Scientific paper

# Synthesis of Some Novel Bis[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazine Derivatives for Antimicrobial Evaluation

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*Received: 06-02-2011*

## Abstract

A new series of novel bis[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **7a**–**j** has been synthesized by the reaction of [5,5'-methylenebis(3-methylbenzofuran-7,5-diyl)]bis[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methanone] (**6**) with a variety of phenacyl bromides in ethanol under reflux for 6 h. All the newly synthesized compounds were tested for *in vitro* activity against certain strains of bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Shigella flexneri*. Compounds **7a**, **7c** and **7g** were highly active against the entire organism employed. Compound **7c** showed the activity higher than the standard drug neomycin, and almost equal to the streptomycin. Compounds **7a**–**j** were also screened for their antifungal activity against *Aspergillus niger*, *Candida albicans*, *Aspergillus flavus* and *Rhizopus oryzae*. Compounds with methoxyphenyl moiety **7d** and dichlorophenyl moiety **7f** showed significant activity against the tested fungal strains.

**Keywords:** Bis-heterocycles, bis[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines, synthesis, antimicrobial evaluation.

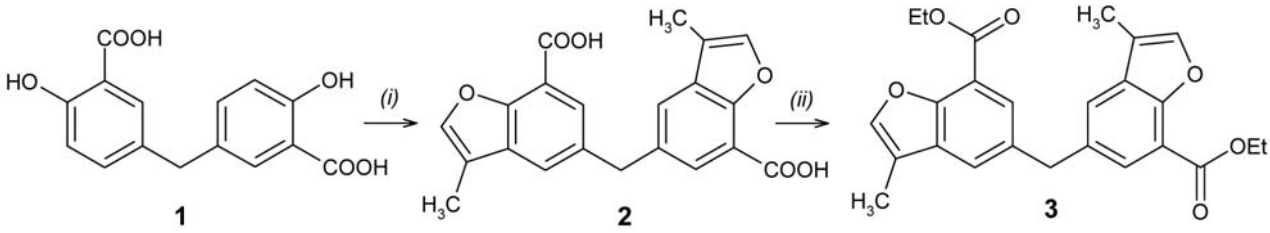
## 1. Introduction

Heterocyclic compounds[1–6] and esp. those containing sulphur and nitrogen atoms possess a wide variety of biological activities and their utility in medicine is very well established.[7–8] Further, the therapeutic effect of 1,2,4-triazole and 1,2,4-triazol-3-one containing compounds have been well studied for a number of pathological conditions including inflammation, cancer, pain, tuberculosis and hypertension.[9–17] In addition, it was reported that 1,3,4-thiadiazine exhibits various biological activities possibly due to the presence of the N–C–S moiety. Moreover, synthesis of triazoles fused to another heterocyclic ring has attracted wide-spread attention due to their diverse applications as antibacterial, antidepressant, antiviral, antitumoral, anti-inflammatory agents, pesticides, herbicides, dyes, lubricant and analytical reagents.[18,19] On the other hand, triazoles fused with six-member ring systems are also found to possess diverse applications in the field of medicine,[20–23] and the commonly known systems are triazolo-pyridines,[24] triazolo-pyridazines,[25] triazolo-pyrimidines,[26] triazolo-triazines,[27] triazolo-pyrazines,[28] and a few monomeric triazolo-thiadiazine.[29] The literature for heterocyclic pharmaceutical agents includes sulphur containing compounds, particularly those incorporating the N–C–S linkage in their skeleton, exhibit a broad spectrum of pharmacological activities such as antimalarial,[30] human immuno virus-1 (HIV-1) inhibitors[31] and antimicrobial.[32] These initial reports and our previous work on biologically active bis-heterocycles[33–38] stimulated us to integrate thiadiazine moiety in a triazole framework, since these systems possess well documented antimicrobial activity. Herein we report the synthesis of a new series of bis-triazolothiadiazines and their antimicrobial activity.

## 2. Results and Discussion

The 5,5'-methylenebis(2-hydroxybenzoic acid) (**1**), has been prepared by the procedure described in the literature.[39] The 5,5'-methylenebis(3-methylbenzofuran-7-car-

boxylic acid) (**2**),[34] intermediate for the synthesis of title compounds, has been prepared by the condensation of **1** with chloroacetone in the presence of $K_2CO_3$ and a catalytic amount of KI at reflux for 12 h followed by cyclization in alc. KOH at reflux for 18 h, in 72% yield. Compound **2** on reaction with absolute ethanol in the presence of a catalytic amount of conc. $H_2SO_4$ at reflux for 3 h, gave the diethyl 5,5'-methylenebis(3-methylbenzofuran-7-carboxylate) (**3**) in 74% yield (Scheme 1). The compounds **2** and **3** were characterized by IR, NMR, MS spectroscopy and elemental analyses.



