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Short communication

Synthesis, antimicrobial and cytotoxic activities of some novel thiazole clubbed 1,3,4-oxadiazoles



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ABSTRACT

A series of thiazole clubbed 1,3,4-oxadiazole derivatives (**5a–I**) have been synthesized and characterized by IR, ^1H NMR, ^{13}C NMR and mass spectral analysis. Synthesized compounds were evaluated for their antimicrobial and cytotoxic activities. The results indicated that, compounds **5c** and **5i** exhibited the most potent antibacterial activity. Compound **5f** was found to be the most potent antifungal agent. The structure activity relationship revealed that the presence of electron withdrawing groups at *para* position of phenyl ring remarkably enhanced the antibacterial activity of synthesized compounds. Further, the results of preliminary MTT cytotoxicity studies on HeLa cells suggested that potent antimicrobial activity of **5b**, **5c**, **5f**, **5h** and **5i** is accompanied by low cytotoxicity.

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1. Introduction

The treatment of infectious diseases still remain a challenging task because of combination of factors such as an alarming increase in number of multi-drug-resistant microbial pathogens and advent of newer infectious diseases such as severe acute respiratory syndrome (SARS), and avian influenza. Despite the availability of a large number of antibiotics and chemotherapeutics, the increasing clinical importance of drug-resistant microbial pathogens have lent additional urgency in microbiological and antifungal research [1–4]. A potential solution to the antibiotic resistance is to design and explore innovative heterocyclic agents with novel mode of actions. In this context, thiazole derivatives have been playing a crucial role in medicinal chemistry. Thiazole nucleus constitutes an integral part of all the available penicillins, which have transformed the bacterial diseases therapy [5]. They display quite a broad spectrum of biological activities [6], which have found applications in the treatment of allergies [7], hypertension [8], inflammation [9], schizophrenia [10], microbial infections [11,12], HIV infections [13], hypnotics [14] and pain [15]. They are also used as new inhibitors of bacterial DNA gyrase B [16]. Further, thiazoles have emerged as new

class of potent antimicrobial agents, which are reported to inhibit bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanisms [17,18].

On the other hand, 1,3,4-oxadiazole heterocycles are very good bioisosteres of amides and esters, which can contribute substantially in enhancing pharmacological activity by participating in hydrogen bonding interactions with the receptors [19]. Potent pharmacological activity of 1,3,4-oxadiazoles can be attributed to the presence of toxophoric $-\text{N}=\text{C}-\text{O}-$ linkage which may react with the nucleophilic centers of the microbial cell [20]. Further, the widespread use of 1,3,4-oxadiazoles as a scaffold in medicinal chemistry established this moiety as a member of the privileged structures class and its derivatives have exhibited a wide range of biological activities such as antibacterial [21], antitubercular [22], vasodilatory [23], antifungal [24], cytotoxic [25], anti-inflammatory and analgesic [26,27], hypolipidemic [28], anticancer [29] and ulcerogenic [30]. Oxadiazole derivatives have been found to possess broad spectrum antimicrobial activity and therefore are useful substructures for further molecular exploration [30]. The most prominent examples of prescribed agents featuring the 1,3,4-oxadiazoles nucleus include the antiretroviral raltegravir [31], antihypertensive nesapidil [32] and the antibiotic furamizole [33] (Fig. 1).

Prompted by above-mentioned observations and in continuation of our search for new, potent, selective, and less toxic antimicrobial agents [34–39], we report herein the synthesis of some

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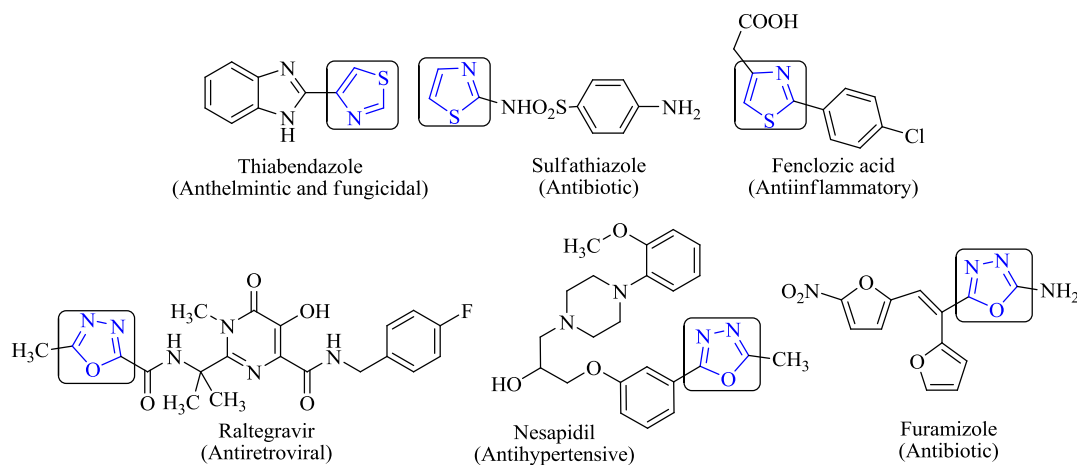


Fig. 1. Commercially available drugs containing thiazole and 1,3,4-oxadiazole nucleus.

novel structural hybrids by combining thiazole and 1,3,4-oxadiazole pharmacophores in single molecular framework in order to investigate their *in vitro* antimicrobial activity. In addition, cytotoxicity studies were also conducted in HeLa cell lines to evaluate the ability of these compounds to inhibit the cell growth. The substitution pattern of 1,3,4-oxadiazole ring was carefully selected in order to confer different electronic environment to the molecules.

