

# Facile Synthesis of Some Novel Derivatives of 1,3,4-Oxadiazole Derivatives Associated with Quinolone Moiety as Cytotoxic and Antibacterial Agents

Sudeesh K<sup>1</sup> and Gururaja R<sup>2\*</sup>

<sup>1</sup>Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu, India

<sup>2</sup>Central Lab Facility, Department of Chemistry, Sarada Vilas College, Mysore, Karnataka, India

## Abstract

A new series of 2-(4-substituted phenyl)-5-(6-substituted-2-chloroquinolin-3-yl)-1,3,4-oxadiazole derivatives were synthesized after refluxing 6-substituted-2-hydroxyquinolin-3-carboxylic acids with different aromatic acid hydrazides in the presence of POCl<sub>3</sub>. The chemical structures of these compounds were confirmed by various physico-chemical methods viz. IR, <sup>1</sup>H-NMR, EI-Mass, C<sup>13</sup>-NMR and elemental analysis. Newly synthesized compounds were screened *in vitro* for their cytotoxic activity against SK-2-MEL cell line. Also, screened *in vitro* for their antimicrobial activity against varieties of gram-positive and gram-negative bacterial strains and fungi strains *Aspergillus niger* and *Rhizopus*. The compound 7a and 7e shows highly significant antimicrobial and compound 7i shows good cytotoxic activity as compared to the standard drug.

**Keywords:** 1,3,4-Oxadiazole; 6-Substituted-2-chloroquinoline; Antimicrobial; Cytotoxic SK-MEL-2

## Introduction

The development of new antimicrobial and cytotoxic agents is one of the fundamental goals in medicinal chemistry. In recent years, there has been a concerned search for the discovery and development of potent and selective cytotoxic and antimicrobial agents. N-heterocyclic ring systems are important for the drug design, among these quino line and 1,3,4-oxadiazole compounds are present in several classes of natural and synthetic biologically active compounds.

1,3,4-Oxadiazole is the important heterocyclic moiety in the medicinal chemistry which has been extensively reported in the literature for the synthesis of new biologically active molecules with antibacterial [1], antifungal [2], anti-inflammatory [3], enzyme inhibition [4], anti-HIV [5], analgesic [6], antitumor [7], antihypertensive [8] and anticonvulsant [9] activities. Examples of drugs containing the 1,3,4-oxadiazole unit currently used in clinical medicine are, Raltegravir, an antiretroviral drug, Nesapidil, an anti-arrhythmic therapy, Furamizole, a nitrofurant derivative that has strong antibacterial activity and Tiodazosin, an antihypertensive drug. On the other hand, quinoline derivatives have been explored for the diverse pharmacological activities such as HIV-integrase inhibitors [10], antitubercular [11], anti-inflammatory [12], analgesics [13], anticancer [14], antimicrobial [15] etc. Attributable to such biological importance, quinoline derivatives have grown to be the synthetic goals of many organic and medicinal chemistry researchers.

In view of these previous findings and of our interest in the fictionalization of quinolinyl oxadiazoles, we report here in on the synthesis of new 1,3,4-oxadiazole derivatives containing quinoline moiety with cytotoxic and antibacterial activities. All the final compounds were characterized on the basis of EI-Mass, <sup>1</sup>H NMR, C<sup>13</sup> NMR and IR analyses (Figure 1).

## Materials and Methods

Melting points of the synthesized compounds were determined in open-glass capillaries using GUNA melting point apparatus and are uncorrected. IR absorption spectra were recorded in the 4000-400 cm<sup>-1</sup> range on a Shimadzu FTIR-8400s using KBr pellets, <sup>1</sup>H-NMR and C<sup>13</sup>-NMR were recorded on Agilent-NMR, 400 MHz spectrophotometer.

EI-Mass spectra were recorded by Agilent-NMR, 400 MHz spectrophotometer. TLC was done on F<sub>254</sub> grade silica-60 from SD Fine. Cytotoxic studies were done in Tata Memorial Centre, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai. Antimicrobial studies were done in the Microbiology Laboratory.

## Synthesis

**General procedure for the synthesis of aromatic ethyl ester, 2(a-c):** A mixture of various 4-substituted aromatic acids 1(a-c), ethyl alcohol (50 mL) and 3 mL of conc. H<sub>2</sub>SO<sub>4</sub> were refluxed for 4-6 hrs. The mixture is poured to crushed ice, separated solid was filtered, dried and re-crystallized from minimum amount of ethyl alcohol. The compound was confirmed by the physical data.