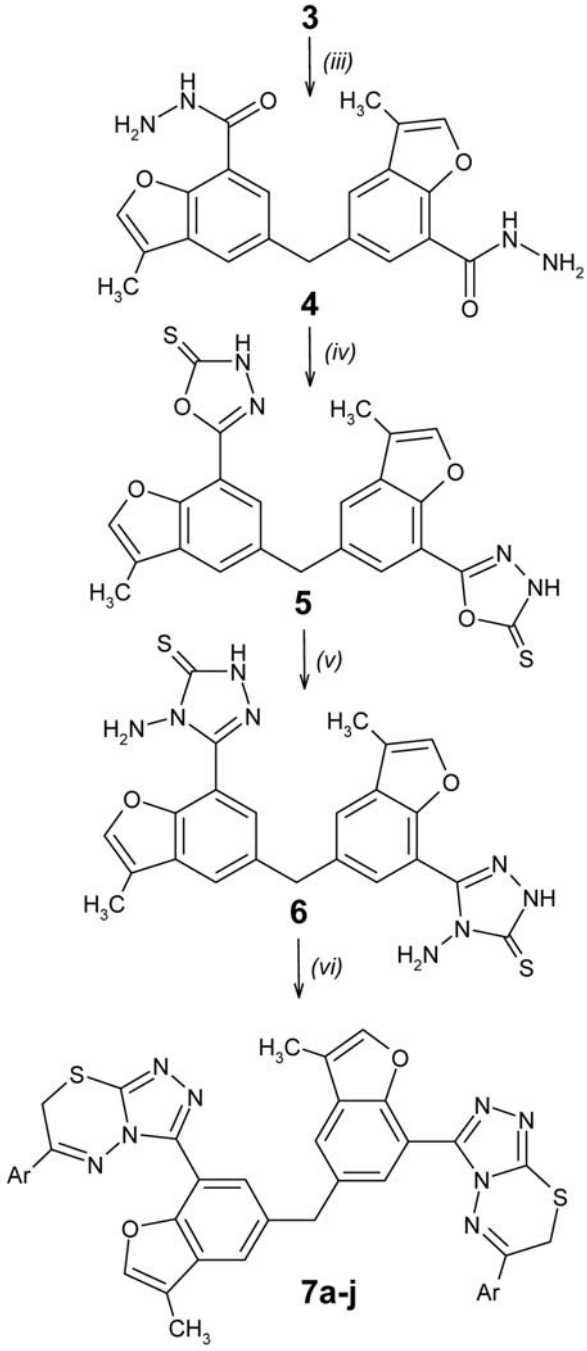

*Reagents and conditions:* (*i*) chloroacetone/$K_2CO_3$/KI/acetone, reflux, 12 h, alc. KOH, reflux 18 h, 72%; (*ii*) EtOH/$H_2SO_4$, reflux 3 h, 74%.

**Scheme 1.** Synthetic route of diethyl 5,5'-methylenebis(3-methylbenzofuran-7-carboxylate) (3)

The IR spectrum of **2** showed characteristic carboxylic acid peak at 1695 $cm^{-1}$ and also the skeletal C–O–C peak of the furan ring at 1043 $cm^{-1}$, while no absorption in the region 3500–3300 $cm^{-1}$ assignable to hydroxyl groups of the starting material **1** was observed. Further, the IR spectrum of **3** showed characteristic ester peaks at 1698 and 1249 $cm^{-1}$. These data can be used to monitor the reaction progress of cyclization of phenolic OH with chloroacetone to form the furan ring, followed by esterification of the carboxylic acid. Phenolic hydroxyl groups of the starting material **1** usually give a sharp peak at δ 9.87 ppm in the $^1$H NMR spectra, so that the absence of a signal in this region and appearance of two new singlets, around 2.39 ppm attributable to $CH_3$ and 7.60 ppm attributable to C-2 proton of furan, counts in favor of the formation of the furan ring. Furthermore, in the $^1$H NMR spectrum of **3** the singlet peak of COOH group of compound **2** (around 11.4 ppm) disappeared as expected and new triplet and quartet peaks attributable to $CH_3$ and $CH_2$ protons appeared around 1.24 and 4.00 ppm, respectively. These signals are confirming the formation of ester derivative **3**. Methyl protons of **3** were located at 2.40 ppm and methylene bridge protons between two phenyl rings were seen around 4.18 ppm. The other aromatic proton signals appeared in the expected region. In the $^{13}$C NMR spectra of compounds **2** and **3** it is obvious that these compounds posses certain symmetry elements and therefore the number of signals observed in the $^{13}$C NMR is lesser than the number of C atoms in the compound **2** and **3**. Further, the signals corresponding to the furan ring in compounds **2** and **3** were observed around δ 119.1 (C-3), 130.5–132.9 (C-3a), 141.5–143.9 (C-2), 151.4–152.7 (C-7a), these signals confirm the formation of the furan ring. The $^{13}$C NMR spectrum of **3** showed signals at δ 16.7 and 64.0 ppm attributable to $CH_3$ and $CH_2$ carbons of ethyl ester group. In brief, spectroscopic data were in complete agreement with those expected.

