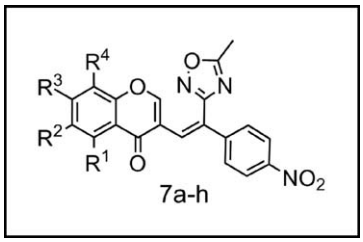
Synthesis and *In Vitro* Antibacterial Assessment of Novel Chromones Featuring 1,2,4-Oxadiazole

Santosh D. Diwakar, Ratnadeep S. Joshi, and Charansing H. Gill*

Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra 431004, India
*E-mail: gillch50@rediffmail.com
Received December 25, 2009
DOI 10.1002/jhet.656
Published online 15 April 2011 in Wiley Online Library (wileyonlinelibrary.com).



In search for a new antibacterial agent with improved antimicrobial spectrum and potency, we designed and synthesized a series of novel 3-((*Z*)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)-vinyl)-4*H*-chromen-4-ones **(7a–h)** by convergent synthesis approach. All the synthesized compounds were assayed for their *in vitro* antibacterial activities against gram-negative and gram-positive bacteria. The preliminary structure-activity relationship to elucidate the essential structure requirements for the antimicrobial activity has been described.

J. Heterocyclic Chem., **48**, 882 (2011).

INTRODUCTION

The treatment of infectious diseases still remain an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multidrug resistant microbial pathogens. Despite a large number of antibiotics and chemotherapeutics available for medical use, emergence of antibiotic resistance to older and newer agents suggest substantial medical need for new classes of antimicrobial agents.

The oxadiazole nucleus is a well studied pharmacophoric scaffold that has emerged as a core structural unit of biologically active synthetic compounds and is often used in drug discovery as hydrolysis-resisting bioisosteric replacements for ester or amide functionalities. Among oxadiazoles, 1,2,4-oxadiazole derivatives have gained importance in medicinal chemistry [1]. The literature suggests that some oxadiazoles with different substituents at different location on the heterocyclic ring results in fungicidal and bactericidal action of varying potencies [2].

It is known that the antimicrobial effect of oxadiazoles heterocycle increases upon conjugation involving a double bond, nitro group, or carbonyl group [3]. Novel *Z*-5-amino-3-[2-(5-nitro-2-furyl) vinyl]-Δ-1,2,4-oxadiazole (SQ 18,506) (Fig. 1) has shown *in vitro* antimicrobial activity against a wide range of bacteria as well as *in vivo* when administered orally to mice infected with *Escherichia coli, Salmonella schottmuelleri, Shigella flexneri, or Klebsiella pneumonia* [4].

Chromones and other related ring systems have several interesting biological activities. Chromones substituted by a heterocyclic ring at the 2 and 3 position are known to exhibit a wide range of biological activity such as antiallergic, antimicrobial, antifungal, anticholesteric, hypolipidemic, and antiblaster action as well as are known to stimulate the central nervous system [5]. On the other hand, chromones derived from ketoamides showed very good selective μ-calpain inhibition [6]. A series of sulfonamide derived chromones, previously reported as inhibitors of carbonic anhydrase, have been found to show *in vitro* antibacterial and antifungal activity [7].

Although 3-formylchromone has emerged as the most valuable synthon for the incorporation of the chromone moiety leading to the synthesis of a variety of heterocycles [8], its synthetic utility is limited due to facile opening of the chromone ring [8–10], hence strategies are being developed to circumvent it [11]. The presence of oxadiazole in conjugation with other active group like chromone molecule has rarely been divulged. Owing to the versatile bioactivities exhibited by chromones and oxadiazole, we directed our efforts to generate libraries of diverse chromones. In continuation of our work on bioactive chromones [12], we now wish to disclose our results towards the synthesis and *in vitro* antibacterial assessment of substituted 3-((*Z*)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4*H* chromen-4-ones by convergent strategies.

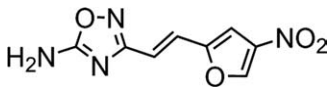


Figure 1. *Z*-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-Δ-1,2,4-oxadiazole (SQ 18,506).

