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Short communication

Synthesis, characterization and evaluation of antibacterial activity of some thiazolo[3,2-*b*][1,2,4]triazole incorporating diphenylsulfone moieties☆

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ABSTRACT

A series of thiazolo[3,2-*b*][1,2,4]triazole incorporating diphenylsulfone moieties were synthesized starting from 5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thioles **3a–c**, X = H, Cl, Br. Thus, alkylation of 1,2,4-triazoles **3** with phenacyl bromide or 4-bromophenacyl bromide afforded S-substituted 1,2,4-triazoles **4**, **5**. These new intermediates **4** and **5**, in the presence of H_2SO_4 (c), were cyclized to 2-[4-(4-X-phenylsulfonyl)phenyl]-6-(4-Y-phenyl)[1,3]thiazolo[3,2-*b*]-[1,2,4]-triazoles **6**, **7** (I) and not to isomeric thiazolo[2,3-*c*][1,2,4]-triazoles **6**, **7** (II). The newly synthesized compounds were characterized by IR, ^1H, ^{13}C NMR and elemental analysis. MS spectra confirmed the formation of thiazolo[3,2-*b*][1,2,4]triazole **6**, **7** (forms I) in detriment of [2,3-*c*] isomeric compounds (forms II). The potential antibacterial effects of the synthesized compounds were investigated using standard bacterial strains: *Acinetobacter baumannii* ATCC 19606, *Citrobacter freundii* ATCC 8090, *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 9027, *Enterococcus faecalis* ATCC 19433, *Staphylococcus aureus* ATCC 12600, *Staphylococcus epidermidis* ATCC 14990, *Bacillus cereus* ATCC 14579.

1. Introduction

Literature survey revealed that many compounds bearing five-membered rings such as triazoles and thiazoles show significant biological activity. Compounds containing 1,2,4-triazole nucleus exhibit antibacterial [1–3], antifungal [1,4,5], anti-tubercular [6], anti-inflammatory [7,8], activities. The thiazole nucleus is also present in various molecules with diverse pharmacological properties, such as antimicrobial [9–11], anti-inflammatory [12], antitumoral [13].

The thiazolo-triazoles are those compounds which contain in their structure two fused rings of thiazole and triazole. These condensed heterocyclic compounds can exist in both the isomer forms: thiazolo[3,2-*b*][1,2,4]triazole and thiazolo[2,3-*c*][1,2,4]triazole. Thiazolo[3,2-*b*][1,2,4]-triazoles possess a broad spectrum of biological activities: antimicrobial [14–16], analgesic, anti-inflammatory [17–20], antipyretic [20], anticancer [21], vasodilatory [22].

Further, diphenylsulfone derivatives were also found to possess antibacterial activity [23,24]. The incorporation of diphenylsulfone moiety into various heterocyclic systems was found to increase their pharmacological activity.

Keeping this observation in view and in continuation of our research on the synthesis of heterocyclic compounds containing nitrogen, sulfur and bicyclic systems with expected biological activity [25–27], this paper presents the synthesis of several new heterocyclic condensed compounds with bridgehead nitrogen from thiazolo[3,2-*b*][1,2,4]triazoles class which contain diphenylsulfone moiety 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*H*-1,2,4-triazole-3-thioles **3a–c** (X = H, Cl, Br), were prepared starting from 4-(4-X-phenylsulfonyl)-benzoic acid hydrazides **1a–c** according to literature [28]. Hydrazides **1a–c** were obtained according to the previously described method [29].

