Full Paper

One-pot Synthesis of 5-{[Bis(azolylmethylthio)]methylene}-pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-triones

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A new class of hitherto unknown trisheterocycles, bisoxadiazolyl/bisthiadiazolyl pyrimidinetriones/thioxopyrimidinediones was prepared in a one-pot reaction and their antimicrobial activity was studied.

Keywords: Oxadiazoles / Pyrimidinetriones / Thiadiazoles

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Introduction

Heterocycles, the largest classical division of organic chemistry are of immense importance biologically, industrially and indeed to that functioning of any developed human society. Over the years five and six membered heterocycles, *viz.*, barbituric acid, thiobarbituric acid, oxadiazole and thiadiazole derivatives have emerged as an interesting class of compounds with wide range of applications in pharmaceutical chemistry. Barbituric acid and its derivatives are known as sedatives, hypnotics since a long time. Substituted barbituric and thiobarbituric acids exhibit analgesic, antipyretic and anti-inflammatory activities [1–3]. 1,3,4-Oxadiazoles display a broad spectrum of biological activities such as anti HIV, antibacterial and antifungal [4, 5]. 1,3,4-Thiadiazoles are associated with diverse biological properties probably due to toxophoric –N=C–S group [6–9]. In fact, the advent of sulfur drugs and the discovery of mesoionic compounds greatly accelerated the rate of progress in the field of thiadiazoles. 5-Unsubstituted 1,3,4-thiadiazoles are used as intermediates in the synthesis of therapeutically potent antibiotic cefazolin [10]. Indeed, we have developed a new class of bis-heterocycles having two different heterocyclic rings and studied their biological activities [11]. However, there are no reports to our knowledge about the synthesis of tris heterocyclic systems. The present communication deals with the synthesis of hitherto unknown trisheterocycles.

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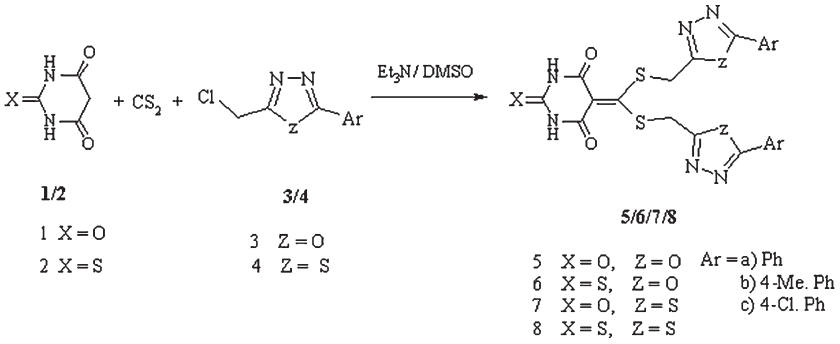
Results and discussion

Chemistry

We report herein a new class of trisheterocyclic systems bisoxadiazolyl/bisthiadiazolyl pyrimidinetriones/thioxopyrimidinediones by the condensation of barbituric acid and thiobarbituric acid with carbon disulfide under base catalysis followed by treatment with 2-chloromethyl-5-aryloxadiazoles (**3**) and 2-chloromethyl-5-arylthiadiazoles (**4**). The compound **3** was prepared by the reaction of chloroacetic acid with benzoic hydrazide in the presence of $POCl_3$ [12]. The **4** was reported by the reaction of *N*-chloroacetyl-*N*-aroylhydrazine with Lawesson's reagent [13]. However, we have achieved the former compound by the interconversion of compound **3** with thiourea in THF.

The desired trisheterocyclic systems 5-(bis((5-aryl-1,3,4-oxadiazol-2-yl)methylthio)methylene)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (**5**) and 5-(bis((5-aryl-1,3,4-oxadiazol-2-yl)methylthio)-methylene)-dihydro-2-thioxopyrimidine-4,6-(1*H*,5*H*)-dione (**6**) were prepared in a one-pot reaction of **1**/**2** with carbon disulfide and **3** in the presence of Et_3N. Adopting similar methodology, 5-(bis((5-aryl-1,3,4-thiadiazol-2-yl)methylthio)-methylene)pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (**7**) and 5-(bis((5-aryl-1,3,4-thiadiazol-2-yl)methylthio)methylene)-dihydro-2-thioxopyrimidine-4,6-(1*H*,5*H*)-dione (**8**) were prepared by treating **1**/**2** with **4** (Scheme 1). The ^{1}H-NMR spectra of **5a**, **6a**, **7a**, and **8a** displayed a sharp singlet at 4.71, 4.68, 4.59, and 4.70 due to methylene protons. A broad singlet was observed



Scheme 1. Synthesis of compounds **5–8**.

at ~10 ppm due to NH which disappeared on deuteration. The structures of the compounds **5–8** were further confirmed by ^{13}C-NMR spectra.

