Scientific paper

Synthesis and Evaluation of Novel Bis[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles as Potent Antimicrobial Agents

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Abstract

A series of novel bis[4-methoxy-3-(6-aryl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methanes **5a**–**l** has been synthesized and characterized *via* IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analyses. All the newly synthesized compounds were screened for their antibacterial activity against *Bacillus subtilis*, *Bacillus sphaericu, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klobsinella aerogenes* and *Chromobacterium violaceum* and antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Compounds **5e**, **5f**, **5h**, **5i**, **5k** and **5l** exhibited potent activity against the test bacteria and fungi, and emerged as potential molecules for further development.

Keywords: Bis(triazolo[3,4-*b*]thiadiazoles), synthesis, antibacterial activity, antifungal activity

1. Introduction

Heterocyclic compounds represent one of the most active classes of compounds possessing a wide spectrum of biological activities, including antibacterial, antifungal, and other biological activities.[1–6] The biological activities of various 1,2,4-triazole derivatives and their *N*-bridged heterocyclic analogs have been widely investigated as antitumor,[7] antiviral,[8] anti-inflammatory,[9] analgesic[10] and antidepressant.[11] It is interesting to use 1,2,4-triazole derivatives as precursor starting material in the synthesis of some important biologically active heterocycles,[12–15] which constitute an important class of organic compounds with diverse biological activities, including antiparasitic, analgesic, antibacterial and anti-inflammatory activites.[16–21] In addition, it was reported that 1,3,4-thiadiazoles exhibit various biological activities such as anti-parkinsonism,[22] hypoglycaemic,[23] anti-histaminic,[24] anticancer,[25] anti-inflammatory,[26] antiasthmatic[27] and antihypertensive.[28] The activity of 1,3,4-thiadiazoles is possibly due to the presence of the =N–C–S moiety.[29]

The triazole system fused to another heterocyclic ring has shown a wide spectrum of biological activities such as antibacterial, antidepressant, antiviral, antitumorial and anti-inflammatory and is a constituent of pesticides, herbicides, dyes, lubricants and also analytical reagents.[30] The commonly known triazole fused to another heterocyclic systems are triazolo-pyridines,[12] triazolo-pyridazines,[13] triazolo-pyrimidines,[14] triazolo-pyrazines,[15] triazolo-triazines[31] and triazolo-thiadiazines.[32] Although there are not many triazole fused to thiadiazole, there is a number of them that are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities.[33–36] However, there is no report on the triazole fused thiadiazole of bis-heterocyclic systems.

In recent years, attention has been increasingly paid to the synthesis of bis-heterocyclic compounds which exhibit various biological activities,[37–40] including antibacterial, fungicidal, tuberculostatic and plant growth regulative properties. Further, recent reports[41] indicate that bis-heterocyclic compounds displayed much better antibacterial activity than the mono heterocyclic compounds.

Owing to the immense importance and varied bioactivities exhibited by triazolo-thiadiazoles and in the continuation of our ongoing research on biologically active bis-

heterocyclics,[42–50] it was thought of interest to accommodate triazole and thiadiazole moieties in a single molecular frame-work and to obtain new bis-heterocyclic compounds with potential biological activity. In the present study we performed the synthesis and biological evaluation of some new bis(triazolo-thiadiazoles). For the synthesis of target compounds, bis(4-amino-1,2,4-triazol-3-thione) has been used as an intermediate because the amino and mercapto groups are ready-made nucleophilic centers for the synthesis of fused heterocyclic compounds.

