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Synthesis and antimicrobial evaluation of isoxazole-substituted 1,3,4-oxadiazoles

https://doi.org/10.1515/hc-2018-0137
Received July 26, 2018; accepted August 14, 2018; previously published online September 20, 2018

Abstract: Synthesis of *N*-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxadiazol-2-yl)acetamides **5a–k** was achieved from readily available materials. The compounds were screened for their *in vitro* antimicrobial activity against representative bacterial and fungal strains. Compounds **5b**, **5d** and **5f** exhibit good activity.

Keywords: 1,3,4-oxadiazole; antimicrobial activity; chloramine-T; isoxazole; oxidative cyclization.

Introduction

The emergence of microbial resistance to drugs is a widespread problem in the treatment of various infections. The identification of novel antibiotics for effective treatment of infections still remains a major challenge to medicinal chemists. The 1,3,4-oxadiazole moiety has become an important structural motif for the development of new drugs because of its biological activities including HIV integrase inhibition [1], anti-inflammatory [2], anticancer [3], antibacterial [4], anticonvulsant [5], analgesic [6], antitubercular [7], antifungal [8] and anti-allergic [9] activities. Some compounds having 1,3,4-oxadiazole derivatives currently used as drugs are raltegravir, an antiretroviral drug [10], zibotentan, an anticancer agent [11], fenadiazole, a hypnotic drug [12], nesapidil, an antihypertensive agent [13] and furamizole, an antibiotic [14], among others [15–24]. In continuation of our work on biologically active isoxazoles [25–27], we now report the synthesis of new compounds bearing 2,5-disubstituted 1,3,4-oxadiazoles that may be developed into practical drugs.

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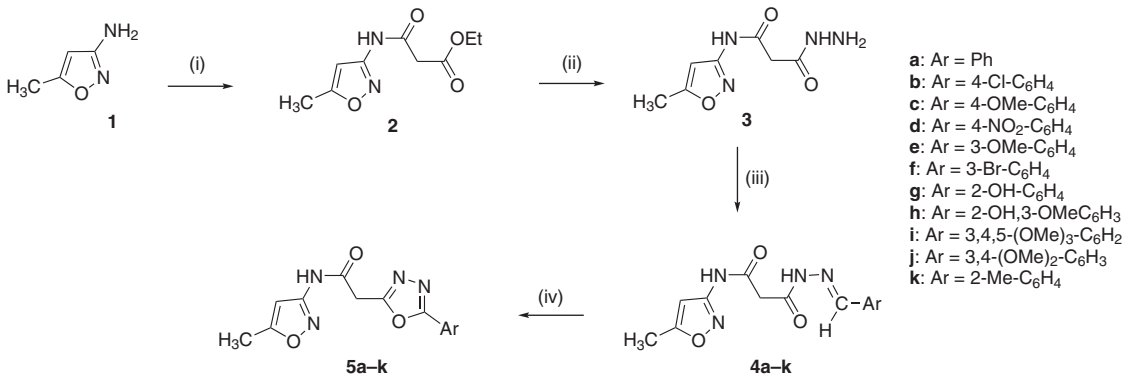
Results and discussion

Chemistry

Synthesis of *N*-(5-methylisoxazol-3-yl)-2-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamides **5a–k** is shown in Scheme 1. Condensation of 3-amino-5-methylisoxazole (**1**) with diethyl malonate in ethanol under reflux afforded ethyl 2-(5-methyl-3-isoxazolylcarbamoyl)acetate [28] (**2**). Treatment of ester **2** with excess of hydrazine hydrate in ethanol furnished 3-hydrazinyl-*N*-(5-methyl-3-isoxazolyl)-3-oxopropanamide (**3**). The hydrazide **3** was condensed with aromatic aldehydes in methanol to furnish (*E/Z*)-3-(2-benzylidenehydrazinyl)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamides **4a–k**. Compounds **4a–k** on treatment with chloramine-T underwent oxidative cyclization to give *N*-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxadiazol-2-yl)acetamides **5a–k**.

The structures of compounds **3–5** were established based on IR, ^{1}H NMR, ^{13}C NMR, ESI-MS and analytical data. In particular, ^{1}H NMR spectra of *N*-acylhydrazones **4a–k** show characteristic double signals for each of the proton around the C=N bond, suggesting that these compounds exist in two isomeric forms. Two geometric isomers may be present in the ratio of 3:1; based on the integration values of proton signals. The results of the NMR experiments are discussed as follows.

Compound **4a** was used for the analysis of nuclear Overhauser effect (nOe) and two-dimensional (2D)-^{1}H NMR-^{13}C heteronuclear multiple bond correlation (HMBC) spectra. In the nOe experiment, irradiation of the methylene protons (10-CH_2) at δ_H 3.72 gives rise to an enhancement for both NH protons, which demonstrates that the methylene group is flanked by two amidic NH groups (Figure 1). The difference in nOe enhancement observed for the two amidic NH protons is due to their spatial arrangement. On irradiation of the NHCO proton at δ_H



Scheme 1 Reagents and conditions: (i) diethyl malonate, ethanol, reflux 12 h, 80%; (ii) $N_2H_4H_2O$, ethanol, reflux 6 h, 92%; (iii) Ar-CHO, methanol, glacial AcOH, reflux 4–6 h, 90–95%; (iv) chloramine-T, ethanol, reflux 4–6 h, 68–76%.

