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Full Paper

Multicomponent Synthesis of Novel 2-Aryl-5-((1-aryl-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazoles using Cu^{I} as Catalyst and their Antimicrobial Evaluation

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A series of novel 2-aryl-5-((1-aryl-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazoles have been synthesised by C–S bond formation and azide–alkyne cyclocondensation between [5-(aryl)-[1,3,4]oxadiazol-2-yl]methanethiol, propargyl bromide, and substituted aryl azides in one pot with an aim to explore their effect on the in vitro growth of microorganisms causing microbial infection. In vitro antibacterial activity was determined against four strains, namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* and antifungal activity against two fungal strains, namely *Aspergillus niger* and *Aspergillus flavus*.

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Introduction

The resistance of many microbial organisms, particularly Gram-positive bacteria *Staphylococcus aureus* and species of the genus *Enterococcus*, towards anti-microbial agents has increased.[1] Microbes have developed resistance against many existing agents and therefore focus has been on the development of alternative new more effective antimicrobial agents with novel modes of action and a broad spectrum of activities. Molecular hybridisation is the approach of designing new chemical entities based on the fusion/coupling of two heterocyclic rings in a single compound wherein both active compounds/units are derived from known bioactive molecules.[2] Pharmacophore hybridisation is believed to be analogous to conventional combination therapy, with the exception that the two drugs are covalently linked and available as a single entity.[3]

The multicomponent reaction (MCR) approach offers major advantages such as atom economy, introduction of structural complexity and diversity, and high efficiency, etc, over conventional linear-type syntheses and have played an important role in modern drug discovery.[4–6] Similarly click chemistry has also played a very significant role in drug discovery, because alkyne and azide components can be incorporated into a wide range of substrates.[7] The combination of MCR with click reactions has been shown to be a powerful strategy to yield complex structures in a single step.[8,9]

1,3,4-Oxadiazoles have been established as a privileged structure class.[10] Oxadiazoles occur frequently in drug-like molecules and act as bioisosteric replacements for ester and amide functionalities thus enhancing the pharmacological activity by participating in hydrogen-bonding interactions with receptors.[11] Substituted 1,3,4-oxadiazoles have been found to exhibit anti-inflammatory,[12–15] hypoglycemic,[16,17] antianxiety, antidepressant,[18] and antimitotic[19] activities. In addition to these, several researchers have also reported antimicrobial activities of 1,3,4-oxadiazoles.[20–24] 1,2,3-Triazoles are also important pharmacologically due to their latent ability for the formation of hydrogen bonds with other active molecules. 1,2,3-Triazoles exhibit antitumour,[25] anti-HIV,[26,27] cytostatic,[28] and anti-bacterial[29] activities. They can also act as gamma-aminobutyric acid[30] and glycosidase inhibitors.[31] This heterocycle has been compared with amide bonds and may serve as a bioisoster of peptide bonds due its electronic features.[32,33] Some of the lead compounds having oxadiazole and triazole moieties (**i**, **ii**, and **iii**) acting as antifungal agents are shown in Fig. 1.[34]

It has also been reported that sulfur is an important bridge for combining two heterocyclic moieties covalently in a single compound.[35] It was believed from a literature survey that oxadiazoles having aryl, alkyl, and one or more methylene groups as a linker exhibit different biological activities like anticancer, anti-inflammatory, and antimicrobial activities by playing an important role in their penetration into the microbial cell.[36,37] Based on the abovementioned observations, we decided to develop a new approach for the synthesis of novel 2-aryl-5-((1-aryl-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazoles by C–S bond formation and an azide–alkyne cycloaddition reaction in one pot and to study their antimicrobial activity on different pathogen strains.

