European Journal of Medicinal Chemistry 44 (2009) 4522–4527

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, characterization and antibacterial activity of 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazoles

Neithnadka Premsai Rai [a,c], Venugopala Katharigatta Narayanaswamy [b], Sheena Shashikanth [a,*], Pirama Nayagam Arunachalam [c]

[a] Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, India
[b] Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Hosur Road, Opp. Lalbagh Main Gate, Bangalore 560 027, Karnataka, India
[c] Syngene International Ltd., Biocon Park, Plot #2 and 3, Bommasandra – Jigani Road, Bangalore 560 099, Karnataka, India

ARTICLE INFO

Article history:
Received 5 January 2009
Received in revised form
17 June 2009
Accepted 19 June 2009
Available online 26 June 2009

Keywords:
2-[1-(5-Chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazoles
Antibacterial activity
Minimum inhibitory concentration

ABSTRACT

In the present investigation a series of novel 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazoles (**4a–j**) were synthesized by cyclization of substituted-benzoic acid *N*′-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazole-4-carbonyl]-hydrazide by using phosphorousoxychloride at 120 °C. The chemical structure of the newly synthesized compounds was characterized by analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR and LC–MS) methods. The title compounds were screened for qualitative (zone of inhibition) and quantitative antibacterial activity (MIC) by agar cup plate and microtitration methods, respectively. Among the synthesized compounds in this series compound 2-[1-(5-chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(4-fluorophenyl)-1,3,4-oxadiazole (**4b**) was found to exhibit significant antibacterial activity with MICs of 22.4, 29.8, 29.6 and 30.0 μg/mL against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*, respectively. The other compounds exhibited moderate activity when compared to standard substance Ampicillin.

1. Introduction

Pyrazole and its derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activities. During the past years, considerable evidence has been accumulated to demonstrate the efficacy of pyrazole derivatives including antibacterial [1], antifungal [2,3], herbicidal [4], insecticidal [5] and other biological activities [6,7]. Up till now, a great variety of these kind of compounds have been synthesized, among which some commercial pesticides have been developed including fripronil (MB46030) [8], ET-751 [9] and pyrazolate (A-544) [10].

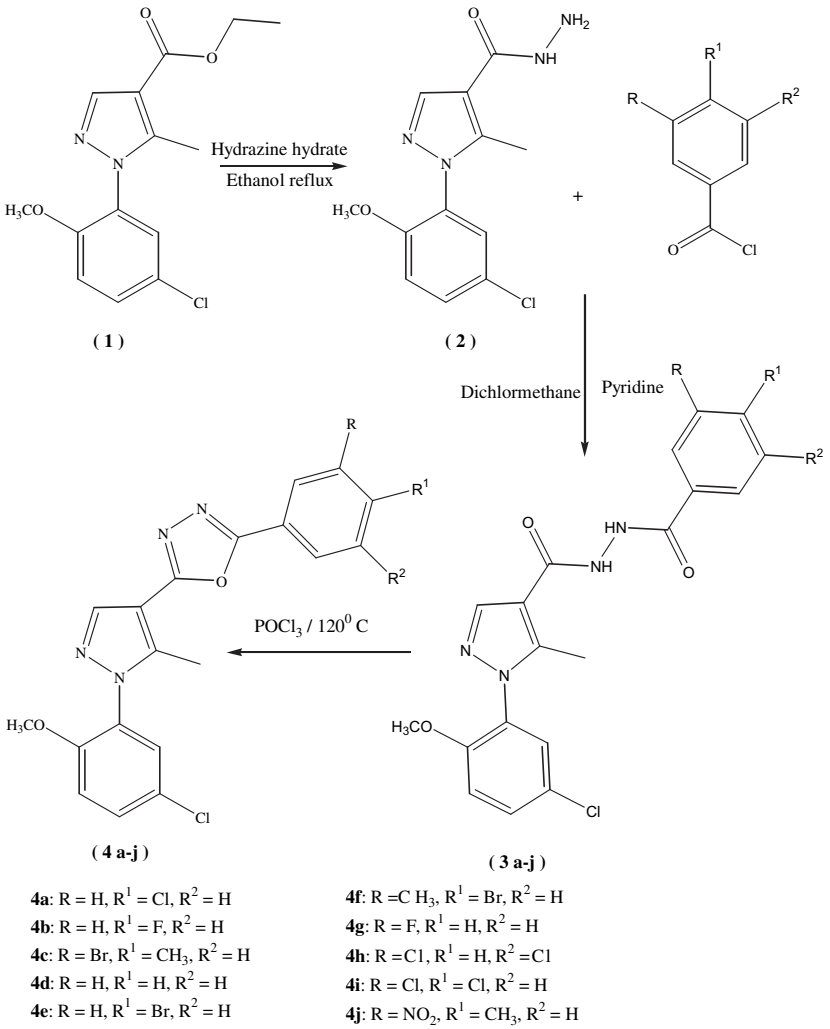
1,3,4-Oxadiazole is associated with potent pharmacological activity due to the presence of toxophoric –N=C–O– linkage [11]. Considerable evidence has been accumulated to demonstrate the efficacy of 1,3,4-oxadiazole, including its use as analgesic [12], antimalarial [13], antiacetylcholine esterase [14], anti-inflammatory [15–17], hyperglycemic [18], antifeedent [19], nervous system depressant [20] and anticonvulsant [21] agent. Furthermore, 1,3,4-oxadiazole exhibits diuretic [22], antipyretic [23], bactericidal [24,25] and antimitotic activity [26]. The synthesis of heterocyclic compounds containing multi-structure in a molecule has received much attention in recent years [27]. Encouraged by these observations and in continuation of our research work on the synthesis of nitrogen and sulfur containing heterocycles for biological activity [28–34] and development of polymorph in bioactive molecules [35], it was considered valuable to integrate two five membered heterocyclic rings together in a molecular frame work to see the additive effect of these rings towards the antibacterial activity.