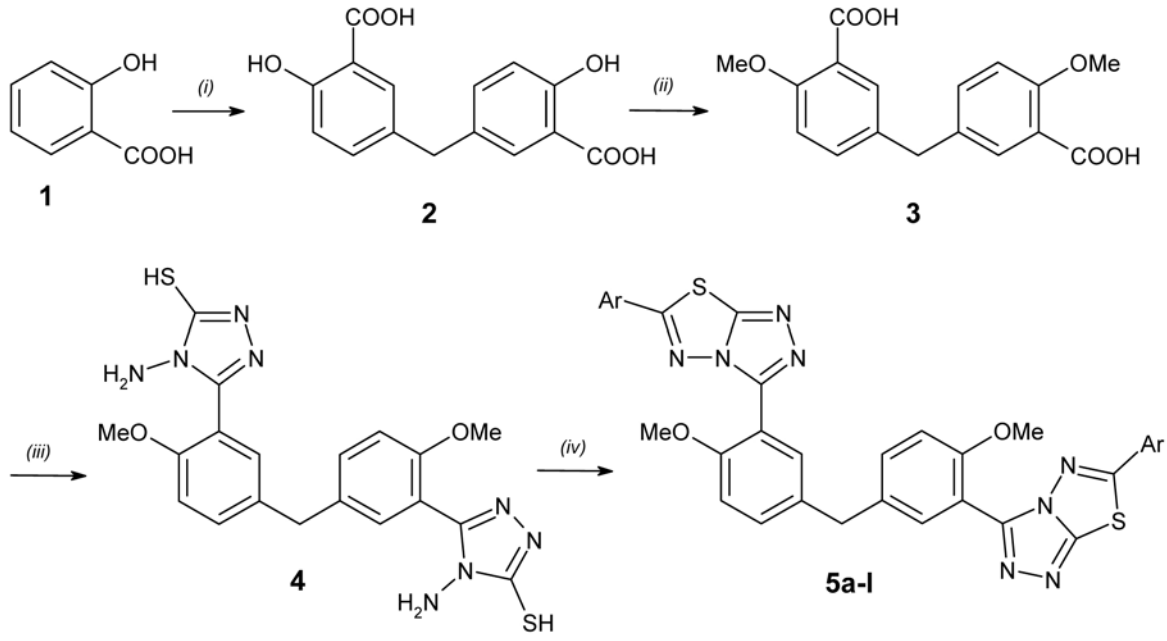
2. Results and Discussion

The compound **2**, required for the synthesis of the title compounds, was prepared according to the procedure described in the literature.[51] The compound **2** on reaction with dimethyl iodide, in the presence of aq. KOH at 80 °C, furnished 5-(3-formyl-4-methoxybenzyl)-2-methoxybenzoic acid (**3**). The condensation of **3** with thiocarbohydrazide at melt temperature for 3 h afforded bis[4-methoxy-3-[4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl]phenyl]methane (**4**) as a yellow solid (Scheme 1).

IR spectrum of compound **4** showed the two absorption bands in the region of 3335–3235 and 2596 cm^{-1} assigned to NH_2 and SH groups, the two absorption bands at 1554 and 1512 cm^{-1} attributable to C=N vibrations, providing a strong evidence for the formation of triazole ring. Its ^1H NMR spectrum showed two signals at δ 2.17 and 5.47 ppm corresponding to –SH and $–NH_2$ protons, respectively. The aromatic protons appeared in the region δ 6.62–9.93 ppm in accord with the structure. ^{13}C NMR spectrum showed signals at δ 156.6 and 134.4 ppm corresponding to the 3-C and 5-C of the triazole moiety, respectively. The other signals observed were at the expected chemical shifts with appropriate integrals. In addition, elemental analysis is also consistent with the structure proposed for **4**.

Compound **4** on reaction with the corresponding aryl/heteroaryl carboxylic acid, in the presence of phosphorus oxychloride at reflux for 10 h, produced bis[4-methoxy-3-(6-aryl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methanes **5a–l** in 68–78% yield (Scheme 1). The elemental analyses, IR, ^1H NMR, ^{13}C NMR and MS spectral data are consistent with the assigned structures.

In the IR spectra of compounds **5a–l** the absence of absorption bands due to –SH and $–NH_2$ stretching frequencies of parent compound **4** revealed the fusing between compound **4** and aryl/heteroaryl carboxylic acid. Appearance of three absorption bands at 1595, 1557 and 1520 cm^{-1} (attributable to C=N vibrations) provides a strong evidence for the fusion of triazole ring. In the ^1H NMR spectra of compounds **5a–l** the disappearance of signals at δ 2.17 and 5.47 ppm (due to SH and NH_2 protons of compound **4**) supports the involvement of these groups in the formation of the thiadiazole ring. In the ^{13}C NMR spectra the signals of triazolo-thiadiazole ring were observed at δ 158.7, 149.0 and 138.2 ppm, respectively.



