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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of some novel 1,2,4-triazole and 1,3,4-oxadiazole derivatives of biological interest

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Abstract In this study, a series of novel [1,2,4]-triazolo-[1,3,4]oxadiazole (**8**), [1,2,4]-triazolo-[1,3,4]oxadiazole thioethers (**9a–b**), [1,2,4]-triazolo-[1,3,4]oxadiazole arylidines (**10a–g**), and bis[1,2,4]-triazole (**11**) derivatives were prepared with the objective of developing better antimicrobial activity. The structures of the compounds were elucidated by spectral analysis. The newly synthesized compounds (**8**), (**9a–b**), (**10a–g**), and (**11**) were screened for their antimicrobial activity. Among all the tested compounds (**10b**) shows significant antibacterial activity.

Keywords [1,2,4]-Triazolo-[1,3,4]oxadiazole · [1,2,4]-Triazolo-[1,3,4]oxadiazole thioethers · [1,2,4]-Triazolo-[1,3,4]oxadiazole arylidines · Bistriazoles and antimicrobial

Introduction

Resistance to number of antimicrobial agents among a variety of clinically significant species of bacteria is becoming increasingly important global problem. There are various problems arising with the use of antimicrobials such as local tissue irritation, interference with wound healing process, hypersensitivity reactions, systemic toxicity, narrow antimicrobial spectrum, and emergence of resistance. So the increasing clinical importance of drug-resistant microbial pathogens has lent additional urgency in antimicrobial research. A wide variety of heterocyclic systems have been explored for developing pharmaceutically important molecules. Among them the nitrogen heterocycles like 1,2,4-triazoles and 1,3,4-oxadiazoles have got greater attention because of their diverse use in pharmaceutical as well as biological field such as antibacterial, antifungal (Sharma *et al.*, 2008), antitubercular (Zahajska *et al.*, 2004), analgesic (Turan-Zitouni *et al.*, 2001), anti-inflammatory (Tozkoparan *et al.*, 2007), anticancer (Rabea *et al.*, 2006), anticonvulsant (Holla *et al.*, 2003), antiviral (Almasirad *et al.*, 2004), insecticidal (Abdel-Aal *et al.*, 2008), antidepressant (Varvaresou *et al.*, 1998), and central nervous system (Modzelewska-Banachiewicz *et al.*, 2004). Some of the compounds containing 1,2,4-triazole nucleus are well known as drugs (Fig. 1), for example, itraconazole and posaconazole are important antifungal drugs (Castellano *et al.*, 2003).

The 1,3,4-oxadiazole is a highly privileged structure the derivatives of which exhibit broad spectrum of biological activities such as anti-inflammatory (Boschelli *et al.*, 1993; Dhansay *et al.*, 2010; Mullaican *et al.*, 1993; Naragund *et al.*, 1994), anticonvulsant (Zarghi *et al.*, 2008), antifungal (Sahin *et al.*, 2002, Shahsafi *et al.*, 1988), hypoglycemic (Ladduwahetty *et al.*, 1996), antibacterial (Chandrakantha *et al.*, 2010; Khanum *et al.*, 2005), anticancer (Aboraia *et al.*, 2006; Li *et al.*, 2006), analgesic (Jayashankar *et al.*, 2009), antiviral (Bhandari *et al.*, 2008; Tan *et al.*, 2006; Kucukguzel *et al.*, 2007), and anthelmintic (Srinivasa *et al.*, 2007) activities. Also some of the

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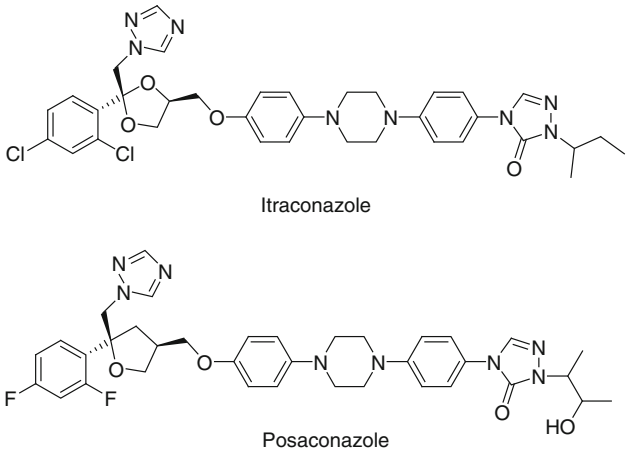


Fig. 1 Drugs containing 1,2,4-triazole nucleus

1,3,4-oxadiazole derivatives act as antimalarial (Foroumadi *et al.*, 2007), muscle relaxants (Yale and Losee, 1966), hypnotic, sedatives (Adelstein *et al.*, 1976), and antimicotic (Coombs 1974) activities. Furamizole (Fig. 2) is a compound which is based upon 1,3,4-oxadiazole ring and has strong antibacterial activity.

In recent years, some 1,2,4-triazoles with Schiff base and 1,2,4-triazoles with thioether derivative have been also found to posses various biological activities such as antibacterial, antifungal (Bayrak *et al.*, 2009; Sureshkumar *et al.*, 2010), antitubercular (Sureshkumar *et al.*, 2010), anti-inflammatory, and molluscicidal (El Shehry *et al.*, 2010) activities.