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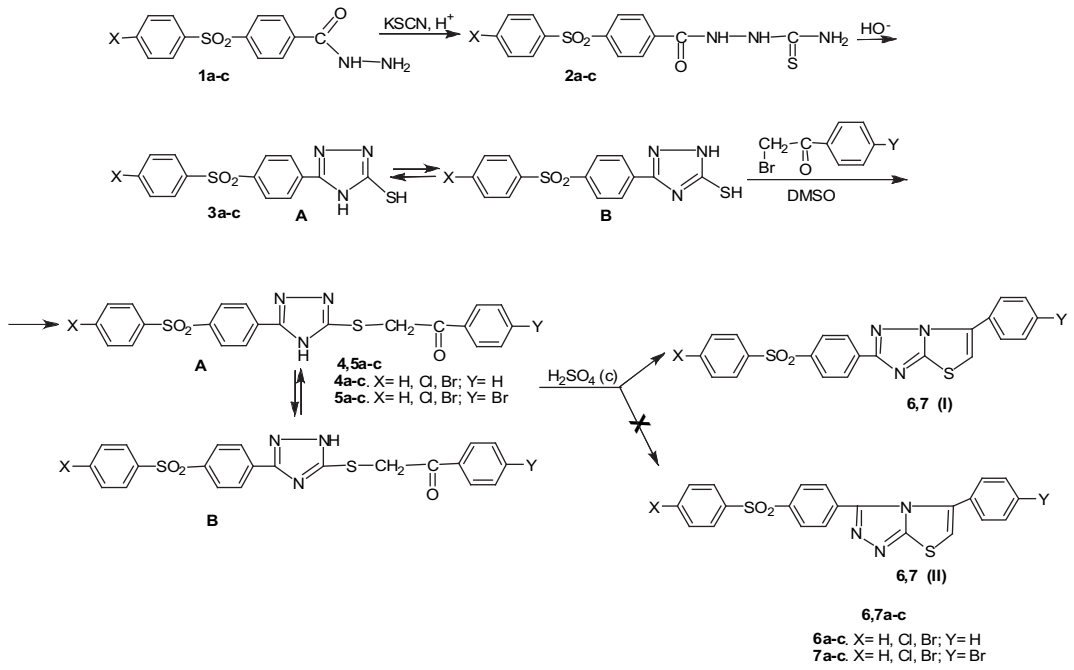


Scheme 1.

The alkylation of 1,2,4-triazoles **3** with phenacyl bromide or 4-bromophenacyl bromide, in the presence of dimethyl sulfoxide, afforded a new series of 5-[4-(4-X-phenylsulfonyl)phenyl]-3-phenacylthio-4*H*-1,2,4-triazole **4** and **5** with good yields (72–88.5%). By reaction of these S-alkylated 1,2,4-triazole derivatives **4**, **5** with sulfuric acid were obtained the thiazolo[3,2-*b*]-[1,2,4]triazole **6**, **7** (I) (with yields varying from 53% to 90%) and not thiazolo[2,3-*c*][1,2,4]triazole isomers **6**, **7** (II). Cyclization of S-substituted 1,2,4-triazoles **4**, **5** in acidic media may occur either at nitrogen N-2 (from intermediate B) or N-4 atom (from intermediate A). The N-2 atom is more likely involved in the cyclization reaction than N-4 atom because of its greater basicity [30,31]. Thus, previous studies indicate that if cyclization take place in acidic media (H_2SO_4, H_3PO_4, HCl, CH_3COOH) isomers [3,2-*b*] are obtained. However, if cyclization takes place in $POCl_3$ media, N-4 would be more basic owing to the formation of [2,3-*c*] isomers [15,19,20,30,31].

3. Biological activity

The synthesized compounds were tested for their in vitro antibacterial activity against the following standard Gram-positive and Gram-negative bacterial strains: *Acinetobacter baumannii* ATCC 19606; *Citrobacter freundii* ATCC 8090; *Escherichia coli* ATCC 11775; *Pseudomonas aeruginosa* ATCC 9027; *Enterococcus faecalis* ATCC 19433; *Staphylococcus aureus* ATCC 12600; *Staphylococcus epidermidis* ATCC 14990; *Bacillus cereus* ATCC 14579 using the paper disc diffusion method [32] (for the qualitative determination) and the serial dilutions in liquid broth method [33] for determination of MIC. Tetracycline and ampicillin were used as control drugs.

4. Results and discussions

4.1. Chemistry

The proposed mechanism of formation of thiazolo-triazoles **6** and **7** (Scheme 2) from S-alkylated 1,2,4-triazoles **4** and **5**, in acidic media, may be proceeded via the nucleophilic attack of nitrogen N-2 atom from intermediate B′ to carbonyl carbon protonated at oxygen atom with formation of intermediate C. By intramolecular dehydration reaction of intermediate C were obtained heterocyclic compounds from thiazolo[3,2-*b*][1,2,4]triazoles **6**, **7** class (Scheme 2).

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

The IR spectra of S-substituted 1,2,4-triazoles **4**, **5a–c** showed a strong band at 1681–1684 cm^{-1} characteristic for carbonyl group. The absorption band due to –NH group appeared at 3265–3304 cm^{-1}, while the –SH peak (2540–2565 cm^{-1}) [28] disappeared.