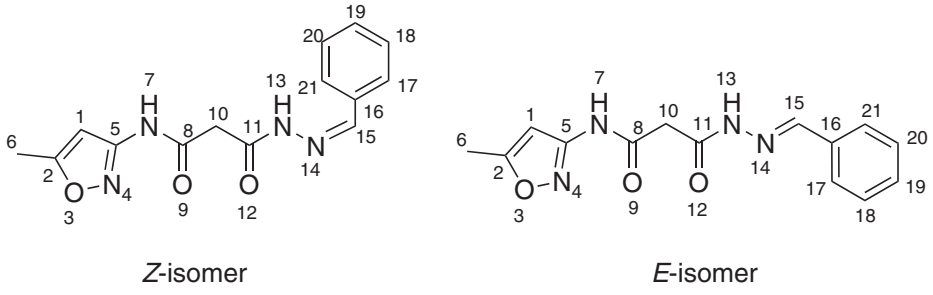


Figure 1 *Z* (minor) and *E* (major) isomers of compound **4a**.

11.07, only a signal for methylene protons is enhanced and irradiation of the olefinic proton NN=CH at δ_H 7.93 gives rise to nOe enhancement for phenyl ring protons and a hydrazine NH proton. Furthermore, irradiation of the other NHN=C proton at δ_H 11.49 gives nOe enhancement for both the methylene protons of the major and minor isomers. There is also a strong enhancement for the olefinic proton of the major isomer while the proton of the minor isomer is unaffected. These results demonstrate that the compounds exist as a mixture of *E*- and *Z*-isomeric forms.

This conclusion was further confirmed by 2D-^{1}H-^{13}C HMBC experiment. HMBC data unambiguously show that the connectivity, as expected, between protons and carbons of the major and minor isomers is intact, and the assignments of chemical shift values for compound **4a** are as follows: correlation of H-6 (δ_H 2.3, 2.4) with C_2 (δ_c 170.0, 170.1) and C_1 (δ_c 96.7); H_7 (δ_H 11.0, 11.1) with C_1 (δ_c 96.6) and C_8 (δ_c 166.3, 165.9); H_{13} (δ_H 11.5, 11.6) with C_{15} (δ_c 143.5, 147.3) and C_{10} (δ_c 43.0, 43.7) and C_{11} (δ_c 163.0 and 169.1) and H_{15} (δ_H 7.9, 8.2) with C_{16} (δ_c 134.5) and C_{17} and C_{21} (δ_c 127.3, 127.6). The key points from HMBC data are the correlations between H_7-C_1, H_{13}-C_{15} and H_{10}-C_8 and H_{10}-C_{11}, which indicate the absence of keto-enol tautomerism. On the basis of both 1D and 2D NMR data, it can be concluded that these compounds exist in the mixture of *Z* and *E* geometrical isomers.

The IR spectrum of *N*-(5-methylisoxazol-3-yl)-2-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (**5a**) shows a characteristic band at 1237 cm^{-1} due to C-O-C stretching vibration confirming the formation of 1,3,4-oxadiazole ring. ^{1}H NMR spectrum of **5a** does not exhibit signals due to the CH=N proton, and the NH protons of the hydrazone, which are present in its precursor **4a** at δ 7.93, 8.16, 11.49 and 11.55, confirming the oxadiazole ring formation. The absence of azomethine carbon signals at δ 143.5 and 147.3 in ^{13}C NMR spectrum of **5a** also supports the formation of the oxadiazole ring. The mass spectrum of **5a** also agrees with its structure by exhibiting the protonated molecular ion $[M+H]^+$ peak at *m/z* 285.

Antibacterial activity

Compounds **5a–k** exhibit good antibacterial activity in comparison to the activity of the standard drug ciprofloxacin (Table 1). Some of the compounds exhibit excellent minimum inhibitory concentration values (MIC). Compounds **5b** and **5f** are highly active. The exceptional activity of compound **5d** may be due to the presence of a

Table 1 Antibacterial activity of *N*-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxadiazol-2-yl)acetamides **5a–k**.

Compound	Minimum inhibitory concentration (MIC)[a,b]					
	Bacterial strains					
	P. aeruginosa	*K. aerogenes*	*C. violaceum*	*B. subtilis*	*B. sphaericus*	*S. aureus*
5a	20	18	18	19	17	15
5b	11	11	8	9	8	7
5c	17	15	13	16	18	14
5d	10	8	7	6	8	6
5e	10	16	15	15	13	14
5f	13	11	14	11	12	10
5g	18	14	16	16	15	15
5h	19	16	20	17	18	21
5i	21	18	21	19	17	18
5j	23	20	22	18	16	15
5k	22	23	20	18	17	16
Ciprofloxacin	30	25	25	20	20	25

[a]Negative control (acetone) – no activity. [b]Concentration in μg/mL.

nitro group on the phenyl ring. The remaining compounds **5a**, **5c**, **5e**, **5g**, **5h**, **5i**, **5j** and **5k** show moderate activity. However, the degree of inhibition varies both with the test compound and with the bacteria used in the present investigation.

