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Short communication

Synthesis and antimicrobial evaluation of some fused heterocyclic [1,2,4]triazolo [3,4-b][1,3,4]thiadiazole derivatives

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ABSTRACT

A series of fused 1,2,4-triazoles with diphenylsulfone moiety are prepared utilizing 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiol **1** (X = H, Br). The latter on reaction with aromatic isothiocyanate in DMF, aromatic acid in $POCl_3$ and CDI in dioxane gives five membered fused triazole derivatives **2a-c**, **3a-c**, **4a-g**, **5a-g** and **6a,b**. The structures of newly synthesized compounds were confirmed on the basis of their elemental analysis and spectral data results (IR, ^{1}H-and ^{13}C NMR). New synthesized compounds were screened for their antimicrobial activities. The preliminary results revealed that some of the compounds exhibited promising antimicrobial activities.

1. Introduction

In the past decades, the problem of multidrug resistant microorganisms has reached on alarming level around the world, and the synthesis of new anti-infective compounds has become an urgent need for the treatment of microbial infections. The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important agents, which mainly displaying antimicrobial activities [1–4]. Moreover, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. For example, a triazolo-thiadiazole system may be viewed as a cyclic analog of two very important components: thiosemicarbazide and biguanide [5], which often display antimicrobial, anticancer, antitubercular, antiinflammatory, analgesic or anticonvulsant activities [6–11]. For this reason and in continuation to our efforts directed toward the synthesis of new heterocyclic compounds with anticipated biological activities, in this paper we proposed to synthesized a new series of this condensed system, which combine these two biolabile components (1,2,4-triazole and 1,3,4-thiadiazole) in a ring together to give a compact and planar structure and evaluated them for their antimicrobial profile after subtle structural modification.

2. Chemistry

The ambient nucleophilic centers present in 3-substituted-4-amino-5-mercapto-1,2,4-triazoles render them as useful synthons for the synthesis of various N-bridged heterocycles. In this paper, the key intermediates, 4-amino-5-[4-(4-X-phenylsulfonyl) phenyl]-4*H*-1,2,4-triazole-3-thiols **1** (X = H, Br) were prepared from corresponding substituted benzoic acid hydrazides according to literature [12].

The resulted triazoles **1** further converted to 3-[4-(4-X-phenylsulfonyl)phenyl]-6-N-(substituted-phenyl)amino-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazoles **2a-c**, **3a-c** by reacting with aryl isothiocyanates in the presence of DMF (Scheme 1).

Cyclocondensation of the SH and NH_2 functions of **1** with various substituted aromatic acids in the presence of phosphorus oxychloride afforded a series of 3-[4-(4-X-phenylsulfonyl)phenyl]-6-(substituted-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles **4a–g**, **5a–g**. The ring closure reaction with $POCl_3$ as the cyclization agent may have an esterification-addition-elimination mechanism (Scheme 2). Phosphorus oxychloride activates the carbonyl group

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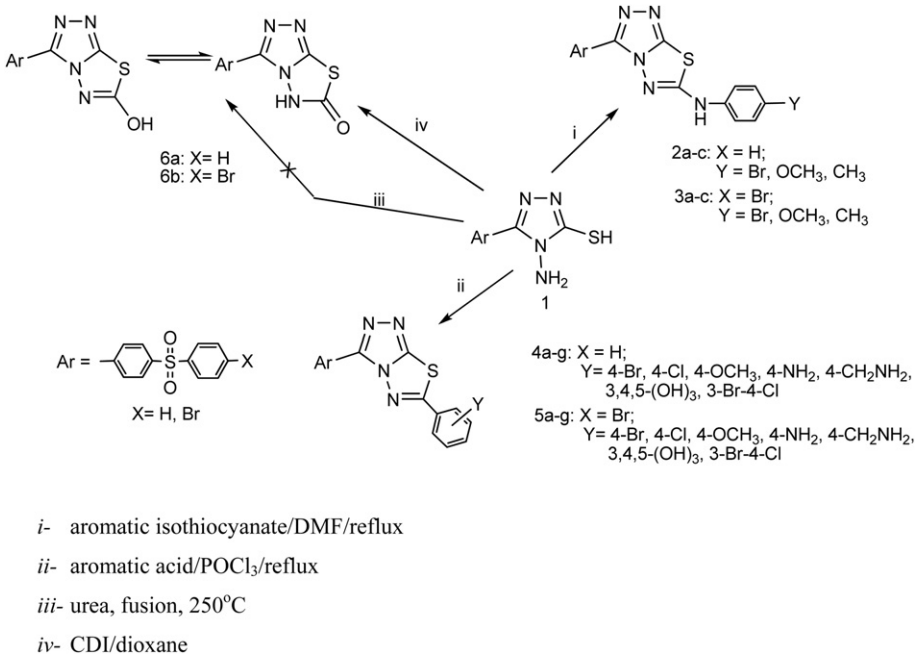


Scheme 1. Synthesis of the title compounds.

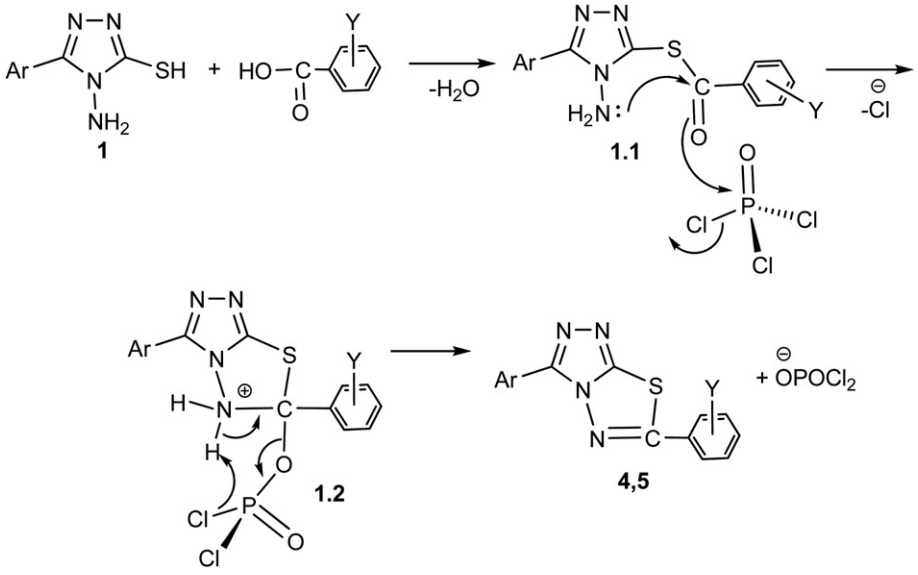
of intermediary **(1.1)** obtained by esterification of aromatic acid with thiol group of triazole **1**, which will be easier attacked by the unshared pair of electrons of the amino-triazole group nitrogen. Thus, an ionic intermediate **(1.2)** could be formed by the addition reaction of $POCl_3$ with the carbonyl group of compound (**1.1**), followed by removal of a chloride anion. Subsequently, after removal of a proton and the electron transfer, the leaving group $^{-}OPOCl_2$ comes off with the electron pair from the C–O bond and the reaction leading to the ring closure product **4/5**.