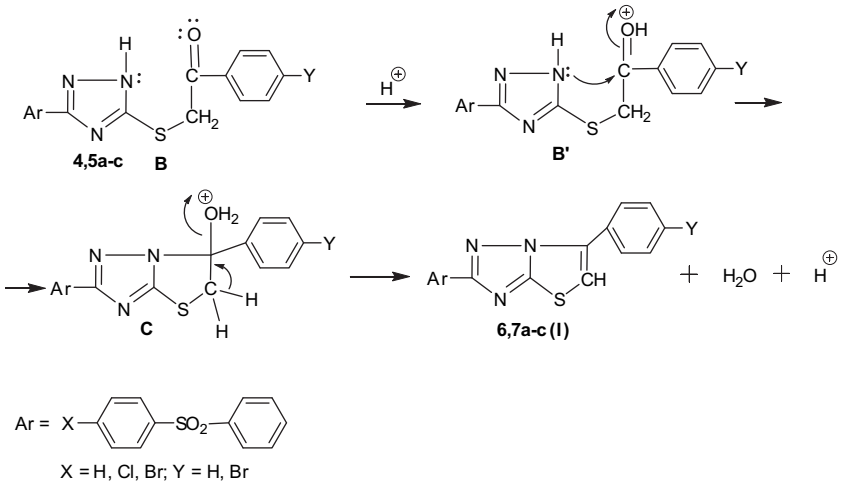
In the ^{1}H NMR spectra of the S-alkylated derivatives **4** and **5**, the signal corresponding to protons of the methylene group appeared as singlet at 4.81–4.95 ppm.

The attributions of the signals ^{13}C NMR of **4**–**7** resulted from the ^{2}D-HETCOR spectra. In compounds **4** and **5**, the C═O signal is observed at ~193 ppm and the $-CH_2-$ group resonated at ~39 ppm. The C(3) and C(5) heterocyclic carbon resonated at ~153 ppm and ~152 ppm, respectively.

Thiazolo[3,2-*b*][1,2,4]triazole **6**, **7a–c** show, in their IR spectra, the disappearance of NH and C═O vibration bands. The absorption band at 1600–1603 cm^{-1} is due to the presence of –C═N– stretch of the triazole and thiazole ring system.

In the ^{1}H NMR spectra of thiazolotriazole, methyne proton appears as a singlet at 7.96–8.06 ppm, in agreement with data reported for analogous compounds [15,20,34], while the signal of the methylene proton (4.81–4.95 ppm) from compounds **4**, **5** disappeared. The phenyl or *p*-phenylene protons from phenacyl or 4-bromophenacyl groups were seen at the expected chemical shifts and integral values (see Section 6).

The ^{13}C NMR spectrum of **6**, **7** displayed no signals belonging to C═O and $-CH_2$ groups; instead, new signal corresponding to methyne carbon (–S–CH═C<) was observed at 111.10–112.19 ppm. Moreover, new signals observed at 130.09–131.72 ppm and 163.59–164.11 ppm could be attributed to the heterocyclic carbon C(6) of



Scheme 2.

the thiazole ring system and to heterocyclic carbon which belongs to both triazole and thiazole ring nucleus, respectively.

The IR and NMR spectral data being very similar in isomers [2,3-*c*] and [3,2-*b*], the differentiation between them is not possible based on these. Presence of [3,2-*b*] or [2,3-*c*] isomer can be explained by mass spectra. Literature indicates that the presence of the signal deriving from the loss of a N_2 molecule $[M-28]^+$ is characteristic of thiazolo[2,3-*c*][1,2,4]triazole [20,35,36]. In the mass spectra of these new compounds the signal corresponding to N_2 less is not present which indicates the formation of thiazolo[3,2-b][1,2,4]triazole isomers **6,7 (I)**. Molecular ion $[M+H]^+$ peak of condensed heterocyclic compounds **6a**–**c** and **7a**–**c** was observed at different intensities in positive ionization mode and confirmed the molecular weights. All the compounds which have a halogen atom in their molecule show in their mass spectrum the characteristic peaks corresponding to isotopic distribution (^{35}Cl and ^{37}Cl or ^{7}Br and ^{81}Br isotopes).

4.2. Antibacterial activity

The results of antibacterial screening of newly prepared compounds **4a**–**c**, **5a**–**c**, **6a**–**c** and **7a**–**c** expressed as the MIC values, compared with the starting triazoles **3a**–**c**, and control (tetracycline and ampicillin), are summarized in Table 2.

In the studied series, the tested 3-phenacylthio-triazoles **4**, **5a**–**c** exhibited better activity against the used strains (MIC values: 64, 128, 256 μg/mL) compared to the "parent" triazole **3a**–**c** (MIC values: 256, 512, 1024 μg/mL). These results confirmed the data collected from literature [20,37–39] and our expectations according to which the presence of C=O group and another aromatic ring increase the potency of triazole nuclei.

The transformation into thiazolo-triazoles **6**, **7a**,**b** almost cancelled the results previously obtained, demonstrating that only the presence of diphenylsulfone moiety are the most important factor for their antibacterial activity and not the presence of

Table 1
Characterization data of compounds **4**–**7**.

