Synthesis and evaluation of some novel precursors of oxazolidinone analogues of chloroquinoline for their antimicrobial and cytotoxic potential

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Abstract. Some 3-(3-(7-chloroquinolin-4-ylamino)propyl-2-imino-5-(4-chloro/nitro/methoxy benzyldene) oxazolidin-4-one **4(a–c)** and 4-(3-(7-chloroquinolin-4-ylamino) propyl)-2(4-chloro/nitro/methoxy benzyldene)-1,6-diox-4,9 diazaspiro[4,4]nonane-3,8-dione **5(a–c)** derivatives were synthesized using appropriate synthetic route. The newly prepared compounds **5a–c** demonstrated inhibitory effects on the growth of a MCF7 (hormone-dependant breast carcinoma cell line), HT29 (colon carcinoma cell line on leukemia). The MCF7 cell line was found to be very susceptible towards compound **5a** with IC₅₀ values of 16 µg/ml. Similarly, the HT29 cell line was found to be moderately susceptible towards compounds **5a** and **5c** with IC 50 values of 32 and 49 µg/ml, respectively.

Keywords. Synthesis; 4-7-dichloroquinoline; 1-3-diaminopropane; oxazolidinone; antimicrobial activity and cytotoxicity study.

1. Introduction

Heterocyclic chemistry has great importance to the medicinal chemists because large numbers of heterocyclic compounds are being used as therapeutic agents and these compounds are also essential for the human life as oxazolidinones. Oxazolidinones are a novel class of synthetic antimicrobial agents^{1,2} which have now entered phase III clinical trials. The most important results of extensive studies (synthesis, spectral, structural characterization and applications in various biological systems) of oxazolidinone derivatives are reviewed. A large number of oxazolidinone are known but still there is a need to develop new derivatives in different fields for biomedical applications and it has recently drawn the attention of numerous research groups, making this area constantly evolve. The most promising feature of oxazolidinone is their oral activity against multidrug-resistant³ which has created tremendous therapeutic problems in recent years. In addition, *in vitro* development of resistance has so far remained below detectable levels. Different from many antibacterial agents used in the treatment of human infections,⁴ oxazolidinones do not block bacterial protein synthesis at the level of polypeptide chain

elongation but rather seem to interfere with initiation of translation.⁵ Both binding of formylmethionine-transfer RNA to initiation complexes as well as release of formylmethionine puromycin from initiation complexes have been reported to be targets for oxazolidinones.^{6,7} The major binding sites of oxazolidinones are the large (50S) ribosomal subunits.⁸ Oxazolidinones were first described by Eleuthère Irénée du Pont de Nemours and Co. in 1987 as a novel class of synthetic antimicrobial agents.⁹ The first representative, DuP 721 (figure 1) showed promising activity against multidrug-resistant Gram-positive bacteria¹⁰ a low occurrence of resistance development,^{11–13} anticancer,^{14–16} antifungal,¹⁷ anticonvulsant,¹⁸ antiinflammatory,^{19,20} anti-tuberculosis^{21,22} anti-HIV^{23–25} and antidiabetic.²⁶ The analogues of oxazolidinone, eperezolid (PNU-100592) and linezolid (PNU-100766) (figure 1) were developed by Pharmacia and Upjohn. Linezolid has now entered phase III clinical testing and is used for the treatment of infections caused by both susceptible and antibiotic-resistant Gram-positive bacteria. (Azolylphenyl)oxazolidinones, in which the morpholine ring of linezolid is replaced with five-membered-ring heterocyclics containing nitrogen and sulphur atoms are new developments with promisingly low minimal inhibitory concentrations towards susceptible bacteria.²⁷

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Figure 1. Linezolid.

Quinoline derivatives occur abundantly in natural products as well as in synthetic compounds of biological interest.²⁸ They are known to exhibit antiallergenic properties,²⁹ antifungal activity,³⁰ hypocholesterolemic,³¹ hypovolemia,³² antibacterial³³ and antiviral activity.³⁴ Thienquinoline and benzothienquinolines have been demonstrated to exhibit antitumour and antimicrobial activities.³⁵

On the basis we describe the synthesis of novel oxazolidinone derivatives with a view of verify this assumption that its incorporation of quinoline could really produce an additive affect on the biological properties of the parent molecule. The present study focuses on synthesis (scheme 1), evaluation of antimicrobial and *in vitro* cytotoxicity properties of N1-(7-chloroquinoline-4-yl)propane-1,3-diamine (**1**), 3-(3-(7-chloroquinoline-4-yl-amino) propyl)-2-imino-oxazolidin-4-one (**3**)-(3-(7-chloroquinolin-4-ylamino)propyl-2-imino-5-(4-chlorobenzylidene)oxazolidin-4-one (**4a**) and 4-(3-(7-chloroquinolin-4-ylamino)propyl)-2(4-nitrobenzylidene)-1,6-diox-4,9-diazaspiro[4,4]nonane-3,8-dione (**5b**).