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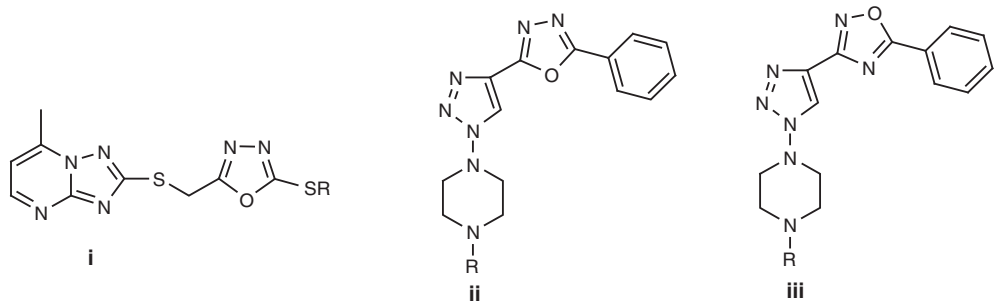
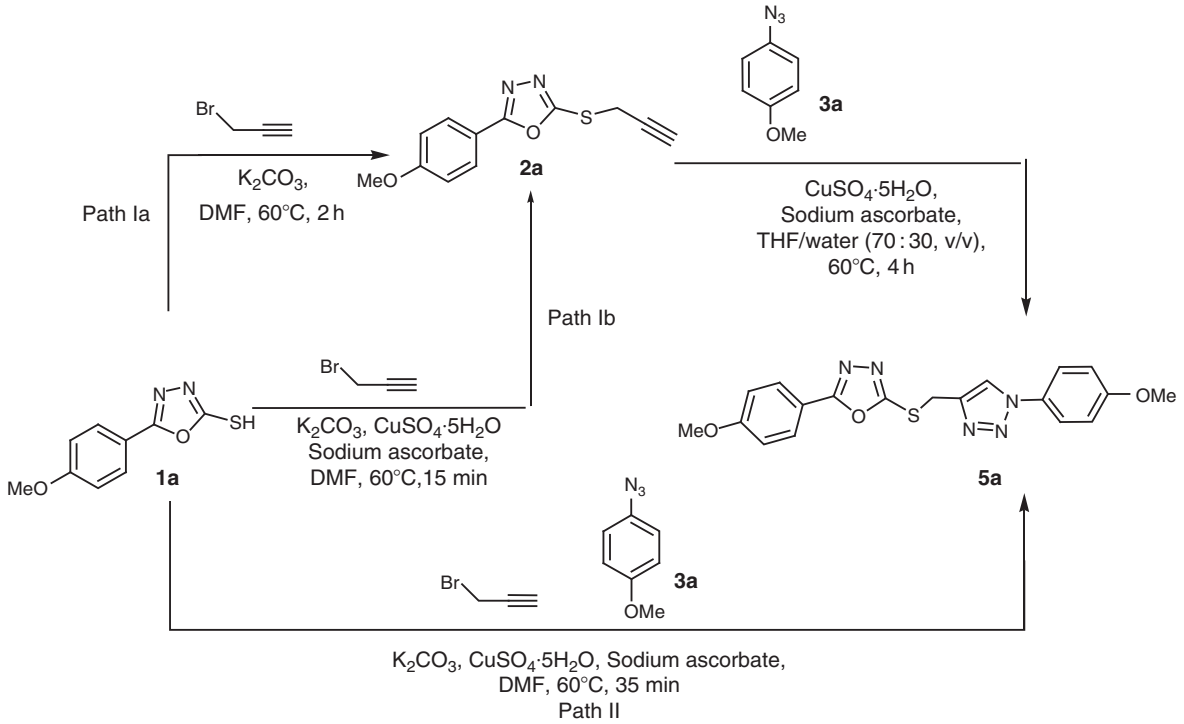


Fig. 1. Some of the lead compounds having antimicrobial activity.



Scheme 1. Synthesis of 2-(4-methoxyphenyl)-5-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazole (**5a**) by different paths.

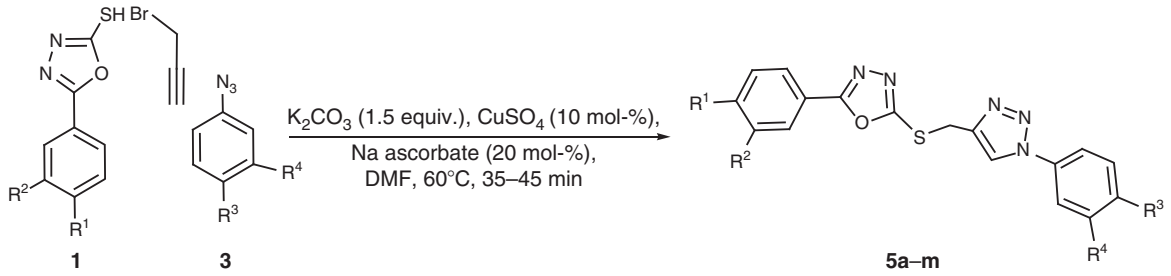
Results and Discussion

Chemistry

The present manuscript reports the synthesis of targeted 2-aryl-5-((1-aryl-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazoles **5a**–**m**, by a one-pot three-component approach using 5-aryl-1,3,4-oxadiazole-2-thiols **1**, substituted phenyl azides **3**, and propargyl bromide, which involves an azide–alkyne cycloaddition reaction and C–S bond formation in the presence of $CuSO_4{\cdot}5H_2O$, sodium ascorbate, and K_2CO_3 in DMF at 60°C. The starting molecules 5-aryl-1,3,4-oxadiazole-2-thiols **1** and substituted phenyl azides **3** were prepared from substituted benzoic acid and substituted amines, respectively, by the reported procedures.[38]

The synthesis of compounds **5a**–**m** can be achieved by different paths as shown in Scheme 1. The reaction of 5-(4-methoxyphenyl)-1,3,4-oxadiazole-2-thiol (**1a**), propargyl bromide, and 1-azido-4-methoxybenzene (**3a**) was attempted by two different paths summarised in Scheme 1. First, we attempted the synthesis of targeted compound 2-(4-methoxyphenyl)-5-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazole (**5a**) by sequential reactions. The first path involved the reaction of **1a** (2.2 mmol) and propargyl bromide (2.8 mmol) in the presence of K_2CO_3 (3.3 mmol) in DMF (10 mL) at 60°C. After 2 h, the reaction was found to be complete. The solid so obtained was filtered and recrystallised from ethanol to afford pure product which was identified as 2-(4-methoxyphenyl)-5-(prop-2-ynylthio)-1,3,4-oxadiazole (**2a**) (90 % yield). Subsequently the intermediate compound **2a** (1.2 mmol) was reacted with 1-azido-4-methoxybenzene (**3a**) (1.2 mmol) in THF/water (70 : 30, v/v) in a round-bottomed flask at 60°C in the presence of an aqueous solution of $CuSO_4{\cdot}5H_2O$ (10 mol-%) and sodium ascorbate (20 mol-%). The reaction was complete after 4 h as monitored by TLC using ethyl acetate/hexane (70 : 30, v/v) as eluent. A crude mass was obtained after work up which was purified by flash chromatography to yield the desired **5a** as a white solid in 85 % yield and an overall yield of 77 % (path Ia).

