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Short communication

Design, synthesis, characterization, and antibacterial activity of {5-chloro-2-[(3-substitutedphenyl-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones

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ABSTRACT

In the present investigation, a series of novel {5-chloro-2-[(3-(substitutedphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**) have been synthesized from 5-(chloromethyl)-3-substitutedphenyl-1,2,4-oxadiazole (**2a**–**i**). The newly synthesized compounds were characterized by IR, NMR (^{1}H and ^{13}C), mass spectral and elemental analysis. The title compounds were investigated for *in-vitro* qualitative (zone of inhibition) and quantitative (MIC) antibacterial activity by agar cup plate and microtitration methods, respectively. The minimum inhibitory concentration and structure activity relationships (SARs) were evaluated. Amongst the synthesized compounds in this series, {5-chloro-2-[(3-(2,5-difluoro-4-methyl-phenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3d**) was found to exhibit significant activity with MICs of 21.5, 22.4, 29.8 and 30.6 μg/mL against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*, respectively.

1. Introduction

The 1,2,4-oxadiazole ring system has received considerable attention in the pharmaceutical industry as heterocyclic amide and ester isosteres [1]. Similar ring systems are present in various biologically interesting compounds such as muscarinic receptor agonists [2], tyrosine kinase inhibitors [3], anti-inflammatory agents [4], selective H_3 receptor antagonists [5], antitumor agents [6], monoamine oxidasea inhibitors [7], anticonvulsant [8], and anti-HIV agents [9]. Synthetic methods generating 1,2,4-oxadiazoles and the chemical properties of these compounds have been reviewed [10].

The efficiency of benzophenone derivatives as chemotherapeutic agents is well established and their chemistry has been extensively studied. A literature survey has revealed that benzophenone derivatives are associated with potent biological activities, such as, inhibition of HIV-1 reverse transcriptase RT [11], anticancer [12] and anti-inflammatory [13]. Benzophenones and their analogues are usually obtained from natural products [14] or by synthetic methods [15]. The importance of these substances is fundamentally due to their diverse biological [16] and chemical [17,18] characteristics and they are frequently used in medicine [19] and in industry [20]. Encouraged by these observations it was envisaged to integrate the 1,2,4-oxadiazole moiety with a benzophenone frame work to study a potential additive effect of the combined molecule towards antibacterial activity.

2. Chemistry

The synthetic route of the novel compounds (**3a**–**i**) is shown in Scheme 1. The title compounds {5-chloro-2-[(3-(substitutedphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**) were synthesized from the 5-(chloromethyl)-3-substitutedphenyl-1,2,4-oxadiazoles (**2a**–**i**). The latter was synthesized from the reaction between equimolar amounts of *N*′-hydroxybenzimidamides (**1a**–**i**) and chloroacetylchloride according to the procedure in literature [21–23]. The synthesis of *N*′-hydroxybenzimidamides (**1a**–**i**) was achieved from the corresponding substituted benzonitriles and hydroxylamine hydrochloride [24]. The synthesized intermediates (**1a**–**i**) and (**2a**–**i**) were characterized by ^{1}H NMR and LCMS whereas the title compounds (**3a**–**i**) were characterized by IR, NMR (^{1}H & ^{13}C-),

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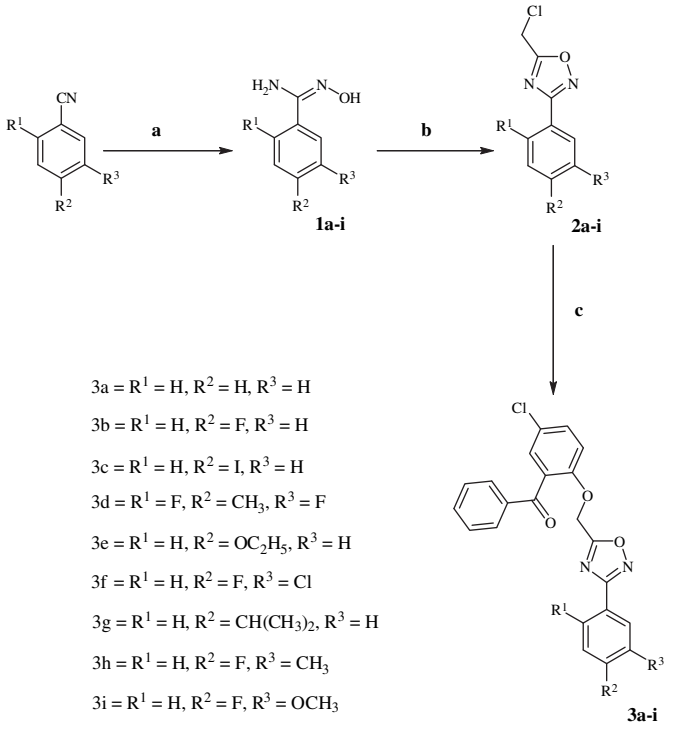