RESULTS AND DISCUSSION

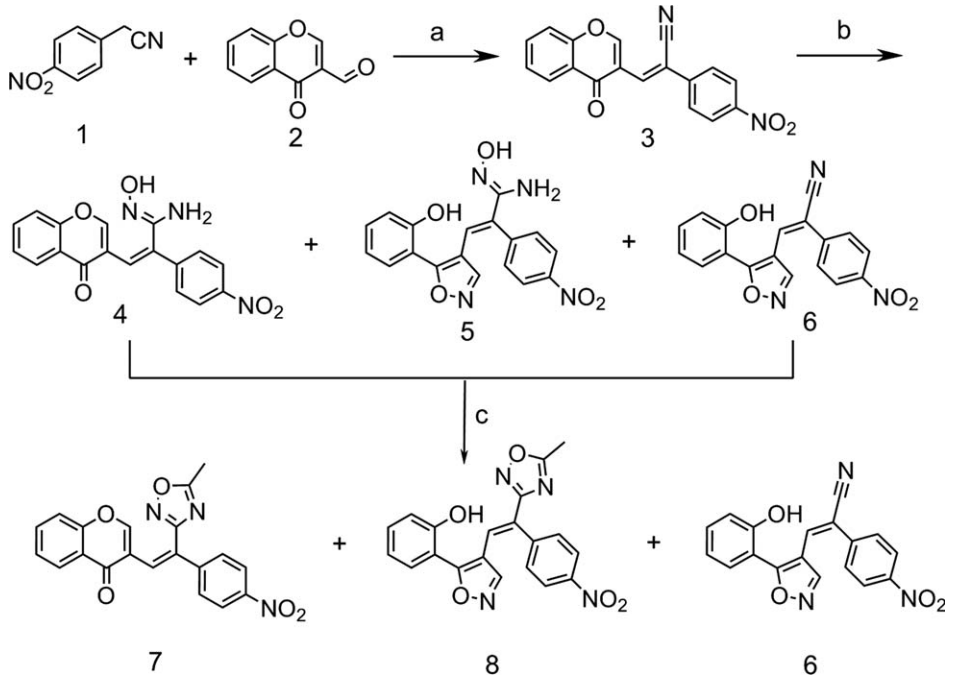
Based on such reports, we designed linear as well as convergent route to synthesize **(7a–h)**. The linear synthetic route employed was evaluated to gain insights and to identify potential hurdles to synthesize the oxadiazolyl chromones **(7)**. The synthesis of compound **(7)** by linear fashion begins with the condensation of *p*-nitrophenylacetonitrile **(1)** [13], which after condensation with 3-formylchromone **(2)** [14] in acetic anhydride by using sodium acetate to afford the nitrile **(3)**. The nitrile **(3)** when treated with hydroxylamine hydrochloride/ $NaHCO_3$ in methanol under reflux afforded amidoxime **(4)** (*m*/*z* 352.2) along with impurities **(5)** (*m*/*z* 367.2) and **(6)** (*m*/*z* 334.5). Since the γ-pyrone system of chromones is highly susceptible towards nucleophilic reagents [8–10], the amidoxime formation proceeds through facile nucleophilic opening of chromone ring system by hydroxylamine hydrochloride and subsequent recyclization to yield isoxazoles **(6)** ($m/z = 334.5$) and **(8)** ($m/z = 391.1$), thereby suppressing the formation of desired oxadiazolyl chromone **(7)** (Scheme 1).

On the contrary, convergent route (Scheme 3) was easy to envisage, was quickly reduced to practice, and additionally prevented the formation of impurities encountered through linear route. In convergent strategy *p*-nitrobenzyl cyanide **(1)** was transformed into 1,2,4-oxadiazole derivative **(11)** (Scheme 2) [15], by reacting amidoxime **(9)** with a suitably activated derivative such as acetyl chloride, to obtain *O*-acetyl amidoxime **(10)**. Cyclodehydration of **(10)** by refluxing in toluene by using molecular sieves led to formation of oxadiazole **(11)**.

