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Original article

Synthesis and antimicrobial activity of novel sulfone-linked bis heterocycles

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Abstract

Novel sulfone-linked bis heterocycles pyrazolines in combination with thiadiazoles, oxadiazoles and triazoles were prepared from *E*-styrylsulfonylacetic acid methyl ester and tested for their antimicrobial activity. The compound **8** showed pronounced activity than the compounds **6** and **7**.

Keywords: 2-Pyrazolines; 1,3,4-Thiadiazoles; 1,3,4-Oxadiazoles; 1,2,4-Triazoles; Antimicrobial activity

1. Introduction

Compounds incorporating heterocyclic ring systems continue to attract considerable interest due to the wide range of biological activities they possess. Amongst them five membered heterocyclic compounds particularly azoles occupy a unique place in the realm of natural and synthetic organic chemistry. 1,3,4-Thiadiazoles find wide application in the design of compounds possessing useful properties [1–3]. 5-Unsubstituted 1,3,4-thiadiazoles are of interest as they are used as intermediates in the synthesis of therapeutically useful antibiotic cefazolin [4]. Nowadays, the most frequently used triazoles are flucanazole and itraconazole. They possess a broad spectrum of antifungal activity and reduced toxicity when compared with the imidazole antifungals [5–10]. Moreover, 1,3,4-oxadiazoles find wide usage as dyes, photosensitive and electrical materials [11]. They also exhibit broad spectrum of biological activities such as HIV, antibacterial and antifungal [12,13]. In addition, pyrazolines have gained importance due to their various chemotherapeutic properties. In fact, celecoxib, a pyrazole derivative is now widely used in the market as an anti-inflammatory drug [14]. In view of these, we aimed the synthesis and bioassay of compounds having both pyrazoline and thiadiazole/oxadiazole/triazole rings.

2. Chemistry

The reactivity of *E*-styrylsufonylacetic acid methyl ester (**1**) towards the development of bis heterocycles, pyrazoline in combination with thiadiazole, oxadiazole and triazole was studied. When *E*-styrylsulfonylacetic acid methyl ester (**1**) was treated with hydrazine hydrate instead of the expected acid hydrazide, a cyclic product was obtained. It seems that initially formed Michael addition product undergoes intramolecular cyclocondensation to 1,1-dioxo-6-phenyl-1λ^6-[1,4,5] thiadiazepan-3-one (**2**). In order to achieve the desired bis heterocycles, the olefin in **1** was first exploited to develop pyrazoline ring. The 1,3-dipolar cycloaddition of diazomethane to **1** at −15 °C for 48 h gave a solid which was identified as (4-phenyl-4,5-dihydro-1*H*-pyrazole-3-sulfonyl)-acetic acid methyl ester (**3**). The articulation of thiadiazole, triazole and oxadiazole rings was made from the ester moiety of **3**. The reaction of **3** with 1 equiv of hydrazine hydrate produced (4-phenyl-4,5-dihydro-1*H*-pyrazole-3-sulfonyl)-acetic acid