Scheme 1. Reagents for the synthesis of title compounds (**3a**–**i**): (a) $NH_2OH{\cdot}HCl$, Na_2CO_3, C_2H_5OH; (b) $ClCH_2COCl$, DIEA, DCE; (c) 2-hydroxy-5-chloro-benzophenone, K_2CO_3, acetone.

LC-MS and also by elemental analysis. The yields of **1a**–**i**, **2a**–**i** and **3a**–**i** were in the range of 55–77, 55–75 and 60–85%, respectively. The physicochemical characteristics of the title compounds are depicted in Table 1.

3. Results and discussion

In the ^{1}H NMR spectra of compounds (**1a**–**i**) $-NH_2$ protons are observed in the range of δ 4.75–5.84. Molecular mass of the compounds (**1a**–**i**) is in compliance with the molecular ion peak (M^+) on LC-MS.

In intermediates (**2a**–**i**), the alkyl protons are observed in the range of δ 4.73–4.77. Melting points for compounds (**2a**, **2b**) and (**2g**) were also consistent with those reported in the literature [21–23], respectively. Molecular mass of the compounds (**2c**–**f**, **2h** and **2i**) is in compliance with the molecular ion peak (M^+) on LC-MS.

In the IR spectra of the title compounds {5-chloro-2-[(3-(substitutedphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**) the carbonyl group (C=O) and C–Cl peaks were observed in the range of 1647–1661 and 726–758cm^{-1}, respectively. Aromatic C=C stretching is observed in the range of 1591–1616, 1475–1495 and 1444–1452. In the ^{1}H NMR spectrum of {5-chloro-2-[(3-(2,5-difluoro-4-methylphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3d**) singlet peak at δ 2.34 was due to methyl group. In LC-MS spectra, molecular ion peaks were in good agreement with proposed molecular weight and elemental analysis results were within ±0.4% of the calculated values of the proposed title compounds (**3a**–**i**).

All the title compounds (**3a**–**i**) were subjected for the determination of partition coefficient by the Shake flask method [25] using *n*-octanol/water system and the values were in the range of 1.8961–5.6726. Screening of the antibacterial activities on two gram negative (*E.c* and *K.p*) and two gram positive bacteria (*B.s* and *S.a*) were performed for a series of {5-chloro-2-[(3-(substitutedphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**). The diameters of the zone of inhibitions corresponding to the MICs are presented in Table 2. From analysis of the results we can conclude that compound {5-chloro-2-[(3-(2,5-difluoro-4-methyl-phenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3d**), the difluoro analog, showed significant MIC at 21.5 μg/mL against *B.s* and moderate activity at 22.4, 29.8, and 30.6 μg/mL against *S.a*, *E.c* and *K.p*, respectively. This activity compares well with the other analogs in the series. The 4-fluoro 3-methoxy analog (**3i**) showed significant activity against *S. a* at 30.0 μg/mL and moderate activities at 29.8, 30.6 and 30.8 μg/mL against *B.s*, *E.c* and *K.p*, respectively. Analog (**3h**) which is 4-fluoro and 3-methyl showed significant activities against *E.c* and *K.p* at 29.6 and 30.0 μg/mL, respectively, and moderate activities against *B.s* and *S.a* at 30.6, 40.0 μg/mL, respectively. 3-Chloro and 4-fluoro analog (**3f**) exhibited significant activity against *B.s* at 29.8 and moderate activities against *S.a*, *E.c* and *K.p* at 30.6, 30.8 and 39.6 μg/mL, respectively. Compound **3e** having ethoxy functional group at fourth position revealed significant activity against *S.a* at 29.8 μg/mL and moderate activity against *K.p*, *B.s* and *E.c* at 40.0, 42.2 and 59.4 μg/mL, respectively. Monofluoro analog **3b** exhibited significant activity against *E.c* at 30.8 μg/mL and moderate activity against *B.s*, *S.a* and *K.p* at 39.6, 40.6 and 42.0 μg/mL, respectively. Whereas unsubstituted analog **3a**, monoiodo analog **3c** and isopropyl analog **3g** exhibited moderate activity against *B.s*, *S.a*, *E.c* and *K.p* when compared to other test samples and standard substance.