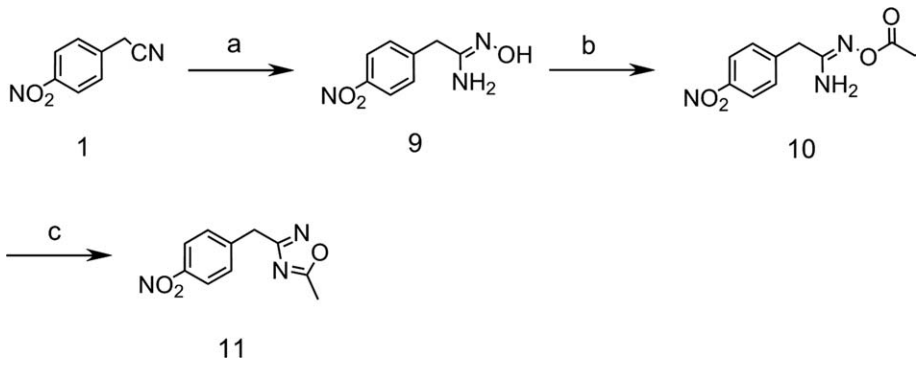
Knoevenagel condensation of active methylene of **(11)** with 3-formylchromone **(2)** [14] in dry pyridine [16] under reflux resulted in the formation of compound **(7a–h)** in 35–76% yield (Scheme 3). The structures of synthesized compounds were confirmed by ^{1}H NMR, IR, and Mass spectral analysis.

All the oxadizolyl chromones **(7a–h)** were assayed for their *in vitro* antibacterial activity by MIC determination against a panel of pathogenic bacterial strains such as *E. coli*, *Staphylococcus aureus* (''Smith''), *S. aureus* (MRSA), *E. faecalis*, and *S. Pneumonia*. The results are furnished in Table 1 indicate that, among the oxadiazolyl chromone analogues, **(7a)**, which is unsubstituted at $\mathbf{R^2}$ of the chromones ring, lacks significant activity. Introduction of **—Cl**, **—Br**, **F**, and **—CH_3** at $\mathbf{R^2}$ of chromone ring, thereby producing analogues **(7b)**, **(7c)**, **(7d)**, and **(7g)** respectively, did not alter the activity profile and continued to demonstrate the lack of activity similar to **(7a)** against the bacterial strains tested. However

Scheme 1. Linear synthesis. Reagents and conditions: (a) Ac_2O, sodium acetate, 80°C; (b) Hydroxylamine hydrochloride, $NaHCO_3$, methanol, reflux; (c) Acetyl chloride, K_2CO_3 in 1,4-dioxane at room temperature, refluxed in toluene with molecular sieves 4Å.



Scheme 2. Synthesis of 5-Methyl-3-(4-nitrobenzyl)-[1,2,4]oxadiazoles **11**. Reagents and conditions: (a) Hydroxylamine hydrochloride, $NaHCO_3$, methanol, reflux, 94%; (b) Acetyl chloride, K_2CO_3, 1,4-dioxane, 66%; (c) Molecular sieves, toluene, reflux, 61%.



(7a) and **(7c)** demonstrated a modest indication of activity against *S. pneumoniae* (MIC 16 μg mL^{-1}).

Introduction of **—Cl** at $\mathbf{R^4}$ of chromones ring, there by producing **(7e)** exhibited enhanced activity as compared to its mono-chloro analogue **(7b)**. Thus **(7e)** showed a further enhancement in the activity by at least twofolds against *S. pneumoniae* and threefolds against *E. faecalis* as compared to **(7b)**.

The improvement in the activity was observed with **(7f)**, having **—CH_3** substitution at $\mathbf{R^3}$ of chromones ring. This chemical modification resulted in an increase in activity by at least twofold against methicillin sensitive strain of *S. aureus* [*S. aureus* (ATCC 13709 ''Smith'')] and *S. pneumoniae* respectively, and onefold against MRSA strain [*S. aureus* 032 (Is an MRSA strain and was obtained as a clinical isolate)], as compared to **(7b)** an unsubstituted $\mathbf{R^3}$ analogue.

Similarly, augmentation in the activity was also noticed after introduction of **—CH_3** at $\mathbf{R^4}$ of chromones ring, thereby producing **(7h)**, a dimethyl analogue of **(7g)**. As compared to **(7g)**, single —CH_3 substitution resulted in an increase in activity by at least threefold against *E. faecalis* and *S. pneumoniae* and twofold against methicillin sensitive strain of *S. aureus* [*S. aureus* (ATCC 13709 ''Smith'')].

Among all the compounds synthesized **(7e)**, **(7f)**, and **(7h)** showed the potential to demonstrate anti-bacterial activity. Of particular interest was the compound **(7f)**, which showed remarkable indication for activity against the multi-drug resistant MRSA strain, comparable to the activity of Gentamicin. Erythromycin, Ampicillin, and Ciprofloxacin were found to be inactive against this methicillin resistant *S. aureus* strain indicating the presence of underlying quinolone as well as macrolide resistance.