The reaction of **1a** and propargyl bromide was also carried out using K_2CO_3 (3.3 mmol), $CuSO_4{\cdot}5H_2O$ (10 mol-%), and sodium ascorbate (20 mol-%) as catalyst at 60°C. The reaction time improved significantly from 2 h to 15 min and the yield of the product also increased from 90 to 95 % (path Ib). The same reaction was also attempted in the presence of $CuSO_4{\cdot}5H_2O$



Scheme 2. Synthesis of compounds **5a**–**m**.

Table 1. Optimisations of reaction conditions for the synthesis 2-(4-methoxyphenyl)-5-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl) methylthio)-1,3,4-oxadiazole (5a)

Entry	Solvent	Time [min]	Temp [°C]	Yield [%]
1	DMF	35	60	94
2	DMF	80	30	78
3	DMF	35	80	89
4	Ethanol	160	60	48[A]
5	*t*-BuOH	120	60	54[A]
6	THF	75	60	70
7	THF/water[B]	90	60	60
8	*t*-BuOH/water[B]	190	60	38[A]

[A]Incomplete reaction.
[B](70 : 30, v/v).

Table 2. Physical data of the synthesised compounds 5a–m

Compound	R^1	R^2	R^3	R^4	Time [min]	Yield [%]
5a	OMe	H	OMe	H	35	94
5b	OMe	H	H	H	37	90
5c	H	H	F	Cl	42	84
5d	H	H	Br	H	40	88
5e	OMe	H	F	Cl	38	86
5f	OMe	H	F	H	40	88
5g	OMe	H	Me	H	35	92
5h	H	H	H	Cl	42	81
5i	H	H	F	H	40	80
5j	OMe	H	H	Cl	38	90
5k	H	H	OMe	H	35	87
5l	H	Cl	OMe	H	38	83
5m	OMe	H	NO_2	H	45	79

(10 mol-%) and sodium ascorbate (20 mol-%) but in the absence of K_2CO_3. This reaction required 60 min for completion. Cu^I polarises the C–Br bond which makes the carbon more susceptible to attack by thiol thus increasing the rate of reaction.

Therefore, keeping in mind the role of Cu^I in both azide–alkyne cycloaddition reactions and also in catalysing C–S bond formation, we decided to attempt both steps in one pot. The reaction of **1a** (1.2 mmol), propargyl bromide (1.2 mmol), and **3a** (1.2 mmol) was carried out in DMF in the presence of K_2CO_3 (1.8 mmol), $CuSO_4{\cdot}5H_2O$ (10 mol-%), and sodium ascorbate (20 mol-%) at 30°C. The reaction was found to be complete after 80 min. The reaction mixture was poured onto crushed ice. The solid so obtained was filtered and purified by flash chromatography to give the target compound **5a** in 78 % yield. The reaction was then repeated at 60°C under otherwise similar conditions in DMF. The reaction was found to be complete in 35 min and the desired compound **5a** was obtained in 94 % yield (path II) (Scheme 2).

No improvement in the reaction time and product yield was observed at higher temperatures. The reaction was also attempted in ethanol, *t*-BuOH, THF, THF/water (70 : 30, v/v), and *t*-BuOH/water (70 : 30, v/v) at 60°C but the best results were obtained in DMF at 60°C as mentioned in Table 1.

A complete spectroscopic study (^{1}H NMR, ^{13}C NMR, and IR spectroscopy and mass spectrometry) of the product **5a** confirmed the formation of the expected 2-(4-methoxyphenyl)-5-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazole (Scheme 2) only. A careful examination of the ^{1}H NMR spectrum of **5a** showed a singlet at δ 8.12 for 1 proton present at the C-5 carbon of the triazole ring. This higher value justifies the substitution of the aryl group on N-1. Similarly a singlet at δ 4.62 confirms the presence of a S–CH_2 group apart from other characteristics signals (see Supplementary Material).

Thus path II was found to be the most efficient as the isolation of intermediate compounds formed during reaction and their purification is not required. It also helps to increase the overall yield of the product. By using Cu^I as a common catalyst in both sequential steps, a multicomponent approach was developed for the synthesis of S-tethered 1*H*-[1,2,3]-triazoles. This approach utilises the well known catalytic activities of Cu^I in both click chemistry and C–S bond formation. Therefore, this strategy can be adopted as a general path for the synthesis of a series of 1,2,3-triazoles. A focussed library of 13 compounds, 2-aryl-5-((1-aryl-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazoles **5a**–**m**, were synthesised (Table 2). All the novel compounds were characterised by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy and electrospray ionisation (ESI) and high resolution mass spectrometry (HRMS). The formation of the triazole ring was confirmed by the resonance of H-5 of the triazole ring in the aromatic region in the ^{1}H NMR spectra. The structures were further supported by the ^{13}C NMR spectra, which confirmed the C-skeleton of the triazole derivatives **5a**–**m**. ESI-MS/HRMS of all compounds showed $[M + Na]^+$, $[M^+]$, or $[M + H]^+$. All these compounds were then screened for their anti-bacterial and anti-fungal activity.