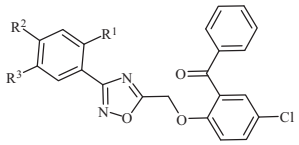
4. Conclusion

We have synthesized several {5-chloro-2-[(3-(substituted-phenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**) and the yields were found to be satisfactory (Table 1). Phenyl group bearing groups such as chloro, fluoro, iodo, methyl, methoxy, ethoxy and isopropyl group were connected to 1,2,4-oxadiazole. The latter was in turn attached to 5-chloro-benzophenone through an ether linkage at the 5th position through a $-CH_2-$ linker. The antibacterial activity of these compounds was determined against two gram positive and gram negative bacterial strains. Compounds **3f** and **3i** exhibited similar pattern of activity against *Bacillus subtilis* whereas all other analogs revealed different spectrum of activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*.

5. Experimental

Chemicals were procured from Aldrich chemical company. Reactions were monitored with thin layer chromatography (TLC) and LCMS. TLC was performed on Merck 60 F-254 silica gel plates and visualization under UV-light using ethyl acetate:*n*-hexane as solvent system. Melting points were determined on a Büchi Melting Point B-545 apparatus and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Nicolet 6700 FT-IR spectrometry. ^{1}H and ^{13}C NMR spectra were recorded on Bruker (300 and 400 MHz) spectrometer instruments, in $CDCl_3$ and DMSO d^6. Chemical shifts were recorded in parts per million downfield from tetramethylsilane. Mass spectra were recorded using a LC-MS Aglilent 1100 series with MSD (Ion trap) using 0.1% aqueous TFA in acetonitrile system on C18-BDS column for 10 min duration and elemental analysis was performed on Thermo Finningan FLASH EA 1112 CHN analyzer. Analysis results were within 0.4% of the calculated value. Column chromatography was performed on silica gel (230–400 mesh) supplied by Acme Chemical Co. (India) for compound purification.

Table 1
Physicochemical characteristics of {5-chloro-2-[(3-(substituted phenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**)



3a-i

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Comp code	R^1	R^2	R^3	M F (M. Wt.)[a]	Yield[b] (%)	m.p. (°C)	Cryst. solvent	Nature of product	*c* log *P*
3a	H	H	H	$C_{22}H_{15}N_2O_3Cl$ (390)	72	108–110	Methanol	White solid	1.8961
3b	H	F	H	$C_{22}H_{14}N_2O_3ClF$ (408)	75	96–97	Methanol	Pale yellow solid	3.1675
3c	H	I	H	$C_{22}H_{14}N_2O_3ClI$ (516)	80	124–126	Methanol	Yellow solid	4.0612
3d	F	CH_3	F	$C_{23}H_{15}N_2O_3ClF_2$ (440)	68	87–89	Ethanol	Pale yellow solid	5.6726
3e	H	OC_2H_5	H	$C_{24}H_{19}N_2O_4Cl$ (434)	75	80–82	Ethanol	Yellow solid	3.9251
3f	H	F	Cl	$C_{22}H_{13}N_2O_3Cl_2F$ (442)	81	85–86	Methanol	Pale yellow solid	4.8762
3g	H	$CH(CH_3)_2$	H	$C_{25}H_{21}N_2O_3Cl$ (432)	76	110–112	Ethanol	White solid	4.2670
3h	H	F	CH_3	$C_{23}H_{16}N_2O_3ClF$ (422)	85	73–74	Ethanol	Pale yellow solid	4.6173
3i	H	F	OCH_3	$C_{23}H_{16}N_2O_4ClF$ (438)	60	84–85	Ethanol	Pale yellow solid	4.1417

[a] Detailed results of elemental analysis are given in Section 5.3.
[b] All the yields are on isolated basis. All the compounds gave satisfactory results for elemental analysis. Purified by silica gel flash column chromatography with ethyl acetate:*n*-hexane (7:3).

5.1. General procedure for the synthesis of *N′*-hydroxybenzimidamide (amidoximes)(***1a***–***i***)

To a solution of hydroxylamine hydrochloride (0.121 mol) and sodium carbonate (0.077 mol) in water (100 mL), a solution of substituted benzonitriles (0.048 mol) in ethanol (50 mL) was added. The reaction mixture was heated at reflux temperature for 8 h. After cooling the reaction mixture, the solvent was removed in vacuum and extracted with ethyl acetate. The combined ethyl acetate layer was washed with water, brine and dried over sodium sulfate and concentrated to obtain a solid which was recrystallized using aqueous ethanol. The characterization data is presented below and compare well with literature.