As discussed above, with the exception of Gentamicin, other antibacterial agents studied here such as Erythromycin, Ampicillin, and Ciprofloxacin did not show appreciable anti-MRSA activity. Till date, only Vancomycin, Linezolid, Tigecycline, and Daptomycin possess indications for the treatment of MRSA infections. Ciprofloxacin is a broad-spectrum quinolone antibacterial used commonly for the treatment of infections caused by Gram-negative pathogens. Erythromycin, Ampicillin, and Gentamicin are primarily used to treat Respiratory Tract Infections (RTI) infections caused by Gram-positive pathogens and certain atypical Gram-negative organisms. Exploratory studies aimed at understanding the mechanism of action of such compounds synthesised previously indicated a possible ''protein biosynthesis inhibition'' mediated mechanism of action [12].

In summary, convergent synthesis of substituted 3-((*Z*)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)-vinyl)-4*H*-chromen-4-ones **(7a–h)** were accomplished by combination of 3-formylchromones **(2)** and 5-Methyl-3-(4-nitrobenzyl)-[1,2,4]oxadiazoles **(11)**.

However, most of these targeted chemical entities exhibited modest spectrum of antibacterial activities against pathogenic bacterial strains. Nevertheless, compounds **(7f)** displayed indication for anti-MRSA activity providing a lead towards the optimization of the series described in this article, with a potential to result into a broad spectrum anti-bacterial agent.

Scheme 3. Convergent synthesis. Reagents and conditions: (a) Pyridine, reflux, 35–76%.

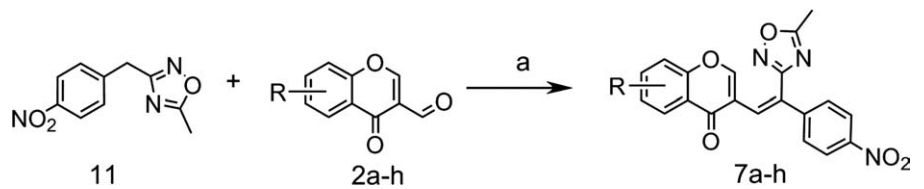
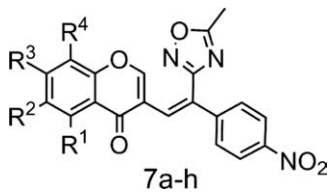


Table 1

Antibacterial activities of substituted 3-((*Z*)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4*H*-chromen-4-ones.



7a-h

					Antibacterial activity (MIC, μg mL^{-1})				
					Microorganism				
Entry	R^1	R^2	R^3	R^4	*S. aureus*[a]	*S. aureus*[b]	*E. faecalis*	*S. pneumoniae*	*E. coli*
7a	H	H	H	H	>32	>32	>32	16	>32
7b	H	Cl	H	H	>32	>32	>32	>32	>32
7c	H	Br	H	H	>32	>32	>32	16	>32
7d	H	F	H	H	>32	>32	>32	>32	>32
7e	H	Cl	H	Cl	32	>32	4	8	>32
7f	H	Cl	CH_3	H	8	16	>32	8	>32
7g	H	CH_3	H	H	>32	>32	>32	>32	>32
7h	H	CH_3	H	CH_3	8	>32	4	4	>32
Erythromycin					0.5	>32	2	0.12	>32
Ampicillin					0.25	>32	0.5	0.06	>32
Gentamicin					0.25	0.5	>32	>32	>32
Ciprofloxacin					0.12	>32	0.5	2	>32

[a] *S. aureus* (ATCC 13709 ''Smith'').
[b] *S. aureus* 032 (Is a MRSA strain and was obtained as a clinical isolate).

In conclusion, the present study revealed that a range of diverse substituents on chromones ring, at position **R^2**, **R^3**, and **R^4** modulate the antibacterial activity. Modest activity of the compounds described in this work suggest that the current linkages employed are obviously not optimum and therefore further work in the optimization of the linkage might lead to an improvement in the antibacterial profile.

EXPERIMENTAL

The melting points were determined on a Veego apparatus and are uncorrected. Infrared spectra were recorded on a Bruker spectrophotometer in a KBr disc, and the absorption bands are expressed in cm^{-1}. ^{1}H-NMR spectra were recorded on a Varian AS 400 MHz spectrometer in $CDCl_3$/DMSO-*d*-6, chemical shifts (δ) are in ppm relative to TMS, and coupling constants (*J*) are expressed in hertz (Hz). Mass spectra were taken on a Macro mass spectrometer (Waters) by electro-spray method (ES).