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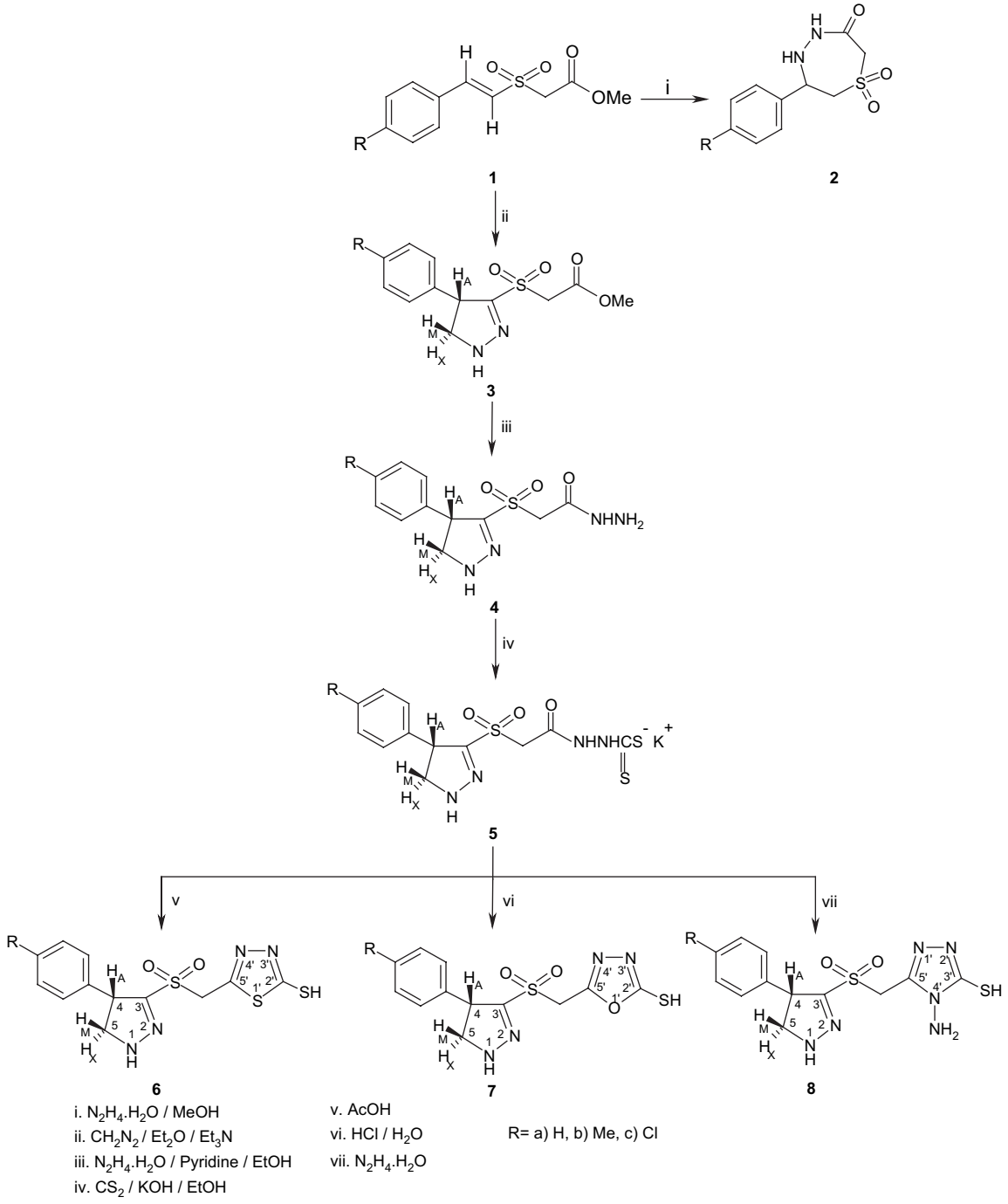
hydrazide (**4**). The potassium dithiocarbazate of acid hydrazide (**5**) was prepared from **4** on treatment with carbon disulfide in the presence of potassium hydroxide under ultrasonic conditions. This on refluxion in acetic acid cyclized to 5′-(4-phenyl-4,5-dihydro-1*H*-pyrazole-3-sulfonylmethyl)-[1′,3′,4′]thiadiazole-2′-thiol (**6**). On the other hand, acid catalyzed hydrolysis of **5** resulted in 5′-(4-phenyl-4,5-dihydro-1*H*-pyrazole-3-sulfonylmethyl)-[1′,3′,4′]oxadiazole-2′-thiol (**7**). The compound **5** on treatment with hydrazine hydrate produced 4′-amino-5′-(4-phenyl-4,5-dihydro-1*H*-pyrazole-3-sulfonylmethyl)-4*H*-[1′,2′,4′]triazole-3′-thiol (**8**) (Scheme 1).

3. Biology

3.1. Antimicrobial activity

The synthesized compounds were tested for their *in vitro* antimicrobial activity against the Gram-positive bacteria



Scheme 1.

Staphylococcus aureus, *Bacillus subtilis*, the Gram-negative bacteria *Escherichia coli*, *Klebsiella pneumoniae* and fungi *Fusarium solani*, *Curvularia lunata* and *Aspergillus niger*. The primary screen was carried out by agar disc-diffusion method [15] using nutrient agar medium. The minimal inhibitory concentration for the most active compounds **6c**, **8a** and **8c** against the same microorganisms used in the preliminary screening was carried out using microdilution susceptibility method [16]. Chloramphenicol and ketoconazole were used as control drugs. The observed data on the antimicrobial activity of the compounds and control drugs are given in Tables 1–3.

4. Results, discussion and conclusion

In the present work, 1,3,4-thiadiazoles, 1,3,4-oxadiazoles and 1,2,4-triazoles were synthesized. The structures of the compounds were elucidated by spectral data. The ^{1}H NMR spectrum of **2a** displayed a singlet and two multiplets at δ 4.35, 4.17–4.22 and 3.84–3.89 ppm for C_2–H, C_6–H and C_7–H, respectively. A broad singlet was observed at δ 10.58 ppm for NH which disappeared on deuteration. The ^{13}C NMR spectrum of this compound displayed signals at δ 60.6, 165.7, 58.9 and 66.8 ppm for C_2, C_3, C_6 and C_7. The mass spectrum of **2a** showed $M^{+\bullet}$ peak at *m/z* 240, which is in agreement with its chemical composition.

The ^{1}H NMR spectrum of **3a** showed an AMX splitting pattern for the pyrazoline ring protons. The doublet of doublets observed at δ 4.48, 3.76 and 3.52 ppm were assigned to H_A, H_M and H_X. Two singlets were observed at δ 4.36 and 3.66 ppm for methylene and methoxy protons as well as signals due to aromatic protons. The ^{13}C NMR spectrum showed signals at δ 38.4, 44.7, 49.8, 52.2, 152.6 and 171.6 ppm for the carbons C_4, C_5, SO_2–CH_2, OCH_3, C_3 and CO, respectively. The ^{1}H NMR spectrum of **4a** displayed broad signals in the region δ 9.51 and 5.16 ppm for NH and NH_2 which disappeared on deuteration, apart from the signals due to pyrazoline ring protons.

The ^{1}H NMR spectra of compounds **6–8** displayed a singlet in the region 10.21–10.31 ppm for SH besides the signals due to pyrazoline ring protons. Apart from this, **8a** showed signals at 5.62 ppm for NH_2 which disappeared on deuteration. The ^{13}C NMR spectra of these compounds exhibited signals at δ 38.8, 44.7, 49.4, 144.6, 154.7 and 168.5 ppm for the carbons C_4, C_5, SO_2–CH_2, $C_{3'}$, C_3, $C_{5'}$, respectively.