2. Results and discussion

2.1. Chemistry

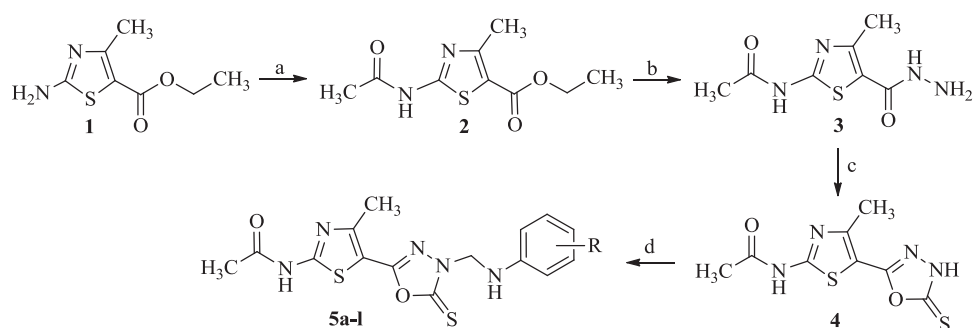
The reaction sequences employed for synthesis of the target compounds **5a–I** are outlined in Scheme 1. Ethyl 2-amino-4-methylthiazole-5-carboxylate (**1**) was taken as starting material and reacted with acetic anhydride to afford ethyl 2-acetamido-4-methylthiazole-5-carboxylate (**2**), which on further reaction with hydrazine hydrate in absolute ethanol yielded intermediate *N*-(5-(hydrazine carbonyl)-4-methylthiazol-2-yl)acetamide (**3**). The intermediate obtained thus, was refluxed with carbon disulfide in the presence of potassium hydroxide in ethanol (99.5%) to yield intermediate *N*-(4-methyl-5-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)acetamide (**4**). Mannich condensation of intermediate **4** with 36% formaldehyde and an appropriately substituted aniline derivative in ethanol (99.5%) furnished the desired compounds **5a–I**. Both analytical and spectral data of the synthesized compounds **5a–I** were fully in agreement with proposed structures.

Formation of titled compounds **5a–I** was confirmed by characteristic IR spectrum absorption bands in the range of 3330–3350 cm^{-1} , 3200–3250 cm^{-1} , 2810–2840 cm^{-1} , 2750–2800 cm^{-1} and 1660–1690 cm^{-1} corresponding to $-\text{NH}$ stretching of secondary amine, $-\text{NH}$ stretching of amide, $-\text{CH}_3$, $-\text{CH}_2-$ and $>\text{C}=\text{O}$ of amide respectively. Singlets at around δ 2.03–2.10, 2.40–2.50, 4.15–4.25, 4.35–4.45 and 9.10–9.20 ppm in ^1H NMR were due to $-\text{CH}_3$ in anilide group, Het- CH_3 , secondary amine, $-\text{CH}_2-$ and $-\text{NH}$ amide group respectively. The aromatic ring protons were observed at δ 6.20–8.05 ppm and *J* value were found to be in accordance with substitution pattern on phenyl ring. Characteristic peaks at around δ 168.5–168.9 and 177.0–177.3 ppm in ^{13}C NMR confirmed the presence of $>\text{C}=\text{O}$ and $>\text{C}=\text{S}$ groups respectively. The mass spectrum of **5a–I** revealed that observed molecular ion peaks were in agreement with molecular weight of respective compound.

2.2. Antimicrobial studies

2.2.1. Antibacterial studies

All the synthesized compounds **5a–I** were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442 (Gram-positive), *Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 1688 (Gram-negative) by conventional broth microdilution method using chloramphenicol as a control drug for antibacterial activity [40]. The results of the antimicrobial studies are presented in Table 1. In general, compounds **5a–I** demonstrated better antibacterial activity than antifungal activity. Compounds **5c** and **5i** emerged as the



Where R = -2-F, -3-F, -4-F, -2-OCH₃, -3-OCH₃, -4-OCH₃, -2-NO₂, -3-NO₂, -4-NO₂, -2-CH₃, -3-CH₃, -4-CH₃

Scheme 1. Synthetic routes for the compounds **5a–I**. Reagents and conditions: (a) $(\text{CH}_3\text{CO})_2\text{O}$, reflux, 1 h; (b) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}$, EtOH, reflux, 5 h; (c) CS_2 , KOH, EtOH, reflux, 12 h; (d) $\text{R-C}_6\text{H}_4\text{NH}_2$, HCHO, reflux, 3h.