General procedure for synthesis of 9 and 11. A mixture of 4-nitrobenzyl cyanide **1** (6.5 g, 40 mmol), $NH_2OH{\cdot}HCl$ (14 gm, 200 mmol), $NaHCO_3$ (20 gm, 240 mmol) and methanol (100 mL) was refluxed 6 hr with vigorous stirring. The solvent was evaporated under vacuum, and ice-cold water (100 mL) was added to the crude reaction mass. The solid that precipitated was collected by filtration and dried under vacuum to give 7.4 g of 2-(4-nitrophenyl)acetamidoxime **9** as a white solid.

To a stirred solution of amidoxime (7.4 g, 39 mmol) in 1,4-dioxane (100 mL), powder K_2CO_3 (5.8 g, 41.7 mmol) was added. After 15 min, acetyl chloride (3 g, 39 mmol) was added and stirred at room temperature for 3 hr. After completion of the reaction, the solvent was evaporated under vacuum, and ice cold water (100 mL) was added to the crude reaction mass. The solid that precipitated was collected by filtration and dried under vacuum to give 6 g of *O*-acetyl amidoxime **10** as a white powder.

A suspension of *O*-acetyl amidoxime **10** in toluene (50 mL) with freshly dried molecular sieves (4 Å) was refluxed for 10 hr. After completion of reaction, the reaction mixture was cooled to room temperature and filtered. The product obtained was purified by column chromatography using mobile phase $CHCl_3$:MeOH (9:1) to afford 3.37 g of pure 5-Methyl-3-(4-nitrobenzyl)-[1,2,4]oxadiazoles **11** as a yellow solid.

2-(4-Nitrophenyl)acetamidoxime (9). Yield: 94.6%; mp 189–190°C; IR (KBr): 3456, 3449, 3116, 2845, 1672, 1603, 1509, 1353, 1112, 944, 899, 709 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*-6): 3.40 (s, 2H, CH_2), 5.50 (bs, 2H, NH_2), 7.50–7.55 (d, 2H, ArH, J = 8.4 Hz), 8.12–8.20 (d, 2H, ArH, J = 8.4 Hz), 8.97 (s, 1H, OH); ESI (+ve): 196.2.

5-Methyl-3-(4-nitrobenzyl)-[1,2,4]oxadiazoles (11). Yield: 61%; mp 68°C; IR (KBr): 1638, 1605, 1587, 1512, 1395, 1352, 1280, 1233, 853, 730 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 2.56 (s, 3H, CH_3), 4.14 (s, 2H, CH_2), 7.45–7.52 (d, 2H, ArH, J = 8.2 Hz), 8.15–8.20 (d, 2H, ArH, J = 8.2 Hz); ESI (+ve): 220.1.

General procedure for synthesis of 7(a–h). A mixture of 3-formylchromone **2** (150 mg, 0.86 mol), 5-Methyl-3-(4-nitrobenzyl)-[1,2,4]oxadiazole **11** (177 mg, 0.86 mol), and dry pyridine (2 mL) was refluxed for 24 hr with stirring under nitrogen. The reaction mixture was cooled to room temperature, slowly poured into 20 mL of 2*N* HCl. The pale-yellow product which then precipitated out was filtered and air dried. The product obtained was purified by column chromatography

using mobile phase hexane:ethyl acetate (9:1) to afford pure **7**. Compounds **7a–h** synthesized by this method are listed in Table 1. The physical, analytical, and spectral data of final compounds are given in the following text.

3-((Z)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4H-chromen-4-one (7a). Yield: 54%; mp 205–207°C; IR (KBr): 1654, 1615, 1595, 1571, 1518, 1466, 1353, 1315, 1260, 1224, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H, oxadiazole-CH_3), 7.30–7.45 (3H, 1 vinylic-CH and 2 ArH), 7.53–7.58 (d, 2H, J = 8.6 Hz, ArH), 7.63–7.70 (m, 1H, ArH), 7.99 (s, 1H, C2—H), 8.20–8.25 (d, 1H, J = 7.6 Hz, ArH), 8.26–8.30 (d, 2H, J = 8.6 Hz, ArH); ESI (+ve): 376.2; Anal. Calcd. for $C_{20}H_{13}N_3O_5$ (375.33): C, 64.00; H, 3.49; and N, 11.20. Found: C, 64.12; H, 3.52; and N, 11.23.