5: Ar = **a**) phenyl; **b**) 2-chlorophenyl; **c**) 4-chlorophenyl; **d**) 4-methylphenyl; **e**)4-hydroxyphenyl; **f**) 4- methoxyphenyl; **g**) 3-nitrophenyl; **h**) 4-nitrophenyl; **i**) benzyl; **j**) 4-chlorobenzyl; **k**) 3-pyridyl; **l**) 2- pyrazinyl

Reagents and conditions: (*i*) CH_2O, H_2SO_4, reflux; (*ii*) MeI, K_2CO_3, DMF, rt; (*iii*) Thiocarbohydrazide, heat; (*iv*) Ar-COOH, $POCl_3$, reflux.

Scheme 1. Synthetic route of bis[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazoles

The other signals were observed at the expected chemical shifts with appropriate integrals. Elemental analyses are also consistent with the structures proposed for compounds **5a–l**.

3. Antibacterial Evaluation

All the newly synthesized compounds **5a–l** were screened for their antibacterial activity against Gram-positive bacteria *viz. Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 11) and *Staphylococcus aureus* (MTCC 96), and Gram-negative bacteria *viz. Pseudomonas aeruginosa* (MTCC 741), *Klobsinella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC 2656) by disc diffusion method.[52] For the antibacterial assay, standard inoculums (1–2 × 10^7 c.f.u/mL 0.5 McFarland standards) were introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The discs measuring 6.26 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in the nutrient agar medium. The plates were inverted and incubated for 24 h at 37 °C. The inhibition zones were measured and compared with the standard drug streptomycin. The MZI data are presented in Table 1.

The antibacterial screening revealed that all the tested compounds **5a–l** showed moderate to good inhibition towards all the tested strains. Compounds **5e**, **5f**, **5i**, and **5l** exhibited potent inhibitory activity compared to the standard drug at the tested concentrations. The results revealed that the presence 4-hydroxyphenyl or 4-methoxyphenyl or benzyl or pyrazinyl on N–C–S moiety of thiadiazole might be the reason for the significant inhibitory activity. Also, the presence of a hydroxyl group in the molecules would enhance the inhibitory activity as shown by **5e**. Presence of 4-chlorophenyl (in **5c**) and 4-nitrophenyl moiety (in **5h)** did not show significant inhibition. The comparison of MZI values (in mm) of the selected compounds **5** and standard drug against different bacteria is presented in Figure 1.

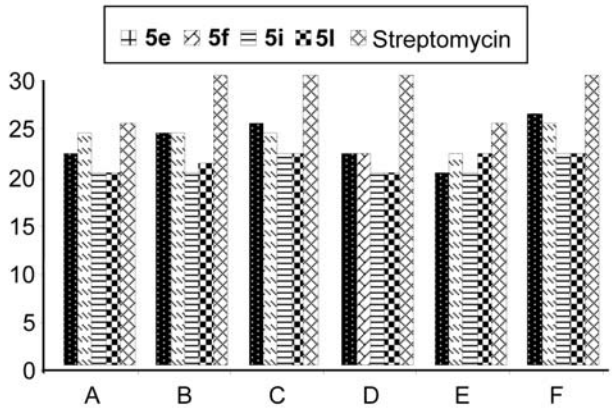


Fig. 1. Comparison of MZI values (in mm) of selected compounds and standard drugs at 50 μg/mL against different bacteria. A) *B. subtilis*; B) *B. sphaericus*; C) *S. aureus*; D) *P. aeruginosa*; E) *K. aerogenes*; F) *C. Violaceum*

