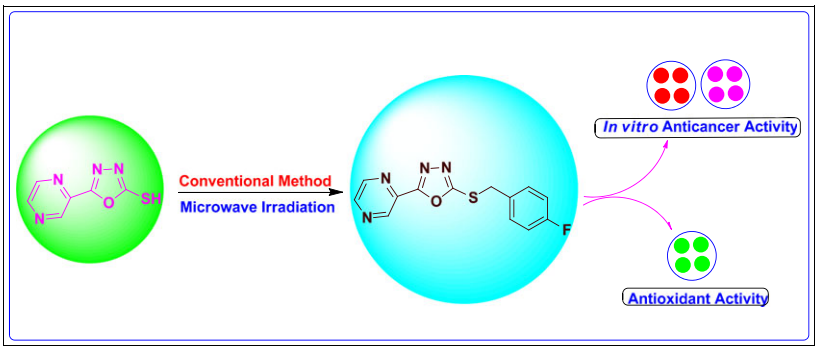
A Facile Synthesis of Substituted 2-(5-(Benzylthio)-1,3,4-oxadiazol-2-yl) pyrazine Using Microwave Irradiation and Conventional Method with Antioxidant and Anticancer Activities

Sanjeev R. Patil,[a] Aniket P. Sarkate,[a]* Kshipra S. Karnik,[a] Ashish Arsondkar,[b] Vrushali Patil,[b] Jaiprakash N. Sangshetti,[c] Anil S. Bobade,[b] and Devanand B. Shinde[d]

[a]Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431004, MS, India
[b]Haffkine Institute for Training, Research and Testing, Parel, Mumbai 400012, MS, India
[c]Y. B. Chavan College of Pharmacy, Roza Baug, Aurangabad 431004, MS, India
[d]Shivaji University, Vidyanagar, Kolhapur 416004, MS, India
*E-mail: dbsaniket09@gmail.com
Received August 24, 2018
DOI 10.1002/jhet.3464
Published online 00 Month 2019 in Wiley Online Library (wileyonlinelibrary.com).



A series of novel substituted 2-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)pyrazine derivatives (**6a**–**n**) were synthesized under microwave irradiation and conventional conditions with less reaction time with good to excellent yields. All the synthesized compounds were screened for antioxidant and anticancer activities. Out of the 14 prepared derivatives, compounds **6f** and **6m** were most potent and active with antioxidant and anticancer activities, respectively. Also, the developed technique was simple, easy, and less time consuming.

J. Heterocyclic Chem., **00,** 00 (2019).

INTRODUCTION

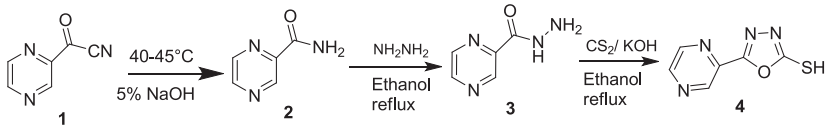
Oxadiazole is a lead moiety in the designing of potent bioactive molecules [1]. The oxadiazole scaffold is a central part of biologically active compounds with various applications and pharmacological properties like antibacterial [2], antifungal [3], antitubercular [4], insecticidal [5], anticonvulsant [6], anticancer [7], antiviral [8], anti-inflammatory [9], antidiabetic [10], and immunosuppressive [11]. The 1,3,4-oxadiazole ring system has been identified as the main core of many bioactive molecules. For the discovery of new lead structures in drug discovery, based on high throughput screening, synthetic methodologies are required that deliver highly diverse derivatives in a timely manner. Under these circumstances, microwave-assisted chemistry appears to be a promising synthetic method [12]. Utility of microwave irradiation [13–15] to carry out organic reaction has now become a regular feature. The main benefits of performing the reaction under microwave conditions are the significant rate enhancements and the higher product yields with minimum time requirement. Here, we wish to report the development and implementation of the methodologies allowing for the synthesis of some novel 2-[(4-fluorobenzyl)thio-5-(pyrazin-2-yl)1,3,4-oxadiazole substituted derivatives. The oxadiazole has been known for over 50 years, so there have been several attempts to design antimicrobial and anticancer agents based on this heterocycle [16–18]. 1,3,4-Oxadiazole heterocycles are very good bioisosteres of amides and esters and can contribute substantially to increasing pharmacological activity by participating in hydrogen-bonding interactions with receptors [19].

In continuation of our work [20–22], on the synthesis of bioactive compounds, we have synthesized some 1,3,4-oxadiazole analogues. The synthetic protocols employed for the synthesis of oxadiazole derivatives **3** and **4** are presented in Schemes 1, 2, and 3, respectively.

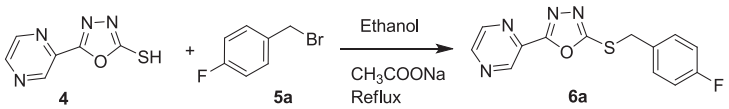
RESULTS AND DISCUSSION

The first part of the study was aimed at optimizing the reaction conditions. The screening of model reaction of 2-[(4-fluorobenzyl)thio-5-(pyrazin-2-yl)1,3,4-oxadiazole **6a** (Scheme 2; Table 1) was performed. We have developed

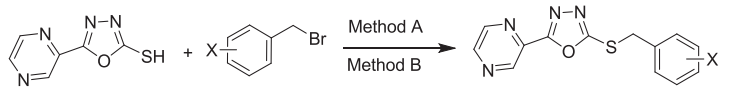
Scheme 1. Synthesis of 5-(pyrazin-2-yl)-1,3,4-oxadiazole-2-thiol (**4**).



Scheme 2. Screening of model reaction 2-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (**6a**). Reaction condition: compound (**3**) (1.0 mmol), benzyl bromide (**5a**) (1.1 mmol), CH_3COONa (1.5 mmol), solvent 1 mL, RT 20–150 min.



Scheme 3. Synthesis of substituted 2-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (**6a**–**n**). Reaction condition: Method A: microwave-assisted synthesis: sodium acetate, ethanol, 40°C, 3–4 min. Method B: conventional synthesis: sodium acetate, ethanol, room temperature, 20–50 min.