5.1.1. *N′*-Hydroxybenzimidamide (***1a***)
Yield 70%; m.p. 72.5–73.6 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 5.82 (bs, 2H), 7.35–7.39 (m, 3H), 7.67.70 (m, 2H), 9.66 (bs, 1H); MS: $m/z = 137.1$ (M^+).

5.1.2. 4-Fluoro-*N′*-hydroxybenzimidamide (***1b***)
Yield 55%; m.p. 63.5–64.7 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 5.84 (bs, 2H), 7.19 (m, 2H), 7.68 (m, 2H), 9.63 (s, 1H); MS: $m/z = 155.1$ (M^+).

5.1.3. *N′*-Hydroxy-4-iodobenzimidamide (***1c***)
Yield 72%; m.p. 155–161 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 5.84 (bs, 2H), 7.45 (d, 2H), 7.73 (d, 2H), 9.72 (s, 1H); MS: $m/z = 263.0$ (M^+).

Table 2
In-vitro antibacterial activity data of {5-chloro-2-[(3-(substituted phenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**).

Compound	Control	Zone of inhibition in mm (MIC in μg/mL)			
		B. subtilis	*S. aureus*	*E. coli*	*K. pneumonia*
3a	–	14(41.0)	09(68.0)	10(64.2)	10(62.0)
3b	–	15(39.6)	14(40.6)	16(30.8)	13(42.0)
3c	–	16(30.8)	13(41.6)	10(62.2)	12(46.0)
3d	–	27(21.5)	25(22.4)	19(29.8)	17(30.6)
3e	–	13(42.2)	19(29.8)	11(59.4)	15(40.0)
3f	–	19(29.8)	17(30.6)	16(30.8)	15(39.6)
3g	–	14(40.6)	15(39.6)	11(60.8)	10(62.0)
3h	–	17(30.6)	15(40.0)	20(29.6)	18(30.0)
3i	–	19(29.8)	18(30.0)	17(30.6)	16(30.8)
Ampicillin	–	32(3.28)	31(3.36)	30(3.88)	30(4.00)

5.1.4. 2,5-Difluoro-*N′*-hydroxy-4-methylbenzimidamide (***1d***)
Yield 65%; m.p. 73.4–74.6 °C; ^{1}H NMR (DMSO-d_6) δ ppm: 2.23 (s, 3H), 5.83 (bs, 2H), 7.18–7.26 (m, 2H), 9.73 (s, 1H); MS: $m/z = 187.2$ (M^+).

5.1.5. 4-Ethoxy-*N′*-hydroxybenzimidamide (***1e***)
Yield 75%; m.p. 109.1–110.2 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 1.40–1.43 (t, 3H, CH_3), 4.02–4.07 (q, 2H, OCH_2), 4.88 (bs, 2H), 6.87–6.92 (d, 2H), 7.52–7.56 (d, 2H); MS: $m/z = 181.2$ (M^+).

5.1.6. 3-Chloro-4-fluoro-*N′*-hydroxybenzimidamide (***1f***)
Yield 69%; m.p. 73.1–74.2 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 4.74 (bs, 2H), 7.24–7.29 (m, 1H), 7.97–8.00 (m, 1H), 8.16–8.18 (m, 1H); MS: $m/z = 189.1$ (M^+).

5.1.7. *N′*-Hydroxy-4-isopropylbenzimidamide (***1g***)
Yield 71%; m.p. 60.1–61.2 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 1.24–1.26 (d, 6H, 2× CH_3), 2.89–2.96 (q, $J = 6.83$ Mz, 1H), 4.90 (bs, 2H), 7.24–7.26 (m, 2H), 7.54–7.56 (m, 2H); MS: $m/z = 179.11$ (M^+).

5.1.8. 4-Fluoro-*N′*-hydroxy-3-methylbenzimidamide (***1h***)
Yield 77%; m.p. 84.5–85.6 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 2.31 (s, 3H, CH_3), 4.9 (bs, 2H), 6.99–7.03 (m, 1H), 7.39–7.47 (m, 2H); MS: $m/z = 169.0$ (M^+).