Table 1
Antimicrobial screening results of compounds **5a–I**.

Entry	R	Minimum inhibitory concentration (MIC), µg/mL						
		Gram-positive bacteria ^a				Fungi ^c		
		<i>Sa</i>	<i>Sp</i>	<i>Ec</i>	<i>Pa</i>	<i>Ca</i>	<i>An</i>	<i>Ac</i>
5a	–2-F	250	100	250	500	500	>1000	500
5b	–3-F	50	100	50	50	500	500	>1000
5c	–4-F	12.5	25	12.5	12.5	500	500	>1000
5d	–2-OCH ₃	500	>1000	500	250	500	250	250
5e	–3-OCH ₃	>1000	500	500	500	250	100	500
5f	–4-OCH ₃	500	>1000	500	500	25	25	62.5
5g	–2-NO ₂	100	250	500	250	250	500	500
5h	–3-NO ₂	50	100	50	50	500	100	250
5i	–4-NO ₂	25	25	12.5	12.5	500	250	100
5k	–2-CH ₃	500	250	250	100	500	500	250
5k	–3-CH ₃	500	500	250	500	500	500	500
5l	–4-CH ₃	500	>1000	500	>1000	500	250	250
Chloramphenicol	–	50	50	50	50	–	–	–
Ketoconazole	–	–	–	–	–	50	50	50

^a *Sa* (*Staphylococcus aureus* MTCC 96); *Sp* (*Staphylococcus pyogenes* MTCC 442).

^b *Ec* (*Escherichia coli* MTCC 443); *Pa* (*Pseudomonas aeruginosa* MTCC 1688).

^c *Ca* (*Candida albicans* MTCC 227); *An* (*Aspergillus niger* MTCC 282); *Ac* (*Aspergillus clavatus* MTCC 1323).

most effective antibacterial agents with 2- to 4-fold higher MIC (12.5–25 µg/mL) than the reference drug chloramphenicol. While, compounds **5b** and **5h** exhibited comparable antibacterial activity with the standard drug. From the results of the antimicrobial activity of the synthesized compounds **5a–I**, the following structure activity relationships can be derived: the antibacterial activity was considerably affected by substitution pattern on the phenyl ring and the most active compounds contain electron withdrawing substituent at *para* and *meta* positions of the phenyl ring ($p > m > o$). In contrast, the presence of electron releasing groups on the phenyl ring witnessed a substantial decrease in antibacterial activity for compounds **5d–f** and **5k–I**. The role of electron withdrawing group in improving antimicrobial activity is very well supported by previous studies [41,42]. Compounds **5c** and **5i**, substituted with inductively electron withdrawing fluoro and nitro groups, respectively at *para* position showed the highest antibacterial activity ($F > NO_2$). It is a very well-known fact that electron withdrawing substitution such as fluoro/nitro at the *para* position of the aromatic ring increases the lipophilicity of molecules. This property is directly related to antimicrobial activity as it facilitates a compound to diffuse through the biological membranes and reach to its site of action. The presence of lipophilic substituent at *para* position of phenyl ring provided a positive influence on antibacterial activity. On the other hand, presence of the same functional groups at *meta* position resulted in slight decrease in the antibacterial activity (**5b** and **5h**) but, still produced significant inhibitory action compared to the standard drug chloramphenicol ($F > NO_2$). While, substituting the phenyl ring with fluoro and nitro group at *ortho* position resulted in noticeable decrease in the antibacterial activity of compounds **5a** and **5g** respectively. On the basis of MIC values, it may be concluded that electron withdrawing atom/group such as fluoro and nitro at the *para* position of phenyl ring induced a positive effect while electron donating groups such as methyl and methoxy induced a negative effect on antibacterial activity of compounds **5a–I**.

2.2.2. Antifungal studies

The *in vitro* antifungal activity of synthesized compounds **5a–I** were determined against *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323 by conventional

Table 2

Levels of cytotoxicity induced by selected compounds on HeLa cells at 24 h of drug exposure.

Compounds	IC ₅₀ (µM)
5b	>100
5c	>100
5f	98.60
5h	>100
5i	96.48
Doxorubicin	3.24

The known numbers of cells (1.0×10^4) were incubated for 24 h in a 5% CO₂ incubator at 37 °C in the presence of different concentrations of test compounds. After 24 h of drug incubation the MTT solution was added and supernatant was discarded and 100 µL DMSO was added in each well and absorbance was recorded at 540 nm by ELISA reader.

broth microdilution method. The results indicated that compound **5f** substituted with methoxy group at *para* position of the phenyl ring was found to be the most promising agent against both *C. albicans* and *A. niger* having 2-fold higher MIC (25 µg/mL) in comparison with control drug ketoconazole. The enhanced activity of compound **5f** may be attributed to the presence of electron releasing group at *para* position. The contrasting nature of substitution pattern at *para* position of the phenyl ring of most active antibacterial and antifungal agents indicate that the structural requirements are different for binding of drug to bacterial or fungal targets, respectively [43]. All other compounds showed less inhibition against the tested microorganisms as compared to the standard drug.