Biological Results and Discussion

In Vitro Antibacterial Activity

A total of 13 chemical compounds were screened for their antibacterial and antifungal activity. The tested chemical compounds possessed variable antibacterial activity against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria. However, they did not exhibit any activity against Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*)

bacteria. Positive controls produced significantly sized inhibition zones against the tested bacteria and fungi. However, negative controls did not show an observable inhibitory effect against any of the test organisms as shown in Table 3.

The tested chemical compounds showed a zone of inhibition ranging between 13.6 and 18.6 mm against the Gram-positive bacteria. On the basis of the zone of inhibition produced against the test bacterium, three compounds, namely, **5b**, **5f**, and **5m** were found to be most active against *S. aureus* and *B.subtilis* with a zone of inhibition of 16.3 and 18.6 mm, respectively. However other tested compounds showed moderate antibacterial activity (Table 3, Fig. 2). The minimum inhibitory concentration (MIC) of all compounds **5a**–**m** was measured against Gram-positive bacteria, as shown in Table 4. In the whole series, the MIC of the chemical compounds ranged between 64 and 512 $\mu g\,mL^{-1}$ against Gram-positive bacteria.

Table 3. Antibacterial activity of chemical compounds 5a–m through agar well diffusion method

Compound	Diameter of growth of inhibition zone [mm][A]			
	Staphylococcus aureus	*Bacillus subtilise*	*Escherichia coli*[B]	*Pseudomonas aeruginosa*[B]
5a	14.6	15.3	–	–
5b	16.3	18.6	–	–
5c	14.3	14.6	–	–
5d	13.6	15.3	–	–
5e	14.3	15.0	–	–
5f	15.6	18.3	–	–
5g	13.6	14.3	–	–
5h	14.3	15.6	–	–
5i	14.6	15.3	–	–
5j	15.3	15.6	–	–
5k	14.3	14.6	–	–
5l	13.6	14.3	–	–
5m	16.3	18.6	–	–
Ciprofloxacin	26.6	24.0	25.0	22.0

[A]Values, including diameter of the well (8 mm), are means of three replicates.
[B]No activity.

Four compounds, **5b**, **5f**, **5j**, and **5m** were found to be best as they exhibited the lowest MIC of 128 $\mu g\,mL^{-1}$ against *S. aureus* whereas three compounds, **5b**, **5f**, and **5m** were found to be best against *B. subtilis* with the lowest MIC of 64 $\mu g\,mL^{-1}$ (Table 4, Fig. 3).

Antifungal Activity

All the 13 compounds **5a**–**m** were also tested for their in vitro antifungal activity against two fungal strains, namely, *A. niger* and *A. flavus* species through poisoned food method. The standard drug fluconazole was used for comparison of the antifungal activity shown by the compounds and results were recorded as a percentage of mycelial growth inhibition. It can be inferred from Table 5 that all the compounds **5a**–**m** showed variable antifungal activity against the two pathogens. From a careful comparison of the results, it is observed that three compounds, namely **5l**, **5f**, and **5m** showed high antifungal activity with >50 % inhibition of mycelial growth against the two fungi in comparison with the standard drug. Other

Table 4. Minimum inhibitory concentration (MIC) of compounds 5a–m

Compound	MIC [$\mu g\,mL^{-1}$]	
	Staphylococcus aureus	*Bacillus subtilis*
5a	256	128
5b	128	64
5c	256	256
5d	512	128
5e	256	128
5f	128	64
5g	512	256
5h	256	128
5i	256	128
5j	128	128
5k	256	256
5l	512	256
5m	128	64
Ciprofloxacin	6.25	6.25

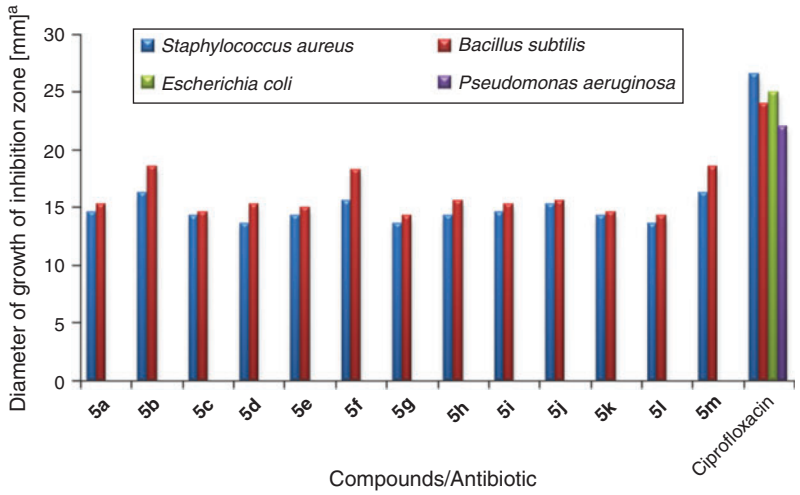


Fig. 2. Comparisons of diameter of growth of inhibition zone (mm) of compounds **5a**–**m** with the standard drug ciprofloxacin. Superscript a represents the zone of inhibition, also including the diameter of the well (8 mm).

compounds also showed good antifungal activities as summarised in Table 5. Comparisons of antifungal activity of all the synthesised compounds with the reference drug in terms of percentage mycelial growth inhibition are also shown in Fig. 4.