Table 1. Antibacterial activity of compounds **5a–l**

Compound	Mean zone inhibition (MZI) (mm)[a]					
	B. subtilis	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
5a	11 ± 0.3	10 ± 0.4	12 ± 0.4	10 ± 0.4	10 ± 0.4	12 ± 0.2
5b	6 ± 0.2	6 ± 0.3	8 ± 1.1	6 ± 0.4	6 ± 0.2	18 ± 1.0
5c	12 ± 0.3	10 ± 0.3	12 ± 0.7	8 ± 0.5	8 ± 0.3	8 ± 1.2
5d	12 ± 0.8	14 ± 1.0	15 ± 1.1	10 ± 0.8	12 ± 1.1	14 ± 0.6
5e	22 ± 0.5	24 ± 0.7	25 ± 1.0	22 ± 0.5	20 ± 0.8	26 ± 1.0
5f	24 ± 1.0	24 ± 1.1	24 ± 0.4	22 ± 1.2	22 ± 1.0	25 ± 0.3
5g	8 ± 0.3	10 ± 0.3	11 ± 0.7	10 ± 0.5	10 ± 0.3	6 ± 1.0
5h	10 ± 0.4	8 ± 0.3	10 ± 1.2	8 ± 0.3	7 ± 1.2	12 ± 1.2
5i	20 ± 1.0	20 ± 1.0	22 ± 1.0	20 ± 1.1	20 ± 1.2	22 ± 0.2
5j	8 ± 0.2	10 ± 0.2	10 ± 0.3	10 ± 0.4	8 ± 0.3	16 ± 0.7
5k	8 ± 0.3	11 ± 0.3	11 ± 0.7	10 ± 0.5	8 ± 0.3	12 ± 1.2
5l	20 ± 1.0	21 ± 1.1	22 ± 0.5	20 ± 1.0	22 ± 0.3	22 ± 0.4
Streptomycin	25 ± 0.5	30 ± 0.5	30 ± 1.0	30 ± 0.6	25 ± 0.8	30 ± 0.5

Streptomycin (50 μg/disc) was used as positive reference and compounds **5a–l** (50 μg/disc).
[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.

4. Antifungal Evaluation

Compounds **5a–j** were also evaluated *in vitro* antifungal activity against four fungi *viz. Candida albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185) and *Trichophyton mentagrophytes* (IFO 40996) by agar diffusion method.[52] For the antifungal assay Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. 20 mL of agar media was poured into each petri-dish, excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. Using agar punch wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. The *C. albicans* was grown for 48 h at 28 °C in YPD broth (1% yeast extract, 2% peptone and 2% dextrose), harvested by centrifugation and then washed twice with sterile distilled water. *A. fumigatus*, *T. rubrum* and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco) and incuba-

ted at 28 °C for two weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculums size of 10^5 spores/mL. The mean inhibition zones were determined and compared with the standard drug amphotericin B (Table 2).

Results of antifungal activity showed that most of the new **5a–l** were active with moderate to good activity. The compounds **5k** and **5l** bearing heterocyclic ring on the carbon of N–C–S fragment of the thiadiazole ring showed highest activity against all the fungi tested. The activity of these compounds is almost equal to the standard. Compounds **5h** and **5i** bearing 4-nitrophenyl and benzyl moieties also showed good inhibition towards *A. fumigatus* and *T. mentagrophytes*. The comparison of MZI values (in mm) of the selected compounds **5** and standard drug against different fungi is presented in Figure 2.

Table 2. Antifungal activity of compounds **5a–l**

Compound	Mean zone inhibition (MZI) (mm)[a]			
	C. albicans	*A. fumigatus*	*T. rubrum*	*T. mentagrophytes*
5a	10 ± 0.6	12 ± 1.4	14 ± 1.0	16 ± 0.8
5b	8 ± 0.4	10 ± 1.2	8 ± 1.1	10 ± 1.2
5c	10 ± 0.3	10 ± 1.6	12 ± 0.9	10 ± 0.6
5d	12 ± 0.3	8 ± 1.6	18 ± 0.9	16 ± 0.6
5e	14 ± 0.7	18 ± 1.0	14 ± 1.0	18 ± 0.5
5f	10 ± 0.6	12 ± 1.2	14 ± 0.6	12 ± 1.0
5g	11 ± 0.6	16 ± 1.2	14 ± 0.6	14 ± 1.0
5h	22 ± 0.9	25 ± 0.8	15 ± 0.7	20 ± 1.0
5i	24 ± 0.8	22 ± 0.6	12 ± 1.0	22 ± 0.7
5j	16 ± 0.6	12 ± 1.2	10 ± 0.6	8 ± 1.0
5k	26 ± 1.0	20 ± 0.6	22 ± 0.5	23 ± 0.4
5l	25 ± 0.6	22 ± 0.7	20 ± 1.1	23 ± 0.7
Amphotericin B	30 ± 1.0	30 ± 1.0	25 ± 1.1	25 ± 0.6

Amphotericin B (100 μg/disc) was used as positive reference and compounds **5a–l** (100 μg/disc).

[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.