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 **5a–c** and **6a–c** were displayed least activity. However, the thiadiazole derivatives **7c**, **8a**, and **8c** showed excellent activity against Gram-positive bacteria (inhibitory zone >25 mm) and good activity against Gram-negative bacteria (inhibitory zone >17 mm). All tested compounds showed moderate (**5a–c** and **6a–c**) to high (**7a–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

Table 1. The *in-vitro* antibacterial activity of compounds **5–8**.

Compound	Concentration (μg)	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*
5a	100	13	13	10	9
	200	14	15	12	11
5b	100	12	10	8	9
	200	14	13	10	10
5c	100	15	16	11	10
	200	17	19	14	12
6a	100	14	13	11	12
	200	16	16	14	13
6b	100	12	10	9	10
	200	14	13	12	12
6c	100	15	16	14	12
	200	18	19	15	14
7a	100	20	19	15	14
	200	22	20	18	16
7b	100	18	15	12	10
	200	21	17	14	12
7c	100	21	22	15	15
	200	25	27	19	17
8a	100	22	24	19	17
	200	25	26	22	18
8b	100	21	19	17	15
	200	22	22	18	17
8c	100	28	29	23	21
	200	32	34	25	22
Chloramphenicol	100	35	38	40	42
	200	39	41	44	45

Table 2. The *in-vitro* antifungal activity of compounds **5–8**.

Compound	Concentration (μg)	Zone of inhibition (mm)		
		F. solani	*C. lunata*	*A. niger*
5a	100	14	12	10
	200	16	15	12
5b	100	13	13	9
	200	14	15	11
5c	100	17	16	10
	200	18	18	13
6a	100	15	13	12
	200	18	16	14
6b	100	13	11	10
	200	14	12	12
6c	100	16	15	12
	200	19	19	15
7a	100	17	18	16
	200	19	22	18
7b	100	16	16	14
	200	20	18	17
7c	100	23	22	17
	200	26	24	19
8a	100	25	27	22
	200	28	29	23
8b	100	22	18	18
	200	24	22	20
8c	100	32	34	27
	200	35	36	30
Ketoconazole	100	38	41	36
	200	42	44	39

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 unit. It was also observed that the compounds with barbituric acid moiety showed less activity when compared with those having thiobarbituric acid unit. In fact, the compounds thiadiazole in combination with thiobarbituric acid **8** exhibited greater inhibitory effect. 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**.

Conclusion

A new class of hitherto unknown trisheterocyclic systems bisoxadiazolyl/thiadiazolyl pyrimidinetriones/thioxopyrimidinediones was prepared in a one-pot reaction in high yields. The antimicrobial studies of trisheterocycles revealed that compounds having thioxopyrimidinedione in combination with bisthiadiazole unit exhibited high activity.

Experimental

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). The IR spectra were recorded on a Thermo Nicolet IR200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^{1}H-NMR spectra were recorded in $CDCl_3$/ DMSO-d_6 on a Bruker-400 spectrometer (400 MHz). The ^{13}C-NMR spectra were recorded in $CDCl_3$/ DMSO-d_6 on a Bruker spectrometer operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard.

*General procedure for preparation of 5-(bis((5-aryl-1,3,4-oxadiazol-2-yl)methylthio)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (5a-c) and 5-(bis((5-aryl-1,3,4-oxadiazol-2-yl)methylthio)-methylene)-dihydro-2-thioxopyrimidine-4,6(1H, 5H)-dione (6a-c) / 5-(bis((5-aryl-1,3,4-thiadiazol-2-yl)methylthio) methylene)pyrimidine-2,4,6(1H,3H,5H)-trione(7a-c) and 5-(bis((5-aryl-1,3,4-thiadiazol-2-yl)methylthio)-methylene)-dihydro-2-thioxopyrimidine-4,6(1H,5H)-dione (**8a–c**)*

To a well stirred solution of barbituric acid/thiobarbituric acid (1.28 g, 10 mmol) in dimethyl sulfoxide (5 mL), triethylamine (2.02 g, 20 mmol) and carbon disulfide (10 mmol) were added in succession. The mixture was stirred for 1 h at room temperature and then 1,3,4-oxadiazole/thiadiazole (3.90 g, 20 mmol) in dimethyl sulfoxide (5 mL) was added. The stirring was continued for 4 h at room temperature and poured into ice water (60 mL). The solid obtained was recrystallized from methanol to afford pure compound.