Compd.	X	Y	Molecular formula	Molecular mass	M.p. (°C)	Yield (%)	Elemental analysis, found (calcd)			
							C	H	N	S
4a	H	H	$C_{22}H_{17}N_3O_3S_2$	435.52	209–211	79.5	60.73 (60.67)	3.86 (3.93)	9.61 (9.65)	14.70 (14.72)
4b	Cl	H	$C_{22}H_{16}ClN_3O_3S_2$	469.96	156–158	84	56.30 (56.22)	3.50 (3.43)	8.88 (8.94)	13.61 (13.65)
4c	Br	H	$C_{22}H_{16}BrN_3O_3S_2$	514.42	140–142	78	51.27 (51.37)	3.06 (3.14)	8.26 (8.17)	12.41 (12.47)
5a	H	Br	$C_{22}H_{16}BrN_3O_3S_2$	514.42	205–207	88.5	51.46 (51.37)	3.20 (3.14)	8.10 (8.17)	12.52 (12.47)
5b	Cl	Br	$C_{22}H_{15}BrClN_3O_3S_2$	548.86	99–101	77	48.07 (48.14)	2.71 (2.75)	7.61 (7.66)	11.64 (11.68)
5c	Br	Br	$C_{22}H_{15}Br_2N_3O_3S_2$	593.31	108–110	72	44.65 (44.54)	2.46 (2.55)	7.15 (7.08)	10.75 (10.81)
6a	H	H	$C_{22}H_{15}N_3O_2S_2$	417.51	224–226	84	63.36 (63.29)	3.56 (3.62)	10.00 (10.06)	15.31 (15.36)
6b	Cl	H	$C_{23}H_{14}ClN_3O_2S_2$	451.95	117–120	53	58.50 (58.47)	3.17 (3.12)	9.25 (9.30)	14.15 (14.19)
6c	Br	H	$C_{22}H_{14}BrN_3O_2S_2$	496.40	147–149	66	53.11 (53.23)	2.76 (2.84)	8.40 (8.46)	12.99 (12.92)
7a	H	Br	$C_{22}H_{14}BrN_3O_2S_2$	496.40	247–249	87	53.29 (53.23)	2.77 (2.84)	8.39 (8.46)	12.89 (12.92)
7b	Cl	Br	$C_{22}H_{13}BrClN_3O_2S_2$	530.84	253–255	90	49.83 (49.78)	2.41 (2.47)	7.85 (7.92)	12.04 (12.08)
7c	Br	Br	$C_{22}H_{13}Br_2N_3O_2S_2$	575.30	254–257	88	46.03 (45.93)	2.37 (2.28)	7.20 (7.30)	11.06 (11.15)

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

Compound	X	Y	Gram-negative bacteria[a]				Gram-positive bacteria[b]			
			Ab	*Cf*	*Ec*	*Pa*	*Ab*	*Cf*	*Pc*	*Ba*
3a	H	–	256	>1024	512	>1024	>1024	512	>1024	1024
4a	H	H	64	128	64	128	256	64	64	128
5a	H	Br	512	512	512	512	512	512	512	512
6a	H	H	64	128	64	128	512	128	64	512
7a	H	Br	64	128	64	128	256	128	64	128
3b	Cl	–	256	>1024	1024	1024	>1024	1024	1024	1024
4b	Cl	H	64	128	64	64	256	64	64	64
5b	Cl	Br	64	128	64	64	256	64	64	64
6b	Cl	H	512	>1024	1024	>1024	>1024	>1024	>1024	>1024
7b	Cl	Br	128	>1024	>1024	>1024	>1024	512	>1024	>1024
3c	Br	–	64	128	64	128	256	64	256	64
4c	Br	H	64	128	64	128	256	128	64	128
5c	Br	Br	64	128	64	128	128	128	64	256
6c	Br	H	64	128	64	128	256	128	64	256
7c	Br	Br	64	128	64	128	256	128	64	256
Control (TE)			**1.5**	**1**	**1.5**	**12**	**32**	**0.19**	**24**	**0.094**
Control (AM)			–	**2**	**2**	–	**0.5**	**0.5**	**0.064**	–

Control: TE = tetracycline; AM = ampicillin.
[a] *Ab* (*Acinetobacter baumannii* ATCC 19606); *Cf* (*Citrobacter freundii* ATCC 8090); *Ec* (*Escherichia coli* ATCC 11775); *Pa* (*Pseudomonas aeruginosa* ATCC 9027).
[b] *Ef* (*Enterococcus faecalis* ATCC 19433); *Sa* (*Staphylococcus aureus* ATCC 12600); *Se* (*Staphylococcus epidermidis* ATCC 14990); *Bc* (*Bacillus cereus* ATCC 14579).

heterocyclic condensed systems. These results are contrary to our expectations, but in concordance with other studies [15].