5.1.9. 4-Fluoro-*N′*-hydroxy-3-methoxybenzimidamide (***1i***)
Yield 67%; m.p. 99.5–100.6 °C; ^{1}H NMR ($CDCl_3$) δ ppm: 3.95 (s, OCH_3), 4.96 (bs, 2H), 7.16–7.20 (m, 1H), 7.64–7.68 (m, 2H); MS: $m/z = 185.16$ (M^+).

5.2. General procedure for the synthesis of 5-(chloromethyl)-3-substitutedphenyl-1,2,4-oxadiazole (***2a***–***i***)

To a solution of *N′*-hydroxy substitutedbenzimidamides (0.0367 mol) in dichloroethane, diisopropylethylamine (DIEA) (0.0733 mol) was added followed by the drop wise addition of chloroacetylchloride (0.0367 mol) at 0 °C. The reaction mixture was stirred for 4 h at room temperature, then heated under reflux conditions overnight (18 h). The reaction mixture was concentrated and diluted with ethyl acetate, washed with water, brine and dried over sodium sulfate. The solution was concentrated to dryness and purified by flash column chromatography using ethyl acetate and

petroleum ether (1:9) as eluent. The characterization data of the intermediates (**2a**–**i**) is presented below and compare well with literature.

*5.2.1. 5-(Chloromethyl)-3-phenyl-1,2,4-oxadiazole (**2a**) [21]*

Yield 55%; Semi solid; ^{1}H NMR ($CDCl_3$) δ ppm: 4.76 (s, 2H), 7.48–7.54 (m, 3H), 8.08–8.11 (m, 2H); MS: $m/z = 195.2$ (M^+).

*5.2.2. 5-(Chloromethyl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (**2b**) [22]*

Yield 66%; Semi solid; ^{1}H NMR ($CDCl_3$) δ ppm: 4.75 (s, 2H), 7.71–7.21 (m, 2H), 8.08–8.11 (m, 2H); MS: $m/z = 213.2$ (M^+).

*5.2.3. 5-(Chloromethyl)-3-(4-iodophenyl)-1,2,4-oxadiazole (**2c**)*

Yield 70%; Semi solid; ^{1}H NMR ($CDCl_3$) δ ppm: 4.74 (s, 2H), 7.79–7.86 (m, 4H); MS: $m/z = 321.1$ (M^+).

*5.2.4. 5-(Chloromethyl)-3-(2,5-difluoro-4-methylphenyl)-1,2,4-oxadiazole (**2d**)*

Yield 62%; Semi solid; ^{1}H NMR ($CDCl_3$) δ ppm: 2.35 (s, 3H), 4.77 (s, 2H), 7.06–7.71 (m, 1H), 7.68–7.72 (m, 1H); MS: $m/z = 245.1$ (M^+).

*5.2.5. 5-(Chloromethyl)-3-(4-ethoxyphenyl)-1,2,4-oxadiazole (**2e**)*

Yield 72%; Semi solid; ^{1}H NMR ($CDCl_3$) δ ppm: 1.43–1.46 (t, 3H, CH_3), 4.07–4.12 (q, 2H, OCH_2), 4.73 (s, 2H), 6.96–6.99 (m, 2H), 7.99–8.02 (m, 2H); MS: $m/z = 239.1$ (M^+).

*5.2.6. 3-(3-Chloro-4-fluorophenyl)-5-(chloromethyl)-1,2,4-oxadiazole (**2f**)*

Yield 75%; Semi solid; ^{1}H NMR ($CDCl_3$) δ ppm: 4.74 (s, 2H), 6.98–6.20 (m, 1H), 8.00–8.03 (m, 1H), 8.18–8.21(m, 1H); MS: $m/z = 247.0$ (M^+).

*5.2.7. 5-(Chloromethyl)-3-(4-isopropylphenyl)-1,2,4-oxadiazole (**2g**) [23]*

Yield 67%; Semi solid; ^{1}H NMR ($CDCl_3$) δ ppm: 1.27–129 (d, 6H, 2× CH_3), 2.93–3.00 (q, $J = 6.83$ Mz, 1H). 4.74 (s, 2H), 7.33–7.35 (m, 2H), 7.99–8.01 (m, 2H); MS: $m/z = 237.1$ (M^+).

*5.2.8. 5-(Chloromethyl)-3-(4-fluoro-3-methylphenyl)-1,2,4-oxadiazole (**2h**)*

Yield 62%; Semi solid; ^{1}H NMR ($CDCl_3$) δ ppm: 2.34 (s, 3H, CH_3), 4.74 (s, 2H), 7.09–7.13 (m, 1H), 7.87–7.95 (m, 2H); MS: $m/z = 227.3$ (M^+).