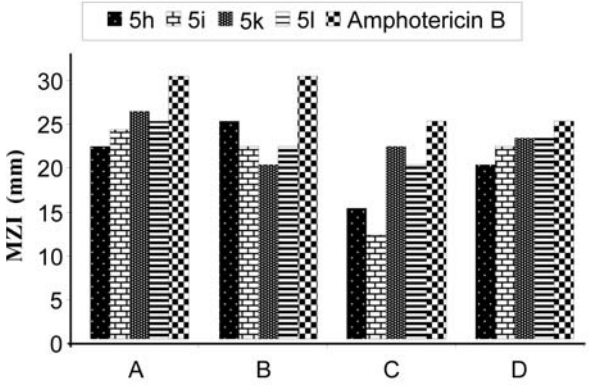


Fig. 2. Comparison of MZI values (in mm) of selected compounds and standard drugs at 100 μg/mL against different fungi. A) *C. albicans*; B) *A. fumigatus*; C) *T. rubrum*; D) *T. mentagrophytes*

5. Experimental

Commercial grade reagents were used as supplied. Solvents except analytical reagent grade were dried and purified according to literature when necessary. Reaction progress and purity of the compounds were checked by thin-layer chromatography (TLC) on pre-coated silica gel F_{254} plates from Merck and compounds visualized either by exposure to UV light or dipping in 1% aqueous potassium permanganate solution. Silica gel chromatographic columns (70–230 mesh) were used for separations. All melting points are uncorrected and measured using Fisher–Johns apparatus. IR spectra were recorded as KBr disks on a Perkin–Elmer FTIR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are reported as δ ppm against TMS as internal reference and coupling constants (J) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H spectrometer. Elemental analyses (C, H, N) determined by a Perkin–Elmer 240 CHN elemental analyzer, were within ± 0.4% of theoretical.

Preparation of 5-(3-formyl-4-methoxybenzyl)-2-methoxybenzoic acid (3): To a solution of **2** (0.01 mol) and K_2CO_3 (0.04 mol) in DMF (16 mL), MeI (0.03 mol) was added. The reaction mixture was stirred for 12 h at room temperature (TLC, EtOAc : petroleum ether, 2:1). The mixture was poured in water (30 mL), and extracted with Et_2O (3 × 20 mL). Washing the organic phase with 2 M NaOH solution, drying over Na_2SO_4 and evaporation of solvent gave compound **3**.

Preparation of bis[4-methoxy-3-[4-amino-5-sulfanyl-4*H*-1,2,4-triazole-3-yl]phenyl]methane (4): A mixture of compound **3** (0.01 mol) and thiocarbohydrazide (0.02 mol) was heated until the contents melted. The reaction was maintained at this temperature for 3 h. The fused mass thus obtained was treated with sodium bicarbonate solution to dissolve the unreacted compound **3**. It was then washed with water and collected by filtration. The product was recrystallized from a mixture of dioxane and ethanol to afford the compound **4** as yellow solid; yield 81%; mp 210–212 °C; IR (KBr) ν 3335–3235, 3072, 2596, 1554, 1512, 1314, 1070 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ2.17 (bs, 2H, SH) 3.81 (s, 6H, OCH_3), 4.00 (s, 2H, CH_2), 5.47 (bs, 4H, NH_2), 6.62 (d, J = 9.1 Hz, 2H, ArH), 7.50 (d, J = 9.1 Hz, 2H, ArH), 9.93 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.0, 56.1, 118.2, 124.2, 129.8, 132.7, 134.4, 153.0, 155.1, 156.6; Anal. Calcd for $C_{19}H_{20}N_8O_2S_2$: C, 49.99; H, 4.42; N, 24.54. Found: C, 49.93; H, 4.45; N, 24.48. MS: *m/z* 456 (M^+).

General procedure for synthesis of bis[4-methoxy-3-(6-aryl[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol)phenyl] methanes (5a–l): To a solution of **4** (0.01 mol) in phosphorus oxychloride (10 mL) was added aryl/heteryl carboxylic acid (0.02 mol). The reaction mixture was refluxed on oil bath for 10 h, the excess $POCl_3$ was distilled off

in vacuo, residual mass was poured over the crushed ice, neutralized with sodium bicarbonate solution and the solid thus separated was filtered, washed thoroughly with water and sodium bicarbonate solution and recrystalized from chloroform-ethanol (10:2) to afford pure compounds **5a–l**.