X= 5a=6a=-4F-$CH_2C_6H_5$, 5b=6b=-3-OCH_3–$CH_2C_6H_5$, 5c=6c=-2-Cl-$CH_2C_6H_5$, 5d=6d=-2-F-$CH_2C_6H_5$, 5e=6e=-2-Cl-6-F-$CH_2C_6H_4$, 5f=6f=-$CH_2C_6H_5$, 5g=6g=-3,4 di F-$CH_2C_6H_4$, 5h=6h=-2,5 di F-$CH_2C_6H_4$, 5i=6i=-4NO_2-$CH_2C_6H_5$, 5j=6j=2CN-$CH_2C_6H_5$, 5k=6k=-3CN-$CH_2C_6H_5$-, 5l=6l=-2,4 di Cl-$CH_2C_6H_4$, 5m=6m=-3-F-$CH_2C_6H_5$, 5m=6n=-4CN-$CH_2C_6H_5$

Table 1

Screening of bases, solvents, reaction time, and yield for the synthesis (**6a**)[a].

Entry	Base	Solvent	Time (min)	Yield[b] (%)
1	Sodium hydroxide	Ethanol	120	50
2	Sodium hydroxide	Water	130	30
3	Sodium hydroxide	DMF	150	35
4	Sodium carbonate	Ethanol	110	40
5	Sodium carbonate	Water	140	30
6	Sodium carbonate	DMF	120	25
7	Triethylamine	Ethanol	100	65
8	Triethylamine	Water	110	40
9	Triethylamine	DMF	130	30
10	**Sodium acetate**	**Ethanol**	120	**95**
11	Sodium acetate	Water	125	35
12	Sodium acetate	DMF	20	80
13	Diethylamine	Ethanol	40	80
14	Diethylamine	Water	60	80
15	Diethylamine	DMF	50	85

The bold texts are indicating the promising results.
DMF, *N*,*N*-dimethylformamide.
[a]All the reaction was carried out in equimolar amounts of each compound in 1 mL of solvent.
[b]Isolated yield.

the protocol for the synthesis of compound **6a** by condensation of compounds **4** and **5a**. After the initial success in ethanol, we screened various solvents, bases, and time and optimized the yield as well; the results are shown in Table 1. The reaction of compound **4** (1 mmol) and compound **5a** (1.2 mmol), catalyzed by various bases and solvents, was selected as a model reaction to optimize the reaction conditions. In terms of the effect of solvents and bases on the condensation reaction, sodium acetate was found to be the better base, and ethanol was found to be the best solvent for the reaction (Table 1, entry 10); in other solvents, including *N*,*N*-dimethylformamide, water were less efficient (Table 1, entries 2, 3, 5, 6, 8, 9 and 11, 12, 14, 15).

Nevertheless, all of these yields were generally low before further optimizations. Ethanol gave the corresponding product in a 50–95% yield, which was the best among these solvents (Table 1, entries 1, 4, 7, and 10). To increase the efficiency of the condensation reaction, the effects of different bases were investigated (Table 1, entries 1–15). Sodium acetate exhibited the best performance with used solvents and gave better yield, (Table 1, entries 10–12). Diethylamine and triethylamine gave lower yields with other solvents but gave better yield in combination with ethanol as a solvent (Table 1, entries 7 and 13). All the reactions were carried out in equimolar amounts of each compound in 1 mL of solvent. Among these reactions, the same amount of the solvent, namely, 1 mL of ethanol, turned out to be the best choice with yields of 50%, 65% and 95% and 80%

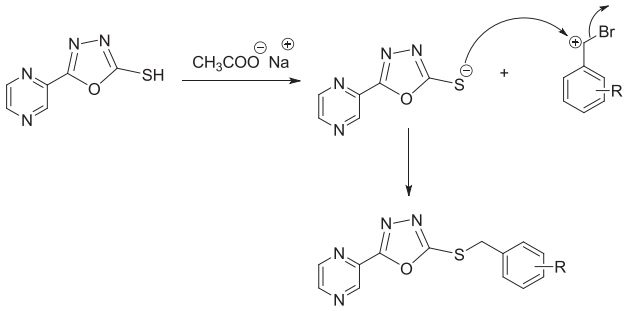
(Table 1, entries 1, 7, 10 and 13). We would like to mention here that ethanol as a solvent with sodium acetate as base was the best choice with a yield of 95% and less time required for the completion of the reaction (Table 1, entry 10). Thus, we decided to carry out the reactions in ethanol with sodium acetate as a base.

Simultaneously, we also performed microwave-assisted reactions. In addition, the reaction time for microwave-assisted reactions was much shorter than the same reactions in all of our studied substrates. As a result, the reaction time was shortened; thermal decomposition was also minimized, resulting in higher isolated yields (Table 2).

The synthesized oxadiazole derivatives by using microwave irradiation and conventional method **6**(**a**–**n**) (Scheme 3; Table 2) are shown. The compound 2-(5-benzylthiol)-1,3,4-oxadiazole-2-yl)pyrazine was prepared *via* condensation between compound **4** [5-(pyrazin-2-yl)-1,3,4-oxadiazole-2-thiol] and substituted compounds **5**(**a**–**n**). Compound **4** was obtained by reaction with compound **3** in ethanol, carbon disulphide, and potassium hydroxide at reflux temperature. The compounds **6**(**a**–**n**) were prepared using sodium acetate as a base with compound **4** and substituted compounds **5**(**a**–**n**).

In this reaction, there was abstraction of –H from –SH, and the negative charge is attacked to benzyl $–CH_2$ to form substituted compounds **6**(**a**–**n**) with leaving HBr.

The plausible reaction mechanism for the synthesis of 2-(5-benzylthiol)-1,3,4-oxadiazole-2-yl)pyrazine is as follows:



The oxadiazole compounds were synthesized by using the microwave irradiation as well as the conventional heating with sodium acetate and ethanol.