Antifungal activity

Compounds **5a–k** are significantly toxic toward all five pathogenic fungi and are lethal even at 100 μg/mL concentration when compared to the standard drug clotrimazole (Table 2). The activity data are indicated as a zone of inhibition at 100 μg/mL concentration. Compounds **5b** and **5f** exhibit high activity and they inhibit the growth of fungi to a remarkable extent, which may be due to the presence of chloro, nitro and bromo substituents on the benzene ring, besides the presence of isoxazole and oxadiazole skeletons. Compound **5d** bearing a nitro group on the benzene ring shows good toxicity against the fungi used. Compounds **5a**, **5c**, **5e**, **5g**, **5h**, **5i**, **5j** and **5k** are moderate in their toxicity and they are less active compared to other compounds in the present study, but better than the standard drug clotrimazole. The degree of spore germination inhibition varies with test compounds and with the type of fungi.

Table 2 Antifungal activity of *N*-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxadiazol-2-yl)acetamides **5a–k**.

Compound	Zone of inhibition (mm)[a,b]				
	Fungal strains				
	A. niger	*C. tropicum*	*R. oryzae*	*F. moniliforme*	*C. lunata*
5a	52.5	46.5	51.0	41.2	50.5
5b	69.1	70.1	72.5	69.8	65.5
5c	56.0	57.0	58.0	61.0	59.5
5d	75.0	77.2	80.5	73.2	69.5
5e	48.0	51.0	48.5	53.2	57.2
5f	63.2	65.0	72.5	60.5	73.5
5g	55.8	58.3	59.1	60.0	61.1
5h	47.2	42.5	40.2	38.5	31.5
5i	39.0	38.3	31.2	37.0	51.0
5j	51.0	55.2	49.8	48.1	59.0
5k	45.5	60.1	55.3	48.1	39.5
Clotrimazole	26.5	30.6	33.5	25.5	35.8

[a]Negative control (acetone) – no activity. [b]Concentration 100 μg/mL.

Conclusions

A simple and efficient protocol for the synthesis of isoxazolyl-2,5-disubstituted 1,3,4-oxadiazoles **5a–k** with potential pharmacological properties is described. Compounds **4a–k** exhibit characteristic ^{1}H NMR spectra indicating the presence of *E* and *Z* geometrical isomers. The title compounds **5a–k** were evaluated for antimicrobial activity. Compounds **5a**, **5d** and **5f** show excellent antimicrobial activity.

Experimental

Melting points were determined on a Cintex melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) analysis was performed on Merck precoated $60F_{254}$ silica gel plates. Visualization was done by exposure to UV light. IR spectra were recorded in KBr pellets on a Perkin-Elmer BX series Fourier-transform infrared (FT-IR) spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker spectrometer in $CDCl_3$ or DMSO-d_6 with tetramethylsilane (TMS) as internal standard. ESI mass spectra were recorded on an Agilent liquid chromatography-mass selective detector (LC-MSD). EA were performed on Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

Synthesis of ethyl 2-(5-methyl-3-isoxazolylcarbamoyl) acetate (2)

A mixture of 3-amino-5-methylisoxazole (**1**, 0.1 mmol) and diethyl malonate (0.1 mmol) in ethanol (20 mL) was heated under reflux for 12 h. The reaction was monitored by TLC analysis. After the mixture was concentrated and cooled, the separated solid product was filtered under suction, dried and crystallized from ethanol; yield 80%; mp 112–113°C; IR: υ_{max} 3266, 1743, 1700 cm^{-1} ; ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 8.0 Hz, 3H, OCH_2-CH_3), 2.41 (s, 3H, isoxazole-CH_3), 3.52 (s, 2H, CH_2), 4.25 (q, J = 8.0 Hz, 2H, OCH_2-CH_3), 6.70 (s, 1H, isoxazole-H), 10.19 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 12.6, 14.0, 42.0, 62.0, 96.6, 157.7, 163.4, 168.2, 170.1; MS: *m/z* 213, (M + H)$^+$. Anal. Calcd for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.92; H, 5.68; N, 13.21.

Synthesis of 3-hydrazino-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (3)

A mixture of compound **2** (0.1 mmol) in ethanol (15 mL) and hydrazine hydrate (0.5 mmol) (98%) was heated under reflux for 6 h, and the progress of the reaction was monitored by TLC. The excess ethanol was removed under reduced pressure and the residue of **3** was washed with cold water and cold methanol and crystallized from methanol; yield 92%; mp 152–153°C; IR: υ_{max} 3211–3337, 3141, 1652 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, isoxazole-CH_3), 3.19 (s, 2H, CH_2), 4.26 (s, 2H, NH_2), 6.57 (s, 1H, isoxazole-H), 9.13 (s, 1H, NH), 10.94 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.5, 42.5, 96.6, 158.3, 165.9, 166.1, 170.0; MS: *m/z* 199.15 (M + H)$^+$. Anal. Calcd for $C_7H_{10}N_4O_3$: C, 42.42; H, 5.09; N, 28.27. Found: C, 42.40; H, 5.08; N, 28.25.