**General procedure for the synthesis of aromatic carbohydrazides, 3(a-c):** To a solution of 2 (a-c) in ethyl alcohol (50 mL) was added with excess of 99% hydrazine hydrate and refluxed for 24 hr on a water bath. Reaction mixture was concentrated and allowed to cool, separated crystals was filtered, dried and re-crystallized from minimum amount of ethyl alcohol. The compound was confirmed by the physical data. 6-substituted-2-chloroquinoline-3-carbaldehyde, 4(a-d) was prepared by a reported method [16]. 6-substituted-2-hydroxyquinoline-3-carbaldehyde, 5(a-d) was prepared by a reported method [17]. 6-substituted-2-hydroxyquinoline-3-carboxylic acid 6(a-d) was prepared by a reported method [18].

**General procedure for the synthesis of 2-(4-substituted phenyl)-5-(6-substituted-2-chloroquinolin-3-yl)-1,3,4-oxadiazole, 7(a-1):** To the equimolar mixture of compounds 3(a-c) and 6(a-d), catalytic

\*Corresponding author: Gururaja R, Department of Chemistry, Sarada Vilas College, Mysore, Karnataka, India, Tel: +917923260076; E-mail: [dr.gururaja@gmail.com](mailto:dr.gururaja@gmail.com)

Received: July 05, 2016; Accepted: July 11, 2017; Published: July 13, 2017

Citation: Sudeesh K, Gururaja R (2017) Facile Synthesis of Some Novel Derivatives of 1,3,4-Oxadiazole Derivatives Associated with Quinolone Moiety as Cytotoxic and Antibacterial Agents. Organic Chem Curr Res 6: 183. doi: [10.4172/2161-0401.1000183](https://doi.org/10.4172/2161-0401.1000183)

Copyright: © 2017 Sudeesh K. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

amount of  $\text{POCl}_3$  was added and it was refluxed for 4-6 hrs. The reaction mixture was poured over crushed ice and neutralized by sodium carbonate solution. The precipitate formed was filtered and dried. The crude product was purified by the alcohol and DMF mixture (Figure 2).

### Cytotoxic activity

Among the synthesized compounds few are selected and its *in-vitro* cytotoxic activity is screened against the SK-MEL-2 cell line. The study is based on the Sulphorhodamine B (SRB) assay [19] water and residual wash solution is removed by a piece of gauze. 100  $\mu\text{l}$  of SRB solution is pipette into each well of the culture plates and allowed to stain for 30 minutes. Residual SRB was removed by using 1% acetic acid and a residual wash is given Table 1. The culture plates are air dried until no moisture is visible. The protein bound dye is solubilized with 100  $\mu\text{l}$  of 10mM Tries base per well. Shake the plates for 10 min to homogenize the dye solution. OD is measured by using ELISA reader at a wavelength of 564 nm and  $\text{IC}_{50}$  values are calculated and tabulated in the Table 2. ADR is used as the standard drug and the compound 7i is showing the good activity against the cell line. The graphical representation is shown below.

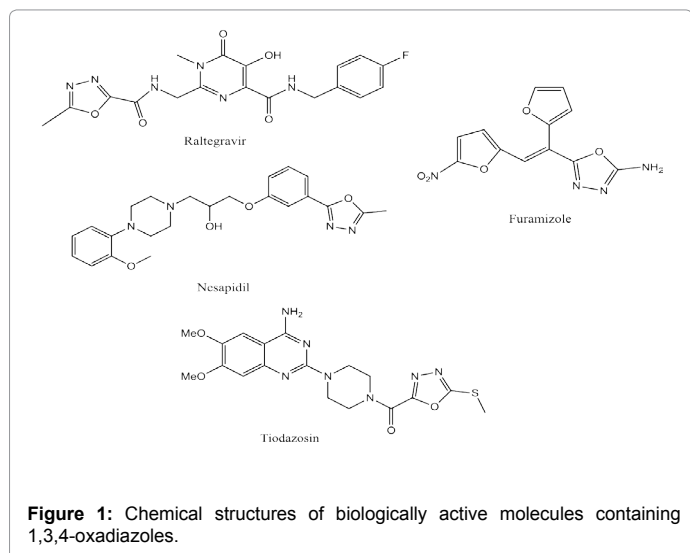


Figure 1: Chemical structures of biologically active molecules containing 1,3,4-oxadiazoles.