2. Chemistry

The synthetic route of title compounds (**4a–j**) is shown in Scheme 1. Intermediate 1-(5-chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazole-4-carboxhydrazide (**2**) was synthesized by refluxing a reaction mixture of ethyl 1-(5-chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazole-4-carboxylate (**1**) and hydrazine hydrate in ethanolic medium. This on reaction with proper substituted benzoyl chlorides in the presence of pyridine yielded the compounds (**3a–j**) which, when heated with phosphorousoxychloride at 120 °C, gave

* Corresponding author. Tel.: +91 0821 2547279.
E-mail address: shashisheena@yahoo.com (S. Shashikanth).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.06.020



4a: R = H, R^1 = Cl, R^2 = H
4b: R = H, R^1 = F, R^2 = H
4c: R = Br, R^1 = CH_3, R^2 = H
4d: R = H, R^1 = H, R^2 = H
4e: R = H, R^1 = Br, R^2 = H
4f: R = CH_3, R^1 = Br, R^2 = H
4g: R = F, R^1 = H, R^2 = H
4h: R = Cl, R^1 = H, R^2 = Cl
4i: R = Cl, R^1 = Cl, R^2 = H
4j: R = NO_2, R^1 = CH_3, R^2 = H

Scheme 1.

the respective title compounds (**4a**–**j**). The intermediate (**1**) was synthesized as its phenyl unsubstituted analog [36].

3. Results and discussion

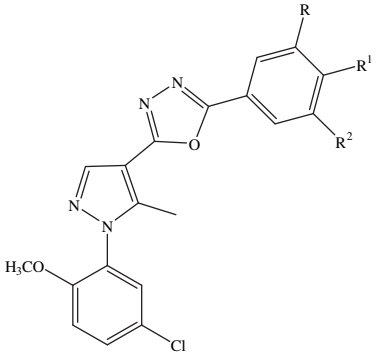
In the ^{1}H NMR spectrum of 1-(5-chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazole-4-carbohydrazide (**2**) two singlet peaks at 2.25 and 3.76 were due to $-CH_3$ and OCH_3 protons respectively. In LC–MS spectra, 280 ion peak was in compliance with molecular weight of the proposed intermediate (**2**). In analogs (**4a**–**j**) $-CH_3$ protons are observed in the range of δ 3.83–3.84. In LC–MS, molecular ion peaks were in good agreement with proposed molecular weight and elemental analysis results were within $\pm$0.4% of the calculated values.

All the title compounds (**4a**–**j**) were subjected for determination of partition coefficient by shake flask method using *n*-octanol/water system and the values are found between 4.26 and 6.42. A screening of antibacterial activities using two Gram negative (*Escherichia coli* and *Klebsiella pneumonia*) and two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) was performed for a series of 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazole analogs (**4a**–**j**). The diameters of the inhibition zones corresponding to the MICs are presented in Table 2. Positive control using only inoculation and negative control using only DMSO in the cavity were carried out. From analysis of Table 2 we can conclude that compound 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-phenyl-[1,3,4]oxadiazole (**4d**) which is unsubstituted showed significant MIC at 29.8 μg/mL against *B. subtilis* and moderate activity at 39.8, 40.6, and 40.6 μg/mL against *S. aureus*, *E. coli* and *K. pneumonia*, respectively. Fluorine incorporated phenyl ring of 1,3,4-oxadiazole displayed varied pharmacological properties such as fluorine atom at para position of phenyl ring showed improved activity at 22.4, 30.6, 29.6 and 30.0 μg/mL against both Gram positive and Gram negative, respectively. Dichloro analogs **4h** and **4i** also exhibited similar pattern of activity where as monochloro analog 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(4-chlorophenyl)-[1,3,4]oxadiazole (**4a**) exhibited moderate activity when compared to 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-phenyl-[1,3,4]oxadiazole (**4d**). Monofluoro analog 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(3-fluorophenyl)-[1,3,4]oxadiazole (**4g**) exhibited better activity against *S. aureus* when compared to other analogs in the series.