*5.2.9. 5-(Chloromethyl)-3-(4-fluoro-3-methoxyphenyl)-1,2,4-oxadiazole (**2i**)*

Yield 72%; Semi solid; ^{1}H NMR ($CDCl_3$) δ ppm: 3.97 (s, CH_3), 4.74 (s, 2H), 7.16–7.20 (m, 1H), 7.64–7.68 (m, 2H); MS: $m/z = 243.2$ (M^+).

*5.3. General procedure for the preparation {5-chloro-2-[(3-(substitutedphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**)*

A mixture of 5-(chloromethyl)-3-substitutedphenyl-1,2,4-oxadiazole (**2a**–**i**) (50 mmol), 2-hydroxy-5-chloro-benzophenone (50 mmol), dried acetone (150 mL) and ultra dried potassium carbonate (100 mmol) was heated under reflux for 8 h, cooled to room temperature and filtered. The filtrate was evaporated to dryness and the residue obtained was purified by column chromatography using ethyl acetate and petroleum ether (3:7) as eluent (60–120 silica gel). The physical characteristics of the title compound (**3a**–**i**) are given in Table 1 and characterization data is presented.

*5.3.1. {5-Chloro-2-[(3-(phenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3a**)*

IR (cm^{-1}): 3063 (ArC–H), 1652 (C=O), 1596, 1483, 1448 (C=C), 1243 (C–O–C), 726 (C–Cl); ^{1}H NMR ($CDCl_3$) δ ppm: 5.27 (s, 2H, OCH_2), 7.05–7.08 (d, $J = 8.60$ Hz, 1H), 7.27–7.58 (m, 8H), 7.81–7.84 (m, 2H), 7.08–8.02 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 62.06, 98.32, 114.90, 125.62, 125.62, 128.01, 128.46, 128.94, 129.79, 131.42, 131.64, 133.51, 136.98, 138.19, 153.67, 167.93, 174.03, 194.08; LC-MS: $m/z = 391.0$ (M^+); Anal. calcd for $C_{22}H_{15}N_2O_3Cl$: C, 67.61; H, 3.87; N, 7.17; Found: C, 67.54; H, 3.95; N, 7.03.

*5.3.2. {5-Chloro-2-[(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3b**)*

IR (cm^{-1}): 3064 (ArC–H), 1652 (C=O), 1591, 1483, 1449 (C=C), 1263 (C–O–C), 743 (C–Cl); ^{1}H NMR ($CDCl_3$) δ ppm: 5.26 (s, 2H, OCH_2), 7.05–7.07 (d, $J = 8.58$ Hz, 1H), 7.14–7.20 (m, 2H), 7.42–7.47 (m, 4H), 7.54–7.59 (m, 1H), 7.81–7.83 (d, $J = 7.2$ Hz, 2H), 8.01–8.05 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 62.02, 114.88, 116.03, 116.26, 122.37, 122.40, 127.94, 128.46, 129.63, 129.72, 129.79, 131.39, 131.63, 133.51, 136.98, 153.69, 163.45, 165.96, 167.64, 173.93, 194.10; LC-MS: $m/z = 409.0$ (M^+); Anal. calcd for $C_{22}H_{14}N_2O_3ClF$: C, 64.64; H, 3.45; N, 6.85; Found: C, 64.52; H, 3.56; N, 6.75.

*5.3.3. {5-Chloro-2-[(3-(4-iodophenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3c**)*

IR (cm^{-1}): 3063 (ArC–H), 1655 (C=O), 1592, 1475, 1449 (C=C), 1260 (C–O–C), 743 (C–Cl), 522 (C–I); ^{1}H NMR ($CDCl_3$) δ ppm: 5.26 (s, 2H, OCH_2), 7.04–7.07 (d, $J = 8.61$ Hz, 1H), 7.41–7.47 (m, 4H), 7.54–7.56 (m, 1H), 7.76–7.86 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 62.06, 98.32, 114.91, 125.62, 128.01, 128.46, 128.94, 129.79, 131.42, 131.63, 133.51, 136.98, 138.19, 153.67, 167.93, 174.03, 194.08; LC-MS: $m/z = 517.0$ (M^+); Anal. calcd for $C_{22}H_{14}N_2O_3ClI$: C, 51.14; H, 2.73; N, 5.42. Found: C, 51.08; H, 2.89; N, 5.32.