Moreover, similar MIC values obtained for **3c**, **4c**, **5c**, **6c** and **7c** demonstrated again that the presence of condensed systems is not the main cause of appearance for antibacterial activity. This behaviour could be attributed to the presence of bromine atom on diphenylsulfone moiety.

The investigations of antibacterial screening data revealed that all of the newly synthesized compounds exhibited poor activity compared to that of the control drugs. Because the MIC values are not spectacular, no statistical calculations were made.

5. Conclusions

This study reports the synthesis and characterization of new heterocyclic condensed systems with bridgehead nitrogen from thiazolo[3,2-*b*][1,2,4]triazole class bearing diphenylsulfone moiety. The title compounds were synthesized as new compounds with expected biological activity and their structures were confirmed successfully by spectral and elemental analyses. The antibacterial data given for the compounds presented in this paper allowed us to state that the diphenylsulfone moiety and halogen atom are in general the main cause for the appearance of their activity. The antibacterial assay against other Gram-negative and Gram-positive strains is in progress, because none of the presented compounds is effective against the tested micro-organisms in comparison with used drugs.

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 FTS-135 BioRad spectrometer and the wave number were given in cm^{-1}. The NMR spectra were registered on a Varian Gemini 300 BB spectrometer working at 300 MHz for a ^1H and 75 MHz for ^{13}C in DMSO-d_6. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The mass spectra of compounds **6b** and **7b** have been acquired with a hybrid quadrupole-time of flight (QqTOF) high resolution mass spectrometer model API QStar Pulsar produced by Applied Biosystems/SCIEX. The instrument was operated in positive ion mode, using an atmospheric pressure pneumatically assisted electrospray ionization interface (ESI, AB model Turboionspray). The voltage of the mass spectrometer (MS) source was set at 5000 V. Molecular ions were detected in full scan over an adequate mass range. Stock solutions were prepared at 1 mg/ml in DMSO. The sample solution (2 μg/ml in water/methanol 1/1, v/v) was introduced in the MS interface by direct infusion, at a flow rate of 20 μl/min, with the help of the built-in Harvard syringe pump. The mass spectra of the compounds **6a**, **c** and **7a**, **c** were registered with a triple quadrupole mass spectrometer Varian 1200L/MS/MS coupled with a high performance liquid chromatograph with Varian ProStar 240 pump and a Varian ProStar 410 automatic injector. An atmospheric pressure chemical ionization interface (APCI) was used in order to obtain the ions. The liquid chromatography was performed on a Hypersil Gold (Thermo) column with pre-column, and the mobile phase was 30% water and 70% methanol.

7. Experimental

7.1. Chemistry

7.1.1. *5-[4-(4-X-phenylsulfonyl)phenyl]-3-(4-Y-phenacylthio)-4H-1,2,4-triazole* ***4***, ***5a–c***

1,2,4-Triazole **3a–c** (1 mmol) dissolved in dimethyl sulfoxide (40 mL) was treated with α-halogeno ketone (1 mmol) and then mixture was stirred at room temperature for 9 h. The reaction mixture was then poured into ice water. The precipitate obtained was filtered, washed with water and then with diethyl ether. The compound was purified by crystallization from ethanol.

Compound **4a**: IR (KBr, ν, cm^{-1}): 3265 (NH), 3095, 3068 (aromatic C–H); 2980, 2920 (aliphatic C–H); 1681 (C=O), 1599, 1449 (C=N + C=C_{aryl}); 1322, 1290, 1158 (SO_2); 1015 (N–N); ^1H NMR (DMSO-d_6, δ ppm): 7.95–8.17 (m, 8H; aromatic protons); 7.53–7.72 (m; 3H; aromatic protons); 4.95 (ws; 2H; –S–C$\underline{H}_2$–CO); ^{13}C NMR (DMSO-d_6, δ ppm): 192.81 (C=O); 153.50 (C3-triazolic ring); 152.85 (C5-triazolic ring); 141.40, 140.40, 135.40, 133.87, 133.68, 129.83, 128.82, 128.39, 127.39, 127.30, 126.92, 126.88 (aromatic ring); 39.77 (–S–$\underline{C}H_2$–CO).