6-Chloro-3-((Z)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4H-chromen-4-one (7b). Yield: 76%; mp 214–216°C; IR (KBr): 1650, 1590, 1563, 1518, 1469, 1351, 1306 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.64 (s, 3H, oxadiazole-CH_3), 7.30–7.35 (d, 1H, J = 8.4 Hz, ArH), 7.37–7.39 (d, 1H, J = 0.8 Hz, vinylic-CH), 7.50–7.56 (d, 2H, J = 8.4 Hz, ArH), 7.60–7.62 (dd, 1H, J = 2.0 and 7 Hz, ArH), 7.94–7.96 (d, 1H, J = 0.8 Hz, C2—H), 8.18–8.20 (d, 1H, J = 2.0 Hz, ArH), 8.25–8.28 (d, 2H, J = 8.4 Hz, ArH); ESI (+ve): 410.3; Anal. Calcd. for $C_{20}H_{12}ClN_3O_5$ (409.79): C, 58.62; H, 2.95; and N, 10.25. Found: C, 58.69; H, 2.93; and N, 10.30.

6-Bromo-3-((Z)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4H-chromen-4-one (7c). Yield: 72%; mp 230–232°C; IR (KBr): 1664, 1598, 1561, 1515, 1464, 1429, 1351, 1302 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.63 (s, 3H, oxadiazole-CH_3), 7.29–7.27 (d, 1H, J = 8.8 Hz, ArH), 7.38 (d, 1H, J = 0.8 Hz, vinylic-CH), 7.52–7.54 (dd, 2H, J = 2.0 and 7.2 Hz, ArH), 7.72–7.75 (dd, 1H, J = 2.4 and 8.8 Hz, ArH), 7.94 (s, 1H, J = 0.8 Hz, C2—H), 8.25–8.29 (dd, 2H, J = 2.0 and 7.2 Hz, ArH), 8.34–8.35 (d, 1H, J = 2.4 Hz, ArH); ESI (+ve): 455.2; Anal. Calcd. for $C_{20}H_{12}BrN_3O_5$ (454.24): C, 52.88; H, 2.66; and N, 9.25. Found: C, 52.77; H, 2.71; and N, 9.30.

6-Fluoro-3-((Z)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4H-chromen-4-one (7d). Yield: 68%; mp 222–224°C; IR (KBr): 1651, 1630, 1590, 1519, 1482, 1351, 1325, 1306 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.63 (s, 3H, oxadiazole-CH_3), 7.34–7.38 (m, 2H, ArH), 7.38 (d, 1H, J = 1.2 Hz, vinylic-CH), 7.52–7.55 (d, 2H, J = 8.8 Hz, ArH),7.83–7.84 (m, 1H, Ar),7.95 (d, 1H, J = 1.2 Hz, C2—H), 8.25–8.28 (d, 2H, J = 8.8 Hz, ArH); ESI (+ve): 394.2; Anal. Calcd. for $C_{20}H_{12}FN_3O_5$ (393.33): C, 61.07; H, 3.08; and N, 10.68. Found: C, 61.10; H, 3.12; and N, 10.69.

6,8-Dichloro-3-((Z)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4H-chromen-4-one (7e). Yield: 66%; mp 210–212°C; IR (KBr): 1696, 1656, 1615, 1557, 1522, 1493, 1467, 1400, 1347, 1266 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H, oxadiazole-CH_3), 7.63–7.66 (dd, 2H, J =2.0 and 7.2 Hz, ArH), 7.76 (s, 1H, vinylic-CH), 7.83–7.84 (d, 1H, J = 2.4 Hz, ArH), 8.18–8.19 (d, 1H, J =2.4 Hz, ArH), 8.36–8.39 (dd, 2H, J =2.0 and 7.2 Hz, ArH), 8.54 (s, 1H, C2—H); ESI (+ve): 444.0; Anal. Calcd. for $C_{20}H_{11}Cl_2N_3O_5$ (443.23): C, 54.08; H, 2.50; and N, 9.46. Found: C, 54.17; H, 2.56; and N, 9.50.