2.2.3. Cytotoxicity studies

In vitro cytotoxicity of compounds **5b**, **5c**, **5f**, **5h** and **5i** were evaluated against human cervical cancer cell line (HeLa) by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay [44], which measures the reduction of tetrazolium bromide salt into a formazan dye by mitochondrial dehydrogenases in treated versus untreated cells. The results are summarized in Table 2. It was observed that none of the tested compounds exhibited any significant cytotoxic effect on HeLa cells, suggesting a great potential for their *in vivo* use as antimicrobial agents.

3. Conclusion

In conclusion, some new structural hybrids of thiazole and 1,3,4-oxadiazole were synthesized and investigated for their antimicrobial property with an anticipation of generating new structural leads serving as potent antimicrobial agents. Many of the synthesized motifs (**5b**, **5c**, **5h** and **5i**), possessing electron withdrawing atom/group such as fluoro and nitro at *para* and *meta* positions were identified as the most potent antibacterial agents. Albeit, it was observed that *para* position was more favorable for enhancing the antibacterial activity. While, compound **5f** with electron releasing group at *para* position came out as the most promising antifungal agent. The potent antimicrobial activity of most active compounds **5b**, **5c**, **5f**, **5h** and **5i** were accompanied with relatively low level of cytotoxicity. The results described here, merits further investigations in our laboratories using a forward chemical genetic approach for finding lead molecules as antimicrobial agents.

4. Experimental

4.1. Chemistry

All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. Melting points

were determined on an electrothermal melting point apparatus and were reported uncorrected. TLC on silica gel plates (Merck, 60, F₂₅₄) was used for purity checking and reaction monitoring. Column chromatography on silica gel (Merck, 70–230 mesh and 230–400 mesh ASTH for flash chromatography) was applied when necessary to isolate and purify the reaction products. Elemental analysis (% C, H, N) was carried out by a Perkin–Elmer 2400 CHN analyzer. IR spectra of all compounds were recorded on a Perkin–Elmer FT-IR spectrophotometer in KBr. ¹H NMR spectra were recorded on Varian Gemini 300 MHz and ¹³C NMR spectra on Varian Mercury-400 100 MHz in DMSO-*d*₆ as a solvent and tetramethylsilane (TMS) as an internal standard. Mass spectra were scanned on a Shimadzu LC-MS 2010 spectrometer. All reactions requiring anhydrous conditions were carried out under nitrogen atmosphere using oven-dried glassware.

4.2. Synthesis of intermediate ethyl 2-acetamido-4-methylthiazole-5-carboxylate (**2**)

Ethyl 2-amino-4-methylthiazole-5-carboxylate (0.01 mol) was taken in a round bottom flask and acetic anhydride (0.02 mol) was added. The reaction mixture was refluxed at 140–150 °C for 1 h and then poured into cold water under constant stirring to get solid product. The mixture was heated to boiling to decompose excess of acetic anhydride and cooled. The solid obtained was filtered, washed with water and dried. The ethyl 2-(acetyl amino)-4-methyl-1,3-thiazole-5-carboxylate was collected and recrystallized from ethanol to get pure white crystals. Yield: 74%; m.p. 158 °C; Anal. obs. C, 47.08%; H, 5.48%; N, 12.44%. Calcd. for C₉H₁₂N₂O₃S: C, 47.35%; H, 5.30%; N, 12.27%.

4.3. Synthesis of intermediate N-(5-(hydrazinecarbonyl)-4-methylthiazol-2-yl)acetamide (**3**)

Intermediate (**2**) (0.01 mol) and 99% hydrazine hydrate (0.015 mol) were taken in a round bottom flask and mixture was refluxed for 10 min. Alcohol was added till both the layers were miscible and refluxing was continued for 5 h. Excess of alcohol and unreacted hydrazine hydrate was distilled out and the contents were poured into a beaker. The solid was recrystallized from ethanol to get pure crystalline product. Yield: 64%; m.p. 172 °C; Anal. obs. C, 38.98%; H, 4.82%; N, 26.37%. Calcd. for C₇H₁₀N₄O₂S: C, 39.24%; H, 4.70%; N, 26.15%.

4.4. Synthesis of intermediate N-(4-methyl-5-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)acetamide (**4**)

A mixture of intermediate (**3**) (0.01 mol), potassium hydroxide (0.01 mol), carbon disulfide (0.02 mol) and ethanol (99.5%) (100 mL) was refluxed for 12 h. The excess solvent was removed by vacuum evaporation, and the residue was dissolved in water and acidified with acetic acid to get solid product. It was filtered, dried and recrystallized from water–ethanol (60–40). Yield: 64%; m.p. 198 °C; Anal. obs. C, 37.65%; H, 3.32%; N, 22.06%. Calcd. for C₈H₈N₄O₂S₂: C, 37.49%; H, 3.15%; N, 21.86%.