*5.3.4. {5-Chloro-2-[(3-(2,5-difluoro-4-methylphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3d**)*

IR (cm^{-1}): 3068 (ArC–H), 2924 (C–H), 1654 (C=O), 1593, 1482, 1452 (C=C), 1264 (C–O–C), 747 (C–Cl); ^{1}H NMR ($CDCl_3$) δ ppm: 2.34 (s, 3H, CH_3), 5.28 (s, 2H, OCH_2), 7.03–7.09 (m, 2H), 7.41–7.47 (m, 4H), 7.54–7.63 (m, 2H), 7.80–7.83 (d, $J = 7.20$ Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 14.86, 14.88, 61.89, 112.62, 112.71, 112.77, 112.85, 114.77, 116.05, 116.08, 116.32, 116.35, 119.03, 119.09, 119.27, 119.32, 127.96, 128.48, 129.76, 130.60, 130.68, 130.79, 130.88, 131.37, 131.62, 133.54, 136.98, 153.60, 154.98, 155.00, 155.84, 155.87, 157.50, 157.53, 158.25, 158.27, 164.62, 164.64, 164.68, 164.70, 173.58, 194.11; LC-MS: $m/z = 441.0$ (M^+); Anal. calcd for $C_{23}H_{15}N_2O_3ClF_2$: C, 62.67; H, 3.43; N, 6.35. Found: C, 62.54; H, 3.56; N, 6.21.

*5.3.5. {5-Chloro-2-[(3-(4-ethoxyphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3e**)*

IR (cm^{-1}): 3075 (ArC–H), 2973 (C–H), 1658 (C=O), 1592, 1476, 1446 (C=C), 1276 (C–O–C), 741 (C–Cl); ^{1}H NMR ($CDCl_3$) δ ppm: 1.43–1.47 (t, 3H, CH_3), 4.06–4.13 (q, 2H, OCH_2), 5.24 (s, 2H, OCH_2), 6.95–6.98 (m, 2H), 7.04–7.07 (d, $J = 8.49$ Hz, 1H), 7.41–7.46 (m, 4H), 7.53–7.56 (m, 1H), 7.80–7.93 (m, 2H), 7.94–7.96 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.64, 61.92, 63.58, 114.71, 114.75, 118.27, 127.76, 128.37, 129.02, 129.67, 129.71, 131.28, 131.53, 133.40, 136.93, 153.66, 161.49, 168.11, 173.39, 194.11; LC-MS: $m/z = 435.2$ (M^+); Anal. calcd for $C_{24}H_{19}N_2O_4Cl$: C, 66.29; H, 4.40; N, 6.44. Found: C, 66.13; H, 4.51; N, 6.15.

*5.3.6. {5-Chloro-2-[(3-(3-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3f**)*

IR (cm^{-1}): 3061 (ArC–H), 1661 (C=O), 1595, 1480, 1448 (C=C), 1260 (C–O–C), 742 (C–Cl); ^{1}H NMR ($CDCl_3$) δ ppm: 5.26 (s, 2H, OCH_2), 7.04–7.07 (d, $J = 8.70$ Hz, 1H), 7.22–7.27 (m, 1H), 7.28–7.47

(m, 4H), 7.55–7.57 (m, 1H), 7.80–7.83 (m, 2H), 7.90–7.95 (m, 1H), 8.08–8.09 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 62.04, 114.90, 117.18, 117.40, 121.95, 122.13, 123.39, 123.43, 127.48, 127.56, 128.04, 128.47, 129.78, 130.05, 131.42, 131.65, 133.97, 153.62, 158.73, 161.26, 166.80, 174.24, 194.05; LC-MS: $m/z = 443.0$ (M^+); Anal. calcd for $C_{22}H_{13}N_2O_3Cl_2F$: C, 59.61; H, 2.96; N, 6.32. Found C, 59.39; H, 3.10; N, 6.23.

*5.3.7. {5-Chloro-2-[(3-(4-isopropylphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3g**)*

IR (cm^{-1}): 3062 (ArC–H), 2960 (C–H), 1651 (C=O), 1593, 1479, 1449 (C=C), 1261 (C–O–C), 758 (C–Cl); ^{1}H NMR ($CDCl_3$) δ ppm: 1.26–1.30 (d, 6H, 2× CH_3), 2.95–2.97 (q, $J = 6.84$ Mz, 1H), 5.25 (s, 2H, OCH_2), 7.04–7.07 (m, 1H), 7.33–7.35 (d, $J = 8.16$ Hz, 2H), 7.41–7.47 (m, 4H), 7.53–7.56 (m, 1H), 7.81–7.84 (m, 2H), 7.94–7.97 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 23.76, 34.20, 61.96, 114.78, 123.63, 127.04, 127.54, 127.83, 128.45, 129.75, 129.79, 131.32, 131.61, 133.49, 137.00, 152.75, 153.70, 168.42, 173.62, 194.19; LC-MS: $m/z = 433.2$ (M^+); Anal. calcd for $C_{25}H_{21}N_2O_3Cl$: C, 69.36; H, 4.89; N, 6.47. Found: C, 69.25; H, 4.95; N, 6.33.