The physical data of the synthesized compounds is presented in Table 2. All the reactions proceeded well in 3–4 min in microwave irradiation to give products in very good yields (92–98%) and 20–50 min using the conventional method to give products in very good yields (72–95%).

Table 3 represents the antioxidant and anticancer activities of the aforementioned synthesized derivatives **6**(**a**–**n**), respectively. It is clear from the aforementioned data that compounds **6b**, **6f**, **6k**, and **6m** gave excellent antioxidant activity than the standard butylated hydroxytoluene. Apart from the aforementioned four compounds, remaining compounds also produced much greater activity when compared with the standard butylated hydroxytoluene.

Table 2

Physical data for synthesized substituted 2-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)pyrazine derivatives **6**(**a**–**n**)[a].

Sr. No.	Substituent (X)	Time (min)		Yield[b] (%)		Melting point (°C)
		MW[c]	Con[d]	MW[c]	Con[d]	
6a	4-F-$CH_2C_6H_5$	4	60	98	95	101–104
6b	3-OCH_3-$CH_2C_6H_5$	4	60	96	80	120–123
6c	2-Cl-$CH_2C_6H_5$	3	65	94	82	100–102
6d	2-F-$CH_2C_6H_5$	3	65	93	80	90–92
6e	2-Cl-6-F-$CH_2C_6H_4$	3	65	90	82	130–133
6f	-$CH_2C_6H_5$	4	65	94	84	91–94
6g	3,4-di-F-$CH_2C_6H_4$	3	60	95	82	121–124
6h	2,5-di-F-$CH_2C_6H_4$	4	65	94	84	86–88
6i	4-NO_2-$CH_2C_6H_5$	4	80	96	80	145–148
6j	2-CN-$CH_2C_6H_5$	3	60	92	76	122–124
6k	3-CN-$CH_2C_6H_5$	3	65	92	72	126–128
6l	2,4-di-Cl-$CH_2C_6H_4$	3	65	94	78	108–110
6m	3-F-$CH_2C_6H_5$	4	65	98	84	95–97
6n	4-CN-$CH_2C_6H_5$	5	60	96	82	142–144

[a]Reaction condition (**6a**–**n**). Compound (**3**) (1 mmol), benzyl halides (**4a**–**n**) (1.2 mmol). Method A: Microwave-assisted synthesis: sodium acetate, ethanol, 40°C, 3–4 min. Method B: Conventional synthesis: sodium acetate, ethanol, room temperature, 20–50 min.
[b]Isolated yields.
[c]Microwave-assisted.
[d]Conventional condition.

Table 3

Antioxidant and anticancer activities of derivatives **6**(**a**–**n**).

Sr. No.	Substituent (X)	Antioxidant activity EC_{50} (μM)	Anticancer activity IC_{50} (μM)
6a	4-F-$CH_2C_6H_5$	54.47	**15.42**
6b	3-OCH_3-$CH_2C_6H_5$	**62.05**	4.60
6c	2-Cl-$CH_2C_6H_5$	57.02	5.44
6d	2-F-$CH_2C_6H_5$	56.72	11.09
6e	2-Cl-6-F-$CH_2C_6H_4$	51.54	11.51
6f	-$CH_2C_6H_5$	**55.71**	**15.54**
6g	3,4-di-F-$CH_2C_6H_4$	49.90	5.80
6h	2,5-di-F-$CH_2C_6H_4$	48.39	4.99
6i	4-NO_2-$CH_2C_6H_5$	49.73	6.83
6j	2-CN-$CH_2C_6H_5$	47.50	**14.56**
6k	3-CN-$CH_2C_6H_5$	**60.86**	10.1
6l	2,4-di-Cl-$CH_2C_6H_4$	55.10	4.670
6m	3-F-$CH_2C_6H_5$	**91.09**	**10.74**
6n	4-CN-$CH_2C_6H_5$	48.42	6.30
Standard		**BHT 11.81 ± 1.11**	**Cisplatin 4.00**

The bold texts are indicating the promising results.
BHT, butylated hydroxytoluene.

Coming to the anticancer activity, compounds **6a**, **6f**, **6j**, and **6m** gave outstanding activity when compared with the standard cisplatin on the MCF-7 breast cancer cell line. All the remaining compounds also gave the comparable anticancer activity with the standard. It can be observed from the aforementioned results that compounds **6f** and **6m** are most versatile as they produced exceptional antioxidant and anticancer activities.

EXPERIMENTAL

Material and methods. Pyrazinamide, hydrazine hydrate, ethanol, and various solvents were commercially available. The major chemicals were purchased from Sigma Aldrich and Avra Labs. Microwave reactions were carried out in Micro SYNTH Labstation of Ethusi Milestone. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm). IR spectra were recorded on a FT-IR (Bruker). Melting points were recorded on SRS Optimelt, a melting point apparatus, and these are uncorrected. ^{1}H NMR spectra were recorded on a 400-MHz Bruker spectrometer, and ^{13}C NMR spectra were recorded on a 100-MHz Bruker spectrometer and are reported as parts per million (ppm) downfield from a tetramethylsilane internal standard. For ^{1}H NMR, chemical shifts were reported in the scale relative to a $CHCl_3$ external standard (0 ppm). The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

Conventional synthesis of pyrazine-2-carboxamide (2). A mixture of pyrazine-2-carbonitrile (**1**) 50 g (0.475 mol) in 5% dilute NaOH solution (500 mL) was heated at 40–45°C for 3 h. The completion of reaction was monitored by TLC (hexane 6: ethyl acetate 4). The reaction mass was cooled to 10–15°C and filtered off under vacuum. Water (50 mL) washing given and suck dried to give pure white solid compound (**2**) 48.2 g (81.65%). mp 223–226°C. IR (KBr, cm^{-1}): 3064.89, 3095.75 (–OH stretch), 2339.65, 2455.38 (–CH stretching), 1710.10 (>C=O stretch); ^{1}HNMR (400 MHz, DMSO-d_6 ppm): δ 8.786–8.798 (dd, 1H, CH-pyrazine), 8.854–8.862 (d, 1H, CH-pyrazine), 9.181–9.186 (d, 1H, CH-pyrazine), 13.749 (s, 1H broad-OH); ^{13}C NMR DEPT (400 MHz, DMSO-d_6 ppm): 143.901 (pyrazine C–N), 144.609 (pyrazine CH), 145.541 (pyrazine CH), 147.696 (pyrazine CH), 165.114 (–C=O); MS: 124.1; *m/z*: 125 (M + H). *Anal*. Calcd for $C_6H_4N_4OS$: C, 39.96; H, 2.22; N, 31.08; S, 17.76%. Found: C, 40.0; H, 2.22; N, 31.11; S, 17.79%.