An attempt to transform the triazole **1** into oxo-analog **6** by fusion with urea at 250 °C for 3h was unsuccessful because the obtained reaction mixture was very complex and the components could not be separated and identified. However, compound **6** was obtained via the interaction of amino-triazole **1** with 1,1′-carbonyldiimidazole (CDI) in dry dioxane.

All new compounds reported here were characterized by elemental analysis, IR, ^{1}H-and ^{13}C NMR data. The physicochemical properties of synthesized compounds are presented in Table 1.

3. Antibacterial activity

The synthesized compounds were tested for their *in vitro* antimicrobial activity against the Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923; *Staphylococcus epidermidis* ATCC 14990; *Enterococcus faecalis* ATCC 29212; *Bacillus cereus* ATCC 13061), Gram-negative bacteria (*Escherichia coli* ATCC 25922; *Enterobacter cloacae* ATCC 49141; *Citrobacter freundii* ATCC 8090; *Acinetobacter baumannii* ATCC 19606; *Pseudomonas aeruginosa* ATCC 27853) and *Candida albicans* ATCC 90028 as fungus, by using the broth dilution



Scheme 2. The proposed mechanism of the reaction in presence of $POCl_3$.

Table 1
Characterization data of the synthesized compounds.

Comp.	X	Y	Molecular formula	Molecular weight (g/mol)	M.p. (°C)	Yield (%)	Elemental analysis[a] calc. (found)		
							C	H	N
2a	H	Br	$C_{21}H_{14}BrN_5O_2S_2$	512.4	217–219	58	49.22 (49.18)	2.75 (2.71)	13.67 (13.63)
2b	H	OCH_3	$C_{22}H_{17}N_5O_3S_2$	463.5	216–218	47	57.00 (56.96)	3.70 (3.65)	15.11 (15.09)
2c	H	CH_3	$C_{22}H_{17}N_5O_2S_2$	447.5	246–250	55	59.04 (59.01)	3.83 (3.79)	15.65 (15.62)
3a	Br	Br	$C_{21}H_{13}Br_2N_5O_2S_2$	591.3	232–234	45	42.66 (42.62)	2.22 (2.17)	11.84 (11.78)
3b	Br	OCH_3	$C_{22}H_{16}N_5O_3S_2$	542.4	201–204	51	48.71 (48.69)	2.97 (2.93)	12.91 (12.88)
3c	Br	CH_3	$C_{22}H_{16}BrN_5O_2S_2$	526.4	180–182	58	50.19 (50.16)	3.03 (3.03)	13.30 (13.28)
4a	H	4-Br	$C_{21}H_{13}BrN_4O_2S_2$	497.3	255–257	69	50.71 (50.67)	2.63 (2.59)	11.26 (11.22)
4b	H	4-Cl	$C_{21}H_{13}ClN_4O_2S_2$	452.9	173–175	65	55.69 (55.64)	2.89 (2.84)	12.37 (12.41)
4c	H	4-OCH_3	$C_{22}H_{16}N_4O_3S_2$	448.5	203–205	68	58.91 (58.89)	3.60 (3.57)	12.49 (12.47)
4d	H	4-NH_2	$C_{21}H_{15}N_5O_2S_3$	433.5	191–194	54	58.18 (58.15)	3.49 (3.52)	16.16 (16.12)
4e	H	4-CH_2NH_2	$C_{22}H_{17}N_5O_2S_2$	447.5	179–182	51	59.04 (59.01)	3.83 (3.79)	15.65 (15.62)
4f	H	3,4,5-$(OH)_3$	$C_{21}H_{14}N_4O_5S_2$	466.4	157–159	58	54.07 (54.04)	3.02 (2.98)	12.01 (12.05)
4g	H	3-Br,4-Cl	$C_{21}H_{12}BrClN_4O_2S_2$	531.8	147–150	48	47.43 (47.40)	2.27 (2.23)	10.53 (10.49)
5a	Br	4-Br	$C_{21}H_{12}Br_2N_4O_2S_2$	576.2	136–139	57	43.77 (43.74)	2.10 (2.04)	9.72 (9.75)
5b	Br	4-Cl	$C_{21}H_{12}BrClN_4O_2S_2$	531.8	172–175	53	47.43 (47.46)	2.27 (2.23)	10.53 (10.50)
5c	Br	4-OCH_3	$C_{22}H_{15}BrN_4O_3S_2$	527.4	187–189	78	50.10 (50.06)	2.87 (2.85)	10.62 (10.59)
5d	Br	4-NH_2	$C_{21}H_{14}BrN_5O_2S_2$	512.4	184–186	64	49.22 (49.19)	2.75 (2.73)	13.67 (13.64)
5e	Br	4-CH_2NH_2	$C_{22}H_{16}BrN_5O_2S_2$	526.4	242–243	66	50.19 (50.22)	3.06 (3.08)	15.18 (15.22)
5f	Br	3,4,5-$(OH)_3$	$C_{21}H_{13}BrN_4O_5S_2$	545.4	212–214	69	46.25 (46.22)	2.40 (2.37)	14.65 (14.60)
5g	Br	3-Br, 4-Cl	$C_{21}H_{11}Br_2ClN_4O_2S_2$	610.7	181–183	71	41.30 (41.27)	1.82 (1.80)	9.17 (9.14)
6a	H	–	$C_{15}H_{10}N_4O_3S_2$	358.4	183–185	70	50.27 (50.24)	2.81 (2.79)	15.63 (15.60)
6b	Br	–	$C_{15}H_9BrN_4O_3S_2$	437.3	198–200	68	41.20 (41.17)	2.07 (2.10)	12.81 (12.85)

[a] elemental analysis value limit = ±0.4% of the theoretical value.

method [13,14] for determination of MIC. Ampicillin, aztreonam and amphotericin were used as control drugs.

4. Results and discussions

4.1. Chemistry

The build up of N-bridged condensed heterocycles, **2a–c** and **3a–c** from **1**, is evidenced by its IR, ^{1}H NMR, ^{13}C NMR. Infrared spectra of these compounds were in accordance with our structural proposal: there is a broad band in 3108–3242 cm^{-1} region, assigned to NH group, new band appears in 1583–1620 cm^{-1} region, which is attributed to stretching frequency of N=C group formed by ring closure for all compounds and additional, for **2,3b,c**, asymmetric and symmetric stretching frequencies of CH_3 group appeared in 2858–2948 cm^{-1} zone. In addition, the ^{1}H NMR spectra contain a characteristic singlet signal around 11 ppm due to the N*H* proton and the new positive signal in 152–155 ppm region in ^{13}C NMR spectra corresponding to quaternary carbon of N=C group.