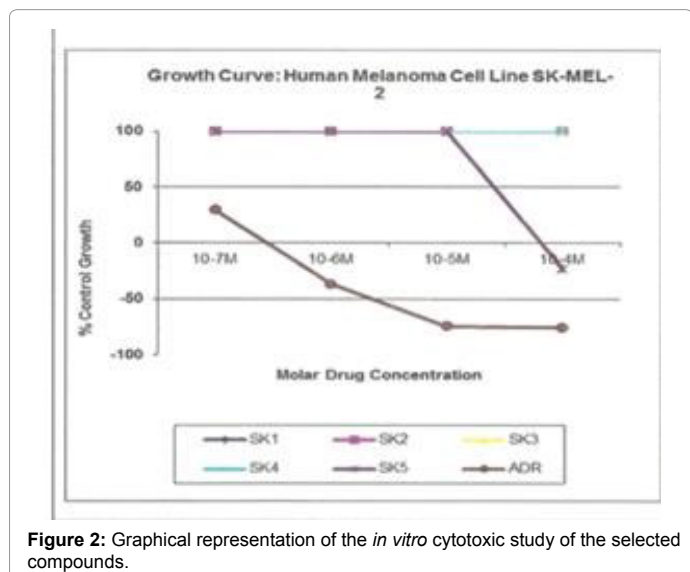


Figure 2: Graphical representation of the *in vitro* cytotoxic study of the selected compounds.

### Antibacterial activity

The *in-vitro* antibacterial activity of the selected synthesized compounds are screened against five bacterial strains namely, *Ralstonia solanacearum*, *Escherichia coli*, *Klebsiella pneumoniae* (Gram negative), *Lactobacillus*, *Bacillus subtilis* (Gram positive). Disc diffusion method was used to determine the Minimum Inhibitory Concentration (MICs  $\mu\text{g}/\text{mL}$ ) in a triplicate experiment. at 10,000 rpm for 5 minutes; a pellet was dissolved in double distilled water and used to inoculate the plates. The autoclaved molten media (20 ml) was poured in each 90 mm Petri plate and allowed to solidify. A circular well of diameter 6 mm was made exactly at the centre of the plates by using cork borer and each well was filled with 0.1 ml of the test solution (10 mg/ml). Chloroamphenicol and DMSO were used as the positive control and negative control respectively. The compounds were tested in triplicate and inhibition zones were measured in mm after 24 hrs of incubation. The results were presented in Table 3.

### Antifungal activity

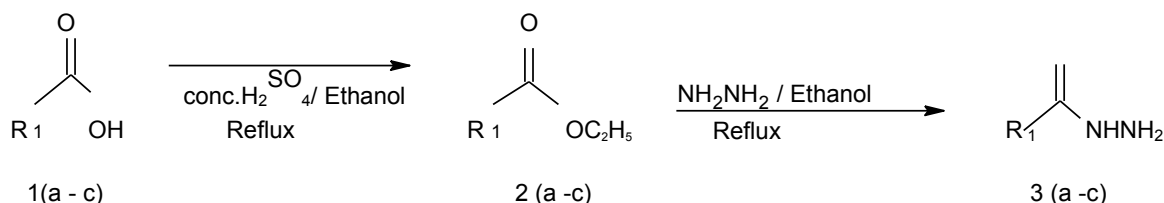
The *in-vitro* antifungal activity of the selected synthesized compounds was carried out against two fungi namely, *Aspergillus niger* and *Rhizopus*. Disc diffusion method [20] was used. were harvested in sterilized normal saline (0.9% NaCl in distilled water) and its concentration was adjusted to  $1 \times 10^6$  /ml with a Haemometer. The autoclaved molten media (20 ml) was poured in each 90 mm sterilized petri plates and allowed to solidify. To study the growth response of fungi species, 0.4 ml of the synthesized compound solution (5 mg/ml) was poured into each p incubation, the fungal growth was measured and compared with the control. The fungal activity of selected synthesized compounds was assessed by comparing the zone of fungal growth in treated plates with that of control plates in mm. For comparison, Nistatine was used as the standard drug. The antifungal data were illustrated in the Table 4.