Table 1
The in vitro antibacterial activity of compounds **6**, **7** and **8**

Compound	Concentration (μg)	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		S. *Aureus*	*B. subtilis*	*E. coli*	*K. pneumoniae*
6a	100	18	20	15	17
	200	21	23	20	20
6b	100	14	16	18	16
	200	17	19	21	19
6c	100	25	28	22	24
	200	29	31	27	26
7a	100	14	13	15	14
	200	16	15	18	16
7b	100	12	13	12	10
	200	15	16	14	12
7c	100	13	15	11	11
	200	17	18	13	14
8a	100	28	31	22	21
	200	30	33	25	24
8b	100	25	27	24	22
	200	28	29	26	24
8c	100	32	34	23	26
	200	35	36	25	28
Chloramphenicol	100	35	38	40	42
	200	39	41	44	45

Table 2
The in vitro antifungal activity of compounds **6**, **7** and **8**

Compound	Concentration (μg)	Zone of inhibition (mm)		
		F. solani	*C. lunata*	*A. niger*
6a	100	22	26	25
	200	24	29	28
6b	100	24	20	22
	200	26	23	24
6c	100	28	23	26
	200	31	26	29
7a	100	17	17	16
	200	19	22	21
7b	100	16	16	17
	200	20	18	21
7c	100	15	16	14
	200	18	19	18
8a	100	39	37	36
	200	44	41	39
8b	100	32	34	35
	200	37	39	38
8c	100	40	39	36
	200	43	42	40
Ketoconazole	100	38	41	36
	200	42	44	39

4.1. Biological results

The results of the final compounds of preliminary antibacterial testing are shown in Table 1. The results revealed that majority of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms. In general, the inhibitory activity against the Gram-positive bacteria was higher than that of the Gram-negative bacteria. The oxadiazole derivatives **7a–c** were displayed least activity. The compounds **6c**, **8a**, **8b** and **8c** showed excellent activity against Gram-positive bacteria (inhibitory zone >25 mm), good activity against Gram-negative bacteria (inhibitory zone >20 mm).

Table 3
The MICs values of the compounds **6a**, **8a** and **8c** against bacteria and fungi

Compound	Minimal inhibitory concentration MIC, μg/ml (mol/l)						
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*	*F. solani*	*C. lunata*	*A. niger*
6c	100 (2.66×10^{-4})	200 (5.33×10^{-4})	200 (5.33×10^{-4})	200 (5.33×10^{-4})	100 (2.66×10^{-4})	100 (2.66×10^{-4})	200 (5.33×10^{-4})
8a	25 (7.38×10^{-5})	25 (7.38×10^{-5})	50 (1.47×10^{-4})	100 (2.95×10^{-4})	100 (2.95×10^{-4})	100 (2.95×10^{-4})	100 (2.95×10^{-4})
8c	12.5 (3.35×10^{-5})	12.5 (3.35×10^{-5})	50 (1.34×10^{-4})	50 (1.34×10^{-4})	25 (6.70×10^{-5})	12.5 (3.35×10^{-5})	25 (6.70×10^{-5})
Chloramphenicol	6.25 (1.93×10^{-5})	6.25 (1.93×10^{-5})	6.25 (1.93×10^{-5})	12.5 (3.86×10^{-5})	–	–	–
Ketoconazole	–	–	–	–	12.5 (2.35×10^{-5})	6.25 (1.17×10^{-5})	6.25 (1.93×10^{-5})

All the tested compounds showed moderate (**7a–c**) to high (**6a–c** and **8a–c**) inhibitory effect towards tested fungi (Table 2).

The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganisms (Table 3). The structure–antimicrobial activity relationship of the synthesized compounds revealed that the compounds having oxadiazole moiety exhibited least activity when compared with compounds having thiadiazole and triazole moieties. Among the substituents on the aryl group, the compounds having 4-chlorophenyl derivatives were the most active. The maximum activity was attained with compound **8c**, having triazole nucleus with chloro substituent in the aryl group.

In conclusion, a new class of bis heterocycles, pyrazoline in combination with thiadiazole, triazole and oxadiazole are developed adopting simple, elegant and well-versed methodologies from a vulnerable substrate, *E*-styrylsulfonylacetic acid methyl ester. We have also evaluated *in vitro* antimicrobial activity of some compounds. The results obtained from antifungal and antibacterial tests together showed that all compounds tested are more active towards fungi than bacteria. The compounds pyrazoline in combination with triazole showed greater antimicrobial activity.

5. Experimental

5.1. Chemistry

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The purity of the compounds was checked by TLC (silica gel H, BDH, ethyl acetate–hexane, 1:3). IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR in KBr pellets. ^{1}H NMR spectra were recorded at 300 MHz on a Varian EM-360 spectrometer.^{13}C NMR spectra were run on a Varian VXR spectrometer operating at 75.5 MHz. All chemical shifts are reported in parts per million from TMS as an internal standard. Mass spectra were recorded on a Joel JMS-D 300 instrument at 70 eV. Elemental analyses were performed using a Perkin–Elmer 240 C elemental analyzer.

The starting compound *E*-styrylsulfonylacetic acid methyl ester **1** was prepared by standard procedure [17].