In the IR spectra of compounds **4,5 a–g,** the absence of absorption bands due to –SH (–C=S) and $-NH_2$ stretching frequencies of parent compounds **1** clearly indicated the fusing between compounds **1** and aromatic acid in the presence of phosphorus oxychloride. All the compounds show absorption peaks for N–N=C in the region of 1255–1269 cm^{-1} and for C–S–C, in the region of 683–711 cm^{-1}. The new band which appears in 1602–1620 cm^{-1} region is attributed to stretching frequency of N=C group of the thiadiazole ring. These data were very similar to previous reports [15–19]. The ^{1}H NMR spectra of compounds **4,5c** showed a singlet at 3.85–3.88 ppm integrating for three protons of the methyl group and for compounds **4,5e** displayed a singlet at 4.36–4.58 ppm corresponding to the methylene protons. The singlet signal observed at 8.57–8.68 ppm integrating for two protons was assigned to $-NH_2$ group. The protons of the some group in **4,5d** appear up field (6.3–6.36 ppm). The other signals of the protons present in the molecule appeared at 6.58–8.30 ppm as expected. As previous reports [20–22], the ^{13}C signals of triazole-C-3 and triazole-C-5 in newly synthesized compounds were observed around 160.48–166.91 ppm and 154.76–160.16 ppm, respectively, while ^{13}C signals derived from C-6 of triazolo-thiadiazole ring of compounds **4,5** were recorded at 150.32–158.81 ppm. The other signals present in ^{13}C NMR spectra of compounds **4,5** were recorded at the expected chemical shifts. Moreover, elemental analyses are consistent with the structures proposed for compounds **4,5** (Table 1).

Analysis of spectral data for compounds **6**, which were obtained via the interaction of amino-triazole **1** with CDI, leads to the conclusion that these compounds can exist in two tautomeric forms: lactame form and lactime form. In the solid state, the stable tautomeric state is lactim form. The IR spectra, recorded in KBr pellets show a broad band at 3398 cm^{-1} (for **6a**) and 3405 cm^{-1} (for **6b**), which can be attributed to OH group and the new stretching frequencies of N=C group formed by thiadiazole ring closure are observed at 1260 cm^{-1} in compound **6b** and 1263 cm^{-1} in compound **6a**. Most important is that there is no band in the wavenumber range 1670–1780 cm^{-1}, where usually carbonyls give a strong peak. The predominant tautomer of **6** was identified as lactame form by means of NMR spectroscopy. Similar to previous reports [23,24], the singlet signal at ~ 9 ppm in the ^{1}H NMR can be attributed to the N*H*-thiadiazolic proton, and the peaks at 175.26 ppm (for **6a**) and 177.63 ppm (for **6b**) in the ^{13}C NMR spectra can be assigned to carbon of carbonyl group in lactame form.

4.2. Antibacterial activity

The newly synthesized compounds were tested for *in vitro* antibacterial activities against five Gram-negative, four Gram-positive bacterial strains and one fungus. Ampicillin, aztreonam and amphotericin were used as reference drug molecules.

The data generated from this study (Table 2) showed that compounds displayed low to moderate activity. The obtained results can be attributed to quite bulky structure of the tested compounds, to the nature of the fragments attached in different positions to these molecules, but they may be associated with the nature of tested bacterial species.

Thus, we can see that none of the tested compounds has inhibitory action against *E. cloacae, C. freundii, A. baumannii, B. cereus, C. albicans*. We can also notice that the exerted action on

Table 2
Antimicrobial activities of the title compounds as MIC values (μg/mL).

Compd.	X	Y	Gram-positive bacteria[a]				Gram-negative bacteria[b]					Fungus
			Sa	**Se**	**Ef**	**Bc**	**Ec**	**Ebc**	**Cf**	**Ab**	**Pa**	**Ca**
2a	H	Br	1024	512	1024	1024	256	512	1024	512	512	1024
2b	H	OCH_3	1024	128	64	256	256	256	256	128	256	256
2c	H	CH_3	1024	512	512	1024	256	512	1024	128	512	256
3a	Br	Br	512	512	128	1024	128	512	1024	512	512	128
3b	Br	OCH_3	512	128	128	1024	256	256	256	128	256	128
3c	Br	CH_3	512	1024	512	1024	256	512	1024	1024	512	128
4a	H	4-Br	512	1024	1024	1024	512	512	1024	1024	512	1024
4b	H	4-Cl	256	512	512	1024	512	512	512	512	512	512
4c	H	4-OCH_3	256	512	512	512	512	512	512	512	256	512
4d	H	4-NH_2	512	64	128	256	256	256	256	128	256	256
4e	H	4-CH_2NH_2	512	512	512	1024	256	512	1024	1024	256	512
4f	H	3,4,5-$(OH)_3$	512	512	32	256	256	512	1024	1024	128	512
4g	H	3-Br,4-Cl	256	128	128	256	128	512	1024	256	128	512
5a	Br	4-Br	256	64	128	1024	256	256	128	256	256	128
5b	Br	4-Cl	128	128	64	256	128	512	512	256	512	1024
5c	Br	4-OCH_3	128	512	512	256	256	1024	512	256	512	512
5d	Br	4-NH_2	256	512	512	128	256	1024	512	1024	512	512
5e	Br	4-CH_2NH_2	512	1024	512	256	256	1024	1024	1024	128	512
5f	Br	3,4,5$(OH)_3$	256	128	64	256	128	256	128	128	128	512
5g	Br	3-Br,4-Cl	64	32	32	128	64	256	1024	512	32	256
6a	H	–	256	512	128	1024	256	256	512	128	512	256
6b	Br	–	256	128	64	1024	256	256	256	256	256	256
Ampicillin			< 2	< 2	< 2	< 2						
Aztreonam							< 2	< 2	< 2	< 2	4	
Amphotericin												2

[a] **Sa** (Staphylococcus aureus ATCC 25923); **Se** (Staphylococcus epidermidis ATCC 14990); **Ef** (Enterococcus faecalis ATCC 29212); **Bc** (Bacillus cereus ATCC 13061).
[b] **Ec** (*Escherichia coli* ATCC 25922); **Ebc** (Enterobacter cloacae ATCC 49141); **Cf** (Citrobacter freundii ATCC 8090); **Ab** (Acinetobacter baumannii ATCC 19606); **Pa** (Pseudomonas aeruginosa ATCC 27853).

Gram-positive bacteria *S. epidermidis* and *E. faecalis* is better than on Gram-negative strains.