2. Experimental

The entire chemicals were purchased from Aldrich Chemical Company (USA) and were used after purification by distillation. The reactions were monitored by pre-coated aluminium silica gel 60F 254 thin-layer plates procured from Merck (Germany). All melting points were measured by capillary apparatus and are uncorrected. All the compounds were routinely checked by IR, ¹H-NMR and mass spectrometries. IR spectra were recorded in KBr on a Perkin-Elmer model 8201 FTIR spectrophotometer. ¹H-NMR spectra were recorded at ambient temperature using a Bruker spectropscopin DPX-300 MHz spectrophotometer in DMSO. The following abbreviations were used to indicate the peak multiplicity s-singlet, d-doublet, t-triplet, and m-multiplet. FAB mass spectra were recorded on a JEOL SX102 mass spectrometer using Argon/Xenon (6 kV, 10 mB) gas. Column chromatography was performed on silica gel (Merck). Anhydrous sodium sulphate was used as a drying agent for the organic phase.

2.1 Preparation of N-(7-chloroquinoline-4-yl)propane-1, 3-diamine (**1**)

A mixture of 4-7-dichloroquinoline (1.96 gm, 15 mmol) and 1-3-diaminopropane (1.68 ml, 20 mmol) in ethanol was refluxed with stirring for 12 h. It was cooled and distilled the solvent. Solid product was dissolved in DCM (80 ml), washed with 5% of sodium

Scheme 1. Schematic presentation of synthesis of some spirooxazolidinones from 4-7-dichloroquinoline.

bicarbonate and brine solution. The organic layer was filtered. The solid product was purified over the column of silica gel and eluted with ethanol as an eluent to give compound **1** (yield 80%), m.p. 90–92°C. IR (KBr) cm^{-1} 3457, 3225 [NH str.], 2928 [C-H str. Ar. H], 1200 [C-N str.], 1559 [NH str.], 786 [C-Cl str.], 1485 [C-H bend. CH_2]. $^1\text{H-NMR}$ (CDCl_3) δ 8.64(d, 1H, CH), 8.00(s, 1H, CH), 7.61 (d, 1H, CH), 7.43 (d, 1H, CH), 6.49 (d, 1H, CH), 4.12(s, 1H, NH), 2.0 (s, 1H, NH), 1.7 (m, 6H, CH). MS: [m/z] 235.71 (M + 1). Anal. Calcd./found for $\text{C}_{12}\text{H}_{14}\text{ClN}_3$: C, 61.15/61.04; H, 5.99/5.92; Cl, 15.04/15.08; N, 17.83/17.76.

2.2 Preparation of 2-chloro-N-(3-(7-chloroquinoline-4-ylamino) propyl) acetamide (**2**)

Compound N-(7-chloroquinoline-4-yl) propane-1, 3-diamine (**1**) (2.35 g, 10 mmol), triethyl amine (1 ml, 1.01 mmol) and chloroacetyl chloride (0.75 ml, 10 mmol) were dissolved in dry ethanol (25 ml). The mixture was stirred for 30 min on ice bath then refluxed with stirring for 4–6 h. Reaction mixture was cooled and solvent was evaporated. Now, solid product was dissolved in DCM and water. Organic layer was extracted. Solvent was distilled and obtained solid was purified over the column of silica gel and eluted with ethanol to give compound **2** (yield 72%), m.p. 80–82°C. IR (KBr) cm^{-1} 1517, 1490 [NH str.], 3200, 2983 [C-H str. CH_2], 798 [C-Cl str.], 2983 [C-H bend CH_2], 1217 [C-N str.], 1700 [CO str.]. $^1\text{H-NMR}$ (CDCl_3) δ 8.74[s, 1H, NH], 8.29[s, 1H, Ar. CH], 8.06 [d, 1H, Ar. CH], 7.63[d, 1H, Ar. CH], 7.41[d, 1H, Ar. CH], 6.68[d, 1H, Ar. CH], 4.23[s, 1H, NH], 3.82[t, 2H, CH_2], 1.24–1.75[m, 6H, CH_2]. MS: [m/z] 312.19 (M + 1); Anal. Calcd./found for $\text{C}_{14}\text{H}_{15}\text{Cl}_2\text{N}_3\text{O}$: C, 53.86/53.81; H, 4.84/4.76; Cl, 22.71/22.60; N, 13.46/13.32; O, 5.12/5.5