4. Conclusion

We have synthesized several substituted 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)

Table 1
Physicochemical characteristics of 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazole analogs (**4a–j**).



(4 a-j)

Compd no.	R	R^1	R^2	MF	M. wt.	Yield[a] (%)	m.p. (°C)	Cryst. solvent	Anal.[b]	log *P*
4a	H	Cl	H	$C_{19}H_{14}Cl_2N_4O_2$	400	75	171.8–172.6	EtOH	C, H, N	4.51
4b	H	F	H	$C_{19}H_{14}ClFN_4O_2$	384	77	162.6–163.7	MeOH–H_2O	C, H, N	4.26
4c	Br	CH_3	H	$C_{20}H_{16}BrClN_4O_2$	460	76	186.2–187.7	EtOH	C, H, N	5.19
4d	H	H	H	$C_{19}H_{15}ClN_4O_2$	366	80	155.2–156.9	EtOH	C, H, N	4.32
4e	H	Br	H	$C_{19}H_{14}BrClN_4O_2$	446	79	177.2–177.7	MeOH	C, H, N	4.88
4f	CH_3	Br	H	$C_{20}H_{16}BrClN_4O_2$	460	69	179.1–180.5	MeOH	C, H, N	5.20
4g	F	H	H	$C_{19}H_{14}ClFN_4O_2$	384	70	144.5–145.6	MeOH–H_2O	C, H, N	4.28
4h	Cl	H	Cl	$C_{19}H_{13}Cl_3N_4O_2$	434	79	148.2–149.4	EtOH	C, H, N	5.61
4i	Cl	Cl	H	$C_{19}H_{13}Cl_3N_4O_2$	434	81	157.0–158.0	EtOH	C, H, N	5.57
4j	NO_2	CH_3	H	$C_{20}H_{16}ClN_5O_4$	425	66	199.5–201.0	CCl_4	C, H, N	6.42

[a] 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 (1:9).
[b] Detailed results of elemental analyses are given in Section 5.4.

-[1,3,4]oxadiazole analogs (**4a–j**) and the yields are found to be satisfactory Table 1. Phenyl group bearing few functional groups such as chloro, fluoro, bromo, methyl and nitro on 1,3,4-oxadiazole which is in turn on pyrazole moiety has been synthesized for antibacterial activity against two Gram positive and Gram negative strains. It was noticed that dichloro analogs **4h** and **4i** revealed similar spectrum of activity than monofluoro analogs **4b** and **4g**. Unsubstituted analog **4d** exhibited moderate activity against *S. aureus*, *E. coli*, and *K. pneumonia* when compared to all other analogs in the series.

5. Experimental

Chemicals were procured from Aldrich Chemical Co. Reactions were monitored and purity of the products was checked by thin layer chromatography (TLC). TLC was performed on Merck 60 F-254 silica gel plates with visualization by UV-light using ethyl acetate and *n*-hexane as solvent system. Melting points were determined on a Büchi Melting Point B-545 apparatus. The IR spectra (in KBr pellets) were recorded on a Nicolet 6700 FT-IR spectrometry. ^{1}H NMR spectra were recorded on Bruker (300 and 400 MHz) spectrometer instruments, in $CDCl_3$. Chemical shifts were recorded in parts per million downfield from tetramethylsilane. Mass spectra were recorded on 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.

5.1. Ethyl 1-(5-chloro-2-methoxyphenyl)-5-methyl-1H-pyrazole-4-carboxylate (*1*)

To a solution of ethyl-2-acetyl-3-(dimethylamino)acrylate [36] (5 g, 0.027 mol) in ethanol (50 mL) was added (5-chloro-2-methoxyphenyl) hydrazine hydrochloride (5.64 g, 0.027 mol) and sodium bicarbonate (4.54 g, 0.054 mol) and it was stirred at room temperature for 4 h. The reaction mixture was concentrated to remove ethanol, diluted with ethylacetate, washed with water, brine solution and dried over sodium sulfate and concentrated to get oily liquid which was purified by column chromatography (60–120 silica gel) using ethyl acetate:petroleum ether as an eluent to get pure white solid (70%); m.p. 120.3–121.2 °C, ^{1}H NMR ($CDCl_3$): δ 1.34–1.37 (t, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 4.28–4.33 (q, 2H, OCH_2–), 6.96–6.98 (d, 1H, $J = 8.9$ Hz, Ar–H), 7.33–7.34 (d, 1H, $J = 2.64$ Hz, Ar–H), 7.39–7.41 (dd, 1H, $J = 2.64$ Hz, Ar-H), 8.02 (s, 1H, Hetero Ar-H); MS: $m/z = 294.7$ (M^+). Anal. Calcd for $C_{14}H_{15}N_2O_3Cl$: C, 57.05; H, 5.13; N, 9.50. Found: C, 56.83; H, 5.27; N, 9.35.