### Analytical data of the compounds

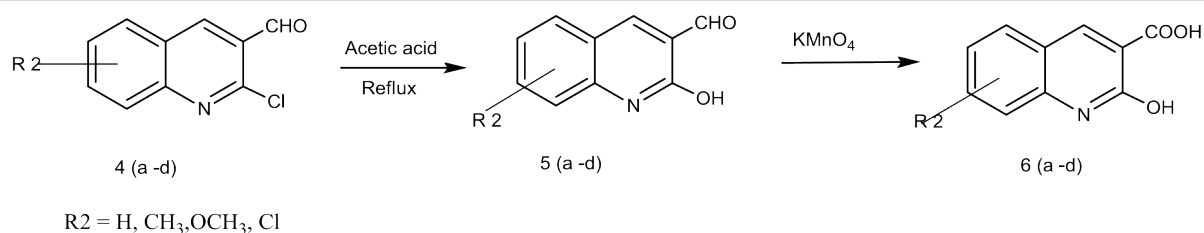
**4.5.1 2-(Phenyl)-5-(2-chloroquinolin-3-yl)-1,3,4-oxadiazole, 7(a):**  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta$  -8.34 (m, 10H, Aromatic-H); IR (KBr,  $\text{cm}^{-1}$ ): 1407(C-O-C), 1095.5(C-Cl), 1481.2(C=N), 1639.4(C=C), 2086.8 (aromatic);  $\text{C}^{13}$  NMR (400 MHz, DMSO- $d_6$ ): 117.58, 123.47, 126.33, 127.31, 128.26, 128.91, 129.45, 129.98, 132.85, 133.66, 142.23, 146.27, 147.82, 161.69 (Carbon atoms of the aromatic ring), 165.15 (carbon of the oxadiazole ring); MS (ESI) m/z: 308.5; Anal. Calculated for  $\text{C}_{17}\text{H}_{10}\text{N}_3\text{OCl}$ : C, 66.31, H, 3.26, N, 13.67; found C, 66.35, H, 3.28, N, 13.65 (Schemes 1-3).

**2-(Phenyl)-5-(6-methyl-2-chloroquinolin-3-yl)-1,3,4-oxadiazole, 7(d):**  $^1\text{H NMR}$  (400 MHz, DMSO):  $\delta$  9-8.3 (m, 8H, Aromatic-)  $\delta$  (- $\text{CH}_3$ );  $\text{C}^{13}$  NMR (400 MHz, DMSO- $d_6$ ): 20.81, 116.50, 117.83, 119.62, 129.81, 133.63, 135.93, 137.94, 146.40, 164.23(Carbon atoms of the aromatic ring), 165.03(carbon of the oxadiazole ring); IR (KBr,  $\text{cm}^{-1}$ ): 1354.09 (C-O-C), 1146.7 (C-Cl), 1485.2 (C=N), 1549 (C=C), 1583 (aromatic); MS(ESI) m/z: 323; Anal. Calculated for  $\text{C}_{18}\text{H}_{12}\text{N}_3\text{OCl}$ : C, 67.19, H, 3.76, N, 13.06; found C, 67.17, H, 3.73, N, 13.08.

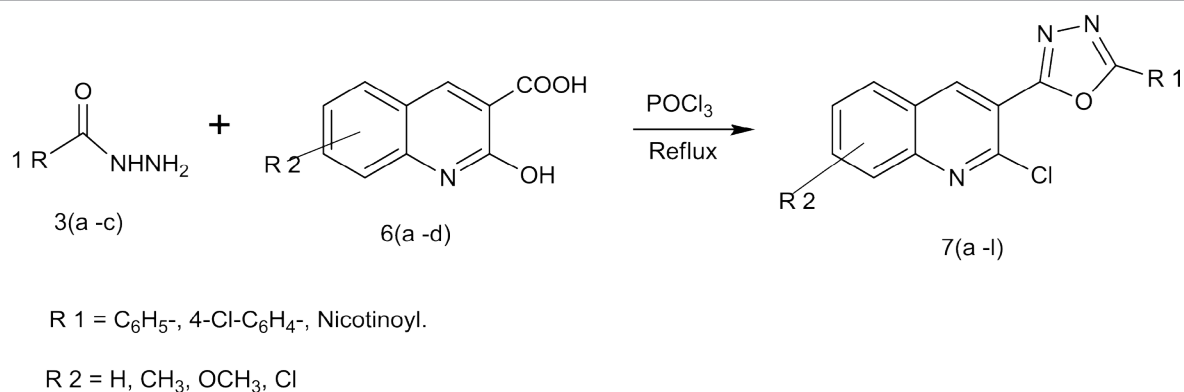
**2-(4-Chlorophenyl)-5-(6-methoxy-2-chloroquinolin-3-yl)-1,3,4-oxadiazole, 7(h):** Yield- 72%, m. p. 269-2  $^1\text{H NMR}$  (400 MHz, DMSO-):  $\delta$  -8.13 (m, 8H, Aromatic-)  $\delta$  9 (-OCH $_3$ );  $\text{C}^{13}$  NMR (400 MHz, MSO- $d_6$ ): 55.81, 128.92, 129.13, 129.98, 130.08, 131.54, 138.20(Carbon atoms of the aromatic ring), 166.80 (carbon of the oxadiazole ring); IR (KBr,  $\text{cm}^{-1}$ ): 1354.09 (C-O-C), 1146.7 (C-Cl), 1485.2 (C=N), 1549 (C=C), 1583