Note that if the tested molecule has at least one halogen atom, antibacterial action is significantly better than in its absence. Thus, the best antibacterial effect has compound **5g** (MIC = 64 μg/mL against *S. aureus* and *E. coli*, MIC = 32 μg/mL against *S. epidermidis*, *E. faecalis* and *P. aeruginosa*) probably due to the cumulative electron-withdrawing effect of the chlorine and bromine atoms which are directly attached to the phenyl ring of the thiadiazole, in addition to the bromine atom attached to the diphenylsulfone moiety. Compounds **5a** and **5b** have also a good antibacterial action (MIC = 64 μg/mL against *S. epidermidis* and *E. faecalis*, respectively). In the serie of the derivatives with unsubstituted diphenylsulfone moiety, compounds **4d** and **4f** showed good activity against *S. epidermidis* (MIC = 64 μg/mL for **4d**) and *E. faecalis*, (MIC = 32 μg/mL for **4f**). This could possibly be due to the presence of the electron donor groups directly attached on condensed thiadiazole ring. The presence of CH_3, OCH_3, CH_2NH_2 to the phenyl ring of the thiadiazole was responsible for the decrease until disappearance of antibacterial activity. From these results it is clear that substituents affect the activity of compounds in different series. All the tested compounds, which are considered active, are less effective than drugs taken as a standard.

5. Conclusions

Novel 3-[4-(4-X-phenylsulfonyl)phenyl]-6-(substituted-phenyl)/6-N-(substituted-phenyl) amino-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles were prepared and screened for their antimicrobial activities. The antibacterial data given for the compounds presented in this paper allowed us to state that the variation of antimicrobial activity may be associated with the nature of tested microorganisms and is due to the chemical structure of the tested compounds. From the obtained results it is clear that substituents affect the activity of compounds in different series. Also, the presence of one or more halogen atom in the structure has considerable increased the biological activity of the molecules. The best antibacterial effect has 6-[(3-bromo-4-chloro)phenyl]-3-[4-(4-bromophenylsulfonyl) phenyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole 5g (MIC = 64 μg/mL against *S. aureus* and *E. coli*, MIC = 32 μg/mL against *S. epidermidis*, *E. faecalis* and *P. aeruginosa*).

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 (in DMSO-d_6, at room temperature) were registered on a Varian Gemini 300 BB apparatus working at 300 MHz for ^{1}H-and 75 MHz for ^{13}C, using TMS as internal standard. The content of C, H, and N were done with ECS-40-10-Costeh micro-dosimeter, after drying the compounds at 105 °C.

6.2. General procedure for synthesis of 3-[4-(4-X-phenylsulfonyl) phenyl]-6-N-(substituted-phenyl)amino-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles ***2a-c***, ***3a-c***

An equimolar mixture (1 mmol) of 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiol (X = H, Br) **1** and aryl isothiocyanate in dimethylformamide (10 mL) was refluxed for 20–22 h. The reaction mixture was cooled to room temperature and then gradually poured on to crushed ice with stirring. The mixture was allowed to stand overnight and the solid separated out was filtered, and washed thoroughly with cold water. The compound so obtained was dried and recrystallized from ethanol.

6.2.1. 3-[4-(phenylsulfonyl)phenyl]-6-N-(4-bromophenyl)amino-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***2a***

IR (KBr, cm^{-1}): 3194 (NH); 3088 (aromatic CH); 1588, 1554, 1471 (C═N + C═C_{aryl}); 1324, 1292, 1156 (SO_2); 1262 (N–N═C); 1011 (N–N); 689 (C–S–C); 575 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 11.04 (s, 1H, NH); 8.21 (d, 2H, *J* = 8.5 Hz, aromatic protons); 8.07 (d, 2H, *J* = 8.5 Hz, aromatic protons); 7.93 (t, 1H, *J* = 8.5 Hz, aromatic proton); 7.70 (d, 2H, *J* = 8.5 Hz; aromatic protons); 7-50-7.72 (m, 4H, aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 162.00 (C3-triazole ring); 156.47 (C5-triazole ring); 153.13 (C═N-thiadiazole ring); 143.03, 139.05, 138.96, 134.87, 116.72 (quaternary aromatic ring carbons); 130.41, 130.06, 129.88, 129.13, 128.57, 128.35, 119.57 (CH-aromatic ring carbons).

6.2.2. 3-[4-(phenylsulfonyl)phenyl]-6-N-(4-methoxyphenyl)amino-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***2b***

IR (KBr, cm^{-1}): 3208 (NH); 3061 (aromatic CH); 2933, 2841 (CH_3); 1618, 1575, 1554, 1501 (C═N + C═C_{aryl}); 1326, 1294, 1158 (SO_2); 1258 (N–N═C); 1009 (N–N); 691 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 10.34 (s, 1H, NH); 8.18 (d, 2H, *J* = 8.7 Hz, aromatic protons); 8.10 (d, 2H, *J* = 8.7 Hz, aromatic protons); 8.00 (dd, 2H, *J* = 8.1; 1.5 Hz, aromatic protons); 7.71 (t, 1H, *J* = 8.1 Hz, aromatic proton); 7.66 (d, 2H, *J* = 8.7 Hz; aromatic protons); 7.56 (t, 2H, *J* = 8.1 Hz, aromatic protons); 7.10 (d, 2H, *J* = 8.7 Hz; aromatic protons); 3.93 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 160.03 (C3-triazole ring); 154.76 (C5-triazole ring); 152.75 (C═N-thiadiazole ring); 153.31, 141.14, 139.03, 135.68, 132.81 (quaternary aromatic ring carbons); 133.68, 128.81, 128.74, 128.62, 128.34, 121.72, 115.18 (CH-aromatic ring carbons); 55.82 (OCH_3).

6.2.3. 3-[4-(phenylsulfonyl)phenyl]-6-N-(4-methylphenyl)amino-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***2c***

IR (KBr, cm^{-1}): 3216 (NH); 3085 (aromatic CH); 2948, 2917 (CH_3); 1614, 1578, 1504 (C═N + C═C_{aryl}); 1326, 1286, 1158 (SO_2); 1264 (N–N═C); 1005 (N–N); 695 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 10.85 (s, 1H, NH); 8.03 (d, 2H, *J* = 8.5 Hz, aromatic protons); 7.96 (d, 2H, *J* = 8.5 Hz, aromatic protons); 7.89 (d, 2H, *J* = 8.5 Hz, aromatic protons); 7.68 (d, 2H, *J* = 8.3 Hz, aromatic protons); 7.52–7.68 (m, 3H, aromatic protons); 2.38 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 159.87 (C3-triazole ring); 154.52 (C5-triazole ring); 153.68 (C═N-thiadiazole ring); 143.08, 140.41, 137.24, 136.84, 131.24 (quaternary aromatic ring carbons); 133.14, 129.61, 128.79, 128.14, 127.95, 127.88, 120.68 (CH-aromatic ring carbons); 20.45 (CH_3).