As a result of remarkable pharmacological efficiency of 1,2,4-triazol and 1,3,4-oxadiazole derivatives, our research group have been focused on biological activity of 1,2,4-triazol nucleus containing 1,3,4-oxadiazoles hoping for the enhanced biological activity. In continuation to this, it is our ongoing project to synthesize bioactive heterocyclic compounds (Patil *et al.*, 2010). We have developed an efficient procedure for synthesis of new heterocyclic systems containing 1,3,4-oxadiazoles clubbed with 1,2,4-triazoles. The structures of synthesized compounds were assigned on the basis of IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data. These compounds were evaluated for their antimicrobial screening on different strains of bacteria and fungi.

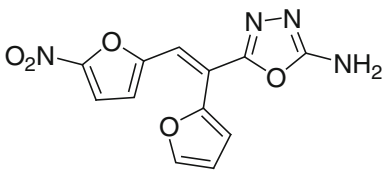


Fig. 2 Furamizole

Synthesis

The synthesis of 3-(4-methoxycarbonyl)phenylsydnone **5** was reported by Kavali *et al.* (2003). The same has been synthesized in excellent yields by minor modifications in excellent yields as depicted in Scheme 1. The compound (**3**) was prepared by treatment of (**1**) with *tert*-butyl bromoacetate in presence of diisopropylethylamine (DIPEA) followed by *tert*-butyl cleavage using trifluoroacetic acid (TFA). The [1,3,4]oxadiazole (**6**) was obtained by the facile one-pot ring conversion of 3-(4-methoxycarbonyl)phenylsydnone (**5**). Compound **6** on treatment with hydrazine hydrate yielded the 4-(4-amino-3-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl)benzoic acid hydrazide (**7**) which was used as a synthon for the current work.

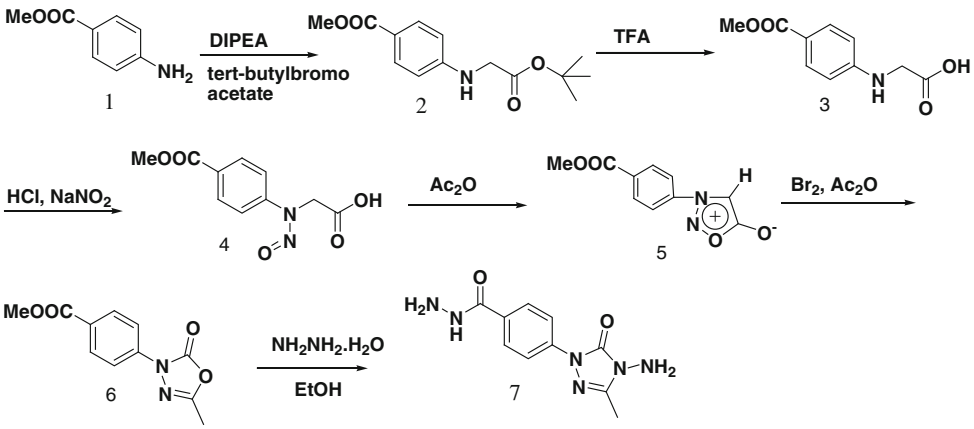
The compound (**8**) was obtained on treatment of (**6**) with hydrazine hydrate in ethanol followed by cyclization using CS_2 in basic media. The reaction of (**8**) with methyl iodide and ethyl bromoacetate in basic media afforded compounds (**9a**) and (**9b**), respectively. The synthesis of Schiff base (**10a–g**) was performed by the treatment of compound (**8**) with corresponding aldehydes in the presence of catalytic amount of sulfuric acid. On the other hand, the reaction of (**8**) with hydrazinehydrate afforded bis-1,2,4-triazole (**11**) in excellent yield. Synthetic route for the preparation of these compounds is shown in Scheme 2.