The intermediate, 5,5'-methylenebis(3-methylbenzofuran-7-carbohydrazide) (**4**) was prepared via hydrazinolysis of **3** with hydrazine hydrate in ethanol at reflux for 4 h, with 70% yield. The compound **4** on reaction with carbon disulfide in the presence of potassium hydroxide in ethanol at reflux for 12 h, followed by acidification afforded the [5,5'-methylenebis(3-methylbenzofuran-7,5-diyl)]bis[(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl) methanone] (**5**) in 71% yield. Further, compound **5** when reacted with hydrazine hydrate in ethanol at reflux for 6 h, resulted in the [5,5'-methylenebis(3-methylbenzofuran-7,5-diyl)]bis[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methanone] (**6**) in 68% yield. Compound **6** has been condensed successively with a variety of phenacyl bromides in ethanol under reflux for 6 h to get the title compounds bis[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazines **7a**–**j** (Scheme 2).

The compounds **4**–**7** were characterized by IR, NMR, MS spectroscopy and elemental analyses. Spectroscopic data were in complete agreement with those expected. IR spectrum of **4** has the amide C=O and $NH_2$ stretching bands at 1670 and 3223–3110 $cm^{-1}$, respectively. The disappearance of amide C=O and $NH_2$ stretching bands of **4** and appearance of strong C–O–C, C=S and C=N stretching bands at about 1133, 1238 and 1612 $cm^{-1}$, respectively, are the evidence for ring closure into **5**. The disappearance of C–O–C stretching band of **5** and appearance of strong $NH_2$ band at 3412–3400 $cm^{-1}$ are evidence for conversion of **5** to **6**. The absence of absorption bands due to $NH_2$ and NH of **6** and the presence of strong C–S–C bands in the region 750–760 $cm^{-1}$ clearly indicates the fusing between **6** and the phenacyl bromides into bis[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **7a**–**j**. In $^1$H NMR spectra all protons showed expected chemical shifts and integrals. Aromatic methyl, methylene and aromatic ring protons were seen at 2.29–2.50, 3.99–4.25 and




**7**: Ar = **a**) phenyl; **b**) 4-methylphenyl; **c**) 4-methoxyphenyl; **d**) 3-methoxypheyl; **e**) 4-chlorophenyl; **f**) 3,4-dichlorophenyl; **g**) 4-bromophenyl; **h**) 4-nitrophenyl; **i**) 3-nitrophenyl; **j**) 4-hydroxyphenyl

*Reagents and conditions:*
(*iii*) $NH_2$-$NH_2$·$H_2O$/EtOH, reflux 4 h, 70%;
(*iv*) $CS_2$/KOH/EtOH, reflux, 12 h, 71%;
(*v*) $NH_2$-$NH_2$·$H_2O$/EtOH, reflux 6 h, 68%;
(*vi*) Ar-$COCH_2Br$/EtOH, reflux 6 h, 62–72%.

**Scheme 2.** Synthetic route of bis[1,2,4]triazolo[3,4-*b*][1,3,4]thia-Diazines **7**

6.90–7.50 ppm, respectively. The NH proton of **5** and **6** was observed at about 9.10–9.15 ppm, and the $NH_2$ protons of **6** were seen at 3.71 ppm. In the $^1$H NMR spectra of compounds **7a**–**j** disappearance of signals corresponding to the $NH_2$ at 3.71 ppm and NH at 9.15 ppm as well as the appearance of the singlet at 3.98 ppm for four protons of $CH_2$–S confirms the cyclization involving the $NH_2$ and C=S groups. In the $^{13}$C NMR spectra of compounds **7a**–**j** the signals of $CH_2$–S and C=N of thiadiazine were observed at about 34.3 and 157.3 ppm, respectively. The signals of triazole ring were observed at about 145.0 and 172.0 ppm.