General procedure for the synthesis of 3-(2-arylidenehydrazino)-*N*-5-(methyl-3-isoxazolyl)-3-oxopropanamides 4a–k

A mixture of hydrazide **3** (0.1 mmol) and an aromatic aldehyde (0.1 mmol) was heated under reflux in methanol (20 mL) in the presence of a catalytic amount of glacial acetic acid for 4–6 h. The progress of the reaction was monitored by TLC. The reaction mixture was cooled, and the separated solid was filtered and crystallized from methanol.

(*E/Z*)-3-(2-Benzylidenehydrazino)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (4a) Yield 90%; mp 160–161°C; IR: υ_{max} 3257, 3220, 1680, 1621 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.33 and 2.36 (s, 3H, H_6), 3.40 and 3.72 (s, 2H, H_{10}), 6.57 and 6.60 (s, 1H, H_1), 7.34 and 7.42 (m, 3H, H_{18}, H_{19}, H_{20}), 7.61 and 7.68 (m, 2H, H_{17}, H_{21}), 7.93 and 8.17 (s, 1H, H_{15}), 11.07 and 11.10 (s, 1H, H_7), 11.49 and 11.55 (s, 1H, H_{13}); ^{13}C NMR (DMSO-d_6): δ 12.6 (C_6), 43.0 and 43.7 (C_{10}), 96.6 (C_1), 127.3, and 127.6 (C_{17} and C_{21}), 129.2 and 129.3 (C_{18} and C_{20}), 130.3 and 130.6 (C_{19}), 134.5 (C_{16}), 143.5 and 147.3 (C_{15}), 158.4 and 158.6 (C_5), 163.0 and 169.1 (C_{11}), 165.9 and 166.3 (C_8), 170.0 and 170.1 (C_2); MS: *m/z* 287.15 (M + H)$^+$. Anal. Calcd for $C_{14}H_{14}N_4O_3$: C, 58.73; H, 4.93; N, 19.57. Found: C, 58.72; H, 4.92; N, 19.56.

(*E/Z*)-3-(2-(4-Chlorobenzylidene)hydrazino)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (4b) Yield 92%; mp 178–180°C; IR: υ_{max} 3225, 1678, 1618 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.34 and 2.38 (2s, 3H, isoxazole-CH_3), 3.41 and 3.70 (2s, 2H, CH_2), 6.59 and 6.61 (2s, 1H, isoxazole-H), 6.80–7.30 (m, 4H, Ar-H), 8.01 and 8.20 (2s, 1H, N=CH), 10.92 and 10.96 (2s, 1H, NH), 11.20 and 11.28 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.7, 43.1, 43.9, 95.7, 130.2, 131.9, 132.9, 133.2, 139.8, 143.2, 147.1, 157.8, 158.2, 162.2, 165.6, 165.8, 169.9, 170.2, 171.4; MS: *m/z* 321 (M + H)$^+$. Anal. Calcd for $C_{14}H_{13}ClN_4O_3$: C, 52.43; H, 4.09; N, 17.47. Found: C, 52.41; H, 4.08; N, 17.45.

(*E/Z*)-3-(2-(4-Methoxybenzylidene)hydrazino)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (4c) Yield 93%; mp 172–173°C; IR: υ_{max} 3230, 1675, 1622 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.33 and 2.36 (2s, 3H, isoxazole-CH_3), 3.40 and 3.72 (2s, 2H, CH_2), 3.78 and 3.80 (2s, 3H, OCH_3), 6.57 and 6.60 (2s, 1H, isoxazole-H), 6.91–7.36 (m, 4H, Ar-H), 7.90 and 8.13 (2s, 1H, N=CH), 11.07 and 11.10 (2s, 1H, NH), 11.10 and 11.56 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.4, 43.0, 43.9, 55.4, 55.6, 96.5, 110.9, 111.2, 116.7, 116.9, 120.3, 120.6, 130.3, 130.4, 135.7, 143.8, 147.5, 158.2, 159.8, 162.0, 163.8, 165.8, 166.3, 169.1, 170.2, 170.4; MS: *m/z* 317 (M + H)$^+$. Anal. Calcd for $C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.10; N, 17.71. Found: C, 56.94; H, 5.09; N, 17.69.

(*E/Z*)-*N*-(5-Methylisoxazol-3-yl)-3-(2-(4-nitrobenzylidene) hydrazino)-3-oxopropanamide (4d) Yield 95%; mp 185–186°C; IR: υ_{max} 3190, 1679, 1617 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.32 and 2.35 (2s, 3H, isoxazole-CH_3), 3.41 and 3.77 (2s, 2H, CH_2), 6.52 and 6.55 (2s, 1H, isoxazole-H), 7.78–7.89 (m, 4H, Ar-H), 8.25 and 8.36 (2s, 1H,

N=CH), 9.60 and 9.62 (2s, 1H, NH), 11.22 and 11.25 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 12.2, 42.9, 43.5, 96.1, 120.8, 131.2, 140.5, 142.9, 146.0, 149.7, 157.2, 163.0, 164.9, 165.2, 168.2, 169.0, 169.8, 170.0; MS: *m/z* 354 $(M+Na)^+$. Anal. Calcd for $C_{14}H_{13}N_5O_5$: C, 50.76; H, 3.96; N, 21.14. Found: C, 50.75; H, 3.95; N, 21.12.