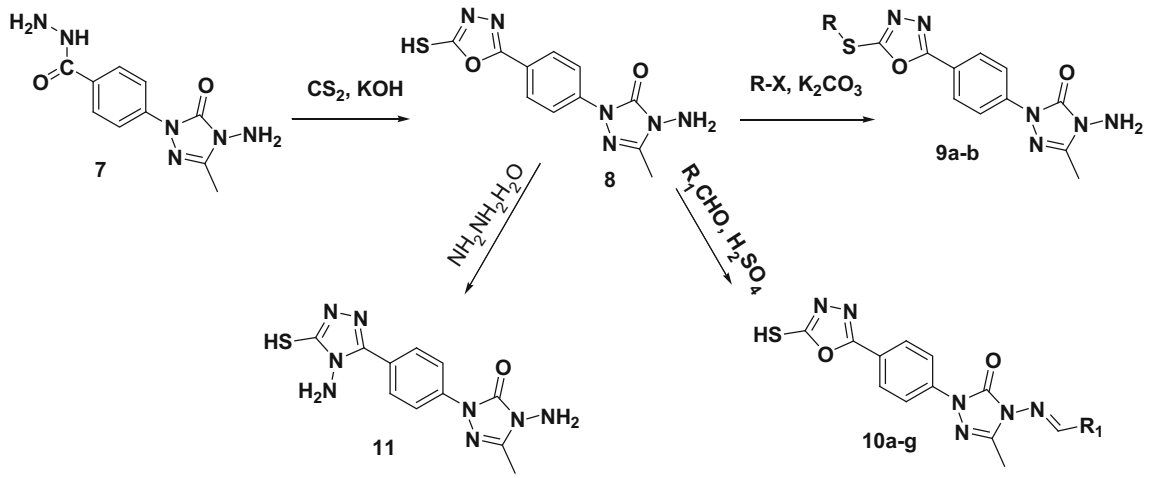
All the synthesized compounds were characterized by their spectral data. The IR spectra of [1,2,4]-triazolo-[1,3,4]oxadiazole (**8**), [1,2,4]-triazolo-[1,3,4]oxadiazole thioethers (**9a–b**), [1,2,4]-triazolo-[1,3,4]oxadiazole arylidines (**10a–g**), and bis[1,2,4]-triazole (**11**) derivatives shows bands in the range of 1,690–1,710 cm^{-1} (C=O of triazolinone) and 1,530–1,540 cm^{-1} (C=N). The compound (**9a**) shows the band at 1,740 cm^{-1} corresponding to C=O of the ester group. The ^{1}H- and ^{13}C-NMR spectrum of (**9a–b**) confirmed the alkylation of (**8**). The '–SCH_3' and '–SCH_2–' proton signals of compounds (**9a**) and (**9b**) were observed as a singlet at δ 2.76 and 4.29 ppm, respectively. The compounds (**9a–b**) gave the stable M+1 ion peaks in mass spectra. The ^{1}H-NMR spectrum of (**10a–g**) confirmed the conversion from (**8**). The prominent singlet for one proton in the range of δ 9.2–10.2 ppm confirms the formation Schiff bases. The compounds (**10a–g**) gave the stable M+1 or M−1 ion peaks in mass spectra. The ^{1}H and ^{13}C-NMR spectrum of (**11**) confirmed the conversion of the oxadiazole ring (**8**) to the bistriazole (**11**). This was further confirmed by mass spectra.

Biological activity

All the newly synthesized compounds were screened for their antimicrobial activity. The results obtained proved

Scheme 1 Synthesis of starting material 4-(4-amino-3-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl)benzoic acid hydrazide (**7**)





9a) R = Me, X = I; 9b) R = CH_2COOEt, ,X = Br; 10a) R_1 = 2-FPh; 10b) R_1 = 3-FPh; 10C) R_1 = 4-FPh; 10d) R_1 = 2,5-diFPh; 10e) R_1 = 2-BrPh; 10f) R_1 = Pyrrol-2-yl; 10g) R_1 = Thiazol-4-yl.

Scheme 2 Synthesis of compounds (**8**), (**9a**–**b**), (**10a**–**g**), and (**11**)

that the antibacterial activities were better than antifungal activities. In general, Table 1 reveals that the higher susceptibilities (lower MICs) were observed with gram-positive and poor susceptibilities with gram-negative bacteria.

The minimum inhibition concentration (MIC) values for 3-fluoro phenyl analogue **10b** showed better activity with standard Streptomycin and Pencillin against *Bacillus subtilis, S. aureus,* and *Escherichia coli.* The bistriazole **11** showed the better antibacterial activity against the *B. subtilis, S. aureus,* However, the other compounds showed the moderate antibacterial activity against Gram-positive bacteria, whereas less activity against Gram-negative bacteria. The graphical representations of these results were depicted in Fig. 3.

The antifungal activity of the new compounds were tested against Ampotericin B as standard drug, and from Table 2 it is evident that the compound **10a** 2-fluoro phenyl showed better activity against *Rhizopus oryza.* The 2,5 diphenyl analogue **10d** showed significant activity against almost all the fungal strains tested. The graphical representations of these results were depicted in Fig. 4. Most of the compounds showed better antimicrobial activity, further optimization of the scaffold and SAR is required for drug development.

Summary

A series of novel [1,2,4]-triazolo-[1,3,4]oxadiazole (**8**), [1,2,4]-triazolo-[1,3,4]oxadiazole thioethers (**9a**–**b**), [1,2,4]-triazolo-[1,3,4]oxadiazole arylidines (**10a**–**g**), and bis[1,2,4]-triazole (**11**) derivatives were synthesized and characterized by spectral studies. The newly synthesized compounds were evaluated for antibacterial and antifungal activity. It is evident from the results that **10b** (R_1 = 3-FPh–) and **11** showed the highest activity against

Table 1 Antibacterial activity of compounds (**8**), (**9a–b**), (**10a–g**), and (**11**)