4.5. General procedure for the synthesis of N-(5-(4-((aryl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)acetamide (**5a–I**)

A mixture of intermediate (**4**) (0.01 mol) and an appropriately substituted aniline derivatives (0.01 mol) was refluxed in ethanol (50 mL) with 36% formaldehyde (0.02 mol) for 3 h. The resulting solid was crystallized from a suitable solvent.

4.5.1. N-(5-(4-((2-fluorophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)acetamide (**5a**)

Yield: 64%, m.p. 178–180 °C; IR (KBr): ν/cm^{-1} 3331 (secondary amine NH), 3211 (secondary amide NH), 3028 (aromatic ring CH), 2831 (CH₃), 2762 (CH₂), 1681 (CO), 1113 (CF). ¹H NMR (DMSO-*d*₆), δ ppm: 2.05 (s, 3H, –NHCOCH₃), 2.44 (s, 3H, –N–C(CH₃)–C–), 4.11 (s, 1H, Ar–NH–CH₂), 4.44 (s, 2H, Het–CH₂–NH), 6.56–7.10 (m, 4H, Ar–H), 9.17 (s, 1H, Het–NH–CO); ¹³C NMR (400 MHz, DMSO-*d*₆), δ ppm: 17.0 (CH₃), 24.1 (COCH₃), 70.6 (CH₂), 115.2, 116.3, 121.3, 125.1, 130.6, 132.4, 154.3 (C–F), 156.6 (C–CH₃), 157.1, 162.9, 168.9 (amide C=O) and 177.1 (C=S); MS (EI): m/z 379.1 (M⁺). Anal. Calcd. For C₁₅H₁₄FN₅O₂S₂ C-47.48, H-3.72, N-18.46; Found: C-47.34, H-3.68, N-18.57%.

4.5.2. N-(5-(4-((3-fluorophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)acetamide (**5b**)

Yield: 61%, m.p. 166–168 °C; IR (KBr): ν/cm^{-1} 3330 (secondary amine NH), 3217 (secondary amide NH), 3023 (aromatic ring CH), 2830 (CH₃), 2769 (CH₂), 1669 (CO), 1111 (CF). ¹H NMR (DMSO-*d*₆), δ ppm: 2.04 (s, 3H, –NHCOCH₃), 2.46 (s, 3H, –N–C(CH₃)–C–), 4.10 (s, 1H, Ar–NH–CH₂), 4.42 (s, 2H, Het–CH₂–NH), 6.54–7.22 (m, 4H, Ar–H), 9.15 (s, 1H, Het–NH–CO); ¹³C NMR (400 MHz, DMSO-*d*₆), δ ppm: 17.2 (CH₃), 24.1 (COCH₃), 70.7 (CH₂), 102.2, 108.9, 109.3, 131.1, 132.6, 149.2, 156.6 (C–CH₃), 157.1, 162.9, 163.7 (C–F), 168.9 (amide C=O) and 177.1 (C=S); MS (EI): m/z 379.1 (M⁺). Anal. Calcd. For C₁₅H₁₄FN₅O₂S₂ C-47.48, H-3.72, N-18.46; Found: C-47.31, H-3.73, N-18.51%.

4.5.3. N-(5-(4-((4-fluorophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)acetamide (**5c**)

Yield: 59%, m.p. 132–134 °C; IR (KBr): ν/cm^{-1} 3329 (secondary amine NH), 3210 (secondary amide NH), 3022 (aromatic ring CH), 2839 (CH₃), 2760 (CH₂), 1678 (CO), 1117 (CF). ¹H NMR (DMSO-*d*₆), δ ppm: 2.04 (s, 3H, –NHCOCH₃), 2.41 (s, 3H, –N–C(CH₃)–C–), 4.13 (s, 1H, Ar–NH–CH₂), 4.44 (s, 2H, Het–CH₂–NH), 7.02–7.05 (m, 4H, Ar–H), 9.16 (s, 1H, Het–NH–CO); ¹³C NMR (400 MHz, DMSO-*d*₆), δ ppm: 17.1 (CH₃), 24.1 (COCH₃), 70.6 (CH₂), 116.3 (2C), 118.9 (2C), 132.5, 143.4, 155.7 (C–F), 156.5 (C–CH₃), 157.1, 162.9, 168.9 (amide C=O) and 177.2 (C=S); MS (EI): m/z 379.1 (M⁺). Anal. Calcd. For C₁₅H₁₄FN₅O₂S₂ C-47.48, H-3.72, N-18.46; Found: C-47.31, H-3.64, N-18.54%.