2.3 Preparation of 3-(3-(7-chloroquinoline-4-yl-amino) propyl)-2-imino-oxazolidin-4-one (**3**)

A mixture of 2-chloro-N-(3-(7-chloroquinoline-4-yl-amino) propyl) acetamide (**2**) (1.66 g, 5 mmol) and potassium cyanate (0.58 mg, 7 mmol) was dissolved in dry acetone (10 ml). The reaction mixture was refluxed on steam bath for 4–6 h. The solvent was distilled to yield a solid, which was purified through a column of silica gel and eluted with ethanol to give compound **3** (yield 65%), m.p. 78–80°C. IR (KBr) cm^{-1} 1578 [NH str.], 3124, 3345 [C-H str. Ar. H], 1678 [NH str.], 1308 [C-Cl str.], 2867 [C-H str. CH_2], 1308 [C-N str.], 1704 [C=O]. $^1\text{H-NMR}$

(CDCl_3) δ 11.7 [s, 1H, NH], 8.65 [d, 1H, Ar. CH], 8.56 [s, 1H, NH], 8.10 [d, 1H, Ar. CH], 7.79 [d, 1H, Ar. CH], 6.69 [d, 1H, Ar. CH], 4.14 [s, 2H, CH], 3.97 [t, 2H, CH_2], 1.39–1.32 [m, 4H, CH_2]. MS: [m/z] 318 (M + 1). Anal. Calcd./found for $\text{C}_{15}\text{H}_{15}\text{ClN}_4\text{O}_2$: C, 56.52/56.42; H, 4.74/4.70; Cl, 11.12/11.02; N, 17.78/17.72; O, 10.04/9.

2.4 Preparation of 3-(3-(7-chloroquinolin-4-ylamino) propyl)-2-imino-5-(4-chloro benzylidene) oxazolidin-4-one (**4a**)

A mixture of compound 3-(3-(7-chloroquinoline-4-yl-amino) propyl)-2-imino-oxazolidin-4-one (**3**) (0.30 g, 0.99 mmol) and sodium acetate (0.10 g, 0.001 mmol) was mixed with p-chlorobenzaldehyde (0.127 g, 1.2 mmol) in dry benzene (20 ml). The reaction mixture was refluxed on water bath for 8 h, cooled at room temperature, filtered and the solid was purified by column chromatography to give compound **4a** (yield 68%), m.p. 76–78°C IR (KBr) cm^{-1} 3187, 2856 [CH str. Ar. H], 1705 [C=O], 1674 [C=C str.], 1567 [NH str. (Amine)], 1456 [NH str. (Imine)], 1389 [C-H bend. CH_2], 1277 [C-N str.] and 767, 678 [C-Cl str.]. $^1\text{H-NMR}$ (CDCl_3) δ 9.26[s, 1H, (NH)], .62[dd, 2H, ArH], 8.21[s, 1H, ArH], 7.51[d, 2H, ArH], 7.34[dd, 2H, ArH], 7.24[s, 2H, C=H], 7.22[dd, 1H, ArH], 6.29[d, 1H, ArH], 4.36[s, 1H, (NH)] and 1.83–2.76[m, 6H, CH_2]. MS: [m/z] 440 (M + 1). Anal. Calcd./found for $\text{C}_{22}\text{H}_{18}\text{Cl}_2\text{N}_4\text{O}_2$: C, 59.88/59.82; H, 4.11/4.08; Cl, 16.07/16.01; N, 12.07/12.02; O, 7.25/7.29.