Scheme 1: Synthetic reaction pathway of the aromatic carbohydrates.



Scheme 2: Synthetic reaction pathway of 6-substituted-2-hydroxyquinoline-3-carboxylic acid.



Scheme 3: Synthetic reaction pathway of 1,3,4-oxadiazole derivatives.

(aromatic); MS (ESI) m/z: 371.8; Anal. Calculated for C<sub>18</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub>Cl<sub>2</sub>: C, 58.08, H, 2.98, N, 11.29; found C, 58.10, H, 2.94, N, 11.32 (Table 1).

## Results and Discussion

### Chemistry

In the present work, 1,3,4-oxadiazole derivatives containing 6-substituted-chloroquinoline moiety 7(a-l) were synthesized and characterized on the basis of spectral (<sup>1</sup>H NMR, <sup>13</sup>C NMR, EI-Mass and IR) analyses. The synthesized compounds were evaluated for their *in-vitro* cytotoxicity against Human Melanoma Cell line SK-MEL-2 and antimicrobial activity against different bacterial and fungi strains. Synthetic chemistry involves conversion of corresponding aromatic acids to respective ethyl esters 2(a-c) and further converted to carbohydrazides 3(a-c) by refluxing with hydrazine hydrate. 6-substituted-2-chloroquinoline-3-carbaldehyde 4(a-d) was converted into 6 substituted-2-hydroxyquinoline-3-carboxylic acid 6(a-d) by hydrolysis with Acetic acid followed by oxidation with KMnO<sub>4</sub>. The two intermediates 3(a-c) and 6(a-d) was refluxed in the presence of catalytic amount of POCl<sub>3</sub> and obtained a series of derivatives of 1,3,4-oxadiazole containing 6-substituted-2-chloroquinoline moiety 7(a-l).

### Biology

Three gram negative and two gram positive bacterial strains were used for the evaluation of the *in-vitro* antibacterial screening of the selected synthesized compounds. Chloroamphenicol was used as the standard. Similarly, two fungi strains were used for the *in-vitro* antifungal screening of the selected compounds. Nistatine was used as the standard. Human Melanoma Cell line SK-MEL-2 was used for the evaluation of *in-vitro* cytotoxic activity using *Sulphorhodamine B* assay. Adriamycin was used as standard drug. The results were illustrated in the Tables 2 and 3 respectively. The compound 7i was the potent anticancer agent against SK-MEL-2 with -23.0 at 10<sup>-4</sup> μM concentration which is comparable with the cytotoxicity of the standard. Compound 7a showed inhibition on *E. coli*. Compound 7e showed the inhibition against the *Rhizopus*. Rest of the compounds showed moderate inhibition on bacterial and fungi strains.

### Conclusion

A new series of 2-(4-substituted phenyl)-5-(6-substituted-2-chloroquinolin-3-yl)-1,3,4-oxadiazole analogues 7(a-l) were synthesized. The synthesized compounds were moderately active as the growth inhibitors of the Human Melanoma Cell Line SK-MEL-2. The synthesized compounds were also moderately active against