Conventional synthesis of pyrazine-2-carbohydrazide (3). A mixture of pyrazine-2-carboxamide (**1**) 25 g (0.203 mol) in ethanol (250 mL) and hydrazine hydrate (98%) 17.38 g (0.347 mol) was refluxed for 12 h. The completion of reaction was monitored by TLC (hexane 6: ethyl acetate 4). The reaction mass was cooled to 10–15°C and filtered off under vacuum. Pyrazin-2-carboxamide is highly soluble in iso-octane at 25–30°C. Iso-octane (25 mL) washing was given and suck dried to give pure white to off-white solid compound (**3**) 20.75 g (80.00%). mp 168–170°C. IR (KBr, cm^{-1}): 3352.4 (–NH_2 stretch), 3230.3 (>NH stretch), 2845 (–OCH_3), 1640.65 (>C=O stretch), 764 (>C–Br); ^{1}HNMR (400 MHz, DMSO-d_6 ppm): δ 4.50 (s, 2H, amine), 8.59 (s, 1H, amide), 8.74 (d, 1H, CH-pyrazine), 9.16 (d, 1H, CH-pyrazine), 9.90 (s, 1H, C=NH); ^{13}C NMR DEPT (400 MHz, DMSO-d_6 ppm):

143.36 (pyrazine C–N), 143.71 (pyrazine C=N), 144.74 (pyrazine C=N), 147.38 (pyrazine C=N), 162.10 (–C=O). MS: 137; *m/z*: 138 (M + H). *Anal*. Calcd for $C_6H_4N_4OS$: C, 39.96; H, 2.22; N, 31.08; S, 17.76%. Found: C, 40.0; H, 2.22; N, 31.11; S, 17.79%.

Conventional synthesis of 5-(pyrazin-2-yl)-1,3,4-oxadiazole-2-thiol (4). Potassium hydroxide 5.75 g (0.10 mol) was dissolved in absolute ethanol (200 mL) at ambient temperature. Then pyrazine-2-carbohydrazide (**2**) 20 g (0.149 mol) was added, and the reaction mass was cooled. Carbon disulfide 12.54 g (0.165 mol) was added in small portions, and the mixture was stirred at reflux temperature for 12 h. The reaction mixture was changed to green color with the evolution of hydrogen sulfide. The completion of reaction was monitored by TLC (chloroform 9.5: methanol 0.5). Ethanol was completely distilled under vacuum below 50°C. The reaction mass was cooled to 25–30°C. Water (50 mL) was added to dissolve salts, and pH 6.0–7.0 was adjusted by concentrated HCl (20 mL). Solid was precipitated out, stirred at 25–30°C for 1 h, and filtered. Water (50 mL) washing was given and dried to give white to off-white solid compound (**4**) 18.28 g (70.00%). Thus, the yield obtained was used in the next step without further purification. mp 208–210°C. IR (KBr, cm^{-1}): 3352.4 (–NH_2 stretch), 3230.3 (>NH stretch), 2845 (–OCH_3), 1640.65 (>C=O stretch), 764 (>C–Br); ^{1}H NMR (400 MHz, DMSO-d_6 ppm): δ 8.76 (s, 1H), 8.80 (s, 1H), 9.19 (s, 1H), 14.82 (s, 1H). ^{13}C NMR DEPT (400 MHz, DMSO-d_6 ppm): 138.58 (pyrazine C–N), 143.45 (pyrazine C=N), 145.28 (pyrazine C=N), 147.29 (pyrazine C=N), 158.32 (–C=O), 178.48 (–C–SH). MS: 178.9; *m/z*: 179.9 (M + H). *Anal*. Calcd for $C_6H_4N_4OS$: C, 39.96; H, 2.22; N, 31.08; S, 17.76%. Found: C, 40.0; H, 2.22; N, 31.11; S, 17.79%.

General procedure for the synthesis of compounds (6a–n).

Method A: Microwave-assisted synthesis. In a 100 mL round bottom flask, the compound **4** (1.0 mmol), benzyl halide **5**(**a**–**n**) (1.2 mmol), and sodium acetate (1.2 mmol) were added to the ethanol (10 mL), and this mixture was subjected to the microwave irradiation (800 W), at 80°C temperature for 3–4 min. The progress of reaction was monitored by TLC (3% ethyl acetate: hexane). After completion of reaction, the reaction mixture was concentrated *in vacuo*. Water (20 mL) was added to the resulting solid, filtered and recrystallized from ethanol to give product **6**(**a**–**n**) (Yield: 92–96%).

Method B: Conventional synthesis. In a 100 mL round bottom flask, the compound **4** (1.0 mmol), benzyl halide **5**(**a**–**n**) (1.2 mmol), and sodium acetate (1.2 mmol) were added to the ethanol (10 mL) and stirred 30–50 min at reflux temperature. The progress of the reaction was monitored by TLC (3% ethyl acetate: hexane). After completion of reaction, the reaction mixture was concentrated *in vacuo*. Water was added (20 mL) to the resulting solid, filtered, and recrystallized from ethanol to give product **6**(**a**–**n**) (Yield: 72–90%).