4.5.4. N-(5-(4-((2-methoxyphenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)acetamide (**5d**)

Yield: 58%, m.p. 139–141 °C; IR (KBr): ν/cm^{-1} 3331 (secondary amine NH), 3208 (secondary amide NH), 3020 (aromatic ring CH), 2831 (CH₃), 2766 (CH₂), 1672 (CO), 1211–1103 (C–O–C). ¹H NMR (DMSO-*d*₆), δ ppm: 2.04 (s, 3H, –NHCOCH₃), 2.44 (s, 3H, –N–C(CH₃)–C–), 4.13 (s, 1H, Ar–NH–CH₂), 3.71 (s, 3H, Ar–OCH₃), 4.44 (s, 2H, Het–CH₂–NH), 6.66–7.02 (m, 4H, Ar–H), 9.16 (s, 1H, Het–NH–CO); ¹³C NMR (400 MHz, DMSO-*d*₆), δ ppm: 17.1 (CH₃), 24.0 (COCH₃), 55.7 (OCH₃), 71.2 (CH₂), 113.5, 114.4, 121.1, 121.7, 132.4, 138.4, 144.5 (C–OCH₃), 156.4 (C–CH₃), 162.9, 168.7 (amide C=O) and 177.1 (C=S); MS (EI): m/z 391.1 (M⁺). Anal. Calcd. For C₁₆H₁₇N₅O₃S₂ C-49.09, H-4.38, N-17.89; Found: C-49.23, H-4.25, N-18.30%.

4.5.5. N-(5-(4-((3-methoxyphenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)acetamide (**5e**)

Yield: 58%, m.p. 139–141 °C; IR (KBr): ν/cm^{-1} 3344 (secondary amine NH), 3223 (secondary amide NH), 3020 (aromatic ring CH), 2833 (CH₃), 2784 (CH₂), 1674 (CO), 1204–1093 (C–O–C). ¹H NMR (DMSO-*d*₆), δ ppm: 2.07 (s, 3H, –NHCOCH₃), 2.44 (s, 3H, –N–C(CH₃)–C–), 3.75 (s, 3H, Ar–OCH₃), 4.15 (s, 1H, Ar–NH–CH₂), 4.44 (s, 2H, Het–CH₂–NH), 6.20–7.30 (m, 4H, Ar–H), 9.20 (s, 1H, Het–

NH–CO); ^{13}C NMR (400 MHz, DMSO- d_6), δ ppm: 17.1(CH₃), 24.0 (COCH₃), 55.7 (OCH₃), 70.9 (CH₂), 105.5, 105.8, 109.2, 110.3, 132.4, 148.5, 156.4 (C–CH₃), 157.0, 161.4 (C–OCH₃), 162.9, 168.7 (amide C=O) and 177.1 (C=S); MS (EI): m/z 391.1 (M⁺). Anal. Calcd. For C₁₆H₁₇N₅O₃S₂ C-49.09, H-4.38, N-17.89; Found: C-49.25, H-4.49, N-17.78%.

4.5.6. *N*-(5-(4-(((4-methoxyphenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)-4-methylthiazol-2-yl)acetamide (**5f**)

Yield: 59%, m.p. 150–152 °C; IR (KBr): ν/cm^{-1} 3339 (secondary amine NH), 3228 (secondary amide NH), 3011 (aromatic ring CH), 2832 (CH₃), 2780 (CH₂), 1670 (CO), 1211–1099 (C–O–C). ^1H NMR (DMSO- d_6), δ ppm: 2.04 (s, 3H, –NHCOCH₃), 2.45 (s, 3H, –N–C(CH₃)–C–), 3.83 (s, 3H, Ar–OCH₃), 4.21 (s, 1H, Ar–NH–CH₂), 4.42 (s, 2H, Het–CH₂–NH), 6.77–7.18 (m, 4H, Ar–H), 9.15 (s, 1H, Het–NH–CO); ^{13}C NMR (400 MHz, DMSO- d_6), δ ppm: 17.1(CH₃), 24.0(COCH₃), 55.8 (OCH₃), 70.8 (CH₂), 115.1 (2C), 115.8 (2C), 132.4, 139.9, 151.7 (C–OCH₃), 156.4 (C–CH₃), 157.0, 162.9, 168.7 (amide C=O) and 177.1 (C=S); MS (EI): m/z 391.1 (M⁺). Anal. Calcd. For C₁₆H₁₇N₅O₃S₂ C-49.09, H-4.38, N-17.89; Found: C-49.17, H-4.52, N-17.98%.

4.5.7. *N*-(4-methyl-5-(4-(((2-nitrophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)acetamide (**5g**)

Yield: 52%, m.p. 155–157 °C; IR (KBr): ν/cm^{-1} 3327 (secondary amine NH), 3224 (secondary amide NH), 3014 (aromatic ring CH), 2833 (CH₃), 2784 (CH₂), 1671 (CO), 1533 (NO₂). ^1H NMR (DMSO- d_6), δ ppm: 2.03 (s, 3H, –NHCOCH₃), 2.46 (s, 3H, –N–C(CH₃)–C–), 4.24 (s, 1H, Ar–NH–CH₂), 4.42 (s, 2H, Het–CH₂–NH), 7.34–8.05 (m, 4H, Ar–H), 9.14 (s, 1H, Het–NH–CO); ^{13}C NMR (400 MHz, DMSO- d_6), δ ppm: 17.0 (CH₃), 24.0 (COCH₃), 69.7 (CH₂), 114.3, 118.1, 126.1, 131.8, 132.4, 135.7, 146.7, 156.4 (C–CH₃), 157.0, 162.9, 168.7 (amide C=O) and 177.1 (C=S); MS (EI): m/z 406.05 (M⁺). Anal. Calcd. For C₁₆H₁₇N₅O₃S₂ C-44.33, H-3.47, N-20.68; Found: C-44.44, H-3.32, N-20.78%.