5.2. 1-(5-Chloro-2-methoxyphenyl)-5-methyl-1H-pyrazole-4-carbohydrazide (*2*)

A mixture of ethyl 1-(5-chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazole-4-carboxylate (10 g, 0.034 mol), ethanol (100 mL), and hydrazine hydrate (1.3 mL, 0.34 mol) was heated to reflux and left overnight. The solid formed was filtered and washed with cold water and ether, to obtain compound **2**. Yield (90%), m.p. 203.2–204.2 °C, ^{1}H NMR ($CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 4.32 (s, 2H, NH_2), 7.25–7.28 (d, 1H, $J = 9.0$ Hz, Ar-H), 7.42–7.43(d, 1H, $J = 2.67$ Hz, Ar-H), 7.55–7.59 (dd, 1H, $J = 2.7$ Hz, Ar-H), 8.0 (s, 1H, Hetero Ar-H), 9.32 (s, 1H, NH); MS: $m/z = 280.0$ (M^+). Anal. Calcd for $C_{12}H_{13}N_4O_2Cl$: C, 51.34; H, 4.67; N, 19.96. Found: C, 51.12; H, 4.78; N, 19.72.

Table 2
In-vitro antibacterial activity data of 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazole analogs (**4a**–**j**).

Compd no.	Control	Zone of inhibition in mm (MIC in μg/mL)			
		B. subtilis	*S. aureus*	*E. coli*	*K. pneumonia*
4a	–	15(39.6)	18(30.6)	14(40.0)	13(41.0)
4b	–	25(22.4)	20(29.8)	21(29.6)	19(30.0)
4c	–	15(39.6)	20(29.8)	14(40.6)	15(39.0)
4d	–	20(29.8)	15(39.8)	14(40.6)	14(40.6)
4e	–	14(40.6)	19(30.0)	15(39.8)	13(41.6)
4f	–	15(39.8)	15(39.8)	17(30.8)	14(40.0)
4g	–	19(30.0)	27(21.5)	18(30.6)	20(29.8)
4h	–	15(31.8)	13(41.0)	25(22.4)	21(29.6)
4i	–	19(30.0)	20(29.8)	27(21.5)	15(39.6)
4j	–	15(39.6)	14(40.6)	17(30.8)	12(42.0)
Ampicillin	–	32(3.28)	31(3.36)	30(3.88)	30(4.00)

5.3. General procedure for the preparation of N′*-(4-substituted benzoyl)-1-(5-chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazole-4-carbohydrazide (**3a**–**j**)*

A mixture of 1-(5-chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazole-4-carboxhydrazide (**2**) (5 g, 0.018 mol), dichloromethane (50 mL), pyridine (1.4 g, 0.018 mol) and substituted benzoyl chlorides (0.018 mol) was stirred overnight at room temperature. Reaction completion was monitored through thin layer chromatography and reaction mixture was concentrated to get solid and as such taken for the next step.

*5.4. General procedure for the preparation of 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazoles (**4a**–**j**)*

Substituted-benzoic acid *N*′-[1-(5-chloro-2-methoxy-phenyl)-1*H*-pyrazole-4-carbonyl]-hydrazide (**3a**–**j**) (6 g, 0.0143 mol) was treated with $POCl_3$ (60 mL) and heated at 120 °C overnight. Reaction completion was monitored through thin layer chromatography and reaction medium was concentrated, then quenched with ice cubes and left aside for 2 h. Reaction mixture was extracted with dichloromethane and combined extract was washed with water, 10% sodium bicarbonate solution and finally with brine solution and concentrated to get solid. The solid obtained was purified by column chromatography using silica gel 60–120 mesh and petroleum ether:ethyl acetate as eluent, to afford 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazoles (**4a**–**j**) in 66–81% yield (Table 1).