R	Comp. no.	MIC of the compounds in μg/mL				
		Gram-positive organisms			Gram-negative organisms	
		B. subtilis	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeroginosa*
	8	37.5	75	150	75	37.5
Me	**9a**	150	37.5	75	75	75
CH_2COOEt	**9b**	37.5	18.75	75	37.5	75
2-FPh	**10a**	75	37.5	150	75	75
3-FPh	**10b**	18.75	9.37	37.5	9.37	75
4-FPh	**10c**	75	150	150	37.5	75
2,5-diFPh	**10d**	37.5	75	18.75	150	75
2-BrPh	**10e**	75	75	75	150	150
Pyrrol-2-yl	**10f**	150	37.5	75	150	37.5
Thiazol-4-yl	**10g**	37.5	75	18.75	37.5	150
	11	18.75	9.37	75	37.5	18.75
(Streptomycin)		6.25	1.56	1.562	3.125	3.125
(Pencillin)		1.526	6.25	3.125	6.25	9.37

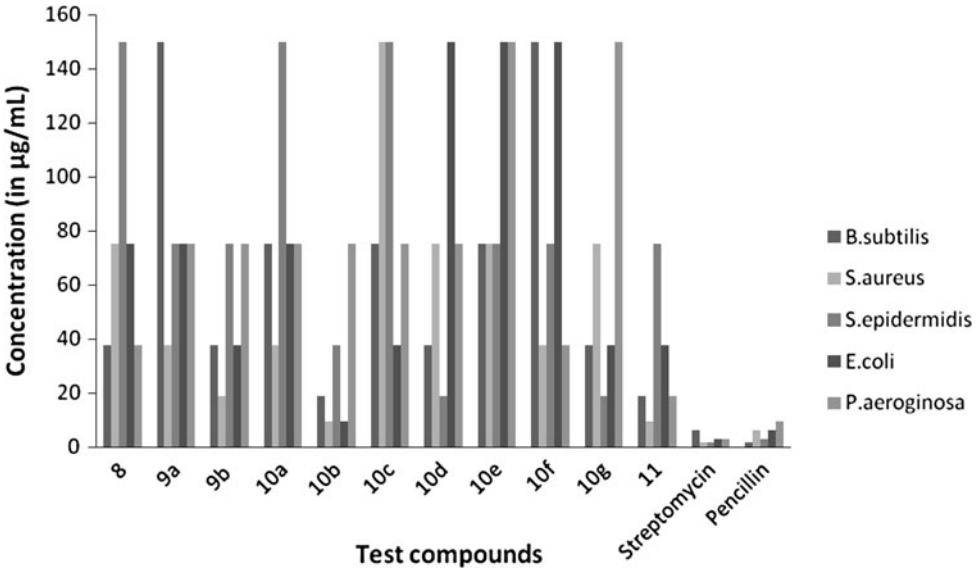


Fig. 3 Antibacterial activity of synthesized compounds

S. aureus. Compounds **10a** 2-fluoro phenyl and 2,5 diphenyl analogue **10d** showed significant activity against almost all the fungal strains tested.

Experimental

Melting points were determined in Buchi B 545 melting point instrument and are presented without corrections. The NMR spectra of the samples in $CDCl_3$ and DMSO-d_6 were recorded on a Bruker Avance 300 or 400 MHz spectrometer using tetra methyl silane as an internal standard. C, H, N, and S analysis were carried out on an Elementor (Vario micro cube). LCMS were recorded on Agilent.

Synthesis of 4-(*tert*-butoxycarbonylmethyl-amino)-benzoic acid methyl ester (**2**)

A mixture of methyl 4-aminobenzoate (0.20 mol), *tert*-butyl bromoacetate (0.22 mol), and DIPEA (0.24 mol) in DMF (300 mL) was stirred at 110 °C for 10 h. The reaction mixture was poured onto ice water (1.5 L) and the precipitated solid was collected by filtration and washed with water. The solid was recrystalised in petroleum ether/DCM to afford compound (**2**) in 90 % yield as an off white solid. (90 %); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.88 (d, 2H, $J = 8.76$ Hz, Ar–H), 6.55 (d, 2H, $J = 8.76$ Hz, Ar–H), 4.73 (brs, 1H, N–H), 3.86 (s, 3H, O–CH_3), 3.84 (s, 2H, N–CH_2), 1.50 (s, 9H, O-*t*-Bu); MS: *m*/*z* 152 [M+1].

Table 2 Antifungal activity of compounds (**8**), (**9a–b**), (**10a–g**), and (**11**)

R	Comp. no.	MIC of the compounds in μg/mL				
		R. oryzae	*A. niger*	*A. flavus*	*C. albicans*	*S. cerevisiae*
	8	75	150	150	37.5	150
Me	**9a**	37.5	150	150	150	150
CH_2COOEt	**9b**	150	150	150	150	150
2-FPh	**10a**	9.37	75	37.5	150	75
3-FPh	**10b**	150	150	150	150	75
4-FPh	**10c**	75	75	75	150	150
2,5-diFPh	**10d**	18.75	37.5	9.37	18.75	150
2-BrPh	**10e**	37.5	150	75	150	150
Pyrrol-2-yl	**10f**	150	150	150	150	150
Thiazol-4-yl	**10g**	37.5	150	150	150	150
	11	18.75	37.5	75	150	150
Amphotericin-B		1.562	1.56	6.25	6.25	6.25