4.5.8. *N*-(4-methyl-5-(4-(((3-nitrophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)acetamide (**5h**)

Yield: 55%, m.p. 165–167 °C; IR (KBr): ν/cm^{-1} 3329 (secondary amine NH), 3225 (secondary amide NH), 3010 (aromatic ring CH), 2838 (CH₃), 2781 (CH₂), 1672 (CO), 1538 (NO₂). ^1H NMR (DMSO- d_6), δ ppm: 2.03 (s, 3H, –NHCOCH₃), 2.44 (s, 3H, –N–C(CH₃)–C–), 4.18 (s, 1H, Ar–NH–CH₂), 4.43 (s, 2H, Het–CH₂–NH), 7.20–7.60 (m, 4H, Ar–H), 9.14 (s, 1H, Het–NH–CO); ^{13}C NMR (400 MHz, DMSO- d_6), δ ppm: 17.1 (CH₃), 24.0 (COCH₃), 70.7 (CH₂), 106.8, 112.1, 119.5, 130.5, 132.4, 148.5, 148.7, 156.4 (C–CH₃), 157.0, 162.9, 168.7 (amide C=O) and 177.1 (C=S); MS (EI): m/z 406.01 (M⁺). Anal. Calcd. For C₁₆H₁₇N₅O₃S₂ C-44.44, H-3.47, N-20.68; Found: C-44.20, H-3.39, N-20.62%.

4.5.9. *N*-(4-methyl-5-(4-(((4-nitrophenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)acetamide (**5i**)

Yield: 60%, m.p. 159–161 °C; IR (KBr): ν/cm^{-1} 3331 (secondary amine NH), 3227 (secondary amide NH), 3010 (aromatic ring CH), 2830 (CH₃), 2782 (CH₂), 1671 (CO), 1524 (NO₂). ^1H NMR (DMSO- d_6), δ ppm: 2.03 (s, 3H, –NHCOCH₃), 2.45 (s, 3H, –N–C(CH₃)–C–), 4.11 (s, 1H, Ar–NH–CH₂), 4.41 (s, 2H, Het–CH₂–NH), 6.72–8.04 (m, 4H, Ar–H), 9.15 (s, 1H, Het–NH–CO); ^{13}C NMR (400 MHz, DMSO- d_6), δ ppm: 17.1 (CH₃), 24.0 (COCH₃), 70.9 (CH₂), 114.4 (2C), 127.5 (2C), 132.4, 136.4, 153.7, 156.4 (C–CH₃), 157.0, 162.9, 168.9 (amide C=O) and 177.1 (C=S); MS (EI): m/z 406.01 (M⁺). Anal. Calcd. For C₁₆H₁₇N₅O₃S₂ C-44.44, H-3.47, N-20.68; Found: C-44.56, H-3.60, N-20.75%.

4.5.10. *N*-(4-methyl-5-(5-thioxo-4-((*o*-tolylamino)methyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)acetamide (**5j**)

Yield: 62%, m.p. 142–144 °C; IR (KBr): ν/cm^{-1} 3331 (secondary amine NH), 3220 (secondary amide NH), 3022 (aromatic ring CH),

2953 (Ar–CH₃), 2840 (Het–CH₃), 2771 (CH₂), 1681 (CO). ^1H NMR (DMSO- d_6), δ ppm: 2.04 (s, 3H, –NHCOCH₃), 2.12 (Ar–CH₃), 2.46 (s, 3H, –N–C(CH₃)–C–), 4.09 (s, 1H, Ar–NH–CH₂), 4.42 (s, 2H, Het–CH₂–NH), 6.63–7.04 (m, 4H, Ar–H), 9.16 (s, 1H, Het–NH–CO); ^{13}C NMR (400 MHz, DMSO- d_6), δ ppm: 17.1 (Het–CH₃), 17.6 (Ar–CH₃), 24.1 (COCH₃), 71.2 (CH₂), 110.6, 121.8 (Ar–C–CH₃), 122.1, 126.5, 127.0, 132.3, 146.5, 156.5 (Het–C–CH₃), 157.0, 162.9, 168.9 (amide C=O) and 177.1 (C=S); MS (EI): m/z 375.08 (M⁺). Anal. Calcd. For C₁₆H₁₇N₅O₂S₂ C-51.18, H-4.56, N-18.65; Found: C-51.30, H-4.40, N-18.52%.