Spectral data. ***3-(5-(4-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6a).*** Cream color solid. Yield: 98%. mp 101–104°C; ES-MS *m/z*: 288, IR ν_{max}/cm^{-1}: 3001.24 (CH–Ar), 1460.11 (C=N), 1246.02 (C–S), 1199.72 (C–N). ^{1}H NMR: δppm 4.44 (s, 2H), 6.99–7.31 (m, 3H, Ar–H), 8.66 (s, 2H, Ar–H), 9.35 (s, 1H, Ar–H). ^{13}C NMR: δppm 36.80 (–S–CH_2–), 56.24 (Ar–OCH_3), 76.37, 77.00, 77.63 (N=C–O), 113.84, 114.63, 121.40, 129.85, 136.52, 139.35, 143.88, 144.51, 146.33, and 159.82 (ArC–O). *Anal*. Calcd for $C_{13}H_9FN_4OS$: C, 54.16; H, 3.147; N, 19.434; S, 11.12%. Found: C, 54.256; H, 3.410; N, 20.088; S, 10.573%.

4-(5-(3-Methoxybenzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6b). Cream color solid. Yield: 96%. mp 120–123°C; ES-MS *m/z*: 302, IR ν_{max}/cm^{-1}: 3016.67, 3057.17 (CH–Ar), 1587.42, 1608.63 (C=C), 1309.67 (–OCH_3), 1269.16 (C–S), 1193.94 (C–N). ^{1}H NMR: δppm = 3.75 (s, 3H, –OCH_3), 4.50 (s, 2H), 6.77–7.20 (m, 4H, Ar–H), 8.67 (s, 2H, Ar–H), 9.36 (s, 1H, Ar–H). ^{13}C NMR: δppm 36.80 (–S–CH_2–), 56.24 (Ar–OCH_3), 76.37, 77.00, 77.63 (N=C–O), 113.84, 114.63, 121.40, 129.85, 136.52, 139.35, 143.88, 144.51, 146.33, and 159.82 (ArC–O). *Anal*. Calcd for $C_{14}H_{12}N_4O_2S$: C, 55.988; H, 4.027; N, 18.655; S, 10.674%. Found: C, 56.043; H, 4.137; N, 19.420; S, 10.233%.

2-(5-(2-Chlorobenzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6c). Cream color solid. Yield: 94%. mp 100–102°C; ES-MS *m/z*: 304, IR ν_{max}/cm^{-1}: 3001.24 (CH–Ar), 1460.11 (C=N), 1246.02 (C–S), 1199.72 (C–N). ^{1}H NMR: δppm 4.70 (s, 2H), 7.25–7.28 (m, 1H, Ar–H), 7.42–7.44 (d, 1H, Ar–H), 7.65–7.68 (d, 1H, Ar–H), 8.74 (s, 2H, Ar–H), 9.43 (s, 1H, Ar–H). ^{13}C NMR: δppm 34.60 (–S–CH_2–), 76.69, 77.00, 77.32 (N=C–O), 127.08, 129.75, 129.81, 131.54, 133.25, 134.41, 139.29, 143.86, 144.56, and 146.36. *Anal*. Calcd for $C_{13}H_9ClN_4OS$: C, 51.236; H, 2.977; N, 18.385; S, 10.52%. Found: C, 51.476; H, 2.095; N, 18.977; S, 10.145%.

2-(5-(2-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6d). Cream color solid. Yield: 93%. mp 90–92°C; ES-MS *m/z*: 288, IR ν_{max}/cm^{-1}: 3076.46 (CH–Ar), 1587.42 (C=C), 1494.83 (C=N), 1197.79 (C–S), 869.90–881.47 (C–N). ^{1}H NMR: δppm 4.70 (s, 2H), 7.25–7.28 (m, 1H, Ar–H), 7.42–7.44 (d, 1H, Ar–H), 7.65–7.68 (d, 1H, Ar–H), 8.74 (s, 2H, Ar–H), 9.43 (s, 1H, Ar–H). ^{13}C NMR: δppm 36.80 (–S–CH_2–), 56.24 (Ar–OCH_3), 76.37, 77.00, 77.63 (N=C–O), 113.84, 114.63, 121.40, 129.85, 136.52, 139.35, 143.88, 144.51, 146.33, and 159.82 (ArC–O). *Anal*. Calcd for $C_{13}H_9FN_4OS$: C, 54.16; H, 3.146; N, 19.434; S, 11.12%. Found: C, 54.11; H, 3.122; N, 19.424; S, 11.1%.

2-(5-(2-Chloro-6-fluorobenzylthio)-1,3,4-oxadiazol-2-yl) pyrazine (6e). Light brown color solid. Yield: 90%. mp 130–133°C; ES-MS *m/z*: 322, IR ν_{max}/cm^{-1}: 3001.24

(CH–Ar), 1460.11 (C=N), 1246.02 (C–S), 1199.72 (C–N). ^{1}H NMR: δppm 4.70 (s, 2H), 7.25–7.28 (m, 1H, Ar–H), 7.42–7.44 (d, 1H, Ar–H), 7.65–7.68 (d, 1H, Ar–H), 8.74 (s, 2H, Ar–H), 9.43 (s, 1H, Ar–H). ^{13}C NMR: δppm 36.80 (–S–CH_2–), 56.24 (Ar–OCH_3), 76.37, 77.00, 77.63 (N=C–O), 113.84, 114.63, 121.40, 129.85, 136.52, 139.35, 143.88, 144.51, 146.33, and 159.82 (ArC–O). *Anal*. Calcd for $C_{13}H_8ClFN_4OS$: C, 48.38; H, 2.5; N, 17.36; S, 9.933%. Found: C, 48.334; H, 2.478; N, 17.35; S, 4.957%.

2-(5-(Benzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6f). Light brown color solid. Yield: 94%. mp 91–94°C; ES-MS *m/z*: 270, IR ν_{max}/cm^{-1}: 3046.60 (CH–Ar), 1540.42 (C=C), 1456.26 (C=N), 1193.94 (C–S). ^{1}H NMR: δppm 4.70 (s, 2H), 7.25–7.28 (m, 1H, Ar–H), 7.42–7.44 (d, 1H, Ar–H), 7.65–7.68 (d, 1H, Ar–H), 8.74 (s, 2H, Ar–H), 9.43 (s, 1H, Ar–H). ^{13}C NMR: δppm 36.80 (–S–CH_2–), 56.24 (Ar–OCH_3), 76.37, 77.00, 77.63 (N=C–O), 113.84, 114.63, 121.40, 129.85, 136.52, 139.35, 143.88, 144.51, 146.33, and 159.82 (ArC=O). *Anal*. Calcd for $C_6H_4N_4OS$: C, 57.76; H, 3.73; N, 20.727; S, 11.86%. Found: C, 57.71; H, 3.7; N, 20.72; S, 11.84%.