Compound **4b**: IR (KBr, ν, cm^{-1}): 3265 (NH); 3093 (aromatic C–H); 2987, 2921 (aliphatic C–H); 1681 (C=O); 1599, 1449 (C=N + C=C_{aryl}); 1324, 1287, 1158 (SO_2); 1013 (N–N); 767 (C–Cl); ^{1}H NMR (DMSO-d_6, δ ppm): 7.96–8.16 (m, 10H, aromatic protons); 7.70 (wt; 2H; J = 7.5 Hz, aromatic protons); 7.56 (tt; 1H; J = 7.5 Hz, aromatic proton); 4.95 (ws; 2H; –S–CH_2–CO); ^{13}C NMR (DMSO-d_6, δ ppm): 193.01 (C=O); 153.43 (C3-triazolic ring); 152.10 (C5-triazolic ring); 140.90, 139.95, 139.60, 135.52, 133.65, 129.95, 128.79, 128.36, 128.23, 128.15, 126.88 (aromatic ring); 39.75 (–S–CH_2–CO).

Compound **4c**: IR (KBr, ν, cm^{-1}): 3273 (NH); 3088 (aromatic C–H); 2991, 2922 (aliphatic C–H); 1681 (C=O); 1599, 1449 (C=N + C=C_{aryl}); 1323, 1288, 1158 (SO_2); 1009 (N–N); 579 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 7.82–8.12 (m, 10H, aromatic protons); 7.56 (wt; 2H; J = 7.4 Hz, aromatic protons); 7.79 (wt; 1H; J = 7.4 Hz, aromatic proton); 4.83 (ws; 2H; –S–CH_2–CO); ^{13}C NMR (DMSO-d_6, δ ppm): 192.52 (C=O); 153.22 (C3-triazolic ring); 153.05 (C5-triazolic ring); 141.05, 140.10, 135.61, 133.65, 132.93, 129.41, 128.82, 128.38, 128.30, 127.79, 126.93, 126.75 (aromatic ring); 39.79 (–S–CH_2–CO).

Compound **5a**: IR (KBr, ν, cm^{-1}): 3304 (NH), 3095 (aromatic C–H); 2976, 2918 (aliphatic C–H); 1682 (C=O), 1584, 1548 (C=N + C=C_{aryl}); 1314, 1289, 1157 (SO_2); 1017 (N–N); 572 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 8.10 (d, 2H, J = 8.7 Hz, aromatic protons); 8.02 (d, 2H, J = 8.7 Hz, aromatic protons); 7.96 (dd, 2H, J = 7.0; 1.3 Hz, aromatic protons); 7.93 (d, 2H, J = 8.7 Hz, aromatic protons); 7.74 (d, 2H, J = 8.7 Hz, aromatic protons); 7.69 (tt, 1H, J = 7.0; 1.3 Hz, aromatic proton); 7.64 (t, 2H, J = 7.0 Hz, aromatic protons); 4.82 (s, 2H, –S–CH_2–CO); ^{13}C NMR (DMSO-d_6, δ ppm): 192.79 (C=O); 153.67 (C3-triazolic ring); 152.80 (C5-triazolic ring); 141.00, 140.87, 134.63, 133.33, 131.51, 129.97, 129.37, 127.80, 127.68, 127.27, 126.99, 126.62 (aromatic ring); 39.93 (–S–CH_2–CO).

Compound **5b**: IR (KBr, ν, cm^{-1}): 3281 (NH), 3091 (aromatic C–H); 2972, 2918 (aliphatic C–H); 1684 (C=O), 1584, 1448 (C=N + C=C_{aryl}); 1321, 1287, 1157 (SO_2); 1012 (N–N); 767 (C–Cl); 584 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 8.08 (d, 2H, J = 8.8 Hz, aromatic protons); 7.98 (d, 2H, J = 8.8 Hz, aromatic protons); 7.95 (d, 2H, J = 8.5 Hz, aromatic protons); 7.85 (d, 2H, J = 8.8 Hz, aromatic protons); 7.76 (d, 2H, J = 8.5 Hz, aromatic protons); 7.68 (d, 2H, J = 8.8 Hz, aromatic protons); 4.86 (s, 2H, –S–CH_2–CO); ^{13}C NMR (DMSO-d_6, δ ppm): 193.24 (C=O); 153.50 (C3-triazolic ring); 152.72 (C5-triazolic ring); 141.30, 139.84, 139.17, 134.84, 131.96, 130.43, 130.04, 128.23, 127.80, 127.11 (aromatic ring); 39.70 (–S–CH_2–CO).