Table 3. Minimum inhibitory concentration of compounds **7c**, **8a**, and **8c**.

Compound	Minimum inhibitory concentration						
	MIC, μg/mL (mol/L)						
	S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*	*F. solani*	*C. lunata*	*A. niger*
7c	100	100	200	200	100	200	200
8a	50	50	100	100	50	50	100
8c	12.5	12.5	50	100	25	12.5	50
Chloramphenicol	6.25	6.25	6.25	12.5	–	–	–
Ketoconazole	–	–	–	–	12.5	6.25	6.25

5-(Bis((5-phenyl-1,3,4-oxadiazol-2-yl)methylthio)-methylene)pyrimidine-2,4,6(1H,3H,5H)-trione ***5a***

Pale yellow solid in 76% yield; m.p.: 145–147°C; IR (KBr) ν_{max} [cm^{-1}]: 3234 (NH), 1656 (*CO*NH), 1637 (C=N), 1624 (C=C); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 4.71 (s, 4H, S–CH_2), 7.30–7.52 (m, 10H, Ar-H), 11.25 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 38.9 (S-CH_2), 105.2 (*C*=C(S)S), 126.3, 127.3, 128.4, 129.6 (aromatic carbons), 151.2 (HN*CO*), 160.2 & 164.8 (C_2 & C_5), 163.7 (C=*CS*(S)), 165.9 (*CO*C=C). Anal. calcd. for $C_{23}H_{16}N_6O_5S_2$: C, 53.07; H, 3.10; N, 16.14. Found: C, 53.18; H, 3.15; N, 16.22.

5-(Bis((5-p-tolyl-1,3,4-oxadiazol-2-yl)-methylthio)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione ***5b***

Yellow solid in 64% yield; m.p.: 132–134°C; IR (KBr) ν_{max} [cm^{-1}]: 3226 (NH), 1663 (*CO*NH), 1635 (C=N), 1623 (C=C); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.36 (s, 6H, Ar-CH_3), 4.65 (s, 4H, S-CH_2), 7.15–7.35 (m, 8H, Ar-H), 11.11 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 24.5 (Ar-CH_3), 36.3 (S-CH_2), 105.7 (*C*=C-(S)S), 123.8, 127.2, 129.4, 137.6 (aromatic carbons), 150.3 (HN*CO*), 160.6 & 164.0 (C_2 & C_5), 164.3 (C=*CS*(S)), 166.2 (*CO*C=C). Anal. calcd. for $C_{25}H_{20}N_6O_5S_2$: C, 54.74; H, 3.67; N, 15.32. Found: C, 54.65; H, 3.73; N, 15.41.

5-(Bis((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-methylthio)methylene)-pyrimidine-2,4,6-(1H,3H,5H)-trione ***5c***

Light brown solid in 73% yield; m.p.: 169–171°C; IR (KBr) ν_{max} [cm^{-1}]: 3229 (NH), 1657 (*CO*NH), 1631 (C=N), 1620 (C=C); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 4.74 (s, 4H, S-CH_2), 7.26–7.45 (m, 8H, Ar-H), 11.86 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 37.5 (S-CH_2), 104.9 (*C*=C(S)S), 124.6, 127.3, 129.3, 133.6 (aromatic carbons), 150.8 (HN*CO*), 161.3 & 165.2 (C_2 & C_5), 161.9 (C=*CS*(S)), 167.3 (*CO*C=C). Anal. calcd. for $C_{23}H_{14}Cl_2N_6O_5S_2$: C, 46.87; H, 2.39; N, 14.26. Found: C, 46.95; H, 2.43; N,14.18.

5-(Bis((5-phenyl-1,3,4-oxadiazol-2-yl)-methylthio)methylene)-dihydro-2-thioxopyrimidine-4,6-(1H,5H)-dione ***6a***

Yellow solid in 70% yield; m.p.: 137–139°C; IR (KBr) ν_{max} [cm^{-1}]: 3238 (NH), 1655 (*CO*NH), 1639 (C=N), 1622 (C=C),1495 (C=S); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 4.68 (s, 4H, S-CH_2), 7.20–7.50 (m, 10H, Ar-H), 11.12 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 37.4 (S–CH_2), 106.2 (*C*=C(S)S), 126.5, 127.6, 128.3, 129.2 (aromatic carbons), 159.2 & 163.6 (C_2 & C_5), 164.6 (C=*CS*(S)), 165.2 (*CO*C=C), 174.3 (C=S). Anal. calcd. for $C_{23}H_{16}N_6O_4S_3$: C, 51.48; H, 3.01; N, 15.66. Found: C, 51.32; H, 3.04; N, 15.57.

5-(Bis((5-p-tolyl-1,3,4-oxadiazol-2-yl)-methylthio)methylene)-dihydro-2-thioxopyrimidine-4,6-(1H,5H)-dione ***6b***

White solid in 65% yield; m.p.: 146–148°C; IR (KBr) ν_{max} [cm^{-1}]: 3235 (NH), 1653 (*CO*NH), 1636 (C=N), 1620 (C=C),1493 (C=S); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.39 (s, 6H, Ar-CH_3), 4.69 (s, 4H, S-CH_2), 7.18–7.40 (m, 8H, Ar-H), 11.37 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 24.3 (Ar-CH_3), 39.2 (S-CH_2), 105.9 (*C*=C-(S)S), 124.1, 127.3, 129.2, 138.1 (aromatic carbons), 160.2 & 164.9 (C_2 & C_5), 165.1 (C=*CS*(S)), 166.7 (*CO*C=C), 172.8 (C=S). Anal. calcd. for $C_{25}H_{20}N_6O_4S_3$: C, 53.18; H, 3.57; N, 14.88. Found: C, 53.07; H, 3.49; N, 14.73.

5-(Bis((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-methylthio)methylene)-dihydro-2-thioxopyrimidine-4,6-(1H,5H)-dione ***6c***

White solid in 69% yield; m.p.: 165–167°C; IR (KBr) ν_{max} [cm^{-1}]: 3239 (NH), 1650 (*CO*NH), 1633 (C=N), 1619 (C=C),1491 (C=S); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 4.73 (s, 4H, S-CH_2), 7.30–7.45 (m, 8H, Ar-H), 11.57 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 39.7 (S-CH_2), 104.2 (*C*=C(S)S), 124.6, 128.0, 129.6, 134.1 (aromatic carbons), 160.2 & 165.0 (C_2 & C_5), 163.8 (C=*CS*(S)), 166.4 (*CO*C=C), 173.6 (C=S). Anal. calcd. for $C_{23}H_{14}Cl_2N_6O_4S_3$: C, 45.62; H, 2.33; N, 13.88. Found: C, 45.55; H, 2.38; N, 13.98.

5-(Bis((5-phenyl-1,3,4-thiadiazol-2-yl)-methylthio)methylene)pyrimidine-2,4,6-(1H,3H,5H)-trione ***7a***

White solid in 77% yield; m.p.: 162–164°C; IR (KBr) ν_{max} [cm^{-1}]: 3234 (NH), 1662 (*CO*NH), 1634 (C=N), 1626 (C=C); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 4.59 (s, 4H, S-CH_2), 7.21–7.50 (m, 10H, Ar-H), 11.32 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 38.2 (S-CH_2), 105.6 (*C*=C(S)S), 127.3, 128.3, 129.2, 133.1 (aromatic carbons), 151.4 (HN*CO*), 162.7 (C=*CS*(S)), 163.2 & 167.3 (C_2 & C_5), 164.8 (*CO*C=C). Anal. calcd. for $C_{23}H_{16}N_6O_3S_4$: C, 49.99; H, 2.92; N, 15.21. Found: C, 50.09; H, 2.86; N, 15.15.

5-(Bis((5-p-tolyl-1,3,4-thiadiazol-2-yl)-methylthio)methylene)pyrimidine-2,4,6-(1H,3H,5H)-trione ***7b***

Pale yellow solid in 69% yield; m.p.: 154–156°C; IR (KBr) ν_{max} [cm^{-1}]: 3231 (NH), 1658 (*CO*NH), 1632 (C=N), 1624 (C=C); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.41 (s, 6H, Ar-CH_3), 4.62 (s, 4H, S-CH_2), 7.19–7.38 (m, 8H, Ar-H), 11.20 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 24.6 (Ar-CH_3), 37.3 (S-CH_2), 105.9 (*C*=C-(S)S), 124.2, 127.6, 128.2, 136.9 (aromatic carbons), 151.4 (HN*CO*), 163.5 (C=*CS*(S)), 163.9 & 169.3 (C_2 & C_5), 165.4 (*CO*C=C). Anal. calcd. for $C_{25}H_{20}N_6O_3S_4$: C, 51.71; H, 3.47; N, 14.47. Found: C, 51.82; H, 3.54; N, 14.56.

5-(Bis((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-methylthio)methylene)-pyrimidine-2,4,6-(1H,3H,5H)-trione ***7c***

White solid in 75% yield; m.p.: 192–194°C; IR (KBr) ν_{max} [cm^{-1}]: 3227 (NH), 1655 (*CO*NH), 1630 (C=N), 1621 (C=C); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 4.64 (s, 4H, S-CH_2), 7.22–7.46 (m, 8H, Ar-H), 11.27 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 39.1 (S-CH_2), 105.3 (*C*=C(S)S), 125.1, 126.9, 130.2, 132.9 (aromatic carbons), 152.2 (HN*CO*), 160.8 (C=*CS*(S)), 164.1 & 168.9 (C_2 & C_5), 167.2 (*CO*C=C). Anal. calcd. for $C_{23}H_{14}Cl_2N_6O_3S_4$: C, 44.45; H, 2.27; N, 13.52. Found: C, 44.52; H, 2.19; N, 13.63.

5-(Bis((5-phenyl-1,3,4-thiadiazol-2-yl)-methylthio)methylene)-dihydro-2-thioxopyrimidine-4,6-(1H,5H)-dione ***8a***

White solid in 69% yield; m.p.: 175–177°C; IR (KBr) ν_{max} [cm^{-1}]: 3236 (NH), 1664 (*CO*NH), 1638 (C=N), 1629 (C=C), 1496 (C=S); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 4.70 (s, 4H, S-CH_2), 7.30–7.51 (m, 10H, Ar-H), 11.34 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 37.9 (S-CH_2), 106.4 (*C*=C(S)S), 126.9, 128.6, 129.8, 133.1 (aromatic carbons), 163.6 (C=*CS*(S)), 165.2 & 168.2 (C_2 & C_5), 166.9 (*CO*C=C), 174.3

(C=S). Anal. calcd. for $C_{23}H_{16}N_6O_2S_5$: C, 48.57; H, 2.84; N, 14.78. Found: C, 48.68; H, 2.87; N, 14.66.

5-(Bis((5-p-tolyl-1,3,4-thiadiazol-2-yl)-methylthio)methylene)-dihydro-2-thioxopyrimidine-4,6-(1H,5H)-dione ***8b***

Pale yellow solid in 65% yield; m.p.: 189–191°C; IR (KBr) ν_{max} [cm^{-1}]: 3232 (NH), 1661 (*CO*NH), 1634 (C=N), 1625 (C=C), 1494 (C=S); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.39 (s, 6H, Ar-CH_3), 4. 65 (s, 4H, S-CH_2), 7.10–7.42 (m, 8H, Ar-H), 11.52 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 23.9 (Ar-CH_3), 39.7 (S-CH_2), 105.3 (*C*=C-(S)S), 127.3, 129.3, 131.2, 136.1 (aromatic carbons), 164.2 (C=*C*S(S)), 166.3 & 169.9 (C_2 & C_5), 167.2 (*CO*C=C), 173.2 (C=S). Anal. calcd. for $C_{25}H_{20}N_6O_2S_5$: C, 50.32; H, 3.38; N, 14.08. Found: C, 50.21; H, 3.29; N, 14.15.

5-(Bis((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-methylthio)methylene)-dihydro-2-thioxopyrimidine-4,6-(1H,5H)-dione ***8c***

Pale yellow solid in 71% yield; m.p.: 202–204°C; IR (KBr) ν_{max} [cm^{-1}]: 3230 (NH), 1659 (*CO*NH), 1631 (C=N), 1622 (C=C), 1490 (C=S); ^{1}H-NMR (DMSO-d_6) δ [ppm]: 4.73 (s, 4H, S-CH_2), 7.29–7.45 (m, 8H, Ar-H), 11.43 (bs, 2H, NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 38.6 (S-CH_2), 104.9 (*C*=C(S)S), 127.8, 129.1, 130.6, 133.9 (aromatic carbons), 164.5 (C=*C*S(S)), 166.9 & 172.3 (C_2 & C_5), 167.8 (*CO*C=C), 174.7 (C=S). Anal. calcd. for $C_{23}H_{14}Cl_2N_6O_2S_5$: C, 43.33; H, 2.21; N, 11.12. Found: C, 43.25; H, 2.18; N, 11.20.

Biological assays

The compounds **5–8** were dissolved in DMSO at different concentrations of 100, 200, and 800 μg/mL.

Cells

Bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, and fungi *Fusarium solani*, *Curvularia lunata*, and *Aspergillus niger* were obtained from NCIM, Pune, India.

Antibacterial and antifungal assays

Preliminary antimicrobial activities of **5–8** compounds were tested by Agar disc-diffusion method [14]. 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 compounds assay 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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