(*E/Z*)-3-(2-(3-Methoxybenzylidene)hydrazino)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (4e) Yield 90%; mp 174–176°C; IR: υ_{max} 3257, 3219, 1677, 1619 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.30 and 2.34 (2s, 3H, isoxazole-CH_3), 3.40 and 3.76 (2s, 2H, CH_2), 3.79 and 3.80 (2s, 3H, OCH_3), 6.58 and 6.60 (2s, 1H, isoxazole-H), 6.95–7.40 (m, 4H, Ar-H), 8.00 and 8.30 (2s, 1H, N=CH), 9.98 and 10.02 (2s, 1H, NH), 11.21 and 11.26 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.2, 43.2, 43.7, 56.0, 96.4, 114.9, 117.2, 122.3, 130.0, 135.0, 144.0, 147.1, 158.0, 159.9, 163.5, 165.2, 165.4, 169.2, 170.2; MS: *m/z* 317 $(M+H)^+$, *m/z* 339 $(M+Na)^+$. Anal. Calcd for $C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.10; N, 17.71. Found: C, 56.98; H, 5.09; N, 17.72.

(*E/Z*)-3-(2-(3-Bromobenzylidene)hydrazino)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (4f) Yield 90%; mp 197–199°C; IR: υ_{max} 3221, 1650, 1600 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.33 and 2.36 (2s, 3H, isoxazole-CH_3), 3.41 and 3.78 (2s, 2H, CH_2), 6.51 and 6.53 (2s, 1H, isoxazole-H), 7.22–7.70 (m, 3H, Ar-H), 8.18 and 8.31 (2s, 1H, N=CH), 11.60 and 11.62 (2s, 1H, NH), 11.78 and 11.80 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 11.9, 42.9, 43.6, 96.2, 122.0, 127.2, 130.9, 131.9, 132.8, 135.6, 144.3, 146.9, 157.2, 157.9, 163.9, 164.7, 165.1, 169.9, 170.6, 170.9; MS: *m/z* 365 $(M+H)^+$; Anal. Calcd for $C_{14}H_{13}BrN_4O_3$: C, 46.05; H, 3.59; N, 15.34. Found: C, 46.02; H, 3.58; N, 15.32.

(*E/Z*)-3-(2-(2-Hydroxybenzylidene)hydrazino)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (4g) Yield 90%; mp 167–169°C; IR: υ_{max} 3308, 3219, 1665, 1617 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.30 and 2.34 (2s, 3H, isoxazole-CH_3), 3.42 and 3.72 (2s, 2H, CH_2), 6.50 and 6.52 (2s, 1H, isoxazole-H), 7.00–7.41 (m, 4H, Ar-H), 8.01 and 8.30 (2s, 1H, N=CH), 9.98 and 10.00 (2s, 1H, NH), 9.38 and 10.80 (2s, 1H, Ar-OH), 11.98 and 12.01 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 11.7, 42.8, 43.2, 95.4, 117.1, 119.2, 122.5, 130.9, 133.2, 143.2, 145.9, 158.0, 160.2, 163.8, 164.5, 165.3, 169.2, 170.4; MS: *m/z* 303 $(M+H)^+$, *m/z* 325 $(M+Na)^+$. Anal. Calcd for $C_{14}H_{14}N_4O_4$: C, 55.63; H, 4.67; N, 18.53. Found: C, 55.65; H, 4.68; N, 18.52.

(*E/Z*)-3-(2-(2-Hydroxy-3-methoxybenzylidene)hydrazino)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (4h) Yield 94%; mp 180–182°C; IR: υ_{max} 3448, 3197, 3155, 1697, 1616 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.35 and 2.38 (2s, 3H, isoxazole-CH_3), 3.40 and 3.71 (2s, 2H, CH_2), 3.79 and 3.80 (2s, 3H, OCH_3), 6.59 and 6.62 (2s, 1H, isoxazole-H), 6.69–7.20 (m, 3H, Ar-H), 8.29 and 8.40 (2s, 1H, N=CH), 9.29 and 10.69 (2s, 1H, Ar-OH), 11.09 (2s, 1H, NH), 11.46 and 11.78 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.5, 42.9, 43.4, 56.3, 96.6, 113.2, 114.2, 118.0, 119.3, 119.5, 121.0, 140.8, 146.3, 147.3, 147.4, 148.4, 158.3, 158.5, 162.7, 165.7, 166.2, 168.7, 169.9, 170.1; MS: *m/z* 333 $(M+H)^+$. Anal. Calcd for $C_{15}H_{16}N_4O_5$: C, 54.21; H, 4.85; N, 16.86. Found: C, 54.19; H, 4.84; N, 16.85.