*5.4.1. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(4-chlorophenyl)-1,3,4-oxadiazole (**4a**)*

IR (cm^{-1}) 3090, 2995, 1614, 1503, 1279. ^{1}H NMR ($CDCl_3$): 2.55 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.01–7.03 (d, 1H, $J = 8.76$, Ar–H), 7.42–7.53 (m, 4H, Ar-H), 8.05–8.08 (d, 2H $J = 8.49$ Ar-H), 8.18 (s, 1H, Hetero Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.55, 56.19, 105.47, 113.16, 122.44, 125.77, 128.05, 128.09, 128.98, 129.45, 130.88, 137.74, 139.47, 142.82, 153.14, 160.54, 162.49. MS: $m/z = 401.0$ (M^+). Anal. Calcd for $C_{19}H_{14}N_4O_2Cl_2$: C, 56.87; H, 3.52; N, 13.96. Found: C, 56.76; H, 3.71; N, 13.87.

*5.4.2. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(4-fluorophenyl)-1,3,4-oxadiazole (**4b**)*

IR (cm^{-1}) 3425, 1621, 1501, 1280. ^{1}H NMR ($CDCl_3$): δ 2.55 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.01–7.03 (d, 1H, $J = 8.76$ Ar-H), 7.20–7.26 (m, 2H, Ar-H), 7.43–7.47 (m, 2H, Ar-H), 8.11–8.18 (m, 3H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.53, 56.19, 105.52, 113.17, 116.30, 116.52, 120.29, 120.32, 125.79, 128.12, 129.00, 129.02, 129.11, 130.87, 139.44, 142.75, 153.16, 160.40, 165.93. MS: $m/z = 385.0$ (M^+). Anal. Calcd for $C_{19}H_{14}N_4O_2ClF$: C, 59.31; H, 3.67; N, 14.56. Found: C, 59.02; H, 3.71; N, 14.43.

*5.4.3. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(3-bromo-4-methylphenyl)-1,3,4-oxadiazole (**4c**)*

IR (cm^{-1}) 3425, 3089, 1621, 1501, 1280. ^{1}H NMR ($CDCl_3$): δ 2.49 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 7.01–7.03 (d, 1H, $J = 8.84$ Ar-H), 7.39–7.48 (m, 3H, Ar-H), 7.96–7.98 (m, 1H, Ar-H), 8.20 (s, 1H, Ar-H), 8.28 (d, 1H $J = 1.5$ Hz, Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.49, 23.10, 56.15, 105.45, 113.15, 123.16, 125.39, 125.76, 128.12, 128.80, 128.95, 130.33, 130.80, 133.89, 139.45, 141.75, 142.75, 153.14, 160.42, 162.04. MS: $m/z = 459.0$ (M^+). Anal. Calcd for $C_{20}H_{16}N_4O_2BrCl$: C, 52.25; H, 3.51; N, 12.19. Found: C, 52.11; H, 3.65; N, 12.09.

*5.4.4. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-phenyl-1,3,4-oxadiazole (**4d**)*

IR (cm^{-1}) 3406, 3003 1699, 1617, 1537, 1505, 1282. ^{1}H NMR ($CDCl_3$): δ 2.56 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.01–7.03 (d, 1H, $J = 8.67$ Hz, Ar-H), 7.43–7.55(m, 5H, Ar-H), 8.11–8.14 (m, 2H, Ar-H), 8.2 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.54, 56.24, 105.62, 113.19, 123.94, 125.76, 126.80, 128.16, 129.03, 130.81, 131.49, 139.49, 142.68, 153.16, 160.33, 163.27. MS: $m/z = 366.0$ (M^+). Anal. Calcd for $C_{19}H_{15}N_4O_2Cl$: C, 62.21; H, 4.12; N, 15.27. Found: C, 62.10; H, 4.24; N, 15.18.

*5.4.5. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(4-bromophenyl)-1,3,4-oxadiazole (**4e**)*

IR (cm^{-1}) 3445, 3086, 1615, 1502, 1279: ^{1}H NMR ($CDCl_3$): δ 2.55 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.01–7.04 (d, 1H, $J = 8.79$, Ar-H), 7.42–7.48(m, 2H, Ar-H), 7.67–7.70 (d, 2H, $J = 8.46$ Hz, Ar-H), 7.98–8.01 (d, 2H, $J = 8.46$ Hz Ar-H), 8.18 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.48, 56.16, 105.41, 113.17, 122.84, 125.75, 126.10, 128.14, 129.94, 130.82, 131.529, 132.35, 139.42, 142.78, 153.12, 160.5, 162.53. MS: $m/z = 445.0$ (M^+). Anal. Calcd for $C_{19}H_{14}N_4O_2BrCl$: C, 51.20; H, 3.17; N, 12.57. Found: C, 51.11; H, 3.19; N, 12.45.