Compound	Molecular formula <sup>a</sup> and Mol. Wt.	R1	R2	(C)	Elemental Analysis % Calculated <sup>b</sup>		
					C	H	N
7a	C <sub>17</sub> H <sub>10</sub> ClON <sub>3</sub> (307.5)	H	H	71 (240)	66.31	3.26	13.67
7b	C <sub>17</sub> H <sub>9</sub> Cl <sub>2</sub> ON <sub>3</sub> (342)	4-Cl	H	78 (260)	59.62	2.61	12.29
7c	C <sub>16</sub> H <sub>9</sub> ClON <sub>4</sub> (308.5)	Nicotinoyl	H	74 (231)	62.25	2.93	18.17
7d	C <sub>18</sub> H <sub>12</sub> ClON <sub>3</sub> (321.5)	H	CH <sub>3</sub>	70 (261)	67.18	3.73	13.06
7e	C <sub>18</sub> H <sub>11</sub> Cl <sub>2</sub> ON <sub>3</sub> (356)	4-Cl	CH <sub>3</sub>	73 (236)	60.65	3.07	11.77
7f	C <sub>17</sub> H <sub>11</sub> ClON <sub>4</sub> (322.5)	nicotinoyl	CH <sub>3</sub>	71 (269)	63.22	3.39	17.33
7g	C <sub>18</sub> H <sub>12</sub> ClO <sub>2</sub> N <sub>3</sub> (337.5)	H	OCH <sub>3</sub>	75 (191)	64.01	3.26	12.41
7h	C <sub>18</sub> H <sub>11</sub> Cl <sub>2</sub> O <sub>2</sub> N <sub>3</sub> (372)	4-Cl	OCH <sub>3</sub>	74 (274)	58.09	2.97	11.28
7i	C <sub>17</sub> H <sub>11</sub> ClO <sub>2</sub> N <sub>4</sub> (338.5)	nicotinoyl	OCH <sub>3</sub>	72 (283)	60.28	3.25	16.55
7j	C <sub>17</sub> H <sub>9</sub> Cl <sub>2</sub> ON <sub>3</sub> (342)	H	Cl	78 (284)	59.66	2.65	12.29
7k	C <sub>17</sub> H <sub>9</sub> Cl <sub>3</sub> ON <sub>3</sub> (376.5)	4-Cl	Cl	75 (>300)	54.19	2.15	11.18
7l	C <sub>16</sub> H <sub>8</sub> C <sub>12</sub> ON <sub>4</sub> (343)	nicotinoyl	Cl	73 (>300)	55.99	2.36	16.34

Table 1: Physical data of the synthesized test compounds, 7(a-l). Solvent for crystallization=Alcohol+DMF.

Compound No.	Human Melanoma Cell Line SK-2-MEL % Control Growth Molar Drug Concentration			
	10 <sup>-7</sup> M	10 <sup>-6</sup> M	10 <sup>-5</sup> M	10 <sup>-4</sup> M
7a	100	100	100	100
7d	100	100	100	100
7e	100	100	100	100
7g	100	100	100	100
7i	100	100	100	-23.0
Adriamycin (Standard drug)	29.3	-37.1	-74.5	-75.6

Table 2: Cytotoxic activity of the selected compounds against the Human Melanoma Cell Line SK-MEL-2.

Compounds	Gram -ve			Gram +ve	
	<i>E. coli</i>	<i>R. solanacearum</i>	<i>K. pneumoniae</i>	<i>Lactobacillus</i>	<i>B. subtilis</i>
7a	14	13	14	12	12
7d	15	16	16	13	15
7e	9	5	8	3	4
7g	6	5	6	4	4
7i	15	16	16	12	13
Chloroamphenicol	14	12	12	10	10

Table 3: *In-vitro* antibacterial screening of the selected synthesized compounds.

Compound No.	<i>Aspergillus niger</i>	<i>Rhizopus</i>
7a	13	13
7d	13	12
7e	7	15
7g	8	7
7i	12	8
Nistatine	14	16

Table 4: *In-vitro* antifungal screening of the selected compounds.

different bacterial and fungi strains. Among the selected compounds, 7i was found to be potent cytotoxic agent. The compounds 7a and 7e exhibited good antibacterial and antifungal agent in our study. These results indicated that substituted 1,3,4-oxadiazole with 6-substituted-2-chloroquinoline moiety may be useful leads for cytotoxic and antimicrobial drug development in the future.

#### Acknowledgements

The authors express their sincere thanks to the Principal, Sarada Vilas College, Mysuru for providing the laboratory facilities. We are also thankful to the Director, Tata Memorial Centre, ACTREC, Mumbai, for carrying out the cytotoxic studies and also to the Director, Vijnan Bhavan, Mysore University, Mysuru for the spectral data.