4.5.11. *N*-(4-methyl-5-(5-thioxo-4-((*m*-tolylamino)methyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)acetamide (**5k**)

Yield: 57%, m.p. 151–153 °C; IR (KBr): ν/cm^{-1} 3330 (secondary amine NH), 3229 (secondary amide NH), 3027 (aromatic ring CH), 2951 (Ar–CH₃), 2830 (Het–CH₃), 2775 (CH₂), 1680 (CO). ^1H NMR (DMSO- d_6), δ ppm: 2.03 (s, 3H, –NHCOCH₃), 2.34 (Ar–CH₃), 2.42 (s, 3H, –N–C(CH₃)–C–), 4.07 (s, 1H, Ar–NH–CH₂), 4.42 (s, 2H, Het–CH₂–NH), 6.52–7.11 (m, 4H, Ar–H), 9.16 (s, 1H, Het–NH–CO); ^{13}C NMR (400 MHz, DMSO- d_6), δ ppm: 17.1 (Het–CH₃), 21.2 (Ar–CH₃), 24.1 (COCH₃), 70.8 (CH₂), 110.5, 113.2117.4, 129.4, 132.4, 132.3, 139.3 (Ar–C–CH₃), 147.5, 156.5 (Het–C–CH₃), 157.0, 162.9, 168.9 (amide C=O) and 177.1 (C=S); MS (EI): m/z 375.08 (M⁺). Anal. Calcd. For C₁₆H₁₇N₅O₂S₂ C-51.18, H-4.56, N-18.65; Found: C-51.00, H-4.48, N-18.76%.

4.5.12. *N*-(4-methyl-5-(5-thioxo-4-((*p*-tolylamino)methyl)-4,5-dihydro-1,3,4-oxadiazol-2-yl)thiazol-2-yl)acetamide (**5l**)

Yield: 61%, m.p. 163–165 °C; IR (KBr): ν/cm^{-1} 3327 (secondary amine NH), 3228 (secondary amide NH), 3029 (aromatic ring CH), 2947 (Ar–CH₃), 2839 (Het–CH₃), 2775 (CH₂), 1687 (CO). ^1H NMR (DMSO- d_6), δ ppm: 2.07 (s, 3H, –NHCOCH₃), 2.34 (Ar–CH₃), 2.43 (s, 3H, –N–C(CH₃)–C–), 4.10 (s, 1H, Ar–NH–CH₂), 4.41 (s, 2H, Het–CH₂–NH), 6.48–7.01 (m, 4H, Ar–H), 9.16 (s, 1H, Het–NH–CO); ^{13}C NMR (400 MHz, DMSO- d_6), δ ppm: 17.1 (Het–CH₃), 21.3 (Ar–CH₃), 24.0 (COCH₃), 70.8 (CH₂), 113.4 (2C), 129.6 (Ar–C–CH₃), 129.8 (2C), 132.3, 144.6, 156.5 (Het–C–CH₃), 157.1, 162.9, 168.9 (amide C=O) and 177.1 (C=S); MS (EI): m/z 375.08 (M⁺). Anal. Calcd. For C₁₆H₁₇N₅O₂S₂ C-51.18, H-4.56, N-18.65; Found: C-51.42, H-4.44, N-18.72%.

4.6. Biological assay

4.6.1. *In vitro* evaluation of antimicrobial activity

All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh. The MICs of synthesized compounds were carried out by broth microdilution method against the standard bacterial strains *S. aureus* MTCC 96, *S. pyogenes* MTCC 442, *E. coli* MTCC 443 and *P. aeruginosa* MTCC 1688 and antifungal activity against the standard fungal strains *C. albicans* MTCC 227, *A. niger* MTCC 282 and *A. clavatus* MTCC 1323. DMSO was used as diluents to get desired concentration of compounds to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the compound concentrations. The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. All the tubes showing no visible growth (same as control tube) were subcultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show: similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity

and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized compound was diluted obtaining 2000 µg/mL concentration as a stock solution. In primary screening 500, 250 and 200 µg/mL concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all microorganisms. The compounds found active in primary screening were similarly diluted to obtain 100, 62.5, 50 and 25 µg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

4.6.2. Preliminary *in vitro* cytotoxic activities (MTT assay)

In vitro cytotoxicity was determined using a standard MTT assay with protocol appropriate for the individual test system. Test compounds were prepared prior to the experiment by dissolving in 0.1% DMSO and diluted with medium. The cells were then exposed to different concentrations of the drugs (1–100 µM) in the volume of 100 µL/well. Cells in the control wells received the same volume of medium containing 0.1% DMSO. After 24 h, the medium was removed and cell cultures were incubated with 100 µL MTT reagent (1 mg/mL) for 5 h at 37 °C. The suspension was placed on micro vibrator for 10 min and absorbance was recorded by the ELISA reader. The experiment was performed in triplicate. Human cancer cell lines, HeLa cells were cultured in MEM medium supplemented with 10% FBS, 1% glutamine and 50 µM/mL gentamicin sulphate in a CO₂ incubator in a humidified atmosphere of 5% CO₂ and 95% air.

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