*5.4.6. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(4-bromo-3-methylphenyl)-1,3,4-oxadiazole (**4f**)*

IR (cm^{-1}) 3117, 3053, 1715, 1627, 1282. ^{1}H NMR ($CDCl_3$): δ 2.46 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.00–7.03 (d, 1H, $J = 8.76$, Ar-H), 7.42–7.48 (m, 2H, Ar-H), 7.68–7.71 (d, 1H, $J = 8.46$ Hz Ar-H), 7.76–7.80 (m, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.19 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.46, 22.90, 56.12, 105.43, 113.11, 122.93, 125.37, 125.72, 128.05, 128.58, 128.64, 130.79, 133.11, 139.05, 139.40, 142.73, 153.09, 160.40, 162.65. MS: $m/z = 459.0$ (M^+). Anal. Calcd for $C_{20}H_{16}N_4O_2BrCl$: C, 52.25; H, 3.51; N, 12.19. C, 52.26; H, 3.53; N, 12.20.

*5.4.7. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(3-fluorophenyl)-1,3,4-oxadiazole (**4g**)*

IR (cm^{-1}) 3074, 2920, 2848, 1698, 1616, 1267. ^{1}H NMR ($CDCl_3$): δ 2.56 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 7.01–7.03 (d, 1H, $J = 8.76$ Hz, Ar-H), 7.23–7.27 (m, 1H, Ar-H), 7.43–7.55 (m, 3H, Ar-H), 7.81–7.83 (d, 1H, $J = 9.1$ Hz, Ar-H), 7.91–7.93 (d, 1H, $J = 7.7$ Hz, Ar-H), 8.20 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.55, 56.19, 105.41, 113.17, 113.19, 113.93, 118.70, 122.58, 125.84, 128.04, 128.97, 130.97, 139.49, 142.90, 153.14, 160.65, 161.62, 162.32, 164.08. MS: $m/z = 385.0$ (M^+). Anal. Calcd for $C_{19}H_{14}N_4O_2ClF$: C, 59.31; H, 3.67; N, 14.56. Found: C, 59.02; H, 3.71; N, 14.43.

*5.4.8. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(3,5-dichlorophenyl)-1,3,4-oxadiazole (**4h**)*

IR (cm^{-1}) 3090, 2995, 1614, 1503, 1279. ^{1}H NMR ($CDCl_3$): 2.56 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 7.01–7.04 (d, 1H, $J = 8.79$ Hz, Ar-H), 7.42–7.48 (m, 2H, Ar-H), 7.61–7.64 (d, 1H, Ar-H),7.95-7.98(dd, 1H, Ar-H), 8.20–8.21 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.51, 56.13, 105.07, 113.13, 124.95, 125.76, 128.42, 127.80, 128.42, 128.97, 130.94, 135.96, 139.47, 142.82, 153.06, 161.09, 168.33. MS: $m/z = 435.0$ (M^+).

Anal. Calcd for $C_{19}H_{13}N_4O_2Cl_3$: C, 59.31; H, 3.67; N, 14.56. Found: C, 59.32; H, 3.70; N, 14.55.

*5.4.9. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(3,4-dichlorophenyl)-1,3,4-oxadiazole (**4i**)*

IR (cm^{-1}) 3090, 2995, 1614, 1503, 1279. ^1H NMR ($CDCl_3$): 2.55 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.01–7.04 (d, 1H, $J = 8.79$ Hz, Ar-H), 7.42–7.48 (m, 2H, Ar-H), 7.61–7.64 (d, 1H, $J = 8.37$ Hz, Ar-H), 7.95–8.20 (m, 2H, Ar-H), 8.21 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.49, 56.12, 105.16, 113.13, 123.62, 125.72, 128.35, 128.87, 128.42, 128.99, 130.87, 133.60, 135.89, 139.42, 142.95, 153.05, 161.41,. MS: $m/z = 435.0$ (M^+). Anal. Calcd for $C_{19}H_{13}N_4O_2Cl_3$: C, 52.38; H, 3.01; N, 12.86. Found: C, 52.40; H, 3.02; N, 12.83.

*5.4.10. 2-[1-(5-Chloro-2-methoxyphenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(3-nitro-4-methylphenyl)-1,3,4-oxadiazole (**4j**)*

IR (cm^{-1}) 3086, 2925, 1618, 1528, 1279. ^1H NMR ($CDCl_3$): 2.56 (s, 3H, CH_3), 2.70 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.01–7.04 (d, 1H, $J = 8.79$ Hz, Ar-H), 7.27–7.47 (m, 2H, Ar-H), 7.53–7.55 (d, 1H, $J = 8.08$ Hz, Ar-H), 8.21 (s, 1H, Ar-H) 8.25–8.27 (m, 1H, Ar-H), 8.66–8.67 (d, 1H, Ar-H); ^{13}C NMR ($CDCl_3$): δ 11.59, 20.63, 56.20, 105.22, 113.18, 122.75, 123.21, 125.79, 128.04, 128.96, 130.57,130.92, 133.84, 136.88, 139.53, 143.04, 149.60, 153.12, 160.91, 161.41,. MS: $m/z = 426.0$ (M^+). Anal. Calcd for $C_{20}H_{16}N_5O_4Cl$: C, 56.41; H, 3.79; N, 16.45. Found: C, 56.40; H, 3.80; N, 16.44.