6-Chloro-7-methyl-3-((Z)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4H-chromen-4-one (7f). Yield: 62%; mp 197–198°C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H, Ar-CH_3), 2.61 (s, 3H, oxadiazole-CH_3), 7.21 (s, 1H, ArH), 7.32 (s, 1H, vinylic-CH), 7.50–7.52 (d, 2H, J = 8.8 Hz, ArH), 7.86 (s, 1H, C2—H), 8.15 (s, 1H, Ar), 8.23–8.25 (d, 2H, J = 8.8 Hz, ArH); ESI (+ve): 424.1; Anal. Calcd. for $C_{21}H_{14}ClN_3O_5$ (423.82): C, 59.52; H, 3.33; and N, 9.91. Found: C, 59.60; H, 3.41; and N, 10.01.

6-Methyl-3-((Z)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4H-chromen-4-one (7g). Yield: 35%; mp 196–198°C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H, CH_3), 2.69 (s, 3H, oxadiazole-CH_3), 7.27 (s, 1H, ArH), 7.38 (s, 1H, vinylic-CH), 7.46–7.49 (dd, 1H, J = 2.0 and 8.4 Hz, ArH), 7.54 (d, 2H, J = 8.4 Hz, ArH), 7.99 (s, 1H, C2—H), 8.02 (s, 1H, ArH), 8.26–8.29 (d, 2H, J = 8.4 Hz, ArH); ESI (+ve): 390.2; Anal. Calcd. for $C_{21}H_{15}N_3O_5$ (389.37): C, 64.78; H, 3.88; and N, 10.79. Found: C, 64.84; H, 3.94; and N, 10.82.

6,8-Dimethyl-3-((Z)-2-(5-methyl-1,2,4-oxadiazol-3-yl)-2-(4-nitrophenyl)vinyl)-4H-chromen-4-one (7h). Yield: 30; mp 168–170°C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H, $ArCH_3$), 2.41 (s, 3H, $ArCH_3$), 2.63 (s, 3H, oxadiazole-CH_3), 7.32 (s, 1H, ArH), 7.43 (s, 1H, vinylic-CH), 7.55–7.57 (d, 1H, J = 8.4 Hz, ArH), 7.86 (s, 1H, ArH), 8.00 (s, 1H, C2—H), 8.27–8.30 (d, 2H, J = 8.4 Hz, ArH); ESI (+ve): 404.2; Anal. Calcd. for $C_{22}H_{17}N_3O_5$: (403.40): C, 65.50; H, 4.25; and N, 10.42. Found: C, 65.57; H, 4.32; and N, 10.45.

In vitro **antibacterial screening procedure.** All the oxadizolyl chromones **(7a–h)** were assayed for their *in vitro* antibacterial activity by MIC determination against a panel of pathogenic bacterial strains such as *E. coli* (ATCC 25922), *S. aureus* (ATCC 13709 ''Smith''), *S. aureus* 032, *E. faecalis* (ATCC 29212), and *S. pneumoniae* (ATCC 49619). *S. aureus* 032 is a methicillin resistant *S. aureus* (MRSA) strain and was obtained as a clinical isolate [17], and remaining strains were obtained from ATCC, USA. *S. aureus* (ATCC 13709 ''Smith'') is a wild type methicillin sensitive strain. Reference strains used as quality control for MIC testing included *S. aureus* ATCC 29213. Erythromycin, Gentamicin, Ampicillin, and Ciprofloxacin were recovered from their commercial preparations. The purities and potencies of the agents recovered from commercial preparations were documented by ascertaining the purity of >98.5% by high-pressure liquid chromatographic analysis and by showing that the MICs of antibacterials were within acceptable limits against quality control strains.

MICs were determined as per CLSI (Clinical and Laboratory Standards Institute) recommendations on Mueller Hinton Agar containing serial twofold dilutions of drugs [18]. For each strain, $\sim 10^4$ CFU were applied per spot using a multipoint inoculator (Applied Quality Services, UK). Incubations were done at 35°C, and growth was scored at 24 hr. In MIC studies, twofold differences were confirmed by a third repetition, and more frequent result were reported. MICs employing *S. pneumoniae* were determined as per CLSI recommendations on Mueller Hinton Agar supplemented with yeast extract (0.25%, Difco, US), glucose (0.5%, Sigma, India) and sheep blood (5%). The incubation was carried out in a CO_2 incubator (5% CO_2) [18]. Dimethyl sulfoxide and potent antibacterial drugs Erythromycin, Ampicillin, Gentamicin, and Ciprofloxacin, were used as solvent control and standards, respectively.

Acknowledgment. The authors are thankful to the Head of the Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, for constant encouragement and providing necessary facilities.

REFERENCES AND NOTES

[1] (a) Kaboudin, B.; Saadati, B. Tetrahedron Lett 2007, 48, 2829; (b) Gaonkar, S. L.; Rai, K. M.; Prabhuswamy, B. Eur J Med Chem 2006, 41, 841; (c) Jochims, J. C. In Comprehensive Heterocyclic Chemistry II; Katritzky, A. R., Rees, C. W., Scriven, E. F. V., Eds.; Pergamon press: New York, 1996; Vol. 4, p 179.

[2] Muhi-eldeen, Z.; Jumaa, G.; Al-kaissi, E.; Nouri, L.; Jordan J. Chemistry 2008, 03, 233.

[3] Tyrkov, A. V.; Sokhenko, L. T. Pharm Chem J 2004, 38, 30.

[4] McRipley, R. J.; Gadebusch, H. H.; Pansy, F.; Semar, R. Antimicrob Ag Chemother 1974, 06, 263.

[5] (a) Ghosh, C. K. Heterocycles 2004, 63, 2875; (b) Nohara, A.; Kuriki, H.; Saijo, T. J Med Chem 1977, 20, 141; (c) Frasinyuk, M. S.; Khilya, V. P. Khim Geterotsikl Soedin 1999, 379, 3; (d) Mateeva, N. N.; Kode, R. N.; Redda, K. K. J Heterocycl Chem 2002, 39, 1251; (e) Tim Cushnie, T. P.; Lamb, A. J. Int J Antimicrob Agents 2005, 26, 343.

[6] Lee, K. S.; Lee, Y. S. Bioorg Med Chem Lett 2005, 15, 2857.

[7] Chohan, Z. H.; Shaikh, A. U.; Supuran, C. T. J Enzyme Inhib Med Chem 2006, 21, 741.

[8] (a) Ghosh, C. K.; Ghosh, C. Indian J Chem 1997, 36B, 968, and references therein; (b) Sabitha, G. Aldrichim Acta 1996, 29, 15, and references therein; (c) Fitton, A. O.; Frost, J. R.; Suschitzky, H.; Houghton, P. G. Synthesis 1977, 2, 133.

[9] (a) Pinto, D. C. G. A.; Silva, A. M. S.; Cavaleiro, J. A. S.; Foces-Foces, C.; Llamas-Saiz, A. L.; Jagerovic, N.; Elguero, J. Tetrahedron 1999, 55, 10187; (b) Sigg, I.; Hass, G.; Winkler, T. Helv Chim Acta 1982, 65, 275; (c) Ghosh, C.; Tewari, N. J Org Chem 1980, 45, 1964; (d) Fitton, A. O.; Houghton, P. G.; Suschitzky, H. Synthesis 1979, 2, 337.

[10] (a) Koña, J.; Fabian, W. M. F.; Zahradnik, P. J Chem Soc Perkin Trans 2 2001, 3, 422; (b) Koña, J.; Zahradnik, P.; Fabian, W. M. F. J Org Chem 2001, 66, 4998.

[11] Borrell, J. I.; Teixidó, J.; Schuler, E.; Michelotti, E. Tetrahedron Lett 2001, 42, 5331.

[12] Diwakar, S. D.; Bhagwat, S. S.; Shingare, M. S.; Gill, C. H. Bioorg Med Chem Lett 2008, 18, 4678.

[13] Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. Vogel's Textbook of Practical Organic Chemistry, Vth ed., Longman Scientific & Technical: Essex, UK, 2004, p 857.

[14] Lacova, L.; Loos, D.; Furdik, M.; Matulova, M.; Hafez, M. Molecules 1998, 03, 149.

[15] Bora, R. O.; Farooqui, M. J Heterocycl Chem 2007, 44, 645.

[16] Ghosh, C. K. J Heterocycl Chem 1983, 20, 1437.

[17] De Souza, N. J.; Gupte, S. V.; Deshpande, P. K.; Desai, V. N.; Bhawsar, S. B.; Yeole, R. D.; Shukla, M. C.; Strahilevitz, J.; Hooper, D. C.; Bozdogan, B.; Appelbaum, P. C.; Jacobs, M. R.; Shetty, N.; Patel, M. V.; Jha, R.; Khorakiwala, H. F. J Med Chem 2005, 48, 5232.

[18] Clinical and Laboratory Standard Institute (Formerly NCCLS). Methods for Dilution for Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard M7-A6; NCCLS: Villanova, PA, 2003.