Among all the tested chemical compounds, compound **5m** showed good antibacterial and antifungal activity and this will be explored further.

Conclusion

In conclusion, the present study offers an application of Cu^{I} in an efficient and convenient synthesis of 2-aryl-5-((1-aryl-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazoles **5a**–**m**. Biological evaluation of these compounds shows that some of the compounds possess good antimicrobial activity.

Experimental

Chemistry

Structures of all of the compounds were identified by their spectroscopic data. Silica gel 60 F_{254} (precoated aluminum plates) from Merck were used to monitor the reaction progress. Melting points were determined on a melting point apparatus and are uncorrected. IR (KBr) spectra were recorded on a Perkin–Elmer FTIR spectrophotometer and the values are expressed as ν_{max} in cm^{-1}. The NMR (^{1}H and ^{13}C) spectra were recorded on a Jeol JNM ECX-400P at 400 and 100 MHz, respectively. The chemical shift values are recorded on the δ scale and the coupling constants (J) are in Hertz. Mass spectra were recorded on a Bruker Micro TOF Q–II.

*Characterisation of Compounds **1***

5-(4-Methoxy-phenyl)-[1,3,4]oxadiazole-2-thiol (**1a**): mp 202°C (lit.[39] 204°C).

5-Phenyl-[1,3,4]oxadiazole-2-thiol (**1b**): mp 216°C (lit.[40] 217–219°C).

5-(3-Chloro-phenyl)-[1,3,4]oxadiazole-2-thiol (**1c**): mp 163°C (lit.[40] 161°C).

*General Procedure for the Synthesis of Compounds **5a**–**m***

A mixture of **1b** (1.0 mmol), propargyl bromide (1.0 mmol), substituted phenyl azide (1.0 mmol), and K_2CO_3 (1.5 mmol) was dissolved in DMF (10 mL) in a 50 mL round-bottomed flask. An aqueous solution of $CuSO_4{\cdot}5H_2O$ (10 mol-%) was added to the above mixture, followed by addition of an aqueous solution of sodium ascorbate (20 mol-%). The reaction mixture was stirred at 60°C on a magnetic stirrer for an appropriate time as shown in Table 2. The progress of the reaction was monitored by TLC using ethyl acetate/petroleum ether (70 : 30, v/v) as eluent. After completion of the reaction, the reaction mixture was poured into a beaker containing crushed ice. The precipitate formed was collected by vacuum filtration. The solid obtained was purified by flash column chromatography using silica gel (230–400 mesh size).

Spectroscopic Data

*2-(4-Methoxyphenyl)-5-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazole (**5a**)*

White solid. Mp 163°C. ν_{max} (KBr)/cm^{-1} 3124, 2994, 1610, 1516, 1475, 1260. δ_H (400 MHz, $CDCl_3$) 8.12 (s, 1H, Ar-H), 7.90–7.88 (m, 2H, Ar-H), 7.58–7.56 (m, 2H, Ar-H), 6.97–6.94 (m, 4H, Ar-H), 4.62 (s, 2H, S–CH_2), 3.84 (s, 3H, OMe), 3.83 (s, 3H, OMe). δ_c (100 MHz, $CDCl_3$) 166.1, 163.1, 162.3, 159.8, 143.5, 130.2, 128.4, 122.2, 121.7, 115.9, 114.6, 114.4, 55.6, 55.4, 26.8. *m/z* (ESI) 395.1050 $[M + H]^+$.

*2-(4-Methoxyphenyl)-5-((1-phenyl-1H-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazole (**5b**)*

White solid. Mp 122°C. ν_{max} (KBr)/cm^{-1} 3134, 2934, 1613, 1504, 1481, 1258. δ_H (400 MHz, $CDCl_3$) 8.21 (s, 1H, Ar-H),

Table 5. Antifungal activity of compounds 5a–m through poisoned food method

Compound	Mycelial growth inhibition [%]	
	Aspergillus niger	*Aspergillus flavus*
5a	41.1	44.4
5b	45.5	48.8
5c	37.7	42.2
5d	33.3	35.5
5e	38.8	41.1
5f	47.7	52.2
5g	33.3	38.8
5h	42.2	45.5
5i	36.6	38.8
5j	43.3	47.7
5k	35.5	41.1
5l	46.6	48.8
5m	51.1	53.3
Fluconazole	81.1	77.7

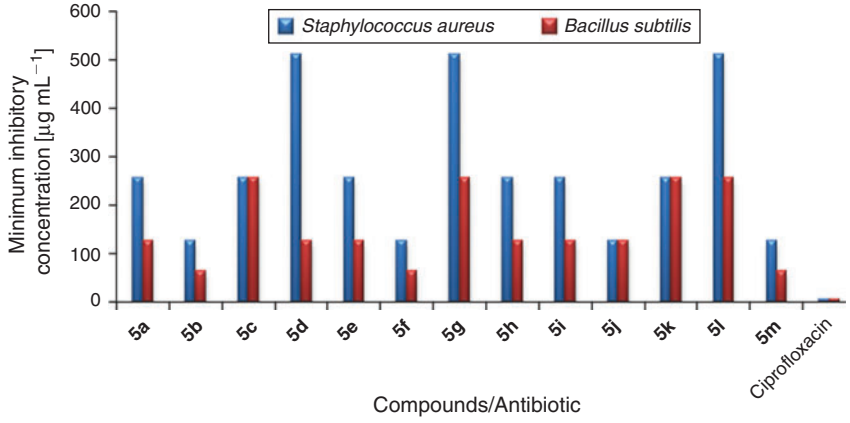


Fig. 3. Comparison of minimum inhibitory concentration of the compounds **5a**–**m**.