5.5. Antibacterial activity (determination of zone of inhibition)

The antibacterial activity [37,38] of the test samples (**4a–j**) was determined by agar cup plate method using four organisms such as *Bacillus subtilis* (NCIM, 2063; ATCC 6633), *Staphylococcus aureus* (NCIM, 2079; ATCC 6538P), *Escherichia coli* (NCIM, 2065; ATCC 8739) and *Klebsiella 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 petri dishes 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 was classified as highly active (>21 mm), moderately active (15–21 mm), least active (12–15 mm) and less than 12 mm was taken as inactive. The antibacterial activity data of 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazole analogs (**4a–j**) are recorded in Table 2.

5.6. Determination of minimum inhibitory concentration

The determination of minimum inhibitory concentration [39,40] was done with four isolates of *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) which were inoculated into Luria broth medium containing 1% tryptone, 0.5% yeast extract and 0.5% sodium chloride. The pH of the medium was adjusted to 7.2 with sterile phosphate buffered saline and incubated at 37° C for 24 h. The optical density of the bacteria from mid-log phase of growth was measured at 540 nm and diluted in fresh medium so as to get an optical density of 0.004 (corresponding to 5×10^5 colony forming units/mL). To each well of the ELISA plate (Corning, USA), 200 μL of diluted bacterial suspension was added and graded concentrations (0.2–500 μg/mL) of the synthesized promising compounds and a standard antibiotic (Ampicillin) in 20% water in dimethyl sulfoxide were added and incubated at 37 °C for 24 h. At the end of incubation the effect of the drugs on the growth of organisms was monitored by measuring the optical density at 540 nm using ELISA reader (Multiscan MS, Labsystems, Helsinki, Finland). The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. The results of 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1*H*-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadiazole analogs (**4a–j**) are recorded in Table 2.

Acknowledgements

Authors NPR are grateful to Dr. Goutham Das, President, Syngene International Ltd., Biocon group of company, Bangalore for giving permission to carryout research work and KNV is thankful to Prof. B.G. Shivananda, Principal, Al-Ameen College of Pharmacy, Bangalore for encouragement.