(*E/Z*)-*N*-(5-Methylisoxazol-3-yl)-3-oxo-3-(2-(3,4,5-trimethoxybenzylidene)hydrazino)propanamide (4i) Yield 94%; mp 199–200°C; IR: υ_{max} 3179, 1679, 1619 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.32 and 2.35 (2s, 3H, isoxazole-CH_3), 3.41 and 3.72 (2s, 2H, CH_2), 3.78 and 3.80 (2s, 9H, $(OCH_3)_3$), 6.51 and 6.52 (2s, 1H, isoxazole-H), 6.80 (s, 2H, Ar-H), 8.09 and 8.29 (2s, 1H, N=CH), 10.97 and 11.11 (2s, 1H, NH), 11.92 and 11.94 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 11.9, 43.0, 44.0, 56.1, 95.9, 108.2, 108.9, 129.2, 129.4, 142.0, 143.2, 146.0, 151.6, 152.0, 157.2, 157.9, 163.6, 164.2, 164.9, 169.7, 170.1; MS: *m/z* 377 $(M+H)^+$. Anal. Calcd for $C_{17}H_{20}N_4O_6$: C, 54.25; H, 5.36; N, 14.89. Found: C, 54.23; H, 5.35; N, 14.88.

(*E/Z*)-3-(2-(3,4-Dimethoxybenzylidene)hydrazino)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (4j) Yield 90%; mp 190–191°C; IR: υ_{max} 3197, 3155, 1648, 1598 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.32 and 2.36 (2s, 3H, isoxazole-CH_3), 3.40 and 3.71 (2s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 6.52 and 6.54 (2s, 1H, isoxazole-H), 6.90–7.30 (m, 3H, Ar-H), 7.92 and 8.10 (2s, 1H, N=CH), 11.32 and 11.34 (2s, 1H, NH), 11.50 and 11.53 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.2, 43.2, 44.1, 56.0, 96.2, 116.4, 116.9, 123.7, 128.2, 128.5, 143.9, 146.8, 151.2, 157.4, 157.8, 158.7, 163.4, 164.7, 165.2, 169.2, 169.9, 170.4; MS: *m/z* 347 $(M+H)^+$; Anal. Calcd for $C_{16}H_{18}N_4O_5$: C, 55.49; H, 5.24; N, 16.18. Found: C, 55.46; H, 5.25; N, 16.20.

(*E/Z*)-3-(2-(2-Methylbenzylidene)hydrazino)-*N*-(5-methylisoxazol-3-yl)-3-oxopropanamide (4k) Yield 90%; mp 204–205°C; IR: υ_{max} 3216, 1650, 1600 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.20 and 2.24 (2s, 3H, Ar-CH_3), 2.32 and 2.35 (2s, 3H, isoxazole-CH_3), 3.41 and 3.72 (2s, 2H, CH_2), 6.50 and 6.51 (2s, 1H, isoxazole-H), 7.20–7.45 (m, 4H, Ar-H), 8.21 and 8.30 (2s, 1H, N=CH), 10.52 and 10.55 (2s, 1H, NH), 11.38 and 11.40 (2s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.2, 19.9, 42.8, 43.9, 95.9, 125.6, 126.4, 126.7, 128.6, 128.9, 130.8, 139.1, 144.0, 147.2, 158.2, 163.1, 165.1, 165.4, 169.6, 170.5; MS: *m/z* 301 $(M+H)^+$. Anal. Calcd for $C_{15}H_{16}N_4O_3$: C, 59.99; H, 5.37; N, 18.66: Found: C, 59.97: H, 5.36: N, 18.64.

General procedure for the synthesis of *N*-(5-methylisoxazol-3-yl)-2-(5-aryl-1,3,4-oxadiazol-2-yl)acetamides 5a–k

A mixture of hydrazone **4** (0.1 mmol), chloramine-T (0.5 mmol) and ethanol (15 mL) was heated under reflux for 4–6 h. The progress of the reaction was monitored with TLC. Afterward, the mixture was poured into water and extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure. After removal of the solvent, the crude product was passed over silica gel column. The product was eluted with 40% ethyl acetate in *n*-hexane.

***N*-(5-Methylisoxazol-3-yl)-2-(5-phenyl-1,3,4-oxadiazol-2-yl)acetamide (5a)** Yield 72%; mp 220–221°C; IR: υ_{max} 3215, 1655, 1237 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.30 (s, 3H, isoxazole-CH_3), 3.42 (s, 2H, CH_2), 6.58 (s, 1H, isoxazole-H), 7.13–7.17 (m, 1H, Ar-H), 7.31–7.35 (m, 2H, Ar-H), 7.47–7.49 (m, 2H, Ar-H), 9.50 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.3, 42.6, 96.4, 126.8, 127.9, 128.1, 128.9, 129.2, 129.9, 156.2, 164.2, 168.0, 169.4, 169.6; MS: *m/z* 285 $(M+H)^+$. Anal. Calcd for $C_{14}H_{12}N_4O_3$: C, 59.15; H, 4.25; N, 19.71. Found: C, 59.13; H, 4.23; N, 19.69.

2-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (5b) Yield 74%; mp 231–232°C; IR: υ_{max} 3220, 1659, 1235 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.56 (s, 3H, isoxazole-CH_3), 3.41 (s, 2H, CH_2), 6.48 (s, 1H, isoxazole-H), 7.15–7.40 (m, 4H, Ar-H), 9.52 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.5, 42.2, 96.0, 125.6, 129.2, 130.2, 134.8, 155.8, 164.0, 167.8, 169.0, 169.8; MS: *m/z* 319 $(M+H)^+$; Anal. Calcd for $C_{14}H_{11}ClN_4O_3$: C, 52.76; H, 3.48; N, 17.58. Found: C, 52.74; H, 3.47; N, 17.56.