5.1.1. Preparation of 1,1-dioxo-6-aryl-1λ^6-[1,4,5] thiadiazepan-3-one **2a–b**

A mixture of **1** (0.25 mmol) and hydrazine hydrate (0.5 mmol) in methanol (15 ml) was refluxed for 10 h. It was cooled and the solid separated was filtered and dried.

5.1.1.1. 1,1-Dioxo-6-phenyl-1λ^6-[1,4,5]thiadiazepan-3-one ***2a***. White crystals (0.91 g, 76%); m.p. 210–212 °C; IR (KBr): 1129, 1322 (SO_2), 1638 (C=O), 3304 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.84–3.89 (m, 2H, C_7-H), 4.17–4.22 (m, 1H, C_6-H), 4.35 (s, 2H, C_2-H), 7.24–7.56 (m, 5H, Ph), 10.58 (br s, 2H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 58.9 (C_6), 60.6 (C_2), 66.8 (C_7), 165.7 (C_3), 128.4, 129.5, 130.8, 131.2 (aromatic carbons); Ms (*m*/*z*): 240 ($M^{+\bullet}$). Anal. Calcd.

for $C_{10}H_{12}N_2O_3S$: C, 49.99; H, 5.03; N, 11.66; Found: C, 50.08; H, 5.08; N, 11.60%.

5.1.1.2. 1,1-Dioxo-6-p-tolyl-1λ⁶-[1,4,5]thiadiazepan-3-one ***2b***. White crystals (1.00 g, 79%); m.p. 225–227 °C; IR (KBr): 1125, 1324 (SO_2), 1640 (C=O), 3310 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 2.34 (s, 3H, Ar-CH_3), 3.71–3.75 (m, 2H, C_7-H), 4.15–4.19 (m, 1H, C_6-H), 4.32 (s, 2H, C_2-H), 7.32–7.66 (m, 4H, Ar), 10.52 (br s, 2H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm): 20.4 (Ar-CH_3), 56.5 (C_6), 58.7 (C_2), 64.2 (C_7), 164.6 (C_3), 128.8, 129.3, 131.8, 133.2 (aromatic carbons); MS (*m/z*) 254 ($M^{+\bullet}$). Anal. Calcd. for $C_{11}H_{14}N_2O_3S$: C, 51.95; H, 5.55; N, 11.02; Found: C, 51.83; H, 5.52; N, 12.00%.

5.1.2. General procedure for the synthesis of (4-aryl-4,5-dihydro-1H-pyrazole-3-sulfonyl)-acetic acid methyl ester ***3a–c***

To a cooled solution of *E*-styrylsulfonylacetic acid methyl ester **1a–c** (5 mmol) in dichloromethane (20 ml), an ethereal solution of diazomethane (40 ml, 0.4 M) and triethylamine (0.12 g) were added. The reaction mixture was kept at −20 to −15 °C for 40–48 h. The solvent was removed under reduced pressure. The resultant solid was purified by column chromatography (hexane–ethyl acetate 4:1).

5.1.2.1. (4-Phenyl-4,5-dihydro-1H-pyrazole-3-sulfonyl)-acetic acid methyl ester ***3a***. Yellow solid (1.01 g, 72%); m.p. 129–131 °C; IR (KBr): 1146, 1325 (SO_2), 1565 (C=N), 1745 (C=O), 3183 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 3.52 (dd, 1H, H_X), 3.66 (s, 3H, OCH_3), 3.76 (dd, 1H, H_M, $J_{MX} = 10.0$ Hz), 4.36 (s, 2H, SO_2CH_2), 4.48 (dd, 1H, H_A, $J_{AM} = 12.6$ Hz, $J_{AX} = 5.5$ Hz), 10.18 (br s, 1H, NH), 7.32–7.58 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ (ppm): 38.4 (C_4), 44.7 (C_5), 49.8 (SO_2CH_2), 52.2 (OCH_3), 152.6 (C_3), 171.6 (C=O), 125.7, 127.9, 128.4, 140.2 (aromatic carbons). Anal. Calcd. for $C_{12}H_{14}N_2O_4S$: C, 51.05; H, 5.00; N, 9.92; Found: C, 51.09; H, 5.02; N, 9.98%.

5.1.2.2. (4-p-Tolyl-4,5-dihydro-1H-pyrazole-3-sulfonyl)-acetic acid methyl ester ***3b***. Yellow solid (0.96 g, 76%); m.p. 142–144 °C; IR (KBr): 1142, 1328 (SO_2), 1568 (C=N), 1742 (C=O), 3174 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2.31 (s, 3H, Ar-CH_3), 3.46 (dd, 1H, H_X), 3.64 (s, 3H, OCH_3), 3.84 (dd, 1H, H_M, $J_{MX} = 10.2$ Hz), 4.34 (s, 2H, SO_2CH_2), 4.46 (dd, 1H, H_A, $J_{AM} = 12.4$ Hz, $J_{AX} = 5.2$ Hz), 10.15 (br s, 1H, NH), 7.14–7.35 (m, 4H, Ar); ^{13}C NMR ($CDCl_3$) δ (ppm): 20.6 (Ar-CH_3), 38.2 (C_4), 44.6 (C_5), 49.6 (SO_2CH_2), 51.9 (OCH_3), 152.5 (C_3), 171.4 (C=O), 129.1, 130.3, 131.8, 133.6 (aromatic carbons). Anal. Calcd. for $C_{13}H_{16}N_2O_4S$: C, 52.69; H, 5.44; N, 9.45; Found: C, 52.64; H, 5.45; N, 9.52%.