6.2.4. 3-[4-(4-bromophenylsulfonyl)phenyl]-6-N-(4-bromophenyl) amino-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole ***3a***

IR (KBr, cm^{-1}): 3108 (NH); 3095 (aromatic CH); 1583, 1534, 1473 (C═N + C═C_{aryl}); 1322, 1290, 1159 (SO_2); 1261 (N–N═C); 1012 (N–N); 693 (C–S–C); 574 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 11.14 (s, 1H, NH); 8.08 (d, 2H, *J* = 8.8 Hz, aromatic protons); 8.02 (d, 2H, *J* = 8.8 Hz, aromatic protons); 7.96 (d, 2H, *J* = 8.8 Hz, aromatic protons); 7.80 (d, 2H, *J* = 8.8 Hz, aromatic protons); 7.61 (d, 2H, *J* = 8.5 Hz; aromatic protons); 7.44 (d, 2H, *J* = 8.5 Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 159.90 (C3-triazole ring); 159.16 (C5-triazole ring); 154.31 (C═N-thiadiazole ring); 141.10, 141.00, 139.47, 129.40, 127.99, 114.63 (quaternary aromatic ring carbons); 133.60, 131.95, 130.20, 129.12, 127.58, 122.25 (CH-aromatic ring carbons).

6.2.5. 3-[4-(4-bromophenylsulfonyl)phenyl]-6-N-(4-methoxyphenyl)amino-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole ***3b***

IR (KBr, cm^{-1}): 3166 (NH); 3078 (aromatic CH); 2928, 2864 (CH_3); 1619, 1579, 1502 (C═N + C═C_{aryl}); 1328, 1292, 1156 (SO_2); 1261 (N–N═C); 1007 (N–N); 690 (C–S–C); 576 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 11.11 (s, 1H, NH); 8.00 (d, 2H, *J* = 8.7 Hz, aromatic protons); 7.97 (d, 2H, *J* = 8.7 Hz, aromatic protons); 7.93 (d, 2H, *J* = 8.7 Hz, aromatic protons); 7.86 (d, 2H, *J* = 8.7 Hz, aromatic proton); 7.83 (d, 2H, *J* = 8.8 Hz; aromatic protons); 7.72 (d, 2H, *J* = 8.8 Hz aromatic protons); 3.76 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 159.53 (C3-triazole ring); 158.74 (C5-triazole ring); 156.29 (C═N-thiadiazole ring); 155.21, 142.13, 139.82, 139.35, 129.70, 124.74 (quaternary aromatic ring carbons); 132.85, 129.28, 128.53, 128.25, 119.92, 115.53 (CH-aromatic ring carbons); 55.41 (OCH_3).

6.2.6. 3-[4-(4-bromophenylsulfonyl)phenyl]-6-N-(4-methylphenyl) amino-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole ***3c***

IR (KBr, cm^{-1}): 3242 (NH); 3089 (aromatic CH); 2920, 2858 (CH_3); 1620, 1582, 1514 (C═N + C═C_{aryl}); 1328, 1288, 1160 (SO_2); 1266 (N–N═C); 1008 (N–N); 694 (C–S–C); 577 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 11.18 (s, 1H, NH); 7.94 (d, 2H, *J* = 8.0 Hz, aromatic protons); 7.87 (d, 2H, *J* = 8.0 Hz, aromatic protons); 7.72 (d, 2H, *J* = 8.0 Hz, aromatic protons); 7.33 (d, 2H, *J* = 8.3 Hz, aromatic protons); 7.33 (d, 2H, *J* = 8.3 Hz; aromatic protons); 7.24 (d, 2H, *J* = 8.3 Hz; aromatic protons); 2.34 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 159.84 (C3-triazole ring); 159.20 (C5-triazole ring); 153.22 (C═N-thiadiazole ring); 141.86, 140.66, 137.54, 131.65, 129.86, 126.85 (quaternary aromatic ring carbons); 132.94, 130.85, 129.85, 129.33, 127.84, 120.31 (CH-aromatic ring carbons); 21.37 (CH_3).

6.3. General procedure for synthesis of 3-[4-(4-X-phenylsulfonyl) phenyl]-6-(substituted-phenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles ***4a–g, 5a–g***

A mixture of 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiol (X = H, Br) **1** (5 mmol) and aromatic acid (5 mmol) in phosphoryl chloride (10 mL) was heated under reflux until hydrogen chloride no longer evolved. The obtained mixture was cooled to room temperature and the viscous material thus formed was added in small portions to a mixture of 20 g of sodium hydroxide, 50 mL of water, and 50 g of ice using a cooling bath. The mixture was kept for 0.5 h at room temperature and adjusted to pH 8 by adding a 2M solution of sodium hydroxide. The obtained precipitate was filtered off, washed on a filter with warm water, dried in air and recrystallized from ethanol.

6.3.1. 6-(4-bromophenyl)-3-[4-(phenylsulfonyl)phenyl]-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole ***4a***

IR (KBr, cm^{-1}): 3088 (aromatic CH); 1602, 1587, 1567, 1520 (C═N + C═C_{aryl}); 1319, 1290, 1156 (SO_2); 1267 (N–N═C); 1008 (N–N); 685 (C–S–C); 568 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.05 (d, 2H, *J* = 8.5 Hz, aromatic protons); 8.01 (d, 2H, *J* = 8.5 Hz, aromatic protons); 7.95 (t, 2H, *J* = 8.0 Hz, aromatic protons); 7.72 (d, 2H, *J* = 8.2 Hz, aromatic protons); 7.65 (d, 2H, *J* = 8.2 Hz; aromatic protons); 7.62 (tt, 1H, *J* = 8.0; 1.8 Hz aromatic proton); 7.50 (t, 2H, *J* = 8.0 Hz; aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 163.45 (C3-triazole ring); 155.28 (C5-triazole ring); 152.68 (C═N-thiadiazole ring); 141.93, 140.26, 143.14, 132.45, 128.53 (quaternary aromatic ring carbons); 132.90, 132.65, 129.53, 128.78, 127.84, 127.13, 126.43 (CH-aromatic ring carbons).