2-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (5c) Yield 74%; mp 226–227°C; IR: υ_{max} 3215, 1685, 1261 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.41 (s, 3H, isoxazole-CH_3), 3.80 (s, 3H, OCH_3), 4.19 (s, 2H, CH_2), 6.55 (s, 1H, isoxazole-H), 6.70 (d, J = 8.0 Hz, 2H, Ar-H), 7.29 (s, J = 8.2 Hz, 2H, Ar-H), 9.25 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.7, 42.1, 56.1, 96.0, 114.2, 116.8, 128.9, 155.9, 160.9, 163.3, 167.8, 169.4, 169.9; MS: m/z 315 $(M+H)^+$. Anal. Calcd for $C_{15}H_{14}N_4O_4$: C, 57.32; H, 4.49; N, 17.83. Found C, 57.34; H, 4.50; N, 17.85.

***N*-(5-Methylisoxazol-3-yl)-2-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)acetamide (5d)** Yield 70%; mp 250–251°C; IR: υ_{max} 3230, 1656, 1230 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.40 (s, 3H, isoxazole-CH_3), 4.05 (s, 2H, CH_2), 6.40 (s, 1H, isoxazole-H), 7.40 (d, J = 8.0 Hz, 2H, Ar-H), 8.10 (d, J = 8.0 Hz, 2H, Ar-H), 9.32 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.8, 41.9, 95.6, 122.8, 129.2, 135.2, 147.9, 155.8, 163.7, 167.2, 168.9, 170.0; MS: m/z 330 $(M+H)^+$. Anal. Calcd for $C_{14}H_{11}N_5O_5$: C, 51.07; H, 3.37; N, 21.27. Found: C, 51.09; H, 3.38; N, 21.29.

2-(5-(3-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (5e) Yield 74%; mp 236–237°C; IR: υ_{max} 3225, 1659, 1232 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.51 (s, 3H, isoxazole-CH_3), 3.80 (s, 3H, OCH_3), 4.00 (s, 2H, CH_2), 6.38 (s, 1H, isoxazole-H), 6.95–7.02 (m, 3H, Ar-H), 7.20–7.25 (m, 1H, Ar-H), 9.25 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.6, 42.5, 56.0, 94.9, 111.6, 115.1, 120.6, 128.5, 130.9, 155.6, 160.2, 164.2, 166.8, 169.2, 169.8; MS: m/z 315 $(M+H)^+$. Anal. Calcd for $C_{15}H_{14}N_4O_4$: C, 57.32; H, 4.49; N, 17.83. Found: C, 57.30; H, 4.48; N, 17.82.

2-(5-(3-Bromophenyl)-1,3,4-oxadiazol-2-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (5f) Yield 72%; mp 240–241°C; IR: υ_{max} 3215, 1650, 1228 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.54 (s, 3H, isoxazole-CH_3), 4.02 (s, 2H, CH_2), 6.42 (s, 1H, isoxazole-H), 7.42–7.70 (m, 4H, Ar-H) 9.56 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 13.0, 42.3, 95.7, 124.2, 125.2, 129.0, 132.6, 132.8, 134.0, 155.8, 163.0, 165.9, 169.1, 169.9; MS: m/z 363 $(M+H)^+$. Anal. Calcd for $C_{14}H_{11}BrN_4O_3$ C, 46.30; H, 3.05; N, 15.43. Found: C, 46.32; H, 3.06; N, 15.44.

2-(5-(2-Hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (5g) Yield 68%; mp 208–210°C; IR: υ_{max} 3221, 1660, 1236 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.31 (s, 3H, isoxazole-CH_3), 4.10 (s, 2H, CH_2), 6.20 (s, 1H, isoxazole-H), 6.50–7.30 (m, 4H, Ar-H), 8.51 (s, 1H, Ar-OH), 9.62 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.2, 42.0, 95.3, 114.2, 117.1, 122.5, 130.2, 132.0, 154.8, 156.2, 163.5, 165.6, 169.8, 170.1; MS: m/z 301 $(M+H)^+$. Anal. Calcd for $C_{14}H_{12}N_4O_4$: C, 56.00; H, 4.03; N, 18.66. Found: C, 56.03; H, 4.01; N, 18.65.

2-(5-(2-Hydroxy-3-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (5h) Yield 75%; mp 248–249°C; IR: υ_{max} 3231, 1652, 1235cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.48 (s, 3H, isoxazole-CH_3), 3.80 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2), 6.45 (s, 1H, isoxazole-H), 6.90–7.18 (m, 3H, Ar-H), 8.71 (s, 1H, Ar-OH), 9.22 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.6, 42.4, 56.1, 95.2, 114.2, 116.5, 122.2, 124.0, 146.2, 152.2, 156.1, 163.9, 165.2, 168.9, 169.8; MS: m/z 331 $(M+H)^+$. Anal. Calcd for $C_{15}H_{14}N_4O_5$: C, 54.55; H, 4.27; N, 16.96. Found: C, 54.58; H, 4.29; N, 16.98.