## 3. Antimicrobial Evaluation

***Antibacterial Activity:*** All the newly synthesized compounds **7a**–**j** were screened for their antibacterial activity against four human pathogenic bacteria *viz*., *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Shigella flexneri*. The zone of inhibition (in mm) at 100 μg/mL concentration was determined using the cup-plate method.[40] For the antibacterial assay standard inoculums (1–2 × $10^7$ c.f.u/mL 0.5 Mc Farland 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 a 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 drugs streptomycin and neomycin and the results are presented in Table 1.

Antibacterial screening data of compounds **7a**–**j** showed that the compounds containing the phenyl moiety (**7a**), methoxyphenyl moiety (**7c**) and bromophenyl moiety (**7g**) at the position 3 of triazolo-thiadiazine ring, were highly active against all the organisms employed. Compound **7c** is highly active against all the test organisms employed and the zone of inhibition is greater than the standard drug neomycin, and is almost equal to the streptomycin. Compounds containing methylphenyl moiety (**7b**) and nitrophenyl moiety (**7i**) at the position 3 of triazolo-thiadiazine ring showed good inhibition against the *E. coli*. Other compounds showed moderate to good activity against the organisms employed. The comparison of zone of inhibition (mm) of the compounds **7a**, **7c** and **7g** with the neomycin against different bacteria is presented in Figure 1.

***Antifungal Activity:*** The compounds **7a**–**j** were also screened for their antifungal activity against *Aspergillus niger*, *Candida albicans*, *Aspergillus flavus* and *Rhizopus oryzae* at a concentration of 500 μg/mL using cup-plate method.[41] The antifungal activity of these compounds was

**Table 1.** Antibacterial activity of compounds **7a–j**

| Compound | Zone of inhibition (mm) at 100 µg/mL | | | |
|---|---|---|---|---|
| | *E. coli* | *K. pneumoniae* | *S. dysenteriae* | *S. flexneri* |
| **7a** | 22 | 21 | 20 | 25 |
| **7b** | 21 | 17 | 17 | 19 |
| **7c** | 27 | 26 | 27 | 26 |
| **7d** | 18 | 14 | 14 | 15 |
| **7e** | 16 | 18 | 18 | 13 |
| **7f** | 15 | 14 | 18 | 11 |
| **7g** | 20 | 21 | 22 | 23 |
| **7h** | 17 | 15 | 13 | 12 |
| **7i** | 23 | 16 | 15 | 12 |
| **7j** | 12 | 12 | 17 | 15 |
| Streptomycin | 30 | 30 | 30 | 30 |
| Neomycin | 20 | 20 | 20 | 20 |

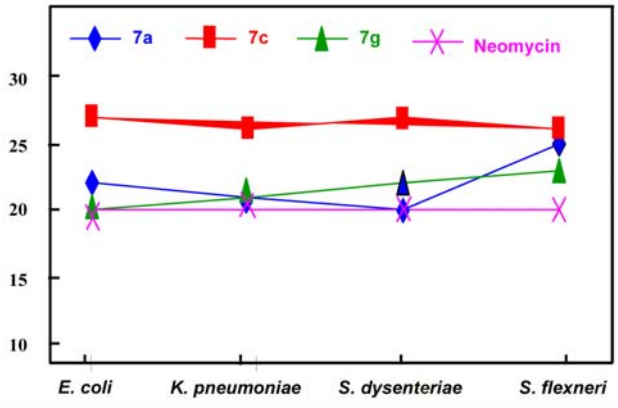



**Fig. 1.** Comparison of antibacterial activity (zone of inhibition, mm) of selected compounds and neomycin

**Table 2.** Antifungal activity of compounds **7a–j**

| Compound | Zone of inhibition (mm) at 100 µg/mL | | | |
|---|---|---|---|---|
| | *A. niger* | *C. albicans* | *A. flavus* | *R. oryzae* |
| **7a** | 18 | 17 | 18 | 14 |
| **7b** | 18 | 17 | 19 | 16 |
| **7c** | 24 | 20 | 21 | 19 |
| **7d** | 25 | 27 | 23 | 22 |
| **7e** | 22 | 16 | 15 | 19 |
| **7f** | 24 | 24 | 22 | 21 |
| **7g** | 16 | 12 | 14 | 17 |
| **7h** | 13 | 17 | 14 | 14 |
| **7i** | 11 | 17 | 17 | 13 |
| **7j** | 15 | 14 | 15 | 16 |
| Griseofulvin | 32 | 32 | 30 | 32 |