6.3.2. 6-(4-chlorophenyl)-3-[4-(phenylsulfonyl)phenyl]-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole ***4b***

IR (KBr, cm^{-1}): 3087 (aromatic CH); 1604, 1582, 1569, 1543 (C═N + C═C_{aryl}); 1320, 1289, 1160 (SO_2); 1267 (N–N═C); 1007 (N–N); 766 (C–Cl); 687 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.27 (d, 2H, *J* = 8.7 Hz, aromatic protons); 8.17 (d, 2H, *J* = 8.7 Hz, aromatic protons); 8.00 (dd, 2H, *J* = 8.4; 1.6 Hz, aromatic protons); 7.91 (t, 1H,

J = 8.5 Hz; aromatic protons); 7.55 (d, 2H, J = 8.5 Hz; aromatic protons); 7.51–7.85 (m, 4H, aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 160.48 (C3-triazole ring); 158.78 (C5-triazole ring); 150.32 (C=N-thiadiazole ring); 143.47, 139.13, 138.04, 131.56, 130.20 (quaternary aromatic ring carbons); 133.99, 133.19, 129.30, 128.89, 128.37, 127.72, 127.58 (CH-aromatic ring carbons).

6.3.3. 6-(4-methoxyphenyl)-3-[4-(phenylsulfonyl)phenyl]-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole ***4c***

IR (KBr, cm^{-1}): 3087 (aromatic CH); 2930, 2867 (CH_3); 1602, 1592, 1518 (C=N + C=C_{aryl}); 1313, 1290, 1159 (SO_2); 1264 (N–N=C); 1005 (N–N); 684 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.01 (d, 2H, J = 8.7 Hz, aromatic protons); 7.95 (d, 2H, J = 8.7 Hz, aromatic protons); 7.86 (d, 2H, J = 8.5, aromatic protons); 7.78 (t, 2H, J = 8.5 Hz, aromatic proton); 7.57 (tt, 1H, J = 8.7; 1.6 Hz; aromatic proton); 7.50 (d, 2H, J = 8.8 Hz, aromatic protons); 7.12 (d, 2H, J = 8.8 Hz; aromatic protons); 3.85 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 166.28 (C3-triazole ring); 159.26 (C5-triazole ring); 154.94 (C=N-thiadiazole ring); 157.31, 142.13, 141.30, 135.78, 132.69 (quaternary aromatic ring carbons); 133.61, 129.53, 129.12, 127.58, 127.12, 126.44, 115.26 (CH-aromatic ring carbons); 55.61 (OCH_3).

6.3.4. 6-(4-aminophenyl)-3-[4-(phenylsulfonyl)phenyl]-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazole ***4d***

IR (KBr, cm^{-1}): 3384, 3188 (NH_2); 3088 (aromatic CH); 1619, 1599, 1585, 1548 (C=N + C=C_{aryl}); 1326, 1288, 1159 (SO_2); 1263 (N–N=C); 1012 (N–N); 707 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.27 (s, 2H, NH_2); 8.03 (d, 2H, J = 8.7 Hz, aromatic protons); 7.96 (d, 2H, J = 8.7 Hz, aromatic protons); 7.89 (dd, 2H, J = 8.6; 1.8 Hz, aromatic protons); 7.76 (d, 2H, J = 8.2 Hz; aromatic protons); 7.71 (t, 2H, J = 8.6 Hz, aromatic protons); 7.57 (t, 1H, J = 8.2 Hz, aromatic proton); 6.66 (d, 2H, J = 8.2 Hz; aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 166.17 (C3-triazole ring); 159.16 (C5-triazole ring); 154.31 (C=N-thiadiazole ring); 151.22, 141.43, 140.66, 135.62, 132.76 (quaternary aromatic ring carbons); 133.59, 129.48, 128.77, 128.53, 128.34, 127.68, 115.10 (CH-aromatic ring carbons).

6.3.5. 6-[(4-aminomethyl)phenyl]-3-[4-(phenylsulfonyl)phenyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***4e***

IR (KBr, cm^{-1}): 3375, 3118 (NH); 3085 (aromatic CH); 2960, 2884 (CH_2); 1619, 1599, 1585 (C=N + C=C_{aryl}); 1327, 1291, 1159 (SO_2); 1259 (N–N=C); 1010 (N–N); 710 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.68 (s, 2H, NH_2); 8.09 (d, 2H, J = 8.6 Hz, aromatic protons); 8.01 (d, 2H, J = 8.6 Hz, aromatic protons); 7.95 (dd, 2H, J = 8.4; 1.8 Hz, aromatic protons); 7.68–7.86 (m, 3H, aromatic protons); 7.50 (d, 2H, J = 8.2 Hz; aromatic protons); 7.28 (d, 2H, J = 8.2 Hz; aromatic protons); 4.36 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 165.45 (C3-triazole ring); 160.16 (C5-triazole ring); 155.28 (C=N-thiadiazole ring); 143.31, 141.61, 141.13, 134.24, 131.35 (quaternary aromatic ring carbons); 134.13, 129.73, 128.43, 128.03, 126.94, 123.17, 116.65 (CH-aromatic ring carbons); 45.93 (CH_2).

6.3.6. 6-[(3,4,5-trihydroxyphenyl)]-3-[4-(phenylsulfonyl)phenyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***4f***

IR (KBr, cm^{-1}): 3384 (OH); 3089 (aromatic CH); 1618, 1593, 1574 (C=N + C=C_{aryl}); 1326, 1291, 1159 (SO_2); 1265 (N–N=C); 1009 (N–N); 700 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.03 (d, 2H, J = 8.2 Hz, aromatic protons); 7.96 (d, 2H, J = 8.2 Hz, aromatic protons); 7.89 (d, 2H, J = 8.2 Hz, aromatic protons); 7.72 (t, 1H, J = 8.2 Hz, aromatic proton); 7.51 (dd, 2H, J = 8.2; 1.2 Hz; aromatic protons); 6,76 (d, 2H, J = 8.1 Hz; aromatic protons); 5.35 (s, 3H, OH); ^{13}C NMR (DMSO-d_6, δ, ppm): 166.48 (C3-triazole ring); 158.81 (C5-triazole ring); 158.75 (C=N-thiadiazole ring); 147.08, 142.12, 139.33, 134.78, 134.23, 134.17, 133.72, (quaternary aromatic ring carbons); 130.12, 129.63, 128.65, 127.73, 127.58, 111.53 (CH-aromatic ring carbons).

6.3.7. 6-[(3-bromo-4-chloro)phenyl]-3-[4-(phenylsulfonyl)phenyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***4g***

IR (KBr, cm^{-1}): 3095 (aromatic CH); 1609, 1596, 1454 (C=N + C=C_{aryl}); 1322, 1290, 1158 (SO_2); 1269 (N–N=C); 1015 (N–N); 766 (C–Cl); 699 (C–S–C); 575 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.13 (d, 2H, J = 8.6 Hz, aromatic protons); 7.95 (dd, 2H, J = 8.7; 1.6 Hz, aromatic protons); 7.86 (d, 1H, J = 8.5 Hz, aromatic proton); 7.78 (d, 1H, J = 8.5 Hz, aromatic proton); 7.61 (t, 2H, J = 8.7 Hz; aromatic protons); 7.57 (d, 2H, J = 8.6 Hz, aromatic protons); 7.42 (dd, 1H, J = 8.5; 2.4 Hz, aromatic proton); ^{13}C NMR (DMSO-d_6, δ, ppm): 165.66 (C3-triazole ring); 158.94 (C5-triazole ring); 154.68 (C=N-thiadiazole ring); 143.10, 140.29, 138.17, 135.78, 134.40, 122.93 (quaternary aromatic ring carbons); 133.33, 128.87, 128.34, 127.93, 127.58, 127.17, 127.02, 126.93 (CH-aromatic ring carbons).