2-(5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (5i) Yield 70%; mp 216–217°C; IR: υ_{max} 3229, 1669, 1222 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.52 (s, 3H, isoxazole-CH_3), 4.04 (s, 2H, CH_2), 3.79–3.80 (s, 9H, $(OCH_3)_3$), 6.54 (s, 1H, isoxazole-H), 6.82 (s, 2H, Ar-H), 8.90 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.4, 41.9, 56.0, 56.2, 56.3, 95.5, 106.1, 106.2, 122.0, 141.2, 150.2, 150.4, 155.9, 163.5, 166.9, 168.9, 169.2; MS: m/z 375 $(M+H)^+$. Anal. Calcd for $C_{17}H_{18}N_4O_6$: C, 54.54; H, 4.85; N, 14.97; Found: C, 54.52; H, 4.83; N, 14.95.

2-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*-(5-methylisoxazol-3-yl)acetamide (5j) Yield 71%; mp 228–229°C; IR: υ_{max} 3232, 1665, 1232 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.51 (s, 3H, isoxazole-CH_3), 4.02 (s, 2H, CH_2), 3.80 (s, 6H, $(OCH_3)_2$), 6.49 (s, 1H, isoxazole-H), 6.80 (m, 2H, Ar-H), 6.99 (d, J = 8.0 Hz, 1H, Ar-H), 9.00 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.6, 42.0, 56.1, 56.2, 95.4, 114.2, 116.5, 121.2, 122.0, 150.2, 150.4, 155.8, 163.8, 166.5, 169.8, 170.1; MS: m/z 345 $(M+H)^+$. Anal. Calcd for $C_{16}H_{16}N_4O_5$: C, 55.81; H, 4.68; N, 16.27. Found: C, 55.79; H, 4.67; N, 16.25.

***N*-(5-Methylisoxazol-3-yl)-2-(5-*o*-tolyl-1,3,4-oxadiazol-2-yl)acetamide (5k)** Yield 76%; mp 250–254°C; IR: υ_{max} 3222, 1668, 1240 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.20 (s, 3H, Ar-CH_3), 2.52 (s, 3H, isoxazole-CH_3), 4.00 (s, 2H, CH_2), 6.45 (s, 1H, isoxazole-H), 7.05–7.40 (m, 4H, Ar-H), 9.02 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.6, 20.2, 42.2, 95.9, 127.2, 128.1, 129.0, 130.2, 138.0, 139.1, 156.0, 162.5, 165.8, 168.8, 170.8; MS: m/z 299 $(M+H)^+$. Anal. Calcd for $C_{15}H_{14}N_4O_3$: C, 60.40; H, 4.73; N, 18.78. Found: C, 60.42: H, 4.74; N, 18.80.

Antibacterial activity

The antibacterial activity was assayed by the broth dilution method [29] and expressed as minimum inhibitory concentration. The nutrient broth medium (HiMedia, 24 g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The medium and test tubes were autoclaved at a pressure of 15 $lb/inch^2$ for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound **5a–k** was dissolved in acetone and the concentration of 100 µg/mL of the test compound was added to the first test tube, which was serially diluted. A fixed volume of 0.5 mL was added to all test tubes, and the tubes were incubated at 37°C for 24 h. Then, the tubes were measured for turbidity. Bacterial strains used were *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella aerogenes* (MTCC 39) *Chromobacterium violaceum* (MTCC 2656) (Gram-negative) and *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 511) and *Staphylococus aureus* (MTCC 96) (Gram-positive). Ciprofloxacin was used as the standard drug for comparison.

Antifungal activity

The antifungal activity was assayed using agar cup bioassay method [30]. The potato dextrose agar (PDA) medium (HiMedia, 39 g) was suspended in distilled water (1 L) and the mixture was heated to boiling until a solution was formed. The medium and Petri dishes were autoclaved at a pressure of 15 $lb/inch^2$ for 20 min. The medium was poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of (week old) culture of the test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. A solution was prepared by dissolving the compound **5a–k** in acetone (100 µg/mL). Agar inoculated cups were scooped out with a 6-mm sterile cork borer, and the lids of the dishes were scooped out with a 6-mm sterile cork borer

and the lids of the dishes were replaced. To each cup, 100 µg/mL concentration of the test solution **5a–k** was added. Controls were maintained with acetone and clotrimazole (100 µg/mL). The treated mixtures and the controls were kept at room temperature for 72–96 h. The inhibition zone was measured as a diameter (mm). Three to four replicates were maintained for each treatment. The fungal strains *Aspergillus niger* (MTCC 282), *Chrysosporium tropicum* (MTCC 2821), *Rhizopus oryzae* (MTCC 262), *Fusarium moniliforme* (MTCC 1848) and *Curvularia lunata* (MTCC 2030) were used.

Acknowledgments: The authors are thankful to Department of Chemistry, University College of Science, Satavahana University, Karimnagar and Department of Chemistry, Siddhartha Degree and P.G. College, Narsampet for providing laboratory facilities. All authors are thankful to Prof. E. Rajanarendar for his valuable suggestions.

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