. The results of the antimicrobial screening reveal that some of the synthesized compounds exhibit good antibacterial and antifungal activities. The antimicrobial screening results are presented in Tables 2 and 3.

Table 2: Antimicrobial screening of compounds 5a-g

Compound code	Concentration µg/ml	Zone of inhibition (mm)			
		Gram +ve			Gram -ve
		Staphylococcus aureus	*Pseudomonas aeruginosa*	*Bacillus subtillis*	*Escherichia coli*
5a	250	10	10	12	08
	500	12	12	15	10
5b	250	11	11	14	07
	500	12	12	17	09
5c	250	07	13	08	06
	500	09	15	09	07
5d	250	11	11	14	09
	500	12	12	16	10
5e	250	08	14	08	06
	500	09	15	09	08
5f	250	11	12	13	10
	500	12	13	14	12
5g	250	11	07	07	07
	500	12	08	08	08
Ciprofloxacin	250	29	30	28	28
	500	34	32	30	31
Ampicillin	250	23	25	21	25
	500	26	28	23	27

Table 3: Antifungal screening of compounds 5a-g

Compound code	Concentration µg/ml	Zone of inhibition (mm)
		C.albicans
5a	250	08
	500	12
5b	250	15
	500	17
5c	250	10
	500	12
5d	250	09
	500	12
5e	250	09
	500	11
5f	250	11
	500	12
5g	250	08
	500	11
Ketoconazole	250	26
	500	30
Fluconazole	250	23
	500	25

RESULTS AND DISCUSSION

The antimicrobial screening revealed that all the compounds tested showed moderate to good antibacterial and antifungal activities against tested pathogenic strains. From the result it was found that compounds **5a, 5b, 5d** and **5f** showed maximum antibacterial activity against *P.aeruginosa.* While compounds **5c** and **5e** have maximum activity against *B. subtilis* and compound **5g** has maximum activity against *S. aureus.* Compound **5f** has maximum activity against Gram Negative bacteria i.e. *Escherichia coli.* Antibacterial activity was compared with ampicillin & ciprofloxacin. The data of bacterial activity is presented in **Table-2.** Antifungal screening data showed that compound **5b** exhibit maximum activity against *C. albicans.* The remaining compounds of the entire series possess moderate to good activity. The antifungal activity was compared with fluconazole and ketoconazole. The data of antifungal activity is presented in **Table 3.** All compounds were less potent than standard drugs ampicillin, ciprofloxacin, fluconazole and ketoconazole.

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