Synthesis of 4-(carboxymethyl-amino)-benzoic acid methyl ester (**3**)

To a solution of compound (**2**) (0.18 mol) in DCM (150 mL) was added TFA (50 mL) at 0–5 °C. The mixture was stirred at room temperature for 12 h. The volatiles were removed under reduced pressure. The residue was poured onto ice water, solid was collected by filtration and dried under vacuum to yield compound (**3**) as an off white solid. (95 %); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.66 (brs, 1H, COOH), 7.68 (d, 2H, $J = 8.60$ Hz, Ar–H), 6.74 (brs, 1H, –NH), 6.58 (d, 2H, $J = 8.6$ Hz, Ar–H), 3.88 (s, 2H, N–CH_2), 3.73 (s, 3H, O–CH_3); MS: *m*/*z* 208 [M−1].

Synthesis of 2-{[(4-methoxy carbonyl) phenyl] (nitroso) amino} acetic acid (**4**)

To a suspension of compound (**3**) (192 mmol) in water (400 mL) was added 3 M HCl (240 mmol) under cooling. The mixture was stirred for 10 min. A solution of sodium nitrite (211 mmol) in water (60 mL) was added dropwise at −5 to 0 °C. After the complete addition, the mixture was slowly warmed to room temperature. Completion of reaction was monitored by TLC. The solid was filtered, washed with water, and dried under vacuum to afford compound (**4**). (88 %); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 13.25 (brs, 1H, COOH), 8.10 (d, 2H, $J = 8.66$ Hz, Ar–H), 7.79 (d, 2H, $J = 8.66$ Hz, Ar–H), 4.82 (s, 2H, N–CH_2), 3.88 (s, 3H, O–CH_3); MS: *m*/*z* 237 [M−1].

Synthesis of 3-(4-methoxycarbonyl)phenylsydnone (**5**)

A mixture of compound (**4**) (169 mmol) in acetic anhydride (100 mL) was stirred at 55 °C for 1 h. Reaction progress was monitored by TLC. The reaction mixture was poured onto ice water and stirred for 30 min. The solid was filtered and dried under vacuum to yield compound (**5**) as an off white solid. (88 %); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.31 (d, 2H, $J = 8.60$ Hz, Ar–H), 7.84 (d, 2H, $J = 8.60$ Hz, Ar–H), 6.79 (s, 1H, sydnone-H), 4.01 (s, 3H, O–CH_3); MS: *m*/*z* 220 [M+1].

Synthesis of 4-(5-methyl-2-oxo-[1,3,4]oxadiazol-3-yl)-benzoic acid methyl ester (**6**)

A mixture of compound (**5**) (137.6 mmol) in acetic anhydride (150 mL) was dropwise added a solution of bromine (15 mL) in acetic anhydride (150 mL) at 0–5 °C. Slowly warmed to room temperature and stirred at 60 °C for 1 h.

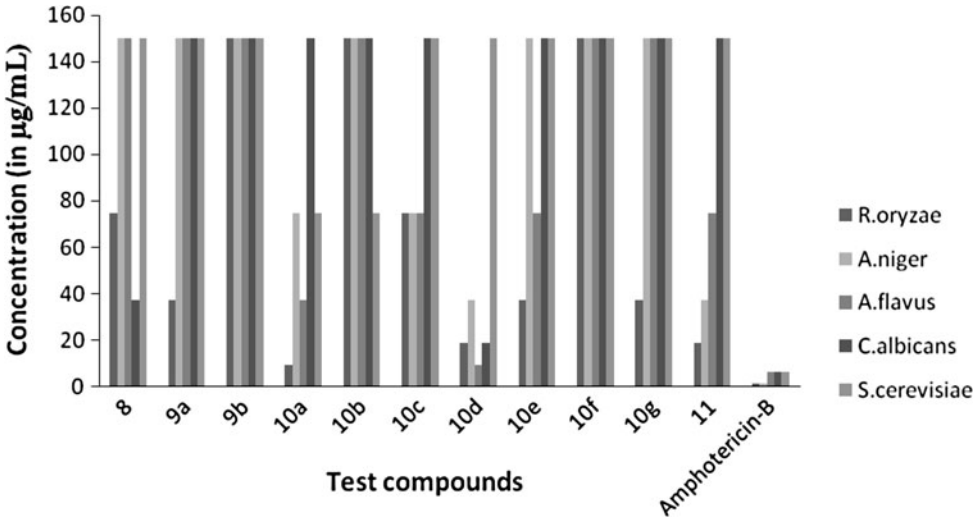


Fig. 4 Antifungal activity of synthesized compounds

After completion of reaction, the reaction mixture was cooled to 0–5 °C and quenched with water (600 mL). Then stirred for 1 h, solid was collected by filtration and dried under vacuum to get compound (**6**) as an off white solid. (80 %). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.12 (d, 2H, $J = 8.88$ Hz, Ar–H), 7.95 (d, 2H, $J = 8.88$ Hz, Ar–H), 3.94 (s, 3H, O–CH_3), 2.39 (s, 2H, triazole–CH_3); MS: *m/z* 235 [M+1].