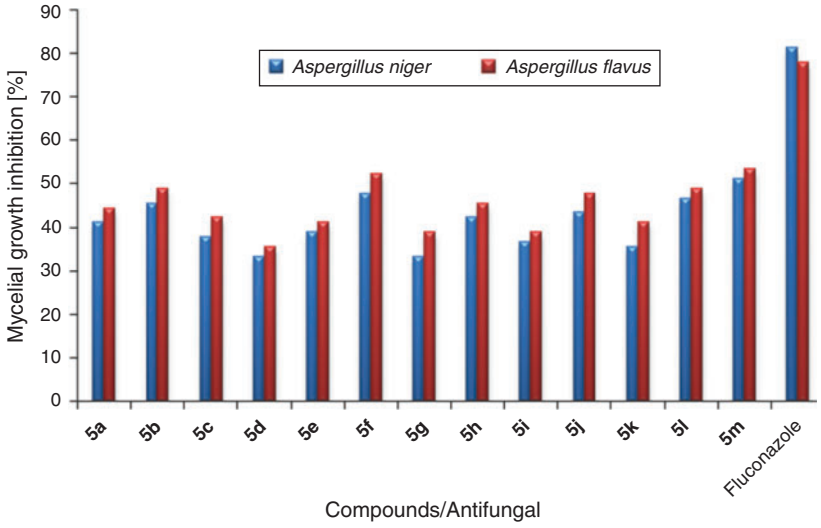


Fig. 4. Graphical representation of antifungal activity of compounds **5a**–**m** with the standard drug fluconazole.

7.90–7.88 (m, 2H, Ar-H), 7.69–7.67 (m, 2H, Ar-H), 7.49–7.39 (m, 3H, Ar-H), 6.96–6.94 (m, 3H, Ar-H), 4.64 (s, 2H, S–CH_2), 3.83 (s, 3H, OMe). δ_c (100 MHz, $CDCl_3$) 166.1, 163.8, 159.8, 143.5, 131.7, 130.2, 129.0, 126.6, 123.3, 122.2, 121.7, 114.6, 55.5, 26.8. *m/z* (ESI) 366.1298 $[M+H]^+$.

*2-((1-(3-Chloro-4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl) methylthio)-5-phenyl-1,3,4-oxadiazole (**5c**)*

White solid. Mp 125°C. ν_{max} (KBr)/cm^{-1} 3146, 3006, 1511, 1472, 1245, 1197. δ_H (400 MHz, $CDCl_3$) 8.21 (s, 1H, Ar-H), 7.97–7.94 (m, 2H, Ar-H), 7.81–7.79 (m, 1H, Ar-H), 7.59–7.46 (m, 4H, Ar-H), 7.28–7.25 (m, 1H, Ar-H), 4.64 (s, 2H, S–CH_2). δ_c (100 MHz, $CDCl_3$) 166.2, 163.7, 159.1, 156.5, 144.1, 131.8, 129.0, 126.5, 123.0, 121.7, 120.2, 120.2, 26.5. *m/z* (ESI) 388.1071 $[M+H]^+$.

*2-((1-(4-Bromophenyl)-1*H*-1,2,3-triazol-4-yl) methylthio)-5-phenyl-1,3,4-oxadiazole (**5d**)*

White solid. Mp 190°C. ν_{max} (KBr)/cm^{-1} 3119, 3093, 2985, 1560, 1492, 1233, 1064. δ_H (400 MHz, $CDCl_3$) 8.22 (s, 1H, Ar-H), 7.97–7.95 (m, 2H, Ar-H), 7.62–7.47 (m, 7H, Ar-H), 4.65 (s, 2H, S–CH_2). δ_c (100 MHz, $CDCl_3$) 166.1, 144.0, 135.7, 132.8, 131.8, 129.0, 126.6122.5, 121.9, 121.4, 26.5. *m/z* (ESI) 413.2883 $[M+H]^+$.

*2-((1-(3-Chloro-4-fluorophenyl)-1*H*-1,2,3-triazol-4-yl) methylthio)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (**5e**)*

White solid. Mp 126°C. ν_{max} (KBr)/cm^{-1} 3145, 2926, 1613, 1508, 1478, 1268, 1175. δ_H (400 MHz, $CDCl_3$) 8.21 (s, 1H, Ar-H), 7.90–7.87 (m, 2H, Ar-H), 7.82–7.79 (m, 1H, Ar-H), 7.60–7.56 (m, 1H, Ar-H), 7.28–7.26 (m, 1H, Ar-H), 6.97–6.95 (m, 2H, Ar-H), 4.63 (s, 2H, S–CH_2), 3.84 (s, 3H, OMe). δ_c (100 MHz, $CDCl_3$) 166.1, 162.9, 162.3, 159.1, 156.6, 144.2, 133.2, 128.3, 123.0, 122.3, 121.7, 120.2, 120.2, 117.6, 117.4, 115.7, 114.4, 55.4, 26.5. *m/z* (ESI) 417.0403 $[M+H]^+$.