Compound **5c**: IR (KBr, ν, cm^{-1}): 3265 (NH), 3088 (aromatic C–H); 2987, 2918 (aliphatic C–H); 1683 (C=O), 1582, 1449 (C=N + C=C_{aryl}); 1322, 1287, 1158 (SO_2); 1017 (N–N); 579 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 8.11 (d, 2H, J = 8.7 Hz, aromatic protons); 8.06 (d, 2H, J = 8.7 Hz, aromatic protons); 7.94 (d, 2H, J = 8, 7 Hz aromatic protons); 7.88 (d, 2H, J = 8.8, aromatic proton); 7.82 (d, 2H, J = 8.8 Hz, aromatic proton); 7.74 (d, 2H, J = 8.7 Hz, aromatic proton); 4.81 (s, 2H, –S–CH_2–CO); ^{13}C NMR (DMSO-d_6, δ ppm): 192.58 (C=O); 153.25 (C3-triazolic ring); 152.84 (C5-triazolic ring); 140.31, 139.89, 134.38, 132.61, 131.56, 130.06, 129.09, 127.88, 127.73, 127.43, 126.66 (aromatic ring); 39.65 (–S–CH_2–CO).

*7.1.2. 2-[4-(4-X-phenylsulfonyl)phenyl]-6-(4-Y-phenyl)[1,3]thiazolo[3,2-b][1,2,4]triazoles **6**, **7a–c***

Compound **4**, **5** (1 mmol) was stirred in 50 mL H_2SO_4 (c) at 0 °C for 3 h and then another 3 h at room temperature. The reaction mixture was poured into ice water and the precipitate obtained was filtered off, washed with water, and recrystallized from C_6H_6:C_2H_5OH (2:1, v:v).

Compound **6a**: IR (KBr, ν, cm^{-1}): 3061 (aromatic C–H); 1603, 1466 (C=N + C=C_{aryl}); 1321, 1283, 1158 (SO_2); 1013 (N–N); ^{1}H NMR (DMSO-d_6, δ ppm): 8.33 (d, 2H, J = 8.7 Hz, aromatic protons); 8.24 (dd, 2H, J = 8.1; 1.4 Hz, aromatic protons): 8.10 (d, 2H, J = 8.7 Hz, aromatic protons); 8.00 (m, 2H, aromatic protons); 7.97 (s, 1H, –S–CH=C<); 7.48–7.75 (m, 6H, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 163.93 (thiazolic and triazolic ring); 157.95 (C2-triazolic ring); 141.74, 140.86, 135.43, 133.87, 129.84, 128.96, 127.38, 127.33, 126.33, 126.08 (aromatic ring); 131.58 (C6-thiazolic ring); 111.60 (–S–CH=C<); MS (APCI) m/z: 418 $[M + H]^+$.

Compound **6b**: IR (KBr, ν, cm^{-1}): 3093 (aromatic C–H); 1603, 1464 (C=N + C=C_{aryl}); 1324, 1280, 1159 (SO_2); 1013 (N–N); 771 (C–Cl); ^{1}H NMR (DMSO-d_6, δ ppm): 8.35 (d, 2H, J = 8.5 Hz, aromatic protons); 8.24 (dd, 2H, J = 8.6; 1.6 Hz, aromatic protons); 8.12 (d, 2H, J = 8.5 Hz, aromatic protons); 8.02 (d, 2H, J = 8.7 Hz, aromatic protons); 7.99 (s, 1H, –S–CH=C<); 7.72 (d, 2H, J = 8.7 Hz, aromatic protons); 7.60 (t, 2H, J = 8.6 Hz, aromatic protons); 7.56 (tt, 1H, J = 8.6; 1.6 Hz, aromatic proton); ^{13}C NMR (DMSO-d_6, δ ppm): 164.01 (thiazolic and triazolic ring); 157.96 (C2-triazolic ring); 141.35, 139.80, 138.91, 135.69, 129.85, 129.63, 129.25, 128.85, 128.10, 127.38, 126.34 (aromatic ring); 131.72 (C6-thiazolic ring); 111.35 (–S–CH=C<); MS (ESI-QqTOF) m/z: 452.0678 $[M + H]^+$; m/z: 454.0649 $[M + H]^+$.

Compound **6c**: IR (KBr, ν, cm^{-1}): 3090 (aromatic C–H); 1603, 1464 (C=N + C=C_{aryl}); 1324, 1279, 1159 (SO_2); 1010 (N–N); 578 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 8.32 (d, 2H, J = 8.6 Hz, aromatic protons); 8.22 (dd, 2H, J = 8.2; 1.6 Hz, aromatic protons); 8.10 (d, 2H, J = 8.6 Hz, aromatic protons); 7.96 (s, 1H, –S–CH=C<); 7.92 (d, 2H, J = 8.8 Hz, aromatic protons); 7.84 (d, 2H, J = 8.8 Hz, aromatic protons); 7.43–7.61 (m, 3H, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 163.85 (thiazolic and triazolic ring); 157.94 (C2-triazolic ring); 141.18, 140.10, 135.62, 132.92, 129.39, 128.92, 128.23, 127.36, 126.30, 126.09 (aromatic ring); 131.56 (C6-thiazolic ring); 111.59 (–S–CH=C<); MS (APCI) m/z: 496 $[M + H]^+$; m/z: 498 $[M + H]^+$.