5.1.2.3. 4-(4-Chlorophenyl-4,5-dihydro-1H-pyrazole-3-sulfonyl)-acetic acid methyl ester ***3c***. Yellow solid (1.25 g, 79%); m.p. 151–153 °C; IR (KBr): 1150, 1332 (SO_2), 1570 (C=N), 1748 (C=O), 3178 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 3.49 (dd, 1H, H_X), 3.68 (s, 3H, OCH_3), 3.88 (dd, 1H, H_M, $J_{MX} = 10.4$ Hz), 4.38 (s, 2H, SO_2CH_2), 4.52 (dd, 1H, H_A, $J_{AM} = 12.9$ Hz, $J_{AX} = 5.9$ Hz), 10.20 (br s, 1H, NH), 7.23–7.41 (m, 4H, Ar); ^{13}C NMR ($CDCl_3$) δ (ppm): 38.6 (C_4), 44.9 (C_5), 50.1 (SO_2CH_2), 54.2 (OCH_3), 152.8 (C_3), 171.8 (C=O), 129.4, 130.5, 135.2, 136.6 (aromatic carbons). Anal. Calcd. for $C_{12}H_{13}ClN_2O_4S$: C, 45.50; H, 4.14; N, 8.84; Found: C, 45.53; H, 4.12; N, 8.91%.

5.1.3. General procedure for the synthesis of (4-aryl-4,5-dihydro-1H-pyrazole-3-sulfonyl)-acetic acid hydrazide ***4a–c***

To a solution of (4-aryl-4,5-dihydro-1*H*-pyrazole-3-sulfonyl)-acetic acid methyl ester **3a–c** (1 mmol) in absolute ethanol, hydrazine hydrate (4.5 mmol) and pyridine (0.4 ml) were added and stirred for 6 h at room temperature. The resultant solid was filtered and recrystallized from ethanol.

5.1.3.1. (4-Phenyl-4,5-dihydro-1H-pyrazole-3-sulfonyl)-acetic acid hydrazide ***4a***. Yellow solid (0.18 g, 65%); m.p. 162–164 °C; IR (KBr): 1135, 1320 (SO_2), 1567 (C=N), 1665 (C=O), 3214 (NH), 3226 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 3.51 (dd, 1H, H_X), 3.83 (dd, 1H, H_M, $J_{MX} = 10.2$ Hz), 4.28 (s, 2H, SO_2CH_2), 4.43 (dd, 1H, H_A, $J_{AM} = 12.5$ Hz, $J_{AX} = 5.6$ Hz), 5.16 (br s, 2H, NH_2), 7.26–7.34 (m, 5H, Ph), 9.51 (br s, 1H, NH), 10.16 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 38.9 (C_4), 44.9 (C_5), 48.6 (SO_2CH_2), 153.5 (C_3), 169.6 (C=O), 128.5, 129.8, 130.1, 132.4 (aromatic carbons). Anal. Calcd. for $C_{11}H_{14}N_4O_3S$: C, 46.80; H, 5.00; N, 19.85; Found: C, 46.85; H, 5.03; N, 19.94%.

5.1.3.2. (4-p-Tolyl-4,5-dihydro-1H-pyrazole-3-sulfonyl)-acetic acid hydrazide ***4b***. Yellow solid (0.20 g, 68%); m.p. 159–161 °C; IR (KBr): 1138, 1330 (SO_2), 1564 (C=N), 1668 (C=O), 3212 (NH), 3224 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 2.27 (s, 3H, Ph-CH_3), 3.49 (dd, 1H, H_X), 3.81 (dd, 1H, H_M, $J_{MX} = 10.1$ Hz), 4.26 (s, 2H, SO_2CH_2), 4.41 (dd, 1H, H_A, $J_{AM} = 12.4$ Hz, $J_{AX} = 5.4$ Hz), 4.98 (br s, 2H, NH_2), 7.18–7.25 (m, 4H, Ar), 9.49 (br s, 1H, NH), 10.14 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 21.2 (Ar-CH_3), 38.7 (C_4), 44.3 (C_5), 48.3 (SO_2CH_2), 153.1 (C_3), 169.4 (C=O), 129.4, 131.6, 133.4, 134.3 (aromatic carbons). Anal. Calcd. for $C_{12}H_{16}N_4O_3S$: C, 48.64; H, 5.44; N, 18.91; Found: C, 48.59; H, 5.40; N, 18.83%.

5.1.3.3. 4-(4-Chlorophenyl-4,5-dihydro-1H-pyrazole-3-sulfonyl)-acetic acid hydrazide ***4c***. Yellow solid (0.22 g, 71%); m.p. 164–166 °C; IR (KBr): 1136, 1335 (SO_2), 1569 (C=N), 1670 (C=O), 3216 (NH), 3229 (NH_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 3.58 (dd, 1H, H_X), 3.85 (dd, 1H, H_M, $J_{MX} = 10.4$ Hz), 4.31 (s, 2H, SO_2CH_2), 4.45 (dd, 1H, H_A, $J_{AM} = 12.6$ Hz, $J_{AX} = 5.7$ Hz), 5.18 (br s, 2H, NH_2), 7.29–7.40 (m, 4H, Ar), 9.56 (br s, 1H, NH), 10.20 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm): 39.4 (C_4), 45.1 (C_5), 48.8 (SO_2CH_2), 153.9 (C_3), 169.8 (C=O), 129.8, 132.4, 133.8, 136.9 (aromatic carbons). Anal. Calcd. for

$C_{11}H_{13}ClN_4O_3S$: C, 41.71; H, 4.14; N, 17.69; Found: C, 41.73; H, 4.12; N, 17.63%.