*2-((1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl) methylthio)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (**5f**)*

Off-white solid. Mp 175°C. ν_{max} (KBr)/cm^{-1} 3135, 2927, 1617, 1502, 1267, 1171, 1031. δ_H (400 MHz, $CDCl_3$) 8.18 (s, 1H, Ar-H), 7.90–7.88 (m, 2H, Ar-H), 7.69–7.64 (m, 2H, Ar-H), 7.19–7.15 (m, 2H, Ar-H), 6.97–6.95 (m, 2H, Ar-H), 4.63 (s, 2H, S–CH_2), 3.84 (s, 3H, OMe). δ_c (100 MHz, $CDCl_3$) 166.1, 163.0, 162.3, 143.9, 128.4, 128.4, 122.6, 122.5, 121.9, 114.6, 114.3, 54.9, 26.6. *m/z* (ESI) 384.1173 $[M+H]^+$.

*2-(4-Methoxyphenyl)-5-((1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazole (**5g**)*

Off-white solid. Mp 142°C. ν_{max} (KBr)/cm^{-1} 3137, 2924, 1611, 1501, 1481, 1254, 1167, 1033. δ_H (400 MHz, $CDCl_3$) 8.16 (s, 1H, Ar-H), 7.89–7.87 (m, 2H, Ar-H), 7.55–7.53 (m, 2H, Ar-H), 7.26 (m, 2H, Ar-H), 6.95–6.93 (m, 2H, Ar-H), 4.63 (s, 2H, S–CH_2), 3.82 (s, 3H, OMe), 2.36 (s, 3H, Ar-Me). δ_c (100 MHz, $CDCl_3$) 166.1, 163.0, 162.3, 143.9, 133.1, 128.4, 122.6, 122.5, 121.8, 116.7, 116.5, 115.8, 114.4, 54.9, 30.9, 26.6. *m/z* (ESI) 380.1438 $[M+H]^+$.

*2-((1-(3-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl) methylthio)-5-phenyl-1,3,4-oxadiazole (**5h**)*

Light pink solid. Mp 135°C. ν_{max} (KBr)/cm^{-1} 3138, 2925, 1596, 1474, 1195, 1047. δ_H (400 MHz, $CDCl_3$) 8.23 (s, 1H, Ar-H), 7.97–7.94 (m, 2H, Ar-H), 7.75–7.74 (m, 1H, Ar-H), 7.60–7.58 (m, 1H, Ar-H), 7.49–7.38 (m, 5H, Ar-H), 4.65 (s, 2H, S–CH_2). δ_c (100 MHz, $CDCl_3$) 166.0, 163.0, 162.2, 143.5, 138.9, 134.4, 130.1, 128.3, 121.5, 120.4, 115.8, 114.4, 55.3, 26.6. *m/z* (ESI) 370.44 $[M+H]^+$.

*2-((1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl) methylthio)-5-phenyl-1,3,4-oxadiazole (**5i**)*

Off-white solid. Mp 145°C. ν_{max} (KBr)/cm^{-1} 3137, 3093, 1514, 1474, 1227, 1191, 1050. δ_H (400 MHz, $CDCl_3$) 8.23 (s, 1H, Ar-H), 7.97–7.94 (m, 2H, Ar-H), 7.75–7.74 (m, 1H, Ar-H), 7.60–7.58 (m, 1H, Ar-H), 7.49–7.38 (m, 5H, Ar-H), 4.65 (s, 2H, S–CH_2). δ_c (100 MHz, $CDCl_3$) 166.0, 163.0, 162.2, 143.5, 138.9, 134.4, 130.1, 128.3, 121.5, 120.4, 115.8, 114.4, 55.3, 26.6. *m/z* (ESI) 354.48 $[M+H]^+$.

*2-((1-(3-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl) methylthio)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (**5j**)*

Light pink solid. Mp 160°C. ν_{max} (KBr)/cm^{-1} 3119, 1615, 1597, 1482, 1266, 1175, 1047. δ_H (400 MHz, $CDCl_3$) 8.12

(s, 1H, Ar-H), 7.96–7.95 (m, 2H, Ar-H), 7.58–7.44 (m, 5H, Ar-H), 4.65 (s, 2H, S–CH_2), 3.82 (s, 3H, OMe). δ_c (100 MHz, $CDCl_3$) 166.0, 163.0, 162.2, 143.5, 138.9, 134.4, 130.1, 128.3, 121.5, 120.4, 115.8, 114.4, 55.3, 26.6. *m*/*z* (ESI) 400.0870 $[M + H]^+$.