2-(5-(3,4-Difluorobenzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6g). Off-white color solid. Yield: 95%. mp 121–124°C; ES-MS *m/z*: 306, IR ν_{max}/cm^{-1}: 3076.46 (CH–Ar), 1587.42 (C=C), 1494.83 (C=N), 1197.79 (C–S), 869.90–881.47 (C–N). ^{1}H NMR: δppm 4.44 (s, 2H), 6.99–7.31 (m, 3H, Ar–H), 8.66 (s, 2H, Ar–H), 9.35 (s, 1H, Ar–H). ^{13}C NMR: δppm 35.62 (–S–CH_2–), 76.37, 77.00, 77.63 (N=C–O), 117.39, 117.73, 118.08, 118.43, 125.38, 139.23, 143.90, 144.54, 146.46 (Ar–F) and 152.56 (Ar–F). *Anal*. Calcd for $C_6H_4N_4OS$: C, 50.979; H, 2.632; N, 18.292; S, 10.467%. Found: C, 51.297; H, 2.405; N, 18.923; S, 11.031%.

2-(5-(2,5-Difluorobenzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6h). Off-white color solid. Yield: 94%. mp 86–88°C; ES-MS *m/z*: 306, IR ν_{max}/cm^{-1}: 3076.46 (CH–Ar), 1587.42 (C=C), 1494.83 (C=N), 1197.79 (C–S), 869.90–881.47 (C–N). ^{1}H NMR: δppm 4.70 (s, 2H), 7.25–7.28 (m, 1H, Ar–H), 7.42–7.44 (d, 1H, Ar–H), 7.65–7.68 (d, 1H, Ar–H), 8.74 (s, 2H, Ar–H), 9.43 (s, 1H, Ar–H). ^{13}C NMR: δppm 36.80 (–S–CH_2–), 56.24 (Ar–OCH_3), 76.37, 77.00, 77.63 (N=C–O), 113.84, 114.63, 121.40, 129.85, 136.52, 139.35, 143.88, 144.51, 146.33, and 159.82 (ArC–O). *Anal*. Calcd for $C_6H_4N_4OS$: C, 50.978; H, 2.632; N, 18.292; S, 10.467%. Found: C, 50.932; H, 2.612; N, 18.283; S, 10.447%.

2-(5-(4-Nitrobenzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6i). Cream color solid. Yield: 96%. mp 145–148°C; ES-MS *m/z*: 315, IR ν_{max}/cm^{-1} 3101.54 (CH–Ar), 1602.85 (C=C), 1350.17 (–NO_2), 1205.51 (C–S), 860.25, 871.82 (C–N). ^{1}H NMR: δppm 4.70 (s, 2H), 7.25–7.28 (m, 1H, Ar–H), 7.42–7.44 (d, 1H, Ar–H), 7.65–7.68 (d, 1H, Ar–H), 8.74 (s, 2H, Ar–H), 9.43 (s, 1H, Ar–H). ^{13}C NMR: δppm 36.80 (–S–CH_2–), 56.24 (Ar–OCH_3), 76.37, 77.00, 77.63 (N=C–O), 113.84, 114.63, 121.40, 129.85, 136.52, 139.35, 143.88, 144.51, 146.33, and 159.82 (ArC–O). *Anal*. Calcd for $C_6H_4N_4OS$: C, 49.52; H, 2.877; N, 22.211; S, 10.168%. Found: C, 49.475; H, 2.854; N, 22.2; S, 10.148%.

2-((5-(Pyrazin-2-yl)-1,3,4-oxadiazol-2-ylthio)methyl) benzonitrile (6j). Cream color solid. Yield: 92%. mp 122–124°C; ES-MS *m/z*: 295, IR ν_{max}/cm^{-1}: 3076.46 (CH–Ar), 1587.42 (C=C), 1494.83 (C=N), 1197.79 (C–S), 869.90–881.47 (C–N). ^{1}H NMR: δppm 4.73 (s, 2H), 7.40–7.44 (m, 1H, Ar–H), 7.66–7.68 (d, 1H, Ar–H), 7.69–7.71 (d, 1H, Ar–H), 7.78–7.80 (s, 2H, Ar–H), 8.72–8.74 (s, 2H), 9.41 (s, 1H, Ar–H). ^{13}C NMR: δppm 34.49 (–S–CH_2–), 76.69, 77.00, 77.32 (N=C–O), 112.80, 116.99 (Ar–CN), 128.72, 130.80, 133.03, 133.20, 139.33, 143.86, 144.56, 146.47, 163.38, and 166.44. *Anal*. Calcd for $C_6H_4N_4OS$: C, 56.94; H, 3.072; N, 23.715; S, 10.856%. Found: C, 56.898; H, 3.005; N, 23.750; S, 11.142%.

3-((5-(Pyrazin-2-yl)-1,3,4-oxadiazol-2-ylthio)methyl) benzonitrile (6k). Off-white color solid. Yield: 92%. mp 126–128°C; ES-MS *m/z*: 295, IR ν_{max}/cm^{-1}: 3076.46 (CH–Ar), 1587.42 (C=C), 1494.83 (C=N), 1197.79 (C–S), 869.90–881.47 (C–N). ^{1}H NMR: δppm 4.73 (s, 2H), 7.40–7.44 (m, 1H, Ar–H), 7.66–7.68 (d, 1H, Ar–H), 7.69–7.71 (d, 1H, Ar–H), 7.78–7.80 (s, 2H, Ar–H), 8.72–8.74 (s, 2H), 9.41 (s, 1H, Ar–H). ^{13}C NMR: δppm 34.49 (–S–CH_2–), 76.69, 77.00, 77.32 (N=C–O), 112.80, 116.99 (Ar–CN), 128.72, 130.80, 133.03, 133.20, 139.33, 143.86, 144.56, 146.47, 163.38, and 166.44. *Anal*. Calcd for $C_6H_4N_4OS$: C, 56.882; H, 3.047; N, 23.703; S, 10.835%. Found: C, 56.898; H, 3.005; N, 23.750; S, 11.142%.