5.1.4. General procedure for the preparation of potassium (4-aryl-4,5-dihydro-1H-pyrazole-3-sulfonylacetyl)-hydrazine-N′-carbodithioate ***5a–c***

To a mixture of potassium hydroxide (2 mmol) and (4-aryl-4,5-dihydro-1*H*-pyrazole-3-sulfonyl)-acetic acid hydrazide **4a–c** (1 mmol) in absolute ethanol (5 ml), carbon disulfide (4 mmol) was added and sonicated for 12 h. The separated solid was filtered and dried.

5.1.5. General procedure for the synthesis of 5′-(4-aryl-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl)-[1′,3′,4′]thiadiazole-2′-thiol ***6a–c***

A mixture of potassium (4-aryl-4,5-dihydro-1*H*-pyrazole-3-sulfonylacetyl)-hydrazine-*N*′-carbodithioate **5a–c** (1 mmol) and acetic acid (4 ml) were refluxed for 24 h. The contents of the flask were cooled and poured into crushed ice. The solid obtained was filtered, dried and recrystallized from 2-propanol.

5.1.5.1. 5′-(4-Phenyl-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl)-[1′,3′,4′]thiadiazole-2′-thiol ***6a***. Yellow solid (0.21 g, 61%); m.p. 202–204 °C; IR (KBr): 1144, 1335 (SO_2), 1566 (C=N), 2558 (SH), 3220 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 3.54 (dd, 1H, H_X), 3.84 (dd, 1H, H_M, $J_{MX} = 10.3$ Hz), 4.51 (dd, 1H, H_A, $J_{AM} = 12.8$ Hz, $J_{AX} = 5.7$ Hz), 4.73 (s, 2H, SO_2CH_2), 7.36–7.68 (m, 5H, Ph), 9.89 (br s, 1H, NH), 10.26 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 39.6 (C_4), 45.4 (C_5), 52.5 (SO_2CH_2), 155.2 (C_3), 160.7 ($C_{2'}$), 168.5 ($C_{5'}$), 127.2, 130.5, 131.7, 133.1 (aromatic carbons); MS (*m/z*) 340.45 ($M^{+\bullet}$). Anal. Calcd. for $C_{12}H_{12}N_4O_2S_3$: C, 42.33; H, 3.55; N, 16.46; Found: C, 42.37; H, 3.56; N, 16.49%.

5.1.5.2. 5′-[4-(p-Tolyl)-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl]-[1′,3′,4′]thiadiazole-2′-thiol ***6b***. Yellow solid (0.23 g, 66%); m.p. 225–227 °C; IR (KBr): 1141, 1328 (SO_2), 1562 (C=N), 2556 (SH), 3224 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 2.28 (s, 3H, Ar-CH_3), 3.52 (dd, 1H, H_X), 3.82 (dd, 1H, H_M, $J_{MX} = 10.2$ Hz), 4.50 (dd, 1H, H_A, $J_{AM} = 12.7$ Hz, $J_{AX} = 5.4$ Hz), 4.68 (s, 2H, SO_2CH_2), 7.28–7.59 (m, 4H, Ar), 9.76 (br s, 1H, NH), 10.22 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.4 (Ar-CH_3), 39.2 (C_4), 45.2 (C_5), 51.9 (SO_2CH_2), 155.0 (C_3), 160.3 ($C_{2'}$), 168.3 ($C_{5'}$), 128.9, 129.1, 134.5, 137.2 (aromatic carbons); MS (*m/z*) 354.47 ($M^{+\cdot}$). Anal. Calcd. for $C_{13}H_{14}N_4O_2S_3$: C, 44.05; H, 3.98; N, 15.81; Found: C, 44.01; H, 3.95; N, 15.88%.

5.1.5.3. 5′-[4-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl]-[1′,3′,4′]thiadiazole-2′-thiol ***6c***. Yellow solid (0.26 g, 69%); m.p. 244–246 °C; IR (KBr): 1146, 1339 (SO_2), 1565 (C=N), 2561 (SH), 3232 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 3.55 (dd, 1H, H_X), 3.88 (dd, 1H, H_M, $J_{MX} = 10.4$ Hz), 4.54 (dd, 1H, H_A, $J_{AM} = 12.9$ Hz, $J_{AX} = 5.8$ Hz), 4.76 (s, 2H, SO_2CH_2), 7.40–7.66 (m, 4H, Ar), 9.95 (br s, 1H, NH), 10.29 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 39.8 (C_4), 45.8 (C_5), 52.8 (SO_2CH_2), 155.5 (C_3), 160.9 ($C_{2'}$), 168.7 ($C_{5'}$), 128.4, 132.3, 134.8, 138.5 (aromatic carbons). MS (*m/z*) 374.89 ($M^{+\bullet}$). Anal. Calcd. for $C_{12}H_{11}ClN_4O_2S_3$: C, 38.45; H, 2.96; N, 14.94; Found: C, 38.47; H, 2.97; N, 14.89%.

5.1.6. General procedure for the synthesis of 5′-(4-aryl-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl)-[1′,3′,4′]oxadiazole-2′-thiol ***7a–c***

Potassium(4-aryl-4,5-dihydro-1*H*-pyrazole-3-sulfonylacetyl)-hydrazine-*N*′-carbodithioate **5a–c** (1 mmol) was dissolved in 6 ml of water and acidified with 1–2 ml of concd. HCl. The regenerated solid was collected by filtration, dried and purified by recrystallization from 2-propanol.

5.1.6.1. 5′-(4-Phenyl-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl)-[1′,3′,4′]oxadiazole-2′-thiol ***7a***. Yellow solid (0.21 g, 64%); m.p. 180–182 °C; IR (KBr): 1138, 1330 (SO_2), 1564 (C=N), 2560 (SH), 3239 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 3.56 (dd, 1H, H_X), 3.82 (dd, 1H, H_M, $J_{MX} = 10.5$ Hz), 4.55 (dd, 1H, H_A, $J_{AM} = 12.7$ Hz, $J_{AX} = 5.6$ Hz), 4.75 (s, 2H, SO_2CH_2), 7.31–7.55 (m, 5H, Ph), 9.92 (br s, 1H, NH), 10.24 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 39.5 (C_4), 45.7 (C_5), 52.6 (SO_2CH_2), 155.5 (C_3), 161.4 ($C_{2'}$), 168.9 ($C_{5'}$), 125.8, 129.9, 132.4, 138.2 (aromatic carbons); MS (*m/z*) 324.38 ($M^{+\bullet}$). Anal. Calcd. for $C_{12}H_{12}N_4O_3S_2$: C, 44.43; H, 3.73; N, 17.27; Found: C, 44.46; H, 3.75; N, 17.22%.

5.1.6.2. 5′-[4-(p-Tolyl)-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl)-[1′,3′,4′]oxadiazole-2′-thiol ***7b***. Yellow solid (0.23 g, 67%); m.p. 210–212 °C; IR (KBr): 1136, 1335 (SO_2), 1562 (C=N), 2564 (SH), 3246 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 2.31 (s, 3H, Ar-CH_3), 3.53 (dd, 1H, H_X), 3.80 (dd, 1H, H_M, $J_{MX} = 10.3$ Hz), 4.51 (dd, 1H, H_A, $J_{AM} = 12.5$ Hz, $J_{AX} = 5.4$ Hz), 4.72 (s, 2H, SO_2CH_2), 7.26–7.66 (m, 4H, Ar), 9.88 (br s, 1H, NH), 10.21 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.7 (Ar-CH_3), 39.3 (C_4), 45.4 (C_5), 52.3 (SO_2CH_2), 155.2 (C_3), 161.1 ($C_{2'}$), 168.7 ($C_{5'}$), 127.5, 128.3, 133.6, 139.8 (aromatic carbons); MS (*m/z*) 338.41 ($M^{+\bullet}$). Anal. Calcd. for $C_{13}H_{14}N_4O_3S_2$: C, 46.14; H, 4.17; N, 16.56; Found: C, 46.18; H, 4.16; N, 16.49%.

5.1.6.3. 5′-[4-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl]-[1′,3′,4′]oxadiazole-2′-thiol ***7c***. Yellow solid (0.26 g, 73%); m.p. 232–234 °C; IR (KBr): 1145, 1338 (SO_2), 1573 (C=N), 2568 (SH), 3248 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 3.58 (dd, 1H, H_X), 3.85 (dd, 1H, H_M, $J_{MX} = 10.4$ Hz), 4.57 (dd, 1H, H_A, $J_{AM} = 12.9$ Hz, $J_{AX} = 5.7$ Hz), 4.78 (s, 2H, SO_2CH_2), 7.39–7.70 (m, 4H, Ar), 9.98 (br s, 1H, NH), 10.27 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 39.7 (C-4), 46.1 (C_5), 52.9 (SO_2CH_2), 155.7 (C_3), 161.8 ($C_{2'}$), 169.1 ($C_{5'}$), 128.1, 131.4, 134.5, 137.6 ppm (aromatic carbons); MS (*m/z*) 358.83 ($M^{+\bullet}$).

Anal. Calcd. for $C_{12}H_{11}ClN_4O_3S_2$: C, 40.17; H, 3.09; N, 15.61; Found: C, 40.20; H, 3.11; N, 15.68%.

5.1.7. General procedure for the preparation of 4′-amino-5′-(4-aryl-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl)-4′H-[1′,2′,4′]triazole-3′-thiol ***8a–c***

To a solution of potassium (4-aryl-4,5-dihydro-1*H*-pyrazole-3-sulfonylacetyl)-hydrazine-*N*′-carbodithioate **5a–c** (1 mmol) in 6 ml of water, hydrazine hydrate (2 mmol) was added and refluxed for 8–9 h. The contents of the flask were cooled, diluted with water and acidified with 2 ml of acetic acid. The separated solid was collected by filtration, dried and recrystallized from 2-propanol.