Bis[4-methoxy-3-(6-phenyl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methane (5a). Yield 72%; mp 196–198 °C; IR (KBr) ν 3034, 2982, 1603, 1594, 1554, 1520, 1470, 1068 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.91 (s, 6H, OCH_3), 4.02 (s, 2H, CH_2), 6.67 (d, *J* = 8.7 Hz, 2H, ArH), 7.40–7.50 (m, 8H, ArH), 7.76 (d, *J* = 8.2 Hz, 4H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.1, 54.1, 118.7, 123.7, 126.7, 130.3, 131.1, 132.4, 133.0, 133.6, 134.7, 138.2, 149.0, 156.6, 158.7; Anal. Calcd for $C_{33}H_{24}N_8O_2S_2$: C, 63.04; H, 3.85; N, 17.82. Found: C, 63.00; H, 3.91; N, 17.78. MS: *m/z* 628 (M^+).

Bis[4-methoxy-3-(6-(2-chlorophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methane (5b). Yield 70%; mp 208–210 °C; IR (KBr) ν 3065, 2967, 1604, 1592, 1554, 1520, 1468, 1070, 685 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.91 (s, 6H, OCH_3), 4.02 (s, 2H, CH_2), 6.67 (d, *J* = 8.7 Hz, 2H, ArH), 7.40–7.50 (m, 6H, ArH), 7.00–7.10 (m, 4H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.1, 54.1, 118.7, 123.6, 128.7, 129.1, 130.1, 130.8, 132.5, 133.0, 133.7, 134.7, 136.7, 138.2, 149.0, 156.6, 158.9; Anal. Calcd for $C_{33}H_{22}Cl_2N_8O_2S_2$: C, 56.82; H, 3.18; N, 16.06. Found: C, 56.86; H, 3.20; N, 16.01. MS: *m/z* 698 (M^+).

Bis[4-methoxy-3-(6-(4-chlorophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methane (5c). Yield 73%; mp 201–203 °C; IR (KBr) ν 3035, 2982, 1603, 1592, 1552, 1520, 1468, 1070, 685 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.91 (s, 6H, OCH_3), 4.02 (s, 2H, CH_2), 6.67 (d, *J* = 8.7 Hz, 2H, ArH), 7.35–7.40 (m, 6H, ArH), 7.66 (d, *J* = 8.1 Hz, 4H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.1, 54.1, 118.6, 123.6, 129.4, 130.3, 131.6, 132.0, 133.0, 134.6, 137.3, 138.2, 149.1, 156.5, 158.9; Anal. Calcd for $C_{33}H_{22}Cl_2N_8O_2S_2$: C, 56.82; H, 3.18; N, 16.06. Found: C, 56.77; H, 3.16; N, 16.10. MS: *m/z* 698 (M^+).

Bis[4-methoxy-3-(6-(4-methylphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methane (5d). Yield 68%; mp 189–191 °C; IR (KBr) ν 3037, 2986, 1604, 1595, 1556, 1520, 1470, 1070 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.26 (s, 6H, CH_3), 3.91 (s, 6H, OCH_3), 4.02 (s, 2H, CH_2), 6.67 (d, *J* = 8.7 Hz, 2H, ArH), 7.20 (d, *J* = 7.5 Hz, 4H, ArH), 7.41 (d, *J* = 8.6 Hz, 2H, ArH), 7.91 (d, *J* = 7.5 Hz, 4H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 23.1, 42.1, 54.1, 118.5, 123.5, 129.8, 130.0, 132.0, 133.0, 133.7, 134.7, 138.2, 140.1, 149.2, 156.6, 158.6; Anal. Calcd for $C_{35}H_{28}N_8O_2S_2$: C, 64.01; H, 4.30; N, 17.06. Found: C, 64.07; H, 4.35; N, 17.00. MS: *m/z* 656 (M^+).

Bis[4-methoxy-3-(6-(4-hydroxyphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methane (5e). Yield 70%; mp 192–194 °C; IR (KBr) ν 3334, 2986, 1602, 1590, 1554, 1518, 1472, 1065 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.91 (s, 6H, OCH_3), 4.00 (s, 2H, CH_2), 5.37 (s, 2H, OH), 6.67 (d, *J* = 8.7 Hz, 2H, ArH), 7.30–7.40 (m, 6H, ArH), 6.92 (d, *J* = 8.3 Hz, 4H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.1, 54.1, 117.0, 118.4, 123.5, 127.1, 130.1, 133.0, 133.7, 134.5, 138.2, 149.1, 156.4, 158.6, 164.3; Anal. Calcd for $C_{33}H_{24}N_8O_4S_2$: C, 59.99; H, 3.66; N, 16.96. Found: C, 60.02; H, 3.65; N, 16.92. MS: *m/z* 660 (M^+).