*5.3.8. {5-Chloro-2-[(3-(4-fluoro-3-methylphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3h**)*

IR (cm^{-1}): 3050 (ArC–H), 2932 (C–H), 1655 (C=O), 1616, 1483, 1450 (C=C), 1259 (C–O–C), 750 (C–Cl); ^{1}H NMR ($CDCl_3$) δ ppm: 2.33 (s, 3H, CH_3), 5.25 (s, 2H, OCH_2), 7.04–7.13 (m, 2H), 7.41–7.46 (m, 4H), 7.53–7.56 (m, 1H), 7.80–7.89 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 14.51, 62.01, 114.86, 115.59, 115.82, 121.94, 121.97, 125.76, 125.94, 126.91, 127.00, 127.89, 128.45, 129.77, 130.81, 130.87, 131.34, 131.63, 133.49, 136.99, 153.70, 162.03, 164.53, 167.76, 173.82, 194.10; LC-MS: $m/z = 423.0$ (M^+); Anal. calcd for $C_{23}H_{16}N_2O_3ClF$: C, 65.33; H, 3.81; N, 6.63. Found C, 65.21; H, 3.94; N, 6.55.

*5.3.9. {5-Chloro-2-[(3-(4-fluoro-3-methoxyphenyl)-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanone (**3i**)*

IR (cm^{-1}): 3075 (ArC–H), 2970 (C–H), 1647 (C=O), 1612, 1495, 1444 (C=C), 1259 (C–O–C), 740 (C–Cl); ^{1}H NMR ($CDCl_3$) δ ppm: 3.95 (s, OCH_3), 5.25 (s, OCH_2), 7.04–7.07 (d, $J = 8.61$ Hz, 1H), 7.13–7.19 (m, 1H), 7.41–7.46 (m, 4H), 7.53–7.64 (m, 3H), 7.79–7.82 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 56.24, 62.00, 112.13, 112.17, 114.89, 116.34, 116.59, 120.55, 120.65, 122.46, 122.51, 127.86, 128.37, 129.67, 131.33, 131.56, 133.41, 136.92, 147.95, 148.10, 152.66, 153.62, 156.00, 167.65, 167.67, 173.87, 193.98; LC-MS: $m/z = 439.0$ (M^+); Anal. calcd for $C_{23}H_{16}N_2O_4ClF$: C, 62.95; H, 3.67; N, 6.38. Found: C, 62.81; H, 3.76; N, 6.21.

6. Antibacterial activity

6.1. Antibacterial activity (determination of zone of inhibition)

The antibacterial activity [26,27] of the test samples (**3a**–**i**) was determined by agar cup plate method using four organisms such as *B. subtilis* (NCIM, 2063; ATCC 6633), *S. aureus* (NCIM, 2079; ATCC 6538P), *E. coli* (NCIM, 2065; ATCC 8739) and *K. pneumoniae* (NCIM, 2957; ATCC 11298) (organisms are recultured) and a standard drug, Ampicillin. This method was based on diffusion of antibacterial component from reservoir bore to the surrounding inoculated nutrient agar medium so that the growth of microorganisms was inhibited as circular zone around the bore. The concentration of test compounds was 100 μg/mL. It was prepared in 20% water in dimethyl sulfoxide (DMSO). The test samples and standard drugs were placed in a bore made in petridishes which contained different organisms and incubated at 37 °C for 24 h. The zone of inhibition around the bore was measured after 24 h. The antibacterial activity data of {5-chloro-2-[(3-substitutedphenyl-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**) are recorded in Table 2. The values reported in the table are the average of three experiments.

6.2. Determination of minimum inhibitory concentration

The determination of minimum inhibitory concentration [28,29] was done with same isolates of organisms and the MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. The results of {5-chloro-2-[(3-substitutedphenyl-1,2,4-oxadiazol-5-yl)-methoxy]-phenyl}-(phenyl)-methanones (**3a**–**i**) are recorded in Table 2.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2010.02.021.

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