References

[1] H.V. Patel, P.S. Fernandez, K.A. Vyas, Indian J. Chem. 29B (1990) 135–141.
[2] K. Sasse, G. Haensler, Schmidt, Ger. Offen. DE 3 (1987) 527; Chem. Abstr. 106 (1987) 133794u.
[3] M.L. Pulido, J.G. Fenyes, Eur. Pat. Appl. EP 467708. Chem. Abstr. 116 (1992) 146150j.
[4] K.M. Morimoto, K. Makino, S. Yamamoto, G.J. Sakato, J. Heterocycl. Chem. 27 (1990) 807–810.
[5] M. Naoto, N. Kazue, I. Tomotoshi, F. Hiroaki, M. Kenichi, T. Hirotaka, O. Yoriko, T. Masahiro, Eur. Pat. Appl. EP 361827 (1990) 120.
[6] A. Pande, V.K. Saxena, Indian J. Chem. 26 B (1987) 390–392.
[7] M. Kopp, J.C. Lancelot, P. Dallemagne, S. Rault, J. Heterocycl. Chem. 38 (2001) 1045–1050.
[8] F. Colliot, K.A. Kukorowski, D.W. Hawkins, D.A. Roberts, Brighton Crop Prot. Conf. Pests Dis. 1 (1992) 29–34.
[9] Y. Miura, M. Ohnishi, T. Mabuchi, I. Yanai, Brighton Crop Prot. Conf. Weeds 1 (1993) 35.
[10] T. Konotsune, K. Kawakubo, T. Honma, JP 8035035, 1980; Chem. Abstr. 93 (1980) 20750.
[11] B. Rigo, D. Couturier, J. Heterocycl. Chem. 22 (1985) 287–288.
[12] I. Angilini, L. Angilini, F. Sparace, British Pat. 1,161,801, 1969. Through Chem. Abstr. 71 (1969) 112936.
[13] C. Baozhen, Q. Weizhong, S. Zhengwu, L. Xinghan, Yiyao Gongye 16 (1985) 305 Through Chem. Abstr. 104 (1986) 186357.
[14] H.K. Misra, Arch. Pharm. 316 (1983) 487.
[15] E. Palaska, G. Sahin, P. Kelicen, N.T. Durlu, G. Altinok, Il Farmaco 57 (2002) 101–107.
[16] M.D. Mullican, M.W. Wilson, D.T. Connor, C.R. Kostlan, D.J. Schrier, R.D. Dyer, J. Med. Chem. 36 (1993) 1090–1099.
[17] K. Raman, K.H. Singh, S.K. Salzman, S.S. Parmar, J. Pharm. Sci. 82 (1993) 167–169.
[18] G. Sahin, P. Palaska, P. Kelicen, R. Demirdmar, G. Altinok, Drug Res. 51 (2001) 478.
[19] Q. Huang, X. Qian, G. Song, S. Cao, Pest Manag. Sci. 59 (2003) 933–939.
[20] . J. Maillard, M. Vincent, R. Morin, M. Benard, Fr. Pat. M 379, 1960. Through Chem. Abstr. 57 (1962) 15251.
[21] P. Tsitsa, A. Papadakko-Valiraki, T. Siatra-Papastaikoudi, Z. Papadopoulou-Daifoiti, A. Vamvakidis, Ann. Pharm. Fr. 47 (1989) 296–303.
[22] K.M.L. Rai, N. Linganna, II Farmaco 55 (2000) 389–392.
[23] L. Mishra, M.K. Said, H. Itokawa, K. Takeya, Bioorg. Med. Chem. 3 (1995) 1241–1245.
[24] S. Papakonstantinou-Garoufalias, P. Marokos, A. Tsantilli-Kakoulidou, A. Chytyroglou-Ladas, Pharmazie 53 (1998) 300–302.
[25] C.S. Andotra, T.C. Langer, Shivakumar, A.N. Sarib, Indian J. Pharm. Sci. 48 (1986) 192–195.
[26] X. Ouyang, E.L. Piatnitski, V. Pattaropong, X. Chen, Y. H-He, A.S. Kiselyov, A. Velankar, J. Kawakami, M. Labelle, L. Smith II, J. Lohman, S.P. Lee, A. Malikzay, J. Fleming, J. Gerlak, Y. Wang, R.L. Rosler, K. Zhou, S. Mitelman, M. Camara, D. Surguladze, J.F. Doody, M.C. Tuma, Bioorg. Med. Chem. Lett. 16 (2006) 1191–1196.
[27] H.S. Chen, Z.M. Li, Y.F. Han, J. Agric. Food Chem. 48 (2000) 5312–5315.
[28] T.D. Venu, S.A. Khanum, A. Firdouse, B.K. Manuprasad, S. Shashikanth, R. Mohamed, B.S. Vishwanth, Bioorg. Med. Chem. Lett. 18 (2008) 4409–4412.
[29] T.D. Venu, S. Shashikanth, S.A. Khanum, S. Naveen, A. Firdouse, M.A. Sridhar, J.S. Prasad, Bioorg. Med. Chem. 15 (2007) 3505–3514.
[30] B.T. Prabhakar, S.A. Khanum, K. Jayashree, B.P. Salimath, S. Shashikanth, Bioorg. Med. Chem. 14 (2006) 435–446.
[31] S.N. Sriharsha, S. Satish, S. Shashikanth, K.A. Raveesha, Bioorg. Med. Chem. 14 (2006) 7476–7481.
[32] S.A. Khanum, S. Shashikanth, S. Umesha, R. Kavitha, Eur. J. Med. Chem. 40 (2005) 1156–1162.

[33] S.A. Khanum, S.K. Murari, B.S. Vishwanth, S. Shashikanth, Bioorg. Med. Chem. Lett. 15 (2005) 4100–4104.
[34] S.K. Murari, S.N. Sriharsha, S. Shashikanth, B.S. Vishwanath, Bioorg. Med. Chem. Lett. 14 (2004) 2423–2425.
[35] P. Munshi, K.N. Venugopala, B.S. Jayashree, T.N.G. Row, Cryst. Growth Des. 4 (2004) 1105–1107.
[36] G.M. Menozzi, L. Mosti, P. Schenone, J. Heterocycl. Chem. 24 (1987) 1669–1675.
[37] M. Kaspady, K.N. Venugopala, M. Raju, G.K. Rao, Lett. Drug Des. Disc. 6 (2009) 21–29.
[38] V. Padmavathi, P. Thriveni, G.S. Reddy, D. Deepti, Eur. J. Med. Chem. 43 (2008) 917–924.
[39] A.P. Oludotun, E.U. Edet, M.S. Santhosh, Eur. J. Med. Chem. 43 (2008) 1095–1104.
[40] S. Kotretsou, M.P. Mingeot-Leclercq, V. Constantinou-Kokotou, R. Brasseur, M.P. Georgiadis, P.M. Tulkens, J. Med. Chem. 38 (1995) 4710–4719.