2.5 Preparation of 3-(3-(7-chloroquinolin-4-ylamino) propyl)-2-imino-5-(4-chloro benzylidene) oxazolidin-4-one (**4b**)

A mixture of compound 3-(3-(7-chloroquinoline-4-yl-amino) propyl)-2-imino-oxazolidin-4-one (**3**) (0.37 g, 0.50 mmol) and sodium acetate (0.10 g, 0.001 mmol) was mixed with p-nitrobenzaldehyde (0.134 g, 1.2 mmol) in dry benzene (20 ml). The reaction mixture was refluxed on water bath for 8 h, cooled at room temperature, filtered and the solid was purified by column chromatography to give compound **4b** (1.32 g, yield 56%), m.p. 115–117°C IR (KBr) cm^{-1} 3196, 2945 [CH str. Ar. H], 1710 [C=O], 1586 [C=C str.], 1569 [NH str. (Imine)], 1508 [NH str. (Amine)], 1500 [N-O str.], 1458 [C-H bend. CH_2], 1356 [C-N str.] and 681 [C-Cl str.]. $^1\text{H-NMR}$ (CDCl_3) δ 9.41[s, 1H, (NH)], 8.45[dd, 2H, ArH], 8.14[dd, 1H, ArH], 8.15[s, 1H, ArH], 7.66[d, 1H, ArH], 7.56[dd, 2H, ArH], 7.43[s, 2H, C=H], 6.39[d, 1H, ArH], 4.21[s, 1H, (NH)], 1.78–2.96[m, 6H (CH_2)] MS: [m/z] 451 (M + 1) Anal.

Calcd./found for $C_{22}H_{18}ClN_5O_4$: C, 58.48/58.49; H, 4.02/4.06; Cl, 7.85/7.92; N, 15.50/15.23, O; 14.16/14.10.

2.6 Preparation of 3-(3-(7-chloroquinolin-4-ylamino)propyl)-2-imino-5-(4-methoxy benzylidene)oxazolidin-4-one (**4c**)

Compound 3-(3-(7-chloroquinoline-4-ylamino)propyl)-2-imino-oxazolidin-4-one (**3**) of (0.30 g, 0.13 mmol) and zinc chloride (0.50 mg, 0.003 mmol) was mixed with p-methoxybenzaldehyde (0.127 g, 0.017 mmol) in glacial acetic acid (20 ml). The reaction mixture was refluxed on sand bath at 120°C for 8 h. Cooled, neutralized with $NaHCO_3$. Obtained white solid was poured in the cold water and filtered. The obtained solid was purified by column chromatography to give **4c**. IR (KBr) cm^{-1} 3278, 2876 [CH str. Ar-H], 1705 [C=O], 1678 [C=C str.], 1578 [NH str. (Imine)], 1545 [NH str. (Amine)], 1487 [C-H bend. CH_2], 1329 [C-O (Ester)], 1142 [C-N str.] and 772 [C-Cl str.]. 1H -NMR ($CDCl_3$) δ 8.41[s, 1H, (NH)], 8.62[d, 1H, ArH], 8.12[s, 1H, ArH], 7.69[d, 2H, ArH], 7.43[s, 1H, =CH], 7.19[dd, 1H, ArH], 6.72[dd, 2H, ArH], 6.49[d, 1H, ArH], 4.11[s, 1H, (NH)], 3.73[s, 3H, CH_3], 1.88–2.15[m, 6H (CH_2)]. MS: [m/z] 436 (M + 1) Anal. Calcd./found for $C_{23}H_{21}ClN_4O_3$: C, 64.59/64.72; H, 7.23/7.45; Cl, 7.06/7.12; N, 13.25/13.32; O, 11.01/11.08.

2.7 Preparation of 4-(3-(7-chloroquinolin-4-ylamino)propyl)-2(4-chlorobenzylidene)-1, 6-diox-4, 9-diazaspiro[4,4]nonane-3,8-dione **5(a)**

Compound 3-(3-(7-chloroquinolin-4-ylamino)propyl)-2-imino-5-(4-chloro benzylidene)oxazolidin-4-one (**4a**) (0.20 g, 0.45 mmol) was mixed with glycolic acid (3.37 g, 0.023 mmol) and dissolved in dry benzene (20 ml). The reaction mixture was refluxed on water bath for 6–7 h, cooled at room temperature, washed with $NaHCO_3$ solution and filtered. The organic layer was dried over anhydrous Na_2SO_4 , concentrated and purified by column chromatography. The product was recrystallized with ethanol to give compound **5a** (yield 54%), m.p. 95–97°C IR (KBr) cm^{-1} 1500 [NH str.], 3272, 3049 [C-H str. Ar-H], 763, 680 [C-Cl str.], 1299 [C-N str.], 1454 [C-H bend. CH_2], 1481 [C=C str.], 1735 [C=O]. 1H -NMR ($CDCl_3$) δ 11.5[s, 1H, NH], 4.1[s, 1H, NH], 8.6[d, 3H, Ar.CH], 6.9–7.1[d, 2H, Ar.CH], 7.8[m, 4H, Ar.CH], 3.4[s, 2H, CH_2], 1.5–2.0[m, 6H (CH_2)]. ^{13}C -NMR ($CDCl_3$) δ 151.9 C_1 , 113.0 C_2 , 123.0 C_4 , 126.6 C_5 , 134.5 C_6 , 160.8 C=O, 126.6 C_7 , 38.9 C_{10} , 39.5 C_{11} , 77.5 C_{12} , 38.7 C_{13} , 138.7