*2-((1-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-4-yl) methylthio)-5-phenyl-1,3,4-oxadiazole (**5k**)*

Off-white solid. Mp 125°C. ν_{max} (KBr)/cm^{-1} 3134, 2934, 1613, 1504, 1481, 1258. δ_H (400 MHz, $CDCl_3$) 8.12 (s, 1H, Ar-H), 7.96–7.95 (m, 2H, Ar-H), 7.58–7.44 (m, 5H, Ar-H), 6.97–6.95(m, 2H, Ar-H), 4.65 (s, 2H, S–CH_2), 3.82(s, 3H, OMe). δ_c (100 MHz, $CDCl_3$) 166.1, 163.8, 159.8, 143.4, 131.7, 130.2, 129.0, 126.6, 123.3, 122.2, 121.7, 114.6, 55.5, 30.9. *m*/*z* (ESI) 366.1294 $[M + H]^+$.

*2-(3-Chlorophenyl)-5-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazole (**5l**)*

Light pink solid. Mp 130°C. ν_{max} (KBr)/cm^{-1} 3119, 1615, 1597, 1482, 1266, 1175, 1047. δ_H (400 MHz, $CDCl_3$) 8.15 (s, 1H, Ar-H), 7.95–7.84 (m, 2H, Ar-H), 7.58–7.38 (m, 4H, Ar-H), 6.98–6.95 (m, 2H, Ar-H), 4.65 (s, 2H, S-CH_2), 3.82 (s, 3H, OMe). δ_c (100 MHz, $CDCl_3$) 164.9, 164.4, 159.8, 135.1, 131.7, 130.4, 126.5, 124.9, 124.6, 122.1, 114.6, 55.5, 29.6. *m*/*z* (ESI) 400.0868 $[M + H]^+$.

*2-(4-Methoxyphenyl)-5-((1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methylthio)-1,3,4-oxadiazole (**5m**)*

Light pink solid. Mp 140°C. ν_{max} (KBr) cm^{-1} 3139, 3087, 1610, 1597, 1525, 1505, 1483, 1341, 1261, 1170. δ_H (400 MHz, $CDCl_3$) 8.38–8.37 (s, 2H, Ar-H), 7.95–7.88 (m, 4H, Ar-H), 7.56 (s, 1H, Ar-H), 6.97–6.96 (m, 2H, Ar-H), 4.65 (s, 2H, S–CH_2), 3.85(s, 3H, OMe). δ_c (100 MHz, $CDCl_3$) 166.1, 162.9, 162.3, 149.4, 144.1, 130.7, 128.8, 128.4, 121.5, 120.7, 118.4, 115.7, 114.4, 55.5, 30.9. *m*/*z* (ESI) 410.0868 $[M]^+$.

Antimicrobial Assay

Test Microorganisms

A total of six microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram-positive bacteria (*Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121), two Gram-negative bacteria (*Escherichia coli* MTCC 1652 and *Pseudomonas aeruginosa* MTCC 741), and two fungi (*Aspergillus niger* and *A. flavus*[41]) were used in the present study for evaluation of antimicrobial activity of the chemical compounds. All the bacterial cultures were procured from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The bacteria were subcultured on nutrient agar whereas fungi were subcultured on Sabouraud's dextrose agar.

Antibacterial Activity

The antibacterial activity of 13 chemical compounds was evaluated by the agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of $\sim 1.5 \times 10^6$ cfu mL^{-1}. Mueller Hinton agar medium (20 mL) was poured into each Petri plate and plates were swabbed with a 100 µL inocula of the test microorganisms and kept for 15 min for adsorption. Using a sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µL volume with concentration of 2.0 mg mL^{-1} of each compound reconstituted in dimethyl sulfoxide (DMSO). All the plates were incubated at 37°C for 24 h. The antibacterial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with a zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as a positive control. This procedure was performed in three replicate plates for each organism and the mean values of the diameter of inhibition zones ± standard deviations were calculated.[42]

Determination of MIC of Chemical Compounds

The MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. The MIC of the various compounds against bacterial strains was tested through a modified agar well diffusion method.[42] In this method, a two-fold serial dilution of each chemically synthesised compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 512 to 1 µg mL^{-1}. A 100 µL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 µL of standardised inoculum (10^6 cfu mL^{-1}) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 h and observed for the inhibition zones. The MIC, taken as the lowest concentration of the chemical compound that completely inhibited the growth of the microbe, shown by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin was used as a positive control.

Antifungal Activity

The antifungal activity of the 13 chemical compounds was evaluated by a poisoned food technique.[43] The moulds were grown on Sabouraud's dextrose agar (SDA) at 25°C for 7 days and used as inocula. Molten SDA (45°C, 15 mL) was poisoned by the addition of 100 µL of each compound having a concentration of 2.0 mg mL^{-1} reconstituted in DMSO, poured into a sterile Petri plate, and allowed to solidify at room temperature. The solidified poisoned agar plates were inoculated at the centre with fungal plugs (8 mm diameter) obtained from the colony margins and incubated at 25°C for 7 days. DMSO was used as the negative control whereas fluconazole was used as the positive control. The experiments were performed in triplicate. The diameter of fungal colonies was measured and expressed as the percentage inhibition of myelial growth by the following calculation: percentage inhibition of myelial growth = (dc − dt)/dc × 100 (%), where dc is the average diameter of the fungal colony in negative control sets and dt is the average diameter of the fungal colony in experimental sets.

Supplementary Material

Spectral data are available on the Journal's website.

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