2-(5-(2,4-Dichlorobenzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6l). Off-white color solid. Yield: 94%. mp 108–110°C; ES-MS *m/z*: 339, IR ν_{max}/cm^{-1}: 3091.89 (CH–Ar), 1583.56 (C=C), 1458.18 (C=N), 1199.72 (C–S), 850.50 (C–N). ^{1}H NMR: δppm 4.70 (s, 2H), 7.25–7.28 (m, 1H, Ar–H), 7.42–7.44 (d, 1H, Ar–H), 7.65–7.68 (d, 1H, Ar–H), 8.74 (s, 2H, Ar–H), 9.43 (s, 1H, Ar–H). ^{13}C NMR: δppm 36.80 (–S–CH_2–), 56.24 (Ar–OCH_3), 76.37, 77.00, 77.63 (N=C–O), 113.84, 114.63, 121.40, 129.85, 136.52, 139.35, 143.88, 144.51, 146.33, and 159.82 (ArC–O). *Anal*. Calcd for $C_6H_4N_4OS$: C, 46.033; H, 2.377; N, 16.52; S, 9.451%. Found: C, 45.99; H, 2.358; N, 16.51; S, 9.434%.

2-(5-(3-Fluorobenzylthio)-1,3,4-oxadiazol-2-yl)pyrazine (6m). Off-white color solid. Yield: 98%. mp 95–97°C; ES-MS *m/z*: 288, IR ν_{max}/cm^{-1}: 3076.46 (CH–Ar), 1587.42 (C=C), 1494.83 (C=N), 1197.79 (C–S), 869.90–881.47 (C–N). ^{1}H NMR: δppm 4.54 (s, 2H), 7.00–7.04 (m, 2H, Ar–H), 7.43–7.47 (m, 2H, Ar–H), 8.72 (s, 2H, Ar–H), 9.41 (s, 1H, Ar–H). ^{13}C NMR: δppm 35.93 (–S–CH_2–), 76.68, 77.00, 77.32 (N=C–O), 115.61, 115.83, 130.97, 143.85, 144.51, 146.37, 161.21, 163.09,

163.67, and 166.09 (Ar–F). *Anal*. Calcd for $C_6H_4N_4OS$: C, 54.16; H, 3.147; N, 19.434; S, 11.12%. Found: C, 54.11; H, 3.122; N, 19.424; S, 11.1%.

4-((5-(Pyrazin-2-yl)-1,3,4-oxadiazol-2-ylthio)methyl) benzonitrile (6n). Cream color solid. Yield: 96%. mp 142–144°C; ES-MS *m/z*: 295, IR ν_{max}/cm^{-1}: 3076.46 (CH–Ar), 2223.92 (C=N), 1462.04 (C=N), 1211.30 (C–S). ^{1}H NMR: δppm 4.73 (s, 2H), 7.66–7.68 (d, 2H, Ar–H), 7.69–7.71 (d, 2H, Ar–H), 7.78–7.80 (s, 2H, Ar–H), 8.72–8.74 (s, 1H, Ar–H), 9.41 (s, 1H, Ar–H). ^{13}C NMR: δppm 34.49 (–S–CH_2–), 76.69, 77.00, 77.32 (N=C–O), 112.80, 116.99 (Ar–CN), 128.72, 130.80, 133.03, 133.20, 139.33, 143.86, 144.56, 146.47, 163.38, and 166.44. *Anal*. Calcd for $C_6H_4N_4OS$: C, 56.94; H, 3.072; N, 23.715; S, 10.856%. Found: C, 56.887; H, 3.047; N, 23.703; S, 10.836%.

Biological methods. ***Antioxidant activity.*** All the synthesized compounds were evaluated for antioxidant activity against *DPPH* at different concentrations (20–100 μg/mL using free radical scavenging activity [DPPH method]). In the present study, *in vitro* DPPH (1,1-diphenyl-2-picryl-hydrazil) radical scavenging method [23–25] was used to evaluate the antioxidant potential of 1,2,4-triazole-3-thiol derivatives (**6a**–**n**). The interaction of all tested compounds with the stable free radical DPPH indicates their radical scavenging activity.

ANTICANCER ACTIVITY

Cell lines and cell culture. The cell lines MCF7 (mammary adenocarcinoma) and HeLa, derived from human cervical cancer cells (ATCC No. CCL-2), were obtained from the National Centre for Cell Sciences, Pune, India. Cells were cultured in RPMI-1640 media supplemented with 10% heating activated fetal bovine serum, 1 mM $NaHCO_3$, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. All cell lines were maintained and cultured at 37°C in an atmosphere of 5% CO_2.

Test concentrations. Initially, stock concentration of each compound was prepared in DMSO (8 mg/mL), from which 50 μL of stock was diluted to 1 mL in a culture medium to obtain experimental stock concentration of 400 μg/mL. Different aliquots of experimental stock were added to the cultured cells in the medium (final volume of 200 μL) to attain the required concentration.

Cytotoxicity. In all experiments, different cell lines were seeded at a final density of 2 × 104 cells per well, in 96-well microtiter plates. Cytotoxicity was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, according to the method of Mosmann [26]. Briefly, the cells (2 × 104) were seeded in each well containing 0.1 mL of medium in 96-well plates. After overnight incubation, the cells were treated with different test concentrations of test compounds (5–200 μg/mL) at identical conditions with five replicates each. The cell viability was assessed after 24 h, by adding 10 μL of MTT (5 mg/mL) per well. The plates were incubated at 37°C for additional 3 h. The medium was discarded, and the formazan blue, which formed in the cells, was dissolved with 100 μL of DMSO. The rate of color production was measured at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SoftMax Pro-5.4). The percent inhibition of cell viability was determined with reference to the control (without test compound). The data were subjected to linear regression analysis, and the regression lines were plotted for the best straight-line fit. The IC_{50} (concentration required for 50% inhibition of cell viability) concentrations were calculated using the respective regression equation.

CONCLUSION

In conclusion, we have successfully developed an easy access to a new series of substituted 2-(5-(benzylthio)-1,3,4-oxadiazol-2-yl)pyrazine derivatives **6**(**a**–**n**). The mild reaction conditions, good to excellent yields, easy workup, and easily available substrates make the reactions attractive for the preparation of compounds **6**(**a**–**n**). The synthesized derivatives possess excellent to good antioxidant and anticancer activities. Besides, the technique has the advantage of being simple and allows the synthesis with a minimum reaction time. We believe that these reactions provide a new approach for the synthesis of pyrazine containing moiety.

Acknowledgments. The authors are thankful to the head of the Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, for providing the laboratory facility and Haffkine Institute for Training, Research and Testing, Parel, Mumbai, for providing the activity facility.

REFERENCES AND NOTES

[1] Chandrakantha, B.; Shetty, P.; Nambiyar, V.; Isloor, N.; Isloor, A. M. J Med Chem 2010, 45, 1206.

[2] Wang, P. Y.; Zhou, L.; Zhou, J.; Wu, Z. B.; Xue, W.; Song, B. A.; Yang, S. Bioorg Med Chem Lett 2016, 26, 1217.

[3] Wani, M. Y.; Ahmad, A.; Shiekh, R. A.; Al-Ghamdi, K. J.; Sobral, A. J. F. N. Bioorg Med Chem 2015, 23, 4172.

[4] Patel, N. B.; Purohit, A. C.; Rajani, D. P.; Moo-Puc, R.; Rivera, G. Eur J Med Chem 2013, 62, 677.

[5] Li, Y. H.; Zhu, H. J.; Chen, K.; Liu, R.; Khallaf, A.; Zhang, X. N.; Ni, J. P. Org Biomol Chem 2013, 11, 3979.

[6] Harish, K. P.; Mohana, K. N.; Mallesha, L.; Prasannakumar, B. N. Eur J Med Chem 2013, 65, 276.

[7] Liu, H.–Q.; Wang, D.–C.; Wu, F.; Tang, W.; Ouyang, P.–K. Chin Chem Lett 2013, 24, 929.

[8] Wu, W.; Chen, Q.; Tai, A.; Jiang, G.; Ouyang, G. Bioorg Med Chem Lett 2015, 25, 2243.
[9] Gadegoni, H.; Manda, S. Chin Chem Lett 2013, 24, 127.
[10] Liao, B.-R.; He, H.-B.; Yang, L.-L.; Gao, L.-X.; Chang, L.; Tang, J.; Li, J.-Y.; Li, J.; Yang, F. Eur J Med Chem 2014, 83, 15.
[11] Tang, J.-F.; Lv, X.-H.; Wanga, X.-L.; Sun, J.; Zhang, Y.-B.; Yang, Y.-S.; Gong, H.-B.; Zhu, H.-L. Bioorg Med Chem 2012, 20, 4226.
[12] Tiwari, S. V.; Siddiqui, S.; Saijas, J. A.; Vazquez-Tato, M. P.; Sarkate, A. P.; Lokwani, D. K.; Nikalje, A. P. G. Molecules 2017, 22, 995.
[13] Jadhav, S. A.; Sarkate, A. P.; Shioorkar, M. G.; Shinde, D. B. Syn Comm 2017, 47, 1661.
[14] Sarkate, A. P.; Bahekar, S. S.; Wadhai, V. M.; Ghandge, G. N.; Wakte, P. S.; Shinde, D. B. Synlett 2013, 24, 1513.
[15] Jadhav, S. A.; Shioorkar, M. G.; Chavan, O. S.; Sarkate, A. P.; Shinde, D. B. Syn Comm 2017, 47, 285.
[16] Maftei, C. V.; Fodor, E.; Jones, P. G.; Freytag, M.; Franz, M. H.; Kelter, G.; Fiebig, H. H.; Tamm, M.; Neda, I. Eur J Med Chem 2015, 101, 431.
[17] Maftei, C. V.; Fodor, E.; Jones, P. G.; Daniliuc, C. G.; Franz, M. H.; Kelter, G.; Fiebig, H. H.; Tamm, M.; Neda, I. Tetrahedron 2016, 72, 1185.
[18] Maftei, C. V.; Fodor, E.; Jones, P. G.; Franz, M. H.; Kelter, G.; Fiebig, H.; Neda, I. Beil J Org Chem 2013, 9, 2202.
[19] Guimaraes, C. R. W.; Boger, D. L.; Jorgensen, W. L. J Am Chem Soc 2005, 127, 17377.
[20] Tiwari, S. V.; Saijas, J. A.; Vazquez-Tato, M. P.; Sarkate, A. P.; Lokwani, D. K.; Nikalje, A. P. G. Molecules 2016, 21, 894.
[21] Lokwani, D. K.; Sarkate, A. P.; Shinde, D. B. Med Chem Res 2013, 22, 1415.
[22] Dofe, V. S.; Sarkate, A. P.; Azad, R.; Gill, C. H. Res Chem Int 2017, 43, 7331.
[23] Srivastava, B. K.; Solanki, M.; Mishra, B.; Soni, R.; Jayadev, S.; Valani, D.; Jain, M.; Patel, P. R. Bioorg Med Chem Lett 2007, 17, 1924.
[24] Lohray, B. B.; Lohray, V. B.; Srivastava, B. K.; Kapadnis, P. B.; Pandya, P. Bioorg Med Chem 2004, 12, 4557.
[25] Amr, A. E.; Abdel-Hafez, N. A.; Mohamed, S. F.; Abdalla, M. M. Turk J Chem 2009, 33, 421.
[26] Mosmann, T. J Immunol Methods 1983, 65, 55.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.