Bis[4-methoxy-3-(6-(4-methoxyphenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methane (5f). Yield 76%; mp 186–188 °C; IR (KBr) ν 3062, 2981, 1602, 1591, 1552, 1520, 1470, 1068 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.66 (s, 6H, OCH_3), 3.91 (s, 6H, OCH_3), 4.00 (s, 2H, CH_2), 6.65–6.70 (m, 6H, ArH), 7.30–7.40 (m, 6H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.1, 54.1, 57.5, 117.8, 118.5, 123.6, 128.0, 130.4, 132.3, 133.0, 134.5, 138.2, 149.0, 156.4, 158.6, 162.1; Anal. Calcd for $C_{35}H_{28}N_8O_4S_2$: C, 61.03; H, 4.10; N, 16.27. Found: C, 61.00; H, 4.14; N, 16.30. MS: *m/z* 688 (M^+).

Bis[4-methoxy-3-(6-(3-nitrophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methane (5g). Yield 72%; mp 210–212 °C; IR (KBr) ν 3082, 1602, 1595, 1552, 1518, 1470, 1368, 1065 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.91 (s, 6H, OCH_3), 4.02 (s, 2H, CH_2), 6.67 (d, *J* = 8.7 Hz, 2H, ArH), 7.41 (d, *J* = 8.6 Hz, 2H, ArH), 8.00–8.15 (m, 8H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.1, 54.1, 118.5, 123.5, 124.3, 125.1, 130.5, 131.5, 133.1, 134.4, 135.7, 138.2, 142.7, 149.0, 151.0, 156.4, 158.6; Anal. Calcd for $C_{33}H_{22}N_{10}O_6S_2$: C, 55.15; H, 3.09; N, 19.49. Found: C, 55.11; H, 3.11; N, 19.42. MS: *m/z* 718 (M^+).

Bis[4-methoxy-3-(6-(4-nitrophenyl)[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methane (5h). Yield 74%; mp 214–216 °C; IR (KBr) ν 3081, 1604, 1596, 1552, 1520, 1471, 1370, 1069 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.91 (s, 6H, OCH_3), 4.02 (s, 2H, CH_2), 6.67 (d, *J* = 8.7 Hz, 2H, ArH), 7.41 (d, *J* = 8.6 Hz, 2H, ArH), 7.92 (d, *J* = 8.3 Hz, 4H, ArH), 8.30 (d, *J* = 8.3 Hz, 4H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.1, 54.1, 118.5, 123.4, 125.1, 129.0, 130.6, 133.2, 134.7, 138.2, 138.8, 149.2, 149.8, 156.5, 158.6; Anal. Calcd for $C_{33}H_{22}N_{10}O_6S_2$: C, 55.15; H, 3.09; N, 19.49. Found: C, 55.10; H, 3.05; N, 19.45. MS: *m/z* 718 (M^+).

Bis[4-methoxy-3-(6-benzyl[1,2,4]triazolo[3,4-*b*] [1,3,4] thiadiazol)phenyl]methane (5i). Yield 69%; mp 188–190 °C; IR (KBr) ν 3062, 2958, 1605, 1592, 1550, 1520, 1470, 1069 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.82 (s, 4H, CH_2), 3.91 (s, 6H, OCH_3), 4.00 (s, 2H, CH_2), 6.70–6.67 (m, 6H, ArH), 7.30–7.40 (m, 8H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 36.3, 42.1, 54.1, 118.4, 123.7, 125.7, 129.1, 130.0, 130.4, 133.1, 134.7, 137.8, 139.5, 152.7, 156.5, 160.2; Anal. Calcd for $C_{35}H_{28}N_8O_2S_2$: C, 64.01; H, 4.30; N, 17.06. Found: C, 64.06; H, 4.35; N, 17.00. MS: *m/z* 656 (M^+).