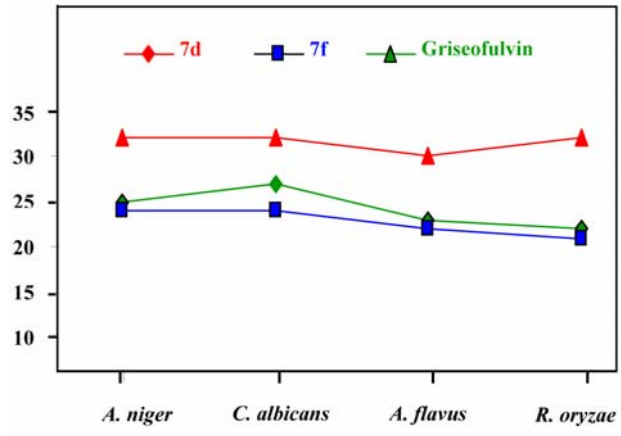



**Fig. 2.** Comparison of antifungal activity (zone of inhibition, mm) of selected compounds and Griseofulvin

compared with the standard drug griseofulvin. The zones of inhibition formed were measured in mm and are presented in Table 2. Antifungal screening data of **7a–j** reveal that the compounds with methoxyphenyl moiety (**7d**) and dichlorophenyl moiety (**7f**) at the position 3 of triazolothiadiazine ring are showing significant activity against all the fungal strains. Compound containing methoxyphenyl moiety (**7c**) is highly active against *A. niger*, *C. albicans* and *A. flavus*. The other compounds showed moderate to good activity against the tested fungal strains. The comparison of zone of inhibition (mm) of the compounds **7d** and **7f** with the griseofulvin against different fungi is presented in Figure 2.

## 4. Experimental

Reagents were of commercial grade and used as supplied. 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. Chromatographic columns with 70–230 mesh silica gel were used for separations. All melting points are uncorrected and measured using Fisher–Johns apparatus. IR spectra were recorded as KBr disk 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 means of a Perkin–Elmer 240 CHN elemental analyzer, were within ±0.4% of calculated values.

**Preparation of 5,5'-methylenebis(3-methylbenzofuran-7-carboxylic acid) (2):** To a stirred solution of **1** (2.88 g, 0.01 mol), anhydrous potassium carbonate (1.40 g, 0.01 mol) and a catalytic amount of potassium iodide in 30 mL dry acetone, was added drop wise a solution of chloroace-

tone (1.75 mL, 0.022 mol) in 20 mL dry acetone at reflux temperature. Reflux was continued for 12 h. The reaction mixture was concentrated to dryness and then transferred into ice water, and the solid separated was collected by filtration. The crude product was dissolved in 100 mL 10% ethanolic potassium hydroxide and further refluxed for 18 h. The excess ethanol was then removed by distillation *in vacuo*, the reaction mixture was poured into ice-cold aq. HCl and the solid collected by filtration, purified by column chromatography using petroleum-ether (60–80 °C) as eluent to give pure **2** as yellow solid; yield 82%; mp 182–184 °C; IR (KBr) ν 3200–3100, 2953, 1695, 1043 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 2.39 (s, 6H, $CH_3$), 3.80 (s, 2H, $CH_2$), 6.85–7.60 (m, 6H, ArH), 11.41 (s, 2H, $CO_2H$); $^{13}C$ NMR ($CDCl_3$): δ 9.2, 42.7, 119.1, 121.2, 124.6, 132.0, 132.9, 135.2, 143.9, 152.7, 172.6; Anal. Calcd for $C_{21}H_{16}O_6$: C, 69.23; H, 4.43. Found: C, 69.18; H, 4.40. MS: *m/z* 365 ($M^+$+1).

**Preparation of diethyl 5,5'-methylenebis(3-methylbenzofuran-7-carboxylate) (3):** To the solution of **2** (3.64 g, 0.01 mol) in 25 mL of absolute ethanol, 2 mL conc. $H_2SO_4$ was added. The mixture was refluxed for 3 h. After completion of the reaction (TLC), the mixture was poured into ice-cold water. Crude product was collected by filtration, washed with 10% $NaHCO_3$ solution, dried and recrystallized from ethanol to get the pure product **3**; yield 74%; mp 162–164 °C; IR (KBr) ν 2943, 1698, 1621, 1513, 1249, 1034 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 1.24 (t, *J* = 3.2 Hz, 6H, $CH_3$), 2.40 (s, 6H, $CH_3$), 4.18 (s, 2H, $CH_2$), 4.00 (q, *J* = 3.2 Hz, 4H, $CH_2$), 7.20 (s, 2H, ArH), 7.80 (s, 2H, ArH), 8.12 (s, 2H, ArH); $^{13}C$ NMR ($CDCl_3$): δ 8.9, 16.7, 42.1, 64.0, 117.1, 119.1, 124.2, 127.3, 129.8, 130.5, 141.5, 151.4, 170.1; Anal. Calcd for $C_{25}H_{24}O_6$: C, 71.42; H, 5.75; N, 22.83. Found: C, 71.40; H, 5.70; N, 22.76. MS: *m/z* 419 ($M^+$ - 1).

**Preparation of 5,5'-methylenebis(3-methylbenzofuran-7-carbohydrazide) (4):** A mixture of compound **3** (4.20 g, 0.01 mol) and hydrazine hydrate (1.22 mL, 0.025 mol) in 20 mL of ethanol was refluxed for 4 h, cooled to room temperature and filtered. The crude product was recrystallized from ethanol to give the new intermediate **4**; yield 70%; mp 158–160 °C; IR (KBr) ν 3223–3110, 1670, 1610, 1395, 741 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 2.29 (s, 6H, $CH_3$), 3.71 (bs, 4H, $NH_2$), 4.25 (s, 2H, $CH_2$), 7.10–7.20 (m, 6H, ArH), 9.25 (bs, 2H, NH); $^{13}C$ NMR ($CDCl_3$): δ 8.9, 42.3, 116.3, 117.3, 121.5, 123.8, 126.9, 134.8, 141.5, 153.2, 169.3; Anal. Calcd for $C_{21}H_{20}N_4O_4$: C, 64.28; H, 5.14; N, 14.28. Found: C, 64.22; H, 5.10; N, 14.22. MS: *m/z* 392 ($M^+$).

**Preparation of [5,5'-methylenebis(3-methylbenzofuran-7,5-diyl)]bis[(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methanone] (5):** A mixture of compound **4** (3.92 g, 0.01 mol), potassium hydroxide (1.12 g, 0.02 mol) and carbon disulfide (1.8 mL, 0.03 mol) in 100 mL of ethanol was heated under reflux with stirring for 12 h. The solvent was distilled *in vacuo*, the residual mass was poured over crushed ice and the alkaline solution neutralized with 10% hydrochloric acid. The precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol to get the pure compound **5**; yield 71%; mp 146–148 °C; IR (KBr) ν 3331, 2902, 1612, 1569, 1238, 1133, 1070 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 2.38 (s, 6H, $CH_3$), 3.99 (s, 2H, $CH_2$), 7.20–7.50 (m, 4H, ArH), 7.85 (s, 2H, ArH), 9.10 (bs, 2H, NH); $^{13}C$ NMR ($CDCl_3$): δ 8.9, 41.7, 117.0, 117.9, 122.0, 125.8, 130.9, 134.3, 141.5, 150.7, 163.5, 170.8; Anal. Calcd for $C_{23}H_{16}N_4O_4S_2$: C, 57.97; H, 3.38; N, 11.76. Found: C, 57.92; H, 3.32; N, 11.75. MS: *m/z* 476 ($M^+$).

**Preparation of [5,5'-methylenebis(3-methylbenzofuran-7,5-diyl)]bis[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methanone] (6):** To a warm solution of compound **5** (4.76 g, 0.01 mol) in 50 mL of ethanol, 80% hydrazine hydrate (1.46 mL, 0.03 mol) was added drop wise and the reaction mixture was heated under reflux for 6 h. The solvent was distilled off *in vacuo,* cooled and the crystals separated were filtered, washed with cold ethanol and recrystallized from chloroform to give the pure compound **6**; yield 68%; mp 167–169 °C; IR (KBr) ν 3400, 3334, 3068, 1625, 1232, 1030 $cm^{-1}$; $^1H$ NMR ($CDCl_3$): δ 2.50 (s, 6H, $CH_3$), 3.71 (bs, 4H, $NH_2$), 3.99 (s, 2H, $CH_2$), 6.90–7.50 (m, 4H, ArH), 7.92 (s, 2H, ