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Short communication

Synthesis, characterization and antibacterial activity of some triazole Mannich bases carrying diphenylsulfone moieties

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ABSTRACT

A series of Mannich bases of 4-substituted 5-[4-(4-X-phenylsulfonyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones, X = H, Cl, Br, **3** and **5** were synthesized and characterized on the basis of IR, NMR and elemental analyses. The potential antibacterial effects of the synthesized compounds were investigated using the *Acinetobacter baumanii* ATCC 19606; *Citrobacter freundii* ATCC 8090; *Pseudomonas aeruginosa* ATCC 9027; *Enterococcus faecalis* ATCC 19433; *Staphylococcus aureus* ATCC 12600; *Staphylococcus epidermidis* ATCC 14990; *Bacillus subtilis* ATCC 6633 strains. Some of them exhibited promising activities against *A. baumanii* and *B. subtilis*.

1. Introduction

Literature survey revealed triazole derivatives which belong to an important group of heterocyclic compounds, that have been the subject of extensive study in the recent past. Diverse biological activities, such as antibacterial, antifungal, anti-inflammatory, antihypertensive and antiviral have been associated with 1,2,4-triazole derivatives [1–8].

Mannich reaction is a three-component condensation reaction involving an active hydrogen containing compound, formaldehyde and a secondary amine [9]. The aminomethylation of aromatic substrates by Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds [10].

In recent years, Mannich bases have gained importance due to their application in pharmaceutical chemistry. They have been found to possess antibacterial, antifungal, anticancer [11,12], antitubercular [13], analgesic and anti-inflammatory [14] properties. Further, diphenylsulfone derivatives were also found to possess antibacterial activity [15,16].

The incorporation of diphenylsulfone moiety into various heterocyclic systems was found to increase their biological activities. We have recently reported a few heterocyclic analogues carrying diphenylsulfone moiety as potent inhibitors of carbonic anhydrase isozymes I, II and IX [17].

Keeping this observation in view and in continuation of our work on the synthesis of biologically active nitrogen and sulphur containing heterocycles [18,19], this paper presents the synthesis of a series of Mannich bases of 4-substituted 5-[4-(4-X-phenylsulfonyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones and the study of their antibacterial activity.

2. Chemistry

The reaction sequences employed for synthesis of title compounds are shown in Scheme 1. The key intermediates, 5-[4-(4-X-phenylsulfonyl)phenyl]-4-*n*-propyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **3a**–**c**, X = H, Cl, Br and 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **5**, X = H, Cl were prepared from substituted benzoic acid hydrazides **1a**–**c** according to the literature [20,21].

Transformation of 4-amino-1,2,4-triazole **5** into Mannich bases is possible after the protection of the exocyclic amino group via condensation, because this nucleus has two susceptible position for aminomethylation: nitrogen N(2) endocyclic atom and NH_2 exocyclic group. The triazole **5** was condensed with various aromatic aldehydes in the presence of glacial acetic acid to afford

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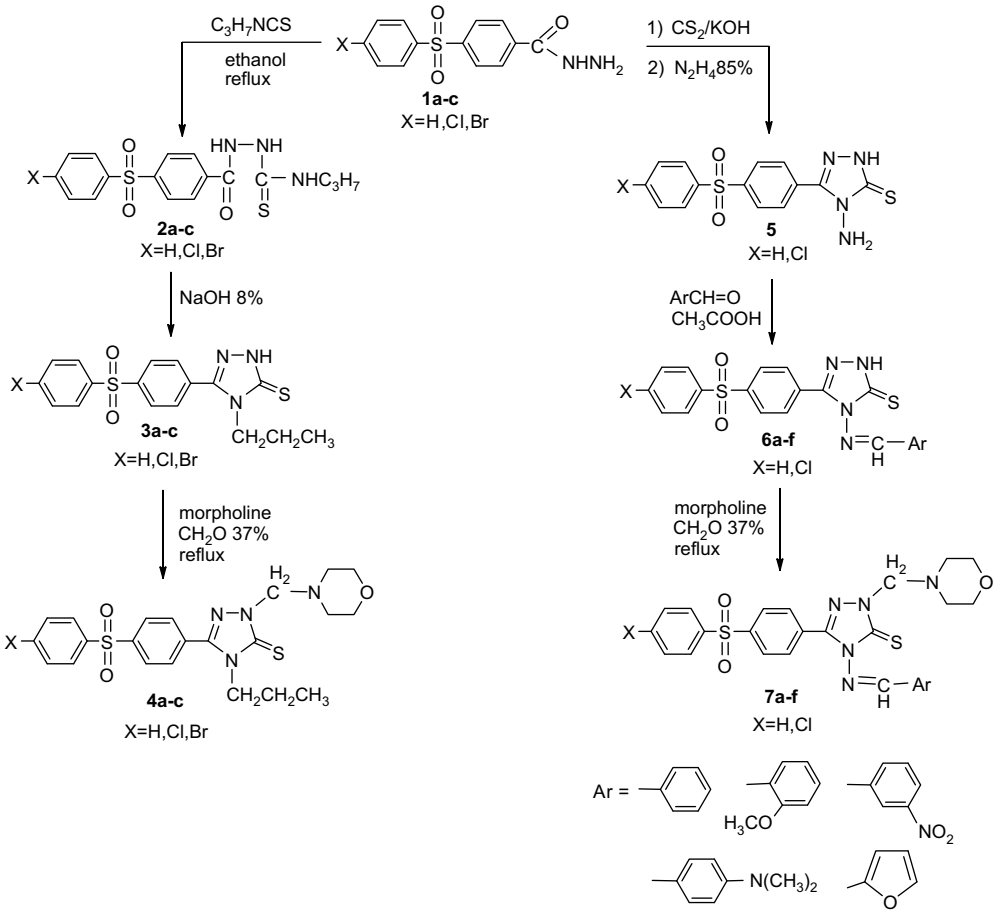


Scheme 1.

a series of 4-(substituted-arylidene)amino-5-[4-(4-X-phenylsulfonyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **6a**–**f**.

The title compounds, 4-arylideneamino-5-[4-(4-X-phenylsulfonyl)phenyl]-2-(morpholin-4-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **7a**–**f** were synthesized in one pot multi-component Mannich reaction involving **6a**–**f**, formaldehyde and morpholine in ethanol medium.

Aminomethylation products **4a**–**c** were synthesized through direct condensation of triazoles **3a**–**c** with morpholine and formaldehyde in methanol medium.

3. Biological activity

The synthesized compounds were tested for their in vitro antibacterial activity against the Gram-positive and the Gram-negative bacteria: *Acinetobacter baumanii* ATCC 19606; *Citrobacter freundii* ATCC 8090; *Pseudomonas aeruginosa* ATCC 9027; *Enterococcus faecalis* ATCC 19433; *Staphylococcus aureus* ATCC 12600; *Staphylococcus epidermidis* ATCC 14990; *Bacillus subtilis* ATCC 6633 using the paper disc diffusion method [22] (for the qualitative determination) and the serial dilutions in liquid broth method [23] for determination of MIC. Chloramphenicol was used as control drug.

4. Results and discussions

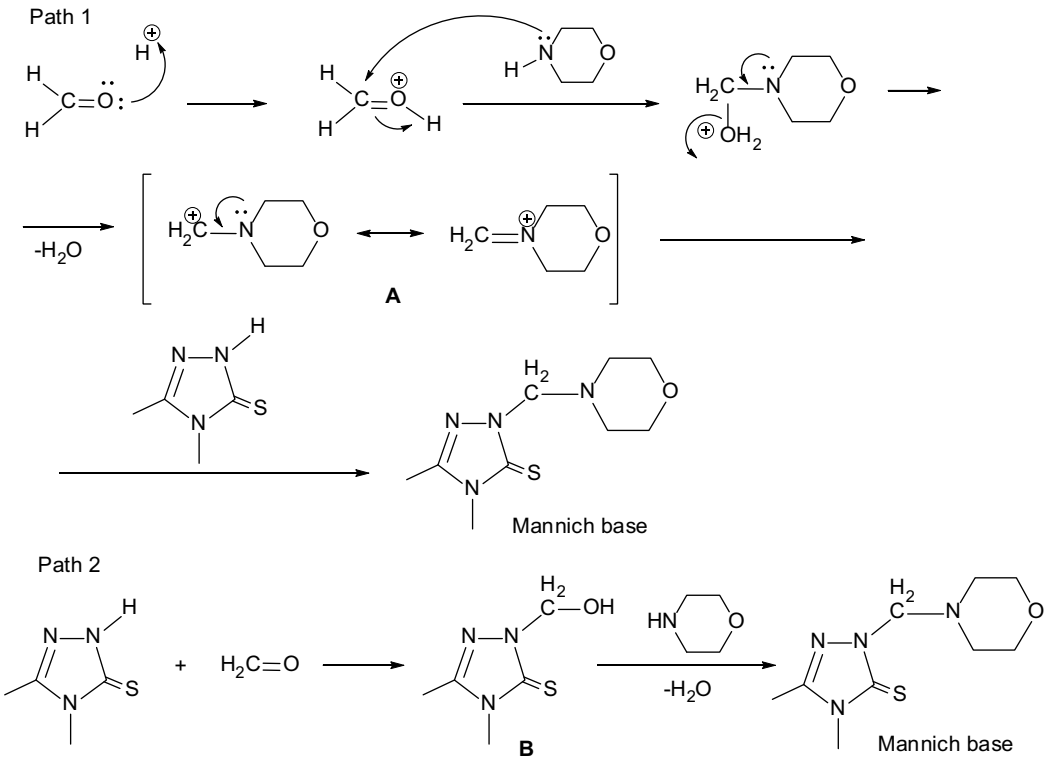
4.1. Chemistry

The Mannich reaction (Scheme 2) consist of the condensation of a substrate (3,4,5-trisubstituted-1,2,4-triazole) possessing at least one active hydrogen with formaldehyde and the secondary amine (morpholine). The condensation can occur using two pathways: first, the amine reacts with formaldehyde to give condensation product (**A**), which attacks the substrate, 3,4,5-trisubstituted-1,2,4-triazole. The reaction does not normally follow the other possible route (path 2); however, some successful reaction between hydroxymethyl derivatives (**B**) and alkylamines to give Mannich base can take place. If the nucleophilicity of the carbanion derived from the labile hydrogen compound is greater than that of amine, formation of a hydroxymethyl derivative (**B**) would be favoured over formation of derivative (**A**).

The structural assignments of new compounds were based on their elemental analysis and spectral (IR, ^{1}H NMR and ^{13}C NMR) data. The characterization data of all the new compounds are summarized in Table 1.

The IR spectrum of compounds **6a**–**f** showed an absorption band at 1600–1611 cm^{-1} indicating the presence of C=N in the ring. The absorption band observed at 3305–3285 cm^{-1} could be attributed to an NH group and indicated the easy thione–thiol tautomerism.

In the ^{1}H NMR spectra of Schiff bases **6a**–**f**, a singlet corresponding to one proton characteristic of the N=CH group was observed at $\delta = 9.21$–10.16 ppm. The AB coupling of the protons of the *para*-substituted phenyl ring resulted in the formation of the doublets at 8.02–8.10 ppm ($J = 8.1$–8.8 Hz) and 8.10–8.14 ppm ($J = 8.1$–8.8 Hz), respectively. The proton of the furan ring in **6e** resonated as two broad doublets at 8.06 ppm ($J = 1.8$ Hz) and 7.40 ppm ($J = 3.5$ Hz), respectively, and as one doublet of doublets at 6.78 ppm ($J = 3.5$; 1.8 Hz), integrating for three protons. The methyl protons of **6b** and **d** appeared as a singlet at $\delta = 3.87$ and 3.02 ppm, respectively.



Scheme 2.

The attributions of the signals ^{13}C NMR of **6a–f** resulted from the ^{2}D-HETCOR spectra. The N=CH signal appears at 153.43–159.44 ppm and the C(3) and C(5) heterocyclic carbon resonated at ~163 and 146–148 ppm, respectively.

In the IR spectra of Mannich bases **4a–c** and **7a–f**, the absorption band at 1247–1243 and 1222–1248 cm^{-1} could be attributed to the C=S functional group. The peaks at 2831–2978 cm^{-1} region were due to the presence of alkyl group ($CH_3^{as,s}$; $CH_2^{as,s}$). The absorption

Table 1
Characterization data of compounds **4a–c**, **6a–f**, **7a–f**

Compound	X	Ar	Molecular formula	Molecular mass	M.p. (°C)	Yield (%)	Elemental analysis found (calcd.)		
							C	H	N
4a	H	–	$C_{22}H_{26}N_4O_3S_2$	458.60	177–179	87	57.72 (57.62)	5.62 (5.71)	12.29 (12.22)
4b	Cl	–	$C_{22}H_{25}ClN_4O_3S_2$	493.04	126–128	87	53.68 (53.59)	5.05 (5.11)	11.44 11.36
4c	Br	–	$C_{22}H_{25}BrN_4O_3S_2$	537.50	125–126	79	49.27 (49.16)	4.62 (4.69)	10.50 (10.42)
6a	H	C_6H_5	$C_{21}H_{16}N_4O_2S_2$	420.51	209–210	67	59.90 (59.98)	3.96 (3.84)	13.26 (13.32)
6b	H	2-CH_3O–C_6H_4	$C_{22}H_{18}N_4O_3S_2$	450.53	262–263	75	58.59 (58.65)	4.12 (4.03)	12.36 (12.44)
6c	H	3-NO_2–C_6H_4	$C_{21}H_{15}N_5O_4S_2$	465.50	219–221	74	54.13 (54.18)	3.30 (3.25)	14.97 (15.04)
6d	H	4-$(CH_3)_2N$–C_6H_4	$C_{23}H_{21}N_5O_2S_2$	463.58	239–240	58	59.51 (59.59)	4.65 (4.57)	15.04 (15.11)
6e	H	Furyl	$C_{19}H_{14}N_4O_3S_2$	410.47	201–202	57	55.52 (55.60)	3.51 (3.44)	13.59 (13.65)
6f	Cl	C_6H_5	$C_{21}H_{15}ClN_4O_2S_2$	454.95	229–230	57	55.38 (55.44)	3.39 (3.32)	12.25 (12.31)
7a	H	C_6H_5	$C_{26}H_{25}N_5O_3S_2$	519.64	185–187	69	60.04 (60.10)	4.91 (4.85)	13.42 (13.48)
7b	H	2-CH_3O–C_6H_4	$C_{27}H_{27}N_5O_4S_2$	549.66	254–256	76	58.96 (59.00)	4.99 (4.95)	12.69 (12.74)
7c	H	3-NO_2–C_6H_4	$C_{26}H_{24}N_6O_5S_2$	564.64	231–233	78	55.26 (55.31)	4.35 (4.28)	14.82 (14.88)
7d	H	4-$(CH_3)_2N$–C_6H_4	$C_{28}H_{30}N_6O_3S_2$	562.71	248–250	76	59.70 (59.76)	5.41 (5.37)	14.88 (14.94)
7e	H	Furyl	$C_{24}H_{23}N_5O_4S_2$	509.60	226–227	78	56.50 (56.57)	4.60 (4.55)	13.69 (13.74)
7f	Cl	C_6H_5	$C_{26}H_{24}ClN_5O_3S_2$	544.08	218–220	64	56.30 (56.36)	4.42 (4.37)	12.59 (12.64)

bands corresponding to the nitro group in **7c** were seen at 1522 and 1345 cm^{-1}.

In the ^1H NMR spectra of compounds **4a–c** and **7a–f**, the >N–CH_2–N< protons resonated as singlet at ~5.15 and 5.05 ppm, respectively, integrating for two protons. The –CH_2–O–CH_2– protons of the morpholine residue appeared as a broad triplet at 3.68 ppm ($J = 4.8$ Hz) in **4a–c** and at 3.50 ppm ($J = 4.4$ Hz) in **7a–f**, while the –CH_2–N–CH_2– protons of the morpholine residue resonated as a triplet at 2.80 ppm ($J = 4.8$ Hz) and 2.63–2.70 ppm ($J = 4.3$ Hz), respectively. The propyl protons of the **4a–c** appeared as a triplet at $\delta = 4.04$–4.08 ppm ($J = 7.3$ Hz), a sextet at δ ~1.72 ppm ($J = 7.3$ Hz) and a triplet at $\delta = 0.84$ ppm ($J = 7.3$ Hz), integrating for seven protons.

The ^{13}C NMR spectrum of Mannich bases (see Section 6) showed signals at $\delta = 169$ and ~148 ppm due to heterocyclic C(3) and C(5) carbons; at $\delta = 69$ ppm due >N–CH_2–N< in **4a–c** and $\delta = 163$–165 ppm; 147–148 ppm and 73 ppm attributed to C(3), C(5) and >N–CH_2–N< in **7a–f**, respectively.

4.2. Antibacterial activity

The results of antibacterial screening of newly prepared compounds **4a–c**, **6a–f**, **7a–f** expressed as the MIC values, comparative with the starting triazoles **3a–c**, **5a**,**b** and chloramphenicol, are summarized in Table 2.

The (4-X-phenylsulfonyl)phenyl moiety in all these derivatives was not a very important factor for their antibacterial activity, since both compounds with X = H, or X = halogen showed similar activity. For the compounds incorporating propyl moiety in the 4-position of triazole ring, derivatives **3a–c**, the antibacterial activity was diminished as compared to the corresponding derivatives **5a**, **b** possessing the more compact amino group in this position.

The transformation of **3a–c** into Mannich bases **4a–c** did not exert any significant modification on the antibacterial activity of the original compounds **3a–c**.

Moreover, it is partly in contradiction with the purpose of Ghannoum and co-workers. considering that both NH_2 and SH groups should be free for antibacterial activity [24]. According to our studies, implication of the NH_2 group in the azomethine function (**6d**, **e**) increases the antibacterial activity: **6d** showed excellent activity against *B. subtilis* (MIC = 64 μg/mL) and **6e** showed good activity against *A. baumanii* and *P. aeruginosa* (MIC = 128 and 256 μg/mL respectively), comparative with triazole **5a** and chloramphenicol. In these cases the increased activity could also be attributed to the presence of $N(CH_3)_2$ group and furyl ring, respectively.

The compounds **7a–f** carrying a morpholino-methyl moiety exhibited varying degrees of inhibition against the tested microorganisms.

Table 2
Antibacterial activities of compounds **3**(**a–c**)–**7**(**a–f**) as MIC values (μg/mL)

Compound	X	Gram-negative bacteria[a]			Gram-positive bacteria[b]			
		Ab	*Cf*	*Pa*	*Ef*	*Sa*	*Se*	*Bs*
3a	H	>1024	>1024	>1024	>1024	>1024	>1024	>1024
3b	Cl	>1024	512	–	>1024	512	–	1024
3c	Br	1024	256	–	>1024	256	–	1024
4a	H	>1024	512	>1024	1024	–	>1024	>1024
4b	Cl	>1024	256	>1024	1024	–	–	>1024
4c	Br	>1024	256	>1024	512	–	–	>1024
5a	H	512	>1024	>1024	>1024	512	128	1024
5b	Cl	128	1024	–	–	1024	512	1024
6a	H	>1024	–	1024	–	512	>1024	–
6b	H	256	–	512	–	128	–	128
6c	H	1024	–	512	–	256	–	512
6d	H	512	–	>1024	–	1024	–	64
6e	H	128	–	256	–	512	–	–
6f	Cl	512	–	512	–	1024	1024	–
7a	H	>1024	>1024	>1024	1024	512	–	512
7b	H	512	–	>1024	>1024	512	–	512
7c	H	512	–	1024	1024	512	–	1024
7d	H	1024	–	>1024	>1024	1024	–	512
7e	H	512	>1024	512	512	512	–	1024
7f	Cl	1024	–	1024	512	1024	–	–
Control		**128**	**128**	**64**	**64**	**64**	**64**	**64**

Note: Control = chloramphenicol; – = no inhibition action.
[a] *Ab* (*Acinetobacter baumanii* ATCC 19606); *Cf* (*Citrobacter freundii* ATCC 8090); *Pa* (*Pseudomonas aeruginosa* ATCC 9027).
[b] *Ef* (*Enterococcus faecalis* ATCC 19433); *Sa* (*Staphylococcus aureus* ATCC 12600); *Se* (*Staphylococcus epidermidis* ATCC 14990); *Bs* (*Bacillus subtilis* ATCC 6633).

5. Conclusions

This study reports the successful synthesis and characterization of new Mannich and Schiff bases of 4-substituted 1,2,4-triazole-3-thiones bearing diphenylsulfone moiety. The antibacterial data given for the compounds presented in this paper allowed us to state that the (phenylsulfonyl)phenyl group bonded to a triazole ring is not a main cause for the appearance of antibacterial activity. Other incorporated fragments to the triazole nucleus could be responsible for the variation of the antibacterial activity. Other investigations are in progress for this class of heterocyclic compounds.

6. Experimental protocols

6.1. Chemistry

Melting points were determined with Boetius apparatus and are uncorrected. The IR spectra (in KBr pellets) were recorded on the Vertex 70 Bruker apparatus. The NMR spectra were registered on a Varian Gemini 300 BB apparatus working at 300 MHz for a ^1H and 75 MHz for ^{13}C using TMS as internal standard.

6.1.1. 5-[4-(4-X-phenylsulfonyl)phenyl]-2-(morpholin-4-ylmethyl)-4-(n-propyl)-2,4-dihydro-3H-1,2,4-triazol-3-thione **4a–c**

1,2,4-Triazole **4a–c** (5 mmol) dissolved in methanol (20 mL) were treated with morpholine (6 mmol) and then with 37% formaldehyde (10 mmol). The mixture was refluxed for 2 h. After cooling with ice, the precipitates were filtered and purified by recrystallization from ethanol.

*6.1.1.1. Compound **4a**.* IR (KBr, cm^{-1}): 3063, 3039 (aromatic C–H); 2978, 2931, 2873, 2834 (CH_2, CH_3); 1580, 1490, 1446 (C=N + $C=C_{aryl}$); 1315, 1282, 1154 (SO_2); 1247 (C=S); 1197 (N–CH_2–N); 1108 (CH_2–O–CH_2); 1009 (N–N); ^1H NMR ($CDCl_3$), δ: 7.72 (d, 2H, $J = 8.4$ Hz, aromatic protons); 8.09 (d, 2H, $J = 8.4$ Hz, aromatic protons); 7.97 (dd, 2H, $J = 8.4$; 1.5, aromatic protons); 7.54 (t, 2H, $J = 8.4$ Hz, aromatic protons); 7.61 (tt, 1H, $J = 8.4$; 1.5, aromatic proton); 4.04 (wt, 2H, $J = 7.3$ Hz, –CH_2–CH_2–CH_3); 1.72 (sx, 2H, $J = 7.3$ Hz, –CH_2–CH_2–CH_3); 0.84 (t, 3H, $J = 7.3$ Hz, –CH_2–CH_2–CH_3); 5.15 (s; 2H, N–CH_2–N); 2.80 (wt, 4H, $J = 4.7$ Hz, morpholine residue); 3.66 (wt, $J = 4.7$ Hz, morpholine residue); ^{13}C NMR ($CDCl_3$), δ: 169.48 (C=S), 148.54 (C5-triazolic ring), 144.00, 140.59, 133.21, 130.52, 129.89, 128.96, 128.87, 127.52, (aromatic ring), 47.12, 21.62, 10.41 (propyl group); 69.62 (N–CH_2–N), 66.76, 50.69 (morpholine residue).

*6.1.1.2. Compound **4b**.* IR (KBr, cm^{-1}): 3089 (aromatic C–H); 2952, 2935, 2857, 2831 (CH_2, CH_3); 1581, 1475, 1434 (C=N + $C=C_{aryl}$); 1321, 1288, 1163 (SO_2); 1243 (C=S); 1199 (N–CH_2–N); 1108 (CH_2–O–CH_2); 1006 (N–N); 762 (C–Cl); ^1H NMR ($CDCl_3$), δ: 7.72 (d, 2H, $J = 8.6$ Hz, aromatic protons); 8.09 (d, 2H, $J = 8.6$ Hz, aromatic protons); 7.92 (d, 2H, $J = 8.8$, aromatic protons); 7.51 (d, 2H,

$J = 8.8$ Hz, aromatic protons); 4.08 (wt, 2H, $J = 7.3$ Hz, –CH_2–CH_2–CH_3); 1.71 (sx, 2H, $J = 7.3$ Hz, –CH_2–CH_2–CH_3); 0.85 (t, 3H, $J = 7.3$ Hz, –CH_2–CH_2–CH_3); 5.16 (s; 2H, N–CH_2–N); 2.81 (wt, 4H, $J = 4.8$ Hz, morpholine residue); 3.68 (wt, 4H, $J = 4.8$ Hz, morpholine residue); ^{13}C NMR ($CDCl_3$), δ: 169.60 (C=S), 148.46 (C5-triazolic ring), 143.61, 140.59, 139.16, 130.86, 129.87, 129.46, 129.33, 128.44 (aromatic ring), 47.18, 21.68, 10.88 (propyl group); 69.70 (N–CH_2–N), 66.82, 50.74 (morpholine residue).

6.1.1.3. Compound ***4c***. IR (KBr, cm^{-1}): 3083 (aromatic C–H); 2952, 2936, 2857, 2831 (CH_2, CH_3); 1572, 1470, 1434 (C=N + C=C_{aryl}); 1322, 1289, 1162 (SO_2); 1243 (C=S); 1220 (N–CH_2–N); 1106 (CH_2–O–CH_2); 1007 (N–N); 573 (C–Br); ^{1}H NMR ($CDCl_3$), δ: 7.74 (d, 2H, $J = 8.7$ Hz, aromatic protons); 8.07 (d, 2H, $J = 8.7$ Hz, aromatic protons); 7.82 (d, 2H, $J = 8.6$, aromatic protons); 7.67 (d, 2H, $J = 8.6$ Hz, aromatic protons); 4.04 (wt, 2H, $J = 7.4$ Hz, –CH_2–CH_2–CH_3); 1.71 (sx, 2H, $J = 7.4$ Hz, –CH_2–CH_2–CH_3); 0.84 (t, 3H, $J = 7.4$ Hz, –CH_2–CH_2–CH_3); 5.15 (s; 2H, N–CH_2–N); 2.81 (wt, 4H, $J = 4.8$ Hz, morpholine residue); 3.68 (wt, 4H, $J = 4.8$ Hz, morpholine residue); ^{13}C NMR ($CDCl_3$), δ: 169.55 (C=S), 148.42 (C5-triazolic ring), 143.51, 139.65, 132.82, 130.83, 129.43, 129.32, 129.13, 128.39 (aromatic ring), 47.15, 21.65, 11.21 (propyl group); 69.65 (N–CH_2–N), 66.77, 50.70 (morpholine residue).

*6.1.2. 4-(Substituted-arylidene)amino-5-[4-(4-X-phenylsulfonyl)phenyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones* ***6a–f***

To the solution of 1,2,4-triazole **5** (10 mmol) in glacial acetic acid (15 mL) an equimolar amount of aromatic aldehyde was added. The obtained suspension was heated until a clear solution was obtained. Then the formed mixture was stirred at room temperature overnight. The precipitated solid obtained after the elimination of glacial acetic acid was washed with cold water and recrystallized from a mixture of ethanol and dioxane (2:1, v/v).

6.1.2.1. Compound ***6a***. IR (KBr, cm^{-1}): 3280, 2746 (NH), 3067 (aromatic C–H), 1602 (CH=N), 1573, 1539, 1499 (C=N + C=C_{aryl}), 1318, 1157 (SO_2), 1222 (C=S), 1016 (N–N); ^{1}H NMR (DMSO-d_6), δ: 8.02 (d, 2H, $J = 8.8$ Hz, aromatic protons); 8.17 (d, 2H, $J = 8.8$ Hz, aromatic protons); 7.96 (dd, 2H, $J = 8.4$; 1.5 Hz, aromatic protons), 7.48–7.63 (m, 6H, aromatic protons), 7.86 (dd, 2H, $J = 8.5$; 1.6 Hz, aromatic protons); 10.16 (s, 1H, CH=N); ^{13}C NMR (DMSO-d_6), δ: 163.40 (C=S), 147.21 (C5-triazole ring), 159.10 (CH=N), 142.61, 140.59, 133.24, 131.86, 131.82, 130.10, 129.12, 129.10, 128.86, 128.74, 128.52, 127.35 (aromatic rings).

6.1.2.2. Compound ***6b***. IR (KBr, cm^{-1}): 3285, 2743 (NH), 3065 (aromatic C–H), 2911, 2823 (CH_3), 1600 (CH=N), 1596, 1541, 1475 (C=N + C=C_{aryl}), 1292, 1155 (SO_2), 1257, 1021 (C_{aryl}–OCH_3), 1225 (C=S), 1012 (N–N); ^{1}H NMR (DMSO-d_6), δ: 8.07 (d, 2H, $J = 8.1$ Hz, aromatic protons); 8.13 (d, 2H, $J = 8.1$ Hz, aromatic protons); 7.96 (dd, 2H, $J = 8.1$; 1.4 Hz, aromatic protons), 7.60–7.70 (m, 5H, aromatic protons), 7.19 (wd, 1H, $J = 8.4$ Hz, aromatic proton); 7.07 (wt, 1H, $J = 8.4$ Hz, aromatic proton); 10.03 (s, 1H, CH=N), 3.87 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6), δ: 162.64 (C=S), 147.22 (C5-triazole ring), 159.44 (CH=N), 161.90, 142.49, 140.53, 134.83, 134.13, 130.26, 129.88, 129.45, 127.70, 127.53, 121.05, 119.92, 112.39 (aromatic rings), 56.06 (CH_3).

6.1.2.3. Compound ***6c***. IR (KBr, cm^{-1}): 3305, 2796 (NH), 3095 (aromatic C–H), 1611 (CH=N), 1476, 1430 (C=N + C=C_{aryl}), 1529, 1350 (NO_2), 1291, 1153 (SO_2), 1231 (C=S), 1025 (N–N); ^{1}H NMR (DMSO-d_6), δ: 8.09 (d, 2H, $J = 8.1$ Hz, aromatic protons); 8.10 (d, 2H, $J = 8.1$ Hz, aromatic protons); 7.98 (dd, 2H, $J = 7.9$; 1.5 Hz, aromatic protons), 7.62–7.70 (m, 3H, aromatic protons), 7.19 (wd, 1H, $J = 8.4$ Hz, aromatic proton); 7.07 (wt, 1H, $J = 8.4$ Hz, aromatic proton); 10.04 (s, 1H, CH=N), 8.83 (t, 1H, $J = 8.1$ Hz, aromatic proton), 8.63 (t, 1H, $J = 8.1$ Hz, aromatic proton), 8.33 (t, 1H, $J = 8.1$ Hz, aromatic proton), 8.27 (t, 1H, $J = 8.1$ Hz, aromatic proton); ^{13}C NMR (DMSO-d_6), δ: 162.81 (C=S), 148.34 (C5-triazole ring), 159.03 (CH=N), 147.32, 142.68, 140.65, 140.48, 134.51, 134.06, 130.98, 129.89, 129.55, 129.10, 127.80, 127.63, 123.19 (aromatic rings).

6.1.2.4. Compound ***6d***. IR (KBr, cm^{-1}): 3284 (NH), 3089 (aromatic C–H), 2993, 2810 (CH_3), 1603 (CH=N), 1586, 1528, 1474 (C=N + C=C_{aryl}), 1289, 1155 (SO_2), 1245 (C=S), 1007 (N–N); ^{1}H NMR (DMSO-d_6), δ: 8.09 (d, 2H, $J = 8.4$ Hz, aromatic protons); 8.14 (d, 2H, $J = 8.4$ Hz, aromatic protons), 7.98 (wd, 2H, $J = 7.8$ Hz, aromatic protons), 7.62–7.70 (m, 3H, aromatic protons), 9.21 (s, 1H, CH=N), 7.69 (d, 2H, $J = 8.9$ Hz, aromatic protons), 6.80 (d, 2H, $J = 8.9$ Hz, aromatic protons), 3.02 (s, 6H, CH_3); ^{13}C NMR (DMSO-d_6), δ: 162.91 (C=S), 146.76 (C5-triazole ring), 153.43 (CH=N), 146.75, 142.40, 140.55, 134.04, 130.72, 130.40, 129.90, 129.15, 127.75, 127.54, 118.31, 111.64, (aromatic rings), 25.26 (CH_3).

6.1.2.5. Compound ***6e***. IR (KBr, cm^{-1}): 3095 (aromatic C–H), 2632 (SH), 1608 (CH=N), 1539, 1472 (C=N + C=C_{aryl}), 1285, 1155 (SO_2), 1017 (N–N), 1104 (C–O–C furyl); ^{1}H NMR (DMSO-d_6), δ: 8.09 (s, 4H, aromatic protons); 8.00 (dd, 2H, $J = 7.1$; 1.7 Hz, aromatic protons); 7.63 (t, 2H, $J = 7.1$ Hz, aromatic protons), 7.71 (tt, 1H, $J = 7.1$; 1.7, aromatic proton), 9.61 (s, 1H, CH=N), 8.06 (wd, 1H, $J = 1.8$ Hz, aromatic proton), 6.78 (dd, 1H, $J = 3.5$, 1.8 Hz, aromatic proton), 7.40 (wd, 1H, $J = 3.5$ Hz, aromatic proton); ^{13}C NMR (DMSO-d_6), δ: 162.68 (C=S), 148.38 (C5-triazole ring), 154.46 (CH=N), 142.59, 140.51, 134.05, 130.13, 129.90, 129.45, 127.75, 127.57 (aromatic rings), 147.17, 146.62, 120.70, 113.15 (furyl ring).

6.1.2.6. Compound ***6f***. IR (KBr, cm^{-1}): 3289, 2740 (NH), 3099 (aromatic C–H), 1603 (CH=N), 1573, 1539, 1499 (C=N + C=C_{aryl}), 1318, 1157 (SO_2), 1222 (C=S), 1016 (N–N), 764 (C–Cl); ^{1}H NMR (DMSO-d_6), δ: 8.10 (d, 2H, $J = 8.8$ Hz, aromatic protons); 8.14 (d, 2H, $J = 8.8$ Hz, aromatic protons); 7.98 (d, 2H, $J = 8.7$ Hz, aromatic protons), 7.67 (d, 2H, $J = 8.7$ Hz, aromatic protons), 9.71 (s, 1H, CH=N); 7.88 (dd, 2H, $J = 7.8$, 1.1 Hz, aromatic protons); 7.55 (t, 2H, $J = 7.8$ Hz, aromatic protons); 7.63 (wt, 1H, $J = 7.8$ Hz, aromatic protons); ^{13}C NMR (DMSO-d_6), δ: 162.90 (C=S), 147.18 (C5-triazole ring), 153.77 (CH=N), 142.21, 139.38, 133.14, 131.86, 131.83, 130.40, 130.14, 129.64, 129.52, 129.38, 128.99, 127.95 (aromatic rings).

*6.1.3. 4-(Substituted-arylidene)amino-5-[4-(4-X-phenylsulfonyl)phenyl]-2-(morpholin-4-yl methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione* ***7a–f***

The Schiff bases **6a–c** (5 mmol) were dissolved in a mixture of ethanol and dioxane (2:1, v/v). Then formaldehyde (37%, 1.5 mL) and morpholine (5 mmol) in ethanol were added to this solution. The mixture was stirred for 3–4 h and kept overnight at room temperature. The separated solid was collected by filtration and recrystallized from a mixture of ethanol and dioxane (2:1, v/v) to yield the compounds **7a–f**.

6.1.3.1. Compound ***7a***. IR (KBr, cm^{-1}): 3089 (aromatic C–H), 2938, 2842 (CH_2, CH_3), 1605 (CH=N), 1583, 1499, 1477 (C=N + C=C_{aryl}), 1293, 1158 (SO_2), 1225 (C=S), 1175 (N–CH_2–N); 1088 (CH_2–O–CH_2); 1012 (N–N); ^{1}H NMR ($CDCl_3$), δ: 8.05 (d, 2H, $J = 8.5$ Hz, aromatic protons); 8.14 (d, 2H, $J = 8.5$ Hz, aromatic protons); 8.00 (dd, 2H, $J = 8.4$; 1.5 Hz, aromatic protons); 7.58–7.70 (m, 3H, aromatic protons); 8.09 (d, 2H, $J = 8.3$; 1.6 Hz, aromatic protons); 7.41 (t, 2H, $J = 8.3$ Hz, aromatic protons); 7.62 (t, 1H, $J = 8.3$ Hz, aromatic proton); 10.10 (s, 1H, CH=N); 5.04 (s, 2H, N–CH_2–N); 2.68 (t, 4H, $J = 4.5$ Hz, morpholine residue); 3.52 (t, $J = 4.5$ Hz, morpholine residue); ^{13}C NMR ($CDCl_3$), δ: 165.08 (C=S), 147.21 (C5-triazole

ring); 154.17 (CH=N); 142.56, 140.98, 134.94, 133.34, 131.78, 130.13, 129.60, 128.95, 128.52, 127.97, 127.65, 125.93 (aromatic rings); 73.74 (N–CH_2–N), 66.80, 50.70 (morpholine residue).

6.1.3.2. Compound ***7b***. IR (KBr, cm^{-1}): 3089 (aromatic C–H), 2939, 2857 (CH_2, CH_3), 1605 (CH=N), 1583, 1499, 1477 (C=N + C=C_{aryl}), 1328, 1158 (SO_2), 1228 (C=S), 1186 (N–CH_2–N); 1088 (CH_2–O–CH_2); 1012 (N–N); 1257, 1022 (C_{arom}–OCH_3); ^{1}H NMR ($CDCl_3$), δ: 8.08 (d, 2H, $J = 8.3$ Hz, aromatic protons); 8.14 (d, 2H, $J = 8.3$ Hz, aromatic protons); 7.95 (dd, 2H, $J = 8.1$; 1.4 Hz, aromatic protons); 7.50 (t, 2H, $J = 8.1$ Hz, aromatic protons); 7.57 (tt, 1H, $J = 8.1$; 1.4 Hz, aromatic proton); 8.10 (d, 1H, $J = 8.3$ Hz, aromatic proton); 7.53 (d, 1H, $J = 8.3$ Hz, aromatic proton); 7.11 (tt, 1H, $J = 8.3$; 1.1 Hz, aromatic protons); 6.90 (d, 1H, $J = 8.3$ Hz, aromatic proton); 10.08 (s, 1H, CH=N); 5.06 (s, 2H, N–CH_2–N); 2.65 (t, 4H, $J = 4.5$ Hz, morpholine residue); 3.50 (wt, 4H, $J = 4.5$ Hz, morpholine residue); 3.85 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$), δ: 164.10 (C=S), 147.42 (C5-triazole ring); 156.28 (CH=N); 159.41, 142.10, 140.53, 133.61, 133.14, 132.95, 130.01, 129.89, 129.45, 127.71, 127.58, 120.33, 118.31, 114.25 (aromatic rings); 73.50 (N–CH_2–N), 66.71, 50.78 (morpholine residue).

6.1.3.3. Compound ***7c***. IR (KBr, cm^{-1}): 3090 (aromatic C–H), 2939, 2848 (CH_2, CH_3), 1606 (CH=N), 1596, 1572, 1475 (C=N + C=C_{aryl}), 1291, 1157 (SO_2), 1230 (C=S), 1175 (N–CH_2–N); 1068 (CH_2–O–CH_2); 1009 (N–N); 1522, 1345 (NO_2); ^{1}H NMR ($CDCl_3$), δ: 8.09 (d, 2H, $J = 8.1$ Hz, aromatic protons); 8.12 (d, 2H, $J = 8.1$ Hz, aromatic protons); 7.96 (wd, 2H, $J = 7.9$ Hz, aromatic protons); 7.62–7.70 (m, 3H, aromatic protons); 8.80 (t, 1H, $J = 8.1$ Hz, aromatic proton); 8.65 (d, 1H, $J = 8.1$ Hz, aromatic proton); 8.45 (d, 1H, $J = 8.1$ Hz, aromatic proton); 7.95 (t, 1H, $J = 8.1$ Hz, aromatic proton); 10.05 (s, 1H, CH=N); 5.06 (s, 2H, N–CH_2–N); 2.63 (t, 4H, $J = 4.4$ Hz, morpholine residue); 3.52 (wt, 4H, $J = 4.4$ Hz, morpholine residue); ^{13}C NMR ($CDCl_3$), δ: 163.86 (C=S), 148.19 (C5-triazole ring); 158.17 (CH=N); 147.80, 142.08, 140.12, 138.48, 134.10,131.46, 130.23, 129.90, 129.75, 129.65, 127.75, 127.60, 125.53, 121.79 (aromatic rings); 73.98 (N–CH_2–N), 66.48, 50.82 (morpholine residue).