5.1.7.1. 4′-Amino-5′-(4-phenyl-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl)-4′H-[1′,2′,4′]triazole-3′-thiol ***8a***. Yellow solid (0.22 g, 65%); m.p. 218–220 °C; IR (KBr): 1135, 1332 (SO_2), 1568 (C=N), 2554 (SH), 3226 (NH), 3240 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 3.48 (dd, 1H, H_X), 3.79 (dd, 1H, H_M, $J_{MX} = 10.4$ Hz), 4.46 (dd, 1H, H_A, $J_{AM} = 12.6$ Hz, $J_{AX} = 5.5$ Hz), 4.66 (s, 2H, SO_2CH_2), 5.62 (br s, 2H, NH_2), 7.30–7.65 (m, 5H, Ph), 10.13 (br s, 1H, NH), 10.27 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 38.8 (C_4), 44.7 (C_5), 49.4 (SO_2CH_2), 144.6 ($C_{3'}$), 154.7 (C_3), 168.5 ($C_{5'}$), 126.7, 127.8, 129.2, 132.9 (aromatic carbons); MS (*m/z*) 338.41 ($M^{+\bullet}$). Anal. Calcd. for $C_{12}H_{14}N_6O_2S_2$: C, 42.59; H, 4.17; N, 24.83; Found: C, 42.61; H, 4.13; N, 24.88%.

5.1.7.2. 4′-Amino-5′-[4-(p-tolyl)-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl]-4′H-[1′,2′,4′]triazole-3′-thiol ***8b***. Yellow solid (0.22 g, 63%); m.p. 234–236 °C; IR (KBr): 1139, 1338 (SO_2), 1572 (C=N), 2557 (SH), 3224 (NH), 3243 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 2.25 (s, 3H, Ar-CH_3), 3.46 (dd, 1H, H_X), 3.75 (dd, 1H, H_M, $J_{MX} = 10.1$ Hz), 4.43 (dd, 1H, H_A, $J_{AM} = 12.5$ Hz, $J_{AX} = 5.1$ Hz), 4.63 (s, 2H, SO_2CH_2), 5.60 (br s, 2H, NH_2), 7.28–7.58 (m, 4H, Ar), 10.11 (br s, 1H, NH), 10.25 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 22.9 (Ar-CH_3), 38.4 (C_4), 44.2 (C_5), 49.1 (SO_2CH_2), 144.3 ($C_{3'}$), 154.5 (C_3), 168.2 ($C_{5'}$), 128.3, 131.4, 136.1, 137.2 (aromatic carbons); MS (*m/z*) 352.44 ($M^{+\bullet}$). Anal. Calcd. for $C_{13}H_{16}N_6O_2S_2$: C, 44.30; H, 4.58; N, 23.85; Found: C, 44.34; H, 4.62; N, 23.91%.

5.1.7.3. 4′-Amino-5′-[4-(4-chlorophenyl)-4,5-dihydro-1H-pyrazole-3-sulfonylmethyl]-4′H-[1′,2′,4′]triazole-3′-thiol ***8c***. Yellow solid (0.26 g, 71%); m.p. 252–254 °C; IR (KBr): 1142, 1343 (SO_2), 1574 (C=N), 2558 (SH), 3229 (NH), 3245 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 3.51 (dd, 1H, H_X), 3.81 (dd, 1H, H_M, $J_{MX} = 10.5$ Hz), 4.49 (dd, 1H, H_A, $J_{AM} = 12.7$ Hz, $J_{AX} = 5.4$ Hz), 4.70 (s, 2H, SO_2CH_2), 5.66 (br s, 2H, NH_2), 7.34–7.80 (m, 4H, Ar), 10.17 (br s, 1H, NH), 10.31 (s, 1H, SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 39.1 (C_4), 44.9 (C_5), 49.8 (SO_2CH_2), 144.8 ($C_{3'}$), 154.9 (C_3), 168.7 ($C_{5'}$), 129.8, 132.5, 134.6, 139.1 (aromatic carbons); MS (*m/z*) 372.86 ($M^{+\bullet}$). Anal. Calcd. for $C_{12}H_{13}ClN_6O_2S_2$: C, 38.66; H, 3.51; N, 22.54; Found: C, 38.62; H, 3.54; N, 22.59%.

5.2. Biological assays

5.2.1. Compounds

The compounds **6–8** were dissolved in DMSO at different concentrations of 100, 200 and 800 μg/ml.

5.2.2. Cells

Bacterial strains *S. aureus*, *B. subtilis*, *E. coli*, *K. pneumoniae* and fungi *F. solani*, *C. lunata* and *A. niger* were obtained from NCIM, Pune, India.

5.2.3. Antibacterial and antifungal assays

Preliminary antimicrobial activities of **6–8** compounds were tested by Agar disc-diffusion method. Sterile filter paper discs (6 mm diameter) moistened with the test compound solution in DMSO of specific concentration 100 μg and 200 μg/disc were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and after 48 h in case of fungi.

The MICs of the compound assays were carried out using microdilution susceptibility method. Chloramphenicol was used as reference antibacterial agent. Ketoconazole was used as reference antifungal agent. The test compounds, chloramphenicol and ketoconazole were dissolved in DMSO at concentration of 800 μg/ml. The twofold dilution of the solution was prepared (400, 200, 100, …, 6.25 μg/ml). The microorganism suspensions were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 and 48 h for bacteria and fungi, respectively. The minimum inhibitory concentrations of the compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no turbidity (i.e. no growth) of inoculated bacteria/fungi.

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