Compound **7a**: IR (KBr, ν, cm^{-1}): 3061 (aromatic C–H); 1603, 1464 (C=N + C=C_{aryl}); 1321, 1280, 1158 (SO_2); 1012 (N–N); 570 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 8.34 (d, 2H, J = 8.5 Hz, aromatic protons); 8.20 (d, 2H, J = 8.6 Hz, aromatic protons); 8.08 (d, 2H, J = 8.5 Hz, aromatic protons); 7.98 (s, 1H, –S–CH=C<); 7.82 (d, 2H, J = 8.6 Hz, aromatic protons); 7.68 (wt, 1H, J = 8.2 Hz, aromatic proton); 7.64 (t, 2H, J = 8.2 Hz, aromatic protons); 7.18 (m, 2H, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 163.59 (thiazolic and triazolic ring); 157.37 (C2-triazolic ring); 141.39, 140.48, 134.83, 133.03, 131.11, 129.05, 127.38, 127.29, 126.74, 122.39 (aromatic ring); 130.09 (C6-thiazolic ring); 111.46 (–S–CH=C<); MS (APCI) m/z: 418 $[M + H]^+$.

Compound **7b**: IR (KBr, ν, cm^{-1}): 3097 (aromatic C–H); 1600, 1464 (C=N + C=C_{aryl}); 1324, 1280, 1159 (SO_2); 1013 (N–N); 773 (C–Cl); 582 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 8.34 (d, 2H, J = 8.4 Hz, aromatic protons); 8.21 (d, 2H, J = 8.6 Hz, aromatic protons); 8.13 (d, 2H, J = 8.4 Hz, aromatic protons); 8.06 (s, 1H, –S–CH=C<); 8.02 (d, 2H, J = 8.7 Hz, aromatic protons); 7.79 (d, 2H, J = 8.6 Hz, aromatic protons); 7.75 (d, 2H, J = 8.7 Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 163.87 (thiazolic and triazolic ring); 157.84 (C2-triazolic ring); 141.23, 139.60, 138.77, 135.43, 131.75, 129.73, 129.12, 128.09, 127.99, 127.24, 126.55, 122.83 (aromatic ring); 130.45 (C6-thiazolic ring); 111.10 (–S–CH=C<); MS (ESI-QqTOF) m/z: 529.9841 $[M + H]^+$; m/z: 531.9817 $[M + H]^+$.

Compound **7c**: IR (KBr, ν, cm^{-1}): 3094 (aromatic C–H); 1602, 1464 (C=N + C=C_{aryl}); 1323, 1279, 1158 (SO_2); 1014 (N–N); 578 (C–Br); ^{1}H NMR (DMSO-d_6, δ ppm): 8.34 (d, 2H, J = 8.5 Hz, aromatic protons); 8.18 (d, 2H, J = 8.7 Hz, aromatic protons); 8.08 (d, 2H, J = 8.5 Hz, aromatic protons); 7.96 (s, 1H, –S–CH=C<); 7.90 (d, 2H, J = 8.7 Hz, aromatic protons); 7.82 (d, 2H, J = 8.7 Hz, aromatic proton); 7.75 (d, 2H, J = 8.7 Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ ppm): 164.11 (thiazolic and triazolic ring); 157.55 (C2-triazolic ring); 141.55, 140.31, 135.79, 132.99, 132.07, 129.44, 128.43, 128.20, 128.16, 127.63, 126.84,

123.19 (aromatic ring); 130.91 (C6-thiazolic ring); 112.19 (–S–CH=C<); MS (APCI) *m/z*: 652 $[M + DMSO + H]^+$; *m/z*: 654 $[M + DMSO + H]^+$; *m/z*: 656 $[M + DMSO + H]^+$; *m/z*: 658 $[M + DMSO + H]^+$ fragmentation by collision at 35 eV: *m/z*: 574 $[M + H]^+$; *m/z*: 576 $[M + H]^+$; *m/z*: 578 $[M + H]^+$; *m/z*: 580 $[M + H]^+$.

7.2. Antibacterial activity

Qualitative determination of antimicrobial activity was done using the disk diffusion method. Suspensions in sterile peptone water from 24 h cultures of micro-organisms 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. Tetracycline and ampicillin were used as reference substances.

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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