C_{16} , 123.2 C_{18} , 127.8 C_{23} , MS: [m/z] 499 (M + 1). Anal. Calcd./found for $C_{24}H_{21}Cl_2N_4O_4$: C, 57.73/57.67; H, 4.04/4.00; Cl, 14.20/14.16; N, 11.22/11.20, O; 12.82/12.70.

2.8 Preparation of 4-(3-(7-chloroquinolin-4-ylamino)propyl)-2(4-nitrobenzylidene)-1, 6-diox-4, 9-diazaspiro[4,4]nonane-3,8-dione **5(b)**

Compound 3-(3-(7-chloroquinolin-4-ylamino)propyl)-2-imino-5-(4-nitro benzylidene)oxazolidin-4-one (**4b**) (3.37 g, 0.12 mmol) was mixed with glycolic acid (3.37 g, 0.023 mmol) and dissolved in dry benzene (20 ml). The reaction mixture was refluxed on water bath for 6–7 h, cooled at room temperature, washed with $NaHCO_3$ solution and filtered. The organic layer was dried over anhydrous Na_2SO_4 , concentrated and purified by column chromatography. The product was recrystallized with ethanol to give compound **5b** (yield 76%), m.p. 129–131°C IR (KBr) cm^{-1} 1512 [NH str.], 3272, 2998 [C-H str. Ar-H], 762, [C-Cl str.], 1290 [C-N str.], 1454 [C-H bend. CH_2], 1600 [C=C str.], 1735 [C=O], 1500 [N-O str.]. 1H -NMR ($CDCl_3$) δ 8.2[d, 3H, Ar.CH], 7.7[d, 2H, Ar.CH], 7.6[m, 4H, Ar.CH], 4.1[s, 1H, NH], 11.5[s, 1H, NH], 3.9[s, 2H, (CH_2)], 1.2–2.0[m, 6H (CH_2)]. ^{13}C -NMR ($CDCl_3$) δ 152.9 C_1 , 104.4 C_2 , 120.5 C_4 , 126.8 C_5 , 127.8 C_6 , 170.7 C=O, 45.6 C_{10} , 40.9 C_{11} , 78.8 C_{12} , 39.9 C_{13} , 149.4 C_{17} , 127.5 C_{23} . MS: [m/z] 542 (M + 1) Anal. Calcd./found for $C_{24}H_{21}ClN_5O_6$: C, 58.48/58.49; H, 4.02/4.06; Cl, 7.85/7.92; N, 15.50/15.23, O; 14.16/14.10.

2.9 Preparation of 4-(3-(7-chloroquinolin-4-ylamino)propyl)-2(4-methoxy benzylidene)-1, 6-diox-4, 9-diazaspiro[4,4]nonane-3,8-dione **5(c)**

A mixture of compound 3-(3-(7-chloroquinolin-4-ylamino)propyl)-2-imino-5-(4-methoxy benzylidene)oxazolidin-4-one (**4c**) (0.30 g, 0.12 mmol), zinc chloride (0.50 mg, 0.001 mmol) and glycolic acid (3.37 g, 0.25 mmol) was dissolved in glacial acetic acid (20 ml). The reaction mixture was refluxed on sand bath for 6–7 h. The temperature of the reaction mixture was raised up to 120°C and maintained. The completion of the reaction checked by TLC. The reaction mixture was cooled at room temperature, neutralized with ammonium hydroxide and filtered. The precipitate was poured in cold water and filtered. The resultant product was purified by column chromatography using silica absorbent system (8:2 (benzene: methanol)). The product was recrystallized with ethanol to give **5c**.