6.3.8. 6-(4-bromophenyl)-3-[4-(4-bromophenylsulfonyl)phenyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***5a***

IR (KBr, cm^{-1}): 3086 (aromatic CH); 1619, 1599, 1573, 1548 (C=N + C=C_{aryl}); 1327, 1291, 1159 (SO_2); 1259 (N–N=C); 1010 (N–N); 698 (C–S–C); 575 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.98 (s, 2H, aromatic protons); 0.7.82 (d, 2H, J = 8.3 Hz, aromatic protons); 7.61 (d, 2H, J = 8.3 Hz, aromatic protons); 7.65 (d, 2H, J = 8.2 Hz; aromatic protons); 7.50–7.58 (m, 4H; aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 164.78 (C3-triazole ring); 154.76 (C5-triazole ring); 153.94 (C=N-thiadiazole ring); 141.30, 139.47, 135.17, 132.45, 129.53, 127.66 (quaternary aromatic ring carbons); 133.79, 133.62, 132.72, 130.20, 129.40, 128.16 (CH-aromatic ring carbons).

6.3.9. 6-(4-chlorophenyl)-3-[4-(4-bromophenylsulfonyl)phenyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***5b***

IR (KBr, cm^{-1}): 3084 (aromatic CH); 1610, 1588, 1574 (C=N + C=C_{aryl}); 1313, 1291, 1160 (SO_2); 1263 (N–N=C); 1005 (N–N); 768 (C–Cl); 683 (C–S–C); 572 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.30 (d, 2H, J = 8.3 Hz, aromatic protons); 8.16 (d, 2H, J = 8.3 Hz, aromatic protons); 7.95 (d, 2H, J = 8.1 Hz, aromatic protons); 7.92 (d, 2H, J = 8.4 Hz; aromatic protons); 7.60 (d, 2H, J = 8.1 Hz; aromatic protons); 7.59 (d, 2H, J = 8.4 Hz; aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 161.89 (C3-triazole ring); 156.66 (C5-triazole ring); 151.16 (C=N-thiadiazole ring); 141.54, 139.40, 138.63, 134.74, 134.18, 128.31 (quaternary aromatic ring carbons); 133.00, 132.48, 129.31, 128.82, 127.71, 127.52 (CH-aromatic ring carbons).

6.3.10. 6-(4-methoxyphenyl)-3-[4-(4-bromophenylsulfonyl) phenyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***5c***

IR (KBr, cm^{-1}): 3088 (aromatic CH); 2937, 2840 (CH_3); 1605, 1589, 1512 (C=N + C=C_{aryl}); 1312, 1288, 1158 (SO_2); 1269 (N–N=C); 1007 (N–N); 687 (C–S–C); 581 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.06 (d, 2H, J = 8.5 Hz, aromatic protons); 7.98 (d, 2H, J = 8.5 Hz, aromatic protons); 7.76 (dd, 2H, J = 8.5, aromatic protons); 7.66 (d, 2H, J = 8.2 Hz, aromatic protons); 7.52 (d, 2H, J = 8.7 Hz; aromatic protons); 7.15 (d, 2H, J = 8.2 Hz, aromatic proton); 3.88 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 165.89 (C3-triazole ring); 158.92 (C5-triazole ring); 153.94 (C=N-thiadiazole ring); 155.87, 140.66, 140.40, 137.03, 133.07, 128.53 (quaternary aromatic ring carbons); 133.48, 129.04, 128.61, 126.98, 125.88, 114.74 (CH-aromatic ring carbons); 55.38 (OCH_3).

6.3.11. 6-(4-aminophenyl)-3-[4-(4-bromophenylsulfonyl)phenyl]-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole ***5d***

IR (KBr, cm^{-1}): 3368, 3220 (NH_2); 3063 (aromatic CH); 1608, 1579, 1541 (C=N + C=C_{aryl}); 1325, 1290, 1158 (SO_2); 1255 (N–N=C); 1012 (N–N); 705 (C–S–C); 580 (C–Br); ^{1}H NMR (DMSO-d_6, δ,

ppm): 6.13 (s, 2H, NH_2); 7.94 (d, 2H, J = 8.8 Hz, aromatic protons); 7.87 (d, 2H, J = 8.8 Hz, aromatic protons); 7.77 (d, 2H, J = 8.8 Hz, aromatic protons); 7.71 (d, 2H, J = 8.8 Hz; aromatic protons); 7.64 (d, 2H, J = 8.6 Hz, aromatic protons); 6.58 (d, 2H, J = 8.6 Hz; aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 165.89 (C3-triazole ring); 159.34 (C5-triazole ring); 154.94 (C=N-thiadiazole ring); 145.88, 142.13, 141.00, 137.60, 131.75, 129.48 (quaternary aromatic ring carbons); 132.72, 130.20, 129.67, 129.12, 128.82, 114.38 (CH-aromatic ring carbons).

6.3.12. 6-[(4-aminomethyl)phenyl]-3-[4-(4-bromophenylsulfonyl) phenyl]-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole **5e**

IR (KBr, cm^{-1}): 3316, 3228 (NH); 3091 (aromatic CH); 2925, 2837 (CH_2); 1620, 1599, 1573 (C=N + C=C_{aryl}); 1326, 1288, 1159 (SO_2); 1263 (N–N=C); 1012 (N–N); 711 (C–S–C); 575 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.68 (s, 2H, NH_2); 8.09 (d, 2H, J = 8.6 Hz, aromatic protons); 8.01 (d, 2H, J = 8.6 Hz, aromatic protons); 7.95 (dd, 2H, J = 8.4; 1.8 Hz, aromatic protons); 7.68–7.86 (m, 3H, aromatic protons); 7.50 (d, 2H, J = 8.2 Hz; aromatic protons); 7.28 (d, 2H, J = 8.2 Hz; aromatic protons); 4.36 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 166.91 (C3-triazole ring); 159.92 (C5-triazole ring); 153.89 (C=N-thiadiazole ring); 143.91, 142.78, 141.40, 135.60, 132.57,128.14 (quaternary aromatic ring carbons); 133.60, 130.53, 129.33, 127.30, 126.94, 126.89 (CH-aromatic ring carbons); 46.13 (CH_2).