Synthesis 4-(4-amino-3-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl)benzoic acid hydrazide (**7**)

A mixture of compound (**6**) (85.4 mmol), hydrazine hydrate (20 mL) in ethanol (200 mL) was stirred at reflux for 4 h. The reaction mixture was cooled, solid formed was collected by filtration, and dried under vacuum to yield compound (**7**) as an off white solid. (86 %); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H, –CONH–), 7.98 (d, 2H, $J = 8.80$ Hz, Ar–H), 7.91 (d, 2H, $J = 8.80$ Hz, Ar–H), 5.42 (s, 2H, triazole–NH_2), 4.48 (brs, 2H, $CONHNH_2$), 2.25 (s, 2H, triazole–CH_3); MS: *m/z* 249 [M+1].

Synthesis of 4-amino-2-[4-(5-mercapto-[1,3,4]oxadiazol-2-yl)phenyl]-2,4-dihydro-[1,2,4]triazol-3-one (**8**)

Potassium hydroxide (120 mmol) was dissolved in ethanol (150 mL), and then compound (**7**) (50 mmol) and carbondisulfide (126 mmol) were added. The reaction mixture was stirred at reflux for 5 h. After the completion of reaction, the mixture was poured onto ice water and neutralized with dilute HCl. The solid precipitated was collected by filtration and dried under vacuum to afford compound (**8**). Reddish brown solid; (70 %), m.p. 265–266 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 14.68 (brs, 1H, oxadiazole–SH), 8.13 (d, 2H, $J = 8.88$ Hz, Ar–H), 7.97 (d, 2H, $J = 8.88$ Hz, Ar–H), 5.44 (brs, 2H, triazole–NH_2) 2.26 (s, 3H, triazole–CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 177.8, 160.6, 151.9, 148.2, 141.2, 127.8, 118.7, 117.9, 11.3; MS: *m/z* 291 [M+1]; Anal. Calculated for $C_{11}H_{10}N_6O_2S$: C. 45.51, H. 3.47, N. 28.95, S. 11.05; Found: C. 45.45, H. 3.46, N. 28.98, S. 11.03 %.

4-Amino-5-methyl-2-[4-(5-methyl-sulfanyl-[1,3,4]oxadiazol-2-yl)-phenyl]-2,4-dihydro-[1,2,4]triazol-3-one (**9a**)

To a solution of compound (**8**) (0.2 mmol) in DMF (5 mL) was added K_2CO_3 (0.3 mmol) followed by methyl iodide (0.4 mmol). The mixture was stirred at RT for 3 h. The reaction mixture was poured onto ice water. The solid obtained was filtered, washed, and dried to afford target compound. Brownish solid; (80 %); m.p. 299–300 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.11 (d, 2H, $J = 8.9$ Hz, Ar–H), 8.05 (d, 2H, $J = 8.9$ Hz, Ar–H), 5.43 (s, 2H, Ar–NH_2), 2.76 (s, 3H, S–CH_3), 2.24 (s, 3H, triazole–CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 165.2, 164.8, 162.7, 151.9, 148.1, 140.9, 128.0, 117.9, 14.81, and 11.3; MS: *m/z* 305 [M+1]; Anal. Calculated for $C_{12}H_{12}N_6O_2S$: C. 47.36, H. 3.97, N. 27.62, S. 10.54; Found: C. 47.43, H. 3.98, N. 27.59, S. 10.50 %.

{5-[4-(4-Amino-3-methyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl)phenyl][1,3,4] oxadiazol-2-ylsulfanyl}-acetic acid ethyl ester (**9b**)

To a solution of compound (**8**) (0.2 mmol) in DMF (5 mL) was added K_2CO_3 (0.3 mmol) followed by ethylbromoacetate (0.205 mmol). The mixture was stirred at RT for 16 h. The reaction mixture was poured onto ice water. The solid obtained was filtered, washed, and dried to afford target compound (**9b**). brownish solid; (71 %); m.p. 189–190 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.12 (d, 2H, $J = 8.91$ Hz, Ar–H), 8.05 (d, 2H, $J = 8.91$ Hz, Ar–H), 5.44 (s, 2H, Ar–NH_2), 4.29 (s, 2H, S–CH_2), 4.16 (q, 2H, $J = 7.12$ Hz, CH_3CH_2), 2.26 (s, 3H, triazole–CH_3), 1.20 (t. 3H, $J = 7.12$ Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 168.2, 165.4, 163.1, 151.9, 148.1, 141.1, 128.1, 119.2, 117.9, 62.1, 34.4, 14.4, and 11.3; MS: *m/z* 377 [M+1]; Anal. Calculated for $C_{15}H_{16}N_6O_4S$: C. 47.87, N. 22.23, S. 8.52; Found: C. 47.82, 4.29, N. 22.26, S. 8.50 %.

General procedure for the synthesis of **10a–g**

A mixture of (**8**) (2 mmol), substituted aldehydes (2.02 mmol) and 1–2 drops of concentrated sulfuric acid in DMSO (5 mL) was stirred at 80 °C for 4–6 h. The completion of reaction was checked by TLC. The reaction mixture was then cooled to RT and poured onto ice water to give a solid product. The solid was collected by filtration and dried. The crude product was recrystallized from suitable solvents to afford the title compounds (**10a–g**).