Bis[4-methoxy-3-(6-(4-chlorobenzyl)[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazol)phenyl]methane (5j). Yield 71%; mp 190–192 °C; IR (KBr) ν 3064, 2966, 1605, 1592, 1552, 1519, 1468, 1070, 686 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.82 (s, 4H, CH_2), 3.91 (s, 6H, OCH_3), 4.00 (s, 2H, CH_2), 6.70–6.67 (m, 6H, ArH), 7.40–7.50 (m, 6H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 36.4, 42.1, 54.1, 118.7, 123.7, 127.4, 130.4, 132.6, 133.0, 134.0, 134.6, 137.0, 137.8, 151.6, 156.5, 160.1; Anal. Calcd for $C_{35}H_{26}Cl_2N_8O_2S_2$: C, 57.93; H, 3.61; N, 15.44. Found: C, 58.00; H, 3.57; N, 15.40. MS: *m/z* 726 (M^+).

Bis[4-methoxy-3-(6-(3-pyridyl)[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazol)phenyl]methane (5k). Yield 78%; mp 184–186 °C; IR (KBr) ν 3060, 2980, 1603, 1594, 1555, 1520, 1469, 1069 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.91 (s, 6H, OCH_3), 4.00 (s, 2H, CH_2), 6.65 (d, J = 8.7 Hz, 2H, ArH), 7.30–7.40 (m, 4H, ArH), 7.81 (d, J = 7.9 Hz, 2H, ArH), 8.60–8.80 (m, 4H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.1, 54.1, 118.4, 123.7, 124.7, 130.3, 133.0, 134.1, 134.5, 137.1, 137.8, 150.0, 151.9, 154.0, 156.5, 159.2; Anal. Calcd for $C_{31}H_{22}N_{10}O_2S_2$: C, 59.04; H, 3.52; N, 22.21. Found: C, 59.00; H, 3.47; N, 22.15. MS: *m/z* 630 (M^+).

Bis[4-methoxy-3-(6-(2-pyrazyl)[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazol)phenyl]methane (5l). Yield 71%; mp 192–194 °C; IR (KBr) ν 3035, 2981, 1602, 1594, 1552, 1519, 1469, 1069 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.91 (s, 6H, OCH_3), 4.02 (s, 2H, CH_2), 6.65 (d, J = 8.7 Hz, 2H, ArH), 7.42 (d, J = 8.6 Hz, 2H, ArH), 8.80–8.90 (m, 6H, ArH), 9.42 (s, 2H, ArH); ^{13}C NMR (DMSO-d_6): δ 42.1, 54.1, 118.3, 123.6, 130.4, 133.1, 134.6, 137.8, 144.2, 145.0, 146.3, 150.0, 156.0, 156.7, 158.7; Anal. Calcd for $C_{29}H_{20}N_{12}O_2S_2$: C, 55.05; H, 3.19; N, 26.57. Found: C, 54.98; H, 3.24; N, 26.51. MS: *m/z* 632 (M^+).

6. Conclusions

A new series of bis[4-methoxy-3-(6-aryl[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol)phenyl]methanes **5a–l** has been synthesized and evaluated for their antimicrobial activity against various bacterial and fungal strains. The screened compounds **5e**, **5f**, **5h**, **5i**, **5k** and **5l** exhibited potent antimicrobial activity compared to standard drug at the tested concentrations. Most of the other compounds also showed appreciable activity against the test bacteria and fungi, and emerged as potential molecules for further development.

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Povzetek

Povzetek: Pripravili smo serijo novih bis[4-metoksi-3-(6-aril[1,2,4]triazolo[3,4-*b*][1,3,4]tiadiazol)fenil]metanov **5a**–**l** in jih karakterizirali z IR, ^{1}H NMR, ^{13}C NMR, MS ter z elementno analizo. Vse nove spojine smo testirali kot potencialna antibakterijska sredstva proti *Bacillus subtilis*, *Bacillus sphaericu, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klobsinella aerogenes* in *Chromobacterium violaceum* ter kot antiglivična sredstva proti *Candida albicans*, *Aspergillus fumigatus*, *Trichophyton rubrum* in *Trichophyton mentagrophytes*. Spojine **5e**, **5f**, **5h**, **5i**, **5k** in **5l** so pokazale močno aktivnost proti preizkušanim bakterijam in glivam ter so obetajoče potencialne molekule za nadaljnje modifikacije.