6.3.13. 6-[(3,4,5-trihydroxyphenyl)]-3-[4-(4-bromophenylsulfonyl) phenyl]-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole **5f**

IR (KBr, cm^{-1}): 3373 (OH); 3088 (aromatic CH); 1620, 1595, 1573, 1552 (C=N + C=C_{aryl}); 1326, 1288, 1158 (SO_2); 1267 (N–N=C); 1010 (N–N); 702 (C–S–C); 573 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.92 (d, 2H, J = 8.5 Hz, aromatic protons); 7.87 (d, 2H, J = 8.5 Hz, aromatic protons); 7.77 (d, 2H, J = 8.5 Hz, aromatic protons); 7.71 (d, 2H, J = 8.5 Hz, aromatic protons); 6,71 (d, 2H, J = 8.2 Hz; aromatic protons); 5.42 (s, 3H, OH); ^{13}C NMR (DMSO-d_6, δ, ppm): 167.62 (C3-triazole ring); 157.93 (C5-triazole ring); 157.72 (C=N-thiadiazole ring); 146.75, 143.15, 142.87, 139.54, 135.28, 134.35, 133.69, 127.28 (quaternary aromatic ring carbons); 133.08, 129.65, 128.49, 127.44, 114.67 (CH-aromatic ring carbons).

6.3.14. 6-[(3-bromo-4-chloro)phenyl]-3-[4-(4-bromophenylsulfonyl) phenyl]-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazole **5g**

IR (KBr, cm^{-1}): 3068 (aromatic CH); 1613, 1592, 1449 (C=N + C=C_{aryl}); 1324, 1287, 1158 (SO_2); 1264 (N–N=C); 1013 (N–N); 762 (C–Cl); 697 (C–S–C); 579 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.09 (s, 4H, aromatic protons); 8.00 (dd, 2H, J = 8.5; 1.6 Hz, aromatic protons); 7.74 (d, 1H, J = 8.8 Hz, aromatic proton); 7.64 (dd, 2H, J = 8.5; 1.6 Hz, aromatic protons); 7.34 (dd, 1H, J = 8.8; 2.2 Hz; aromatic proton); 7.58 (d, 1H, J = 8.8 Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 165.31 (C3-triazole ring); 159.04 (C5-triazole ring); 154.12 (C=N-thiadiazole ring); 142.84, 140.66, 136.14, 135.07, 133.89, 131.79, 128.53 (quaternary aromatic ring carbons); 134.82, 133.60, 129.88, 128.61, 128.12, 122.95, 121.05 (CH-aromatic ring carbons).

6.4. General procedure for synthesis of 3-[4-(4-X-phenylsulfonyl) phenyl]-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazol-6(5H)-one **6a,b**

A mixture of 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiol **1** (0.36 mmol) and *N,N′*-carbonyldiimidazole (0.54 mmol) in dry dioxane (10 mL) was heated under reflux for 4 h. After cooling, the solvent was removed under reduced pressure and the solid product obtained was washed with water, filtered and recrystallized from ethanol.

6.4.1. 3-[4-(phenylsulfonyl)phenyl]-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6(5H)-one **6a**

IR (KBr, cm^{-1}): 3398 (OH), 3089 (aromatic CH); 1616, 1572, 1489 (C=N + C=C_{aryl}); 1324, 1291, 1159 (SO_2); 1263 (N–N=C); 1009 (N–N); 700 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 9.08 (s, 1H, NH); 8.27 (d, 2H, J = 8.5 Hz, aromatic protons); 8.11 (d, 2H, J = 7.8 Hz, aromatic proton); 8.00 (d, 2H, J = 8.5 Hz, aromatic proton); 7.70 (tt, 1H, J = 7.8; 1.5 Hz, aromatic proton); 7.63 (t, 2H, J = 7.8 Hz; aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 175.26 (C=O); 158.75 (C3-triazole ring); 157.89 (C5-triazole ring); 143.51, 139.56, 133.84 (quaternary aromatic ring carbons); 133.97, 129.87, 128.62, 127.57, 124.47 (CH-aromatic ring carbons).

6.4.2. 3-[4-(4-bromophenylsulfonyl)phenyl]-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazol-6(5H)-one **6b**

IR (KBr, cm^{-1}): 3405 (OH), 3091 (aromatic CH); 1617, 1573, 1471 (C=N + C=C_{aryl}); 1326, 1292, 1158 (SO_2); 1260 (N–N=C); 1009 (N–N); 692 (C–S–C); 577 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 9.09 (s, 1H, NH); 8.15 (d, 2H, J = 8.5 Hz, aromatic protons); 8.09 (d, 2H, J = 8.5 Hz, aromatic protons); 7.94 (d, 2H, J = 8.8 Hz, aromatic proton); 7.87 (d, 2H, J = 8.8 Hz, aromatic proton); ^{13}C NMR (DMSO-d_6, δ, ppm): 177.63 (C=O); 159.10 (C3-triazole ring); 158.35 (C5-triazole ring); 143.13, 140.68, 134.35 (quaternary aromatic ring carbons); 133.05, 129.60, 128.45, 127.92 (CH-aromatic ring carbons).

6.5. Antibacterial activity

Determination of minimum inhibitory concentration (MIC, μg/mL) was done using the serial dilutions in liquid broth method. Tests were performed in 96-well round bottom sterile culture plates. Antibacterial and antifungal assays were performed in Mueller-Hinton broth (Merck) and Sabouraud dextrose agar (Difco), respectively. Ampicillin and Aztreonam for bacteria and Amphotericine for the yeast *C. albicans* were used as standard drugs.

For inoculum preparation microbial suspensions of reference strains in saline were made. They were adjusted to the turbidity of 0.5 McFarland standard and were diluted 1:100 in Mueller-Hinton broth/Sabouraud dextrose agar for achieving a density of 1×10^6 CFU/mL. Aliquots of 50 μL of the diluted inoculum were added to each well containing the tested compounds and in the positive growth control well, filled already with 50 μL broth without compound. The sterility control well contained only compound-free broth. The final liquid volume in each well was of 100 μL.

All the test compounds were dissolved in DMSO (2048 μg/mL). Further dilutions of the compounds in the test medium were furnished at the required quantities of the broth used. Ten different dilutions of the test compounds between 1024 and 2 μg/mL were prepared in microplates by serial dilutions from top to bottom. Then all the wells except the 12th wells (positive control) were filled with 50 μL of the standardized strains. These plates were incubated at 37 °C for 18–20 h for bacterial strains and 48 h for *C. albicans*. The lowest concentration of the test compounds inhibiting visible growth of microorganisms was taken as the MIC value. Tests using DMSO as negative control were carried out in parallel and it was determined that the solvent had no antimicrobial activity against any of the test microorganisms. Because the MIC values are not spectacular, no statistical calculations were made.

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