*4-[(2-Fluoro-benzylidine)-amino]-2-[4-(5-mercapto-[1,3,4]oxadiazol-2-yl)phenyl]-5-methyl-2,4-dihydro-[1,2,4]triazol-3-one (**10a**)*

Reddish brown solid; (80 %); m.p. 282–283 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 14.73 (s, 1H, oxadiazole–SH), 9.97 (s, 1H, N=CH), 8.13 (d, 2H, $J = 8.9$ Hz, Ar–H), 8.07–8.04 (m, 1H, Ar–H), 7.99 (d, 2H, $J = 8.9$ Hz, Ar–H), 7.64-7.62 (m, 1H, Ar–H), 7.42–7.35 (m, 2H, Ar–H), 2.44 (s, 3H, triazole–CH_3); MS: *m/z* 397 [M+1]; Anal. Calculated for $C_{18}H_{13}FN_6O_2S$: C. 54.54, H. 3.31, N. 21.20, S. 8.09; Found: C. 54.58, H. 3.32, N. 21.23, S. 8.07 %.

*4-[(3-Fluoro-benzylidine)-amino]-2-[4-(5-mercapto-[1,3,4]oxadiazol-2-yl)phenyl]-5-methyl-2,4-dihydro-[1,2,4]triazol-3-one (**10b**)*

Reddish brown solid; (76 %); m.p. 276–277 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 14.75 (s, 1H, oxadiazole–SH), 9.74 (s, 1H, N=CH), 8.13 (d, 2H, $J = 8.85$ Hz, Ar–H), 7.99 (d, 2H, $J = 8.91$ Hz, Ar–H), 7.74–7.71 (m, 2H, Ar–H), 7.61–7.56 (m, 1H, Ar–H), 7.42–7.37 (m, 1H, Ar–H), 2.43 (s, 3H, triazole–CH_3); MS: *m/z* 397 [M+1]; Anal. Calculated for $C_{18}H_{13}FN_6O_2S$: C. 54.54, H. 3.31, N. 21.20, S. 8.09; Found: C. 54.50, H. 3.30, N. 21.24, S. 8.10 %.

*4-[(4-Fluoro-benzylidine)-amino]-2-[4-(5-mercapto-[1,3,4]oxadiazol-2-yl)phenyl]-5-methyl-2,4-dihydro-[1,2,4]triazol-3-one (**10c**)*

Brownish solid; (77 %); m.p. 272–273 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 14.75 (s, 1H, oxadiazole–SH), 9.70 (s, 1H, N=CH), 8.12 (d, 2H, $J = 8.9$ Hz, Ar–H), 8.00–7.98 (m, 4H, Ar–H), 7.38 (m, 2H, Ar–H), 2.42 (s, 3H, triazole–CH_3); MS: *m/z* 397 [M+1]; Anal. Calculated for $C_{18}H_{13}FN_6O_2S$: C. 54.54, H. 3.31, N. 21.20, S. 8.09; Found: C. 54.60, H. 3.33, N. 21.20, S. 8.10 %.

*4-[(2,5-Difluoro-benzylidine)-amino]-2-[4-(5-mercapto-[1,3,4]oxadiazol-2-yl)phenyl]-5-methyl-2,4-dihydro-[1,2,4]triazol-3-one (**10d**)*

Brownish solid; (79 %); m.p. 255–256 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 14.73 (s, 1H, oxadiazole–SH), 9.94 (s, 1H, N=CH), 8.12 (d, 2H, $J = 8.9$ Hz, Ar–H), 7.80 (m, 1H, Ar–H), 7.48 (m, 2H, Ar–H), 2.45 (s, 3H, triazole–CH_3); MS: *m/z* 413 [M−1]; Anal. Calculated for $C_{18}H_{12}F_2N_6O_2S$: C. 52.17, H. 2.92, N. 20.28, S. 7.74; Found: C. 52.15, H. 2.90, N. 20.31, S. 7.78 %.

*4-[(2-Bromo-benzylidine)-amino]-2-[4-(5-mercapto-[1,3,4]oxadiazol-2-yl)phenyl]-5-methyl-2,4-dihydro-[1,2,4]triazol-3-one (**10e**)*

Reddish brown solid; (75 %); m.p. 288–289 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 14.78 (s, 1H, oxadiazole–SH), 10.16 (s, 1H, N=CH), 8.15 (d, 2H, $J = 8.52$ Hz, Ar–H), 8.09 (m, 1H, Ar–H), 7.99 (d, 2H, $J = 8.52$ Hz, Ar–H), 7.78 (d, 1H, Ar–H), 7.54–7.48 (m, 2H), 2.44 (s, 3H, triazole–CH_3); MS: *m/z* 456 [M], 458 [M+2]; Anal. Calculated for $C_{18}H_{13}BrN_6O_2S$: C. 47.28, H. 2.87, N. 28.38, S. 7.01; Found: C. 47.31, H. 2.86, N. 28.30, S. 6.98 %.

*2-[4-(5-Mercapto-[1,3,4]oxadiazol-2-yl)phenyl]-5-methyl-4-[(1H-pyrrol-2-ylmethylene)-amino]-2,4-dihydro-[1,2,4]triazol-3-one (**10f**)*

Brownish solid; (71 %); m.p. 249–250 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 14.75 (brs, 1H, oxadiazole-SH), 11.77 (s, 1H, pyrrole-NH), 9.30 (s, 1H, N=CH), 8.13 (d, 2H, $J = 8.91$ Hz, Ar–H), 7.9 (d, 2H, $J = 8.91$ Hz, Ar–H), 7.12 (s, 1H, pyrrole–H), 6.75 (s, 1H, pyrrole–H), 6.75 (s, 1H, pyrrole–H), 2.38 (s, 3H, triazole–CH_3); MS: *m/z* 367.4 [M+1]; Anal. Calculated for $C_{16}H_{12}N_7O_2S$: C. 52.45, H. 3.30, N. 26.76; S. 8.75; Found C. 52.44, H. 3.32, N. 26.70, S. 8.74 %.

*2-[4-(5-Mercapto-[1,3,4]oxadiazol-2-yl)phenyl]-5-methyl-4-[(thiazol-4-ylmethylene)-amino]-2,4-dihydro-[1,2,4]triazol-3-one (**10g**)*

Brownish solid; (68 %); m.p. 289–290 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 14.75 (brs, 1H, Oxadiazol–SH), 9.81 (s, 1H, thiazole–H), 9.27 (s, 1H, N=CH), 8.49 (s, 1H, thiazole–H), 8.13 (d, 2H, $J = 8.84$ Hz, Ar–H), 7.99 (d, 2H, $J = 8.84$ Hz, Ar–H), 2.41 (s, 3H, triazole–CH_3); MS: *m/z* 386 [M+1]; Anal. Calculated for $C_{15}H_{11}N_7O_2S_2$: C. 46.74, H. 2.88, N. 25.44, S. 16.44; Found: C. 46.69, H. 2.90, N. 25.47, S. 16.41 %.

Synthesis of 4-amino-2-[4-amino-5-mercapto-4*H*-[1,2,4]triazol-3-yl)-phenyl]-5-methyl-2,4-dihydro-[1,2,4] triazol-3-one (**11**)

A mixture of (**11**) (2 mmol) in hydrazine hydrate (10 mL) was stirred at reflux for 12 h. The reaction mixture was cooled and solid formed was filtered and dried under vacuum to afford compound (**11**) as an off white solid. (70 %); m.p. 284–285 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 14.73 (brs, 1H, triazole-SH), 8.12 (d, 2H, $J = 11.86$ Hz, Ar–H), 2.24 (s, 3H, triazole–CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 167.3, 151.9, 149.4, 147.8, 139.8, 129.4, 122.2, 117.3, 11.3; MS: *m/z* 305 [M+1]; Anal. Calculated for $C_{11}H_{12}N_8OS$: C. 43.41, H. 3.97, N. 36.82, S. 10.54. Found: C. 43.45, H. 3.98, N. 36.77, S. 10.53 %.

Biological activity

The in vitro antimicrobial activity was carried out by disc diffusion method in dimethylformamide (DMF) as solvent. All the newly synthesized compounds were screened for antimicrobial activity against *B. subtilis* (NICM 2063), *S. aureus*(NICM 2079), *S. epidermidis* (NICM 2493), *E. coli* (NICM 2138), *Pseudomonas aeruginosa* (NICM 2036) and the antifungal activity against *Rhizopus oryzae* (NICM 997), *Aspergillus niger* (NICM 572),*A.*

flavus(NICM 524),*Candida albicans* (NICM 3100), and *Saccharomyces cerevisiae* (NICM 3312).

The MIC was determined by using twofold serial dilution method with 64-well micro test plates. Streptomycin and Penicillin for antibacterial activity and Ampotericin B was used for antifungal activity as reference standards to compare the antibacterial and antifungal activities, respectively.

For determining both antibacterial and antifungal activities, the synthesized compounds and the control drugs were dissolved in redistilled dimethyl formamide. Further dilutions were made at the required quantities of 300, 150, 75, 37.5, 18.75, 9.75, 6.25, 3.125, and 1.56 μg/mL, respectively. In order to ensure that the solvent had no effect on bacterial growth, a control test was also performed containing broth supplemented with only DMF at the same dilution used in our experiment. The MIC values were obtained from the lowest concentration of the test compound where the tubes remain clear, indicating that the bacterial growth was completely inhibited at this concentration.

Highlights

- Synthesized novel 1,2,4-triazolo-[1,3,4]oxadiazole, 1,2,4-triazolo-[1,3,4]oxadiazole thioethers, 1,2,4-triazolo-[1,3,4]oxadiazole arylidines, and bis-1,2,4-triazole derivatives
- The new heterocycles were synthesized by simple transformations
- Characterized by spectral data
- All the synthesized compounds were screened for antimicrobial activities
- Compound **10b** was more potent in antibacterial activity.

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