6.1.3.4. Compound ***7d***. IR (KBr, cm^{-1}): 3089 (aromatic C–H), 2935, 2853 (CH_2, CH_3), 1603 (CH=N), 1596, 1526, 1477 (C=N + C=C_{aryl}), 1292, 1159 (SO_2), 1248 (C=S), 1179 (N–CH_2–N); 1088 (CH_2–O–CH_2); 1012 (N–N); 1257, 1022 (C_{arom}–OCH_3); ^{1}H NMR ($CDCl_3$), δ: 8.10 (d, 2H, $J = 8.1$ Hz, aromatic protons); 8.18 (d, 2H, $J = 8.1$ Hz, aromatic protons); 7.98 (wd, 2H, $J = 7.8$ Hz, aromatic proton); 7.48 (t, 2H, $J = 7.8$ Hz, aromatic protons); 7.68 (tt, 1H, $J = 7.8$ Hz, aromatic proton); 7.80 (d, 2H, $J = 8.6$ Hz, aromatic proton); 6.74 (d, 2H, $J = 8.6$ Hz, aromatic proton); 9.50 (s, 1H, CH=N); 5.06 (s, 2H, N–CH_2–N); 2.64 (t, 4H, $J = 4.3$ Hz, morpholine residue); 3.50 (t, 4H, $J = 4.3$ Hz, morpholine residue); 2.90 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$), δ: 164.85 (C=S), 147.09 (C5-triazole ring); 153.48 (CH=N); 151.40, 141.98, 140.48, 133.58, 130.86, 130.05, 129.85, 129.45, 127.53, 127.28, 119.28, 112.07 (aromatic rings); 73.64 (N–CH_2–N), 66.37, 50.80 (morpholine residue); 35.38 (CH_3).

6.1.3.5. Compound ***7e***. IR (KBr, cm^{-1}): 3093 (aromatic C–H), 2940, 2860 (CH_2, CH_3), 1610 (CH=N), 1584, 1503 (C=N + C=C_{aryl}), 1293, 1159 (SO_2), 1232 (C=S), 1175 (N–CH_2–N); 1071 (CH_2–O–CH_2); 1012 (N–N); ^{1}H NMR ($CDCl_3$), δ: 8.06 (s, 4H, aromatic protons); 8.01 (dd, 2H, $J = 7.9$; 1.5 Hz, aromatic protons); 7.58 (t, 2H, $J = 7.9$ Hz, aromatic protons); 7.72 (tt, 1H, $J = 7.9$; 1.5 Hz, aromatic proton); 6.80 (d, 1H, $J = 1.4$ Hz, furyl proton); 6.54 (t, 1H, $J = 1.4$ Hz, furyl proton); 7.84 (wd, 1H, $J = 1.4$ Hz, furyl proton); 9.78 (s, 1H, CH=N); 5.07 (s, 2H, N–CH_2–N); 2.64 (t, 4H, $J = 4.4$ Hz, morpholine residue); 3.51 (t, 4H, $J = 4.4$ Hz, morpholine residue); ^{13}C NMR ($CDCl_3$), δ: 164.14 (C=S), 147.47 (C5-triazole ring); 154.78 (CH=N); 142.76, 139.95, 134.05, 130.42, 129.87, 129.76, 127.88, 127.58 (aromatic rings); 146.16, 142.33, 120.51, 112.33 (furyl ring); 73.12 (N–CH_2–N), 66.78, 50.74 (morpholine residue).

6.1.3.6. Compound ***7f***. IR (KBr, cm^{-1}): 3099 (aromatic C–H), 2989, 2924, 2878, 2835 (CH_2, CH_3), 1603 (CH=N), 1573, 1539, 1499 (C=N + C=C_{aryl}), 1318, 1157 (SO_2), 1222 (C=S), 1186 (N–CH_2–N); 1071 (CH_2–O–CH_2); 1016 (N–N); ^{1}H NMR ($CDCl_3$), δ: 8.08 (d, 2H, $J = 8.5$ Hz, aromatic protons); 8.12 (d, 2H, $J = 8.5$ Hz, aromatic protons); 7.98 (d, 2H, $J = 8.1$ Hz, aromatic protons); 7.70 (d, 2H, $J = 8.1$ Hz, aromatic protons); 8.05 (dd, 2H, $J = 8.2$ Hz, aromatic protons); 7.58 (t, 2H, $J = 8.2$ Hz, aromatic protons); 7.62 (t, 1H, $J = 8.2$ Hz, aromatic proton); 10.10 (s, 1H, CH=N); 5.04 (s, 2H, N–CH_2–N); 2.69 (t, 4H, $J = 4.4$ Hz, morpholine residue); 3.52 (t, $J = 4.4$ Hz, morpholine residue); ^{13}C NMR ($CDCl_3$), δ: 165.86 (C=S), 148.09 (C5-triazole ring); 152.98 (CH=N); 142.50, 140.34, 139.60, 134.94, 132.04, 130.33, 129.68, 129.52, 128.99, 128.70, 127.97, 125.50 (aromatic rings); 73.65 (N–CH_2–N), 66.82, 50.68 (morpholine residue).

6.2. Antibacterial activity

Qualitative determination of antimicrobial activity was done using the disk diffusion method. Suspensions in sterile peptone water from 24 h cultures of microorganisms were adjusted to 0.5 McFarland. Muller–Hinton Petri dishes of 90 mm were inoculated using these suspensions. Paper disks (6 mm in diameter) containing 10 μL of the substance to be tested (at a concentration of 2048 μg/mL in DMSO) were placed in a circular pattern in each inoculated plate. Incubation of the plates was done at 37 °C for 18–24 h. Reading of the results was done by measuring the diameters of the inhibition zones generated by the tested substances using a ruler. Chloramphenicol was used as a reference substance.

Determination of MIC was done using the serial dilutions in liquid broth method. The materials used were 96-well plates, suspensions of microorganism (0.5 McFarland), Muller–Hinton broth (Merck), solutions of the substances to be tested (2048 μg/mL in DMSO). The following concentrations of the substances to be tested were obtained in the 96-well plates: 1024, 512, 256, 128, 64, 32, 16, 8, 4, 2 μg/mL. After incubation at 37 °C for 18–24 h, the MIC for each tested substance was determined by macroscopic observation of microbial growth. It corresponds to the well with the lowest concentration of the tested substance where microbial growth was clearly inhibited.

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