#### References

- Raj K, Narayan B, Ashalatha B, Kumari N, Sarojini B (2007) Synthesis of some

bioactive 2-bromo-5-methoxy-N'-[4-(aryl)-1,3-thiazol-2-yl]benzohydrazide derivatives. *Eur J Med Chem* 42: 425-429.

- Joshi RS, Mandhane PG, Chate AV, Gill CH (2011) Synthesis of Novel Series of Various Substituted 1-(5-(2-p-tolyloxyquinolin-3-yl)-2-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone and its Antibacterial Activity. *J Korean Chem Society* 55: 760-764.
- Singh AK, Parthasarathy R, Lahani M (2012) Synthesis, characterization and anti-inflammatory activity of some 1, 3, 4 -oxadiazole derivatives. *J Chem Pharm Res* 4: 779-782.
- Uto Y, Ueno Y, Kiyotsuka Y, Miyazawa Y, Kunata H, et al. (2010) *Eur J Med Chem* 45: 4785-4796.
- Johns B, Weather HJG, Allen SH, Thompson JB, Garvey EP, et al. (2009) The use of oxadiazole and triazole substituted naphthyridines as HIV-1 integrase inhibitors. Part 1: Establishing the pharmacophore. *Bioorg Med Chem Lett* 19: 1807-1810.

6. Manjunatha K, Poojary B, Lobo PL, Fernandes J, Kumari NS (2010) Synthesis characterization and antimicrobial activity of some disubstituted 1,3,4-oxadiazoles carrying 2-(aryloxymethyl)phenyl moiety. *Eur J Med Chem* 45: 5225-5233.
7. Savaiz FC, Formagio ASN, Barbosa VA, Foglio MA, Carvalho JE (2010) Synthesis in vitro Antiproliferative and Anti-Mycobacterium tuberculosis Activities of Novel  $\beta$ -Carboline Derivatives. *J Bio Chem Soc* 21: 288-298.
8. Bnkar GR, Nandakumar K, Nayak PG, Thakur A, Chamallamudi MR (2009) A preliminary in vitro assessment of a 1,3,4-oxadiazole derivative. *Chem Biol Interact* 181: 377-382.
9. Rajak H, Deshmukh R, Veerasamy R, Sharma AK, Mishra P, et al. (2010) Crystal structure and Hirshfeld surface analysis of 1-(4-bromophenyl)-2-[[5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl]sulfanyl]ethan-1-one. *Acta Crystallogr E Crystallogr Commun Bioorg Med Chem Lett* 20: 4168-4172.
10. Hu L, Yan S, Luo Z, Han X, Wang Y (2012) Design Practical Synthesis and Biological Evaluation of Novel 6-(Pyrazolylmethyl)-4-quinoline-3-carboxylic Acid Derivatives as HIV-1 Integrates Inhibitors. *Molecules* 17: 10652-10666.
11. Mungra DC, Kathrotiya HG, Ldani NK, Patel MP, Patel RG (2012) Imidazole and its derivatives as potential candidates for drug development. *Chinese Chem Lett* 23: 1367.
12. Rajanarendar E, Nagi RM, Rama KS, Ramamurthy K, Reddy MV (2012) Design, Synthesis, Antimicrobial, Anti-Inflammatory and Analgesic Activity of Novel Isoxazolyl Pyrimido[4,5-B]quinolines and Isoxazolyl Chromeno[2,3-D]pyrimidin-4-Ones. *Eur J Med Chem* 55: 273.
13. Giordani A, Mandelli S, Verpillio I, Zanzola S, Tarchino F, et al. (2012) 6-1H-imidazo-quinazoline and quinolines derivatives. new potent analgesics and anti-inflammatory agents. US Patent 8193353.
14. Kumar N, Raj VP, Jayashree BS, Kar SS, Anandam A, et al. (2012) Elucidation of structure-activity relationship of 2-quinolone derivatives and exploration of their antitumor potential through Bax-induced apoptotic pathway. *Chem Bio Drug Des* 80: 291.
15. Mohammed IA, Subrahmanyam EVS (2009) *Acta Pharm Sci* 57: 113.
16. Meth-Cohn O, Narine B, Tarnoski B (1981) *J Chem Perkin Trans I*, p: 1521.
17. Tilakraj T (1987) PhD Thesis, Mysore University, Mysore, India.
18. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45: 493-496.
19. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, et al. (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 82: 1107-1112.
20. Barry AL (1976) The Antimicrobial susceptibility test: Principle and Practice. In: Illus L, Febiger F (eds.), p: 180.