IR (KBr) cm^{-1} 3263[NH str.(Amine)], 3043,2987[C-H str.], 1705[C=O(str.)],1658[C=CH],1500[NH (Amine)], 1458 [C-H bend CH_2], 1278[C-N str.], 1311[C-O(str.)] and 750[C-Cl str.(Aro)]. $^1\text{H-NMR}$ (CDCl_3) δ 11.5[s,1H,NH], 8.5[s, 1H, Ar.H], 8.0[dd,2H, ArH],7.7[d,1H, ArH], 7.5[d,1H, ArH], 7.3[d,1H, ArH], 7.2[dd,2H, ArH], 7.0[d,1H, ArH], 6.6[s, 2H, (C=H)], 4.1[s,1H,NH], 3.0[s,2H, (Ar. CH_2)], 2.4[s,3H, (CH_3)] and 1.8–1.0[m,6H (CH_2)]. $^{13}\text{C-NMR}$ (CDCl_3) δ 152.0 C_1 , 148.7 C_3 , 119.7 C_4 , 126.9 C_5 , 136.0 C_6 , 162.1 $\text{C}=\text{O}$, 126.9 C_7 , 51.4 C_{10} , 46.4 C_{11} , 162.2 C_{12} , 148.7 C_{16} , 101.0 C_{17} , 127.1 C_{18} , 136.0 C_{23} , 67.6 C_{24} . MS: [m/z] 512 (M +1) Anal. Calcd./found for $\text{C}_{25}\text{H}_{24}\text{ClN}_4\text{O}_7$: C, 62.76/62.81; H, 7.37/7.53; Cl, 6.18/6.29; N, 8.51/8.58, O; 16.20/16.28.

3. Antimicrobial activity

The *in vitro* antimicrobial activity of compounds **1**, **3**, **4a** and **5b** was performed using the disk diffusion method. Streptomycin for bacteria and fluconazol for fungal were used as standard drugs. The compounds **1**, **3**, **4a** and **5b** were tested for their antibacterial and antifungal activities by disk-diffusion method using nutrient broth medium [contained (g/l): beef extract 3 g; peptone 5 g; pH 7.0] for bacteria and potato dextrose broth medium [contained (g/l): beef extract 3 g; peptone 5 g; pH 7.0] for fungi. The Gram-positive bacteria and Gram-negative bacteria utilized in this study consisted of *Escherichia coli*, and *Bacillus subtilis* for bacterial species and *Aspergillus niger* and *Fusarium oxysporum* for the fungal species. In the disk-diffusion method, sterile paper disks (0.5 mm) impregnated with compound dissolved in dimethylsulphoxide (DMSO) at concentration 100, 200 and 400 $\mu\text{g/ml}$ were used. Then, the paper disks impregnated with the solution of the tested compounds were placed on the surface of the media inoculated with the microorganism. The plates were incubated at 35°C for 24 h. After incubation, the growth inhibition zones and activity were checked. The outcome of this study is presented in table 1.

4. *In vitro* cytotoxicity assay

MCF7 (hormone-dependant breast carcinoma cell line), HT29 (colon carcinoma cell line) were purchased from the Microbial Type Culture Collection (MTCC), Chandigarh. The viability of the cells was checked before and after treatment by the trypan blue exclusion dye method. Frozen cell stocks were stored in liquid nitrogen (−196°C) prior to use. The MCF7 and

HT29 cells were maintained in RPMI 1640 medium (Sigma), supplemented with 10% fetal bovine serum (FBS, MP Biomedicals, Mumbai), 100 $\mu\text{g/ml}$ Ampicillin (CDH, New Delhi) and 50 $\mu\text{g/ml}$ of fluconazole (MP Biomedicals, Mumbai). The cells were cultured in a 5% CO_2 incubator (Accurate) kept at 37°C in a humidified atmosphere. The culture was subcultured every two to three days as needed and routinely checked under an inverted microscope (IMT-2 Olympus, Japan) for any contamination.

4.1 *In vitro* neutral red cytotoxicity assay

The neutral red cytotoxicity assay was followed as previously described.³⁶ Briefly, cells were detached from the tissue culture flask with 0.25% trypsin-ethylene diamine tetra acetic acid (EDTA) solution and phosphate buffered saline (PBS) solution and washed in culture medium. The cell pellet was obtained by centrifugation at 1000 rpm for 5 min. The density of the viable cells was counted by 0.4% of trypan blue exclusion in a haemocytometer (Rohem). The cells ($1 \times 10^4/\text{well}$) were then plated in a 96-well microtiter plate (Biotron Healthcare) in a volume of 190 μl . The plate was incubated in a CO_2 incubator at 37°C for 24 h to allow the cells to adhere and achieve 60–70% confluence at the time of the addition of the test agents (that is, test extracts and positive control). After 24 h of incubation, the cells were treated with different concentrations (1, 10, 50 and 100 $\mu\text{g/ml}$) of each test agents in three replicate tests. The plates were incubated for 72 h at 37°C in a 5% CO_2 incubator. DMSO was used to dilute the test agents and the final concentration of DMSO in test wells and control wells used was not in excess of 1% (v/v). No effect due to the DMSO was observed 5FU (MP Biomedicals, Mumbai) was used as the positive control. The well containing untreated cells is the negative control. At the end of the incubation period, the media was replaced with medium containing 50 $\mu\text{g/ml}$ neutral red. The plates were incubated for another 3 h to allow for uptake of the vital dye into the lysosomes of viable and uninjured cells. After the incubation period, the media was removed and cells were washed with the neutral red washing solution. The dye was eluted from the cells by adding 200 μl of neutral red resorb solution and further incubated for 30 min at room temperature with rapid agitation on a microtiter plate shaker (Biotron Healthcare). Dye absorbance was measured at 540 nm using ELISA reader (Biotron Healthcare). Three replicate plates were used to determine the cytotoxicity of each test agent. The cytotoxic effect of each

Table 1. Antimicrobial activity of synthesized compounds **1**, **3**, **4a** and **5b**.

Compound	Conc. ($\mu\text{g/ml}$)	<i>E. coli</i>		<i>B. subtilis</i>		<i>A. niger</i>		<i>F. oxysporum</i>	
		Zone of inhibition (mm)	% activity compared to the standard	Zone of inhibition (mm)	% activity compared to the standard	Zone of inhibition (mm)	% activity compared to the standard	Zone of inhibition (mm)	% activity compared to the standard
1	400	14	50.00	16	53.33	53	81.53	16	55.17
	200	12	54.55	11	47.82	44	73.33	11	47.82
	100	10	62.50	7	41.17	–	–	8	44.44
3	400	20	67.85	25	83.33	54	78.346	18	62.06
	200	17	63.64	17	73.91	46	65.00	13	56.52
	100	11.5	59.30	11	64.70	–	–	9	50.00
4a	400	18	64.28	20	66.67	38	58.46	23	72.41
	200	16	72.72	13	56.52	26	43.33	16	60.86
	100	9	56.25	9	52.94	–	–	11	55.56
5a	400	17	65.17	18	48.17	22	49.34	18	43.11
	200	13	53.34	14	41.15	17	33.13	10	31.44
	100	10	41.14	12	32.42	–	–	4	22.20
5b	400	19	71.82	21	70.00	51	83.07	21	79.31
	200	14	77.27	14	60.86	39	76.66	14	69.56
	100	9.5	71.80	10	58.82	–	–	10	61.11
5c	400	16	45.12	15	43.17	20	44.28	16	40.01
	200	11	35.27	10	31.05	14	30.08	9	29.19
	100	7	22.04	7	14.72	–	–	5	19.20
Streptomycin	400	28	100	30	100	–	–	–	–
	200	22	100	23	100	–	–	–	–
	100	16	100	17	100	–	–	–	–
Fluconazol	400	–	–	–	–	65	100	29	100
	200	–	–	–	–	60	100	23	100
	100	–	–	–	–	–	–	18	100

test agent was evaluated based on percentage inhibition values. The percentage of inhibition (%) was calculated according to the following formula:

$$\text{Percentage of inhibition (\%)} = \frac{\text{OD control} - \text{OD sample} \times 100\%}{\text{OD control}}$$

The average of three replicates was then obtained. Cytotoxicity of each test agent is expressed as IC_{50} value. The IC_{50} value is the concentration of test agents that cause 50% inhibition or cell death, averaged from the three experiments, and was obtained by plotting the percentage inhibition versus concentration of test agents.³⁷

5. Results and discussion

A new synthetic method for the preparation of oxazolidinone has been developed and obtained the desired oxazolidinone derivatives in 54–80% yields. All the nine compounds were prepared by refluxing in the presence of solvent (dry ethanol, acetone and benzene). The obtained compounds are stable in the solid state. Characteristic IR bands provide significant indications for the formation of oxazolidinone derivatives. IR spectra of all the compounds showed $1500\text{--}1578\text{ cm}^{-1}$ (NH str.), $1735\text{--}1770\text{ cm}^{-1}$ (C=O str.), $1600\text{--}1475\text{ cm}^{-1}$ (C=C str.), which confirmed the formation of oxazolidinone derivatives. Structure of oxazolidinone derivatives were further confirmed by ^1H -NMR spectra, which proved as a diagnostic tool for the positional elucidation of the proton. Assignments of the signals are based on chemical shift and intensity pattern. The ^1H -NMR spectra showed multiplets in the region 1.5–2.0 ppm for (CH_2) and doublets in the region 8.14–7.3 ppm for aromatic proton in all spectra, respectively. Characteristic peaks were observed in the mass spectra of all compounds which followed the similar fragmentation pattern. The spectra of compounds showed molecular ion peak (M^+) at m/z which confirmed the molecular weight of the oxazolidinone derivatives.

The antibacterial activity was evaluated against two pathogenic strains (*E. coli* and *B. subtilis*). The zone of inhibition and activity index were determined by comparison with the standard drug streptomycin. The outcome of this study is presented in table 1. The antibacterial screening against *E. coli* showed that amongst the compounds **1**, **3**, **4a** and **5b** the compound **5b** displayed highest activity. The compound **1** showed minimum activity amongst all the compounds. The remaining compounds **4a** and **3** showed only moderate activity.

Table 2. *In vitro* cytotoxicity on MCF7 and HT29 cells of compounds **5a–c**.

	Cytotoxicity on MCF7 IC_{50} ($\mu\text{g/mL}$)	Cytotoxicity on HT29 IC_{50} ($\mu\text{g/mL}$)
5-Fluorouracil	1.3 ± 0.76	5.6 ± 1.40
Chloroquinoline	49.4 ± 0.56	56.6 ± 3.09
5a	16.1 ± 3.40	32.4 ± 5.03
5b	46.0 ± 4.17	$54.0 \pm 3.19^*$
5c	38.2 ± 3.08	49.0 ± 2.30

The values expressed are mean $\pm$ standard deviation of triplicate measurements

*Not significant

Contrary to this observation, compound **3** showed highest activity amongst all the compounds screened for this activity against *B. subtilis*.

Similarly, the antifungal activity was evaluated against two pathogenic strains (*A. niger* and *F. oxysporum*). The zone of inhibition and activity index were determined by comparison with the standard drug fluconazole. The outcome of this study is presented in table 1. The antifungal screening against *A. niger* showed that amongst the compounds **1**, **3**, **4a** and **5b**, the compound **5b** displayed highest activity. The compound **4a** showed minimum activity amongst the entire compound. The remaining compounds **1** and **3** showed only moderate activity. Contrary to this observation, compound **5b** showed highest activity amongst all the compounds screened for this activity against *F. oxysporum*.

All the compounds **5a–c** were tested for their biological activities towards MCF7 and HT29 cells lines and the results are shown in table 2. IC_{50} value lower than $50\text{ }\mu\text{g/mL}$ was considered as active for cytotoxicity on MCF7 and HT29 cells. 5-Fluorouracil was used as positive reference compound. The MCF7 cell line was found to be very susceptible towards compound **5a** with IC_{50} values of $16\text{ }\mu\text{g/mL}$ while medium inhibitory activities were observed for compounds **5c** with IC_{50} values of 38 and weak activity was observed for compound **5b** with an IC_{50} value of $46\text{ }\mu\text{g/mL}$, respectively. Similarly, the HT29 cell line was found to be moderately susceptible towards compounds **5a** and **5c** with IC_{50} values of 32 and $49\text{ }\mu\text{g/mL}$, respectively while compound **5b** had no activities ($\text{IC}_{50} > 54.0\text{ }\mu\text{g/mL}$) against the cell line.

6. Conclusion

In conclusion, novel oxazolidinone analogues of chloroquinoline **5(a–c)** displaying potential antibacterial and antifungal activities were synthesized. The results

showed that the addition of the oxazolidinone ring on the quinoline molecule framework increased the antibacterial activity. Among the four compounds, oxazolidinone derivative showed better antibacterial and antifungal activity than the respective drug streptomycin and fuconazol. The compound **5a** was found to be most potent inhibitor of both the cancer cell lines i.e., MCF7 and HT29 cells lines.

The importance of such work lies in the possibility that the new synthesized compound might be a more efficacious drug against various bacterial species for which a thorough investigation regarding the structure–activity relationship, mechanism of action and toxicity in their biological effects which could be helpful in designing more potent antibacterial and antifungal agents for therapeutic use.

Supplementary information

The electronic supporting information can be seen in www.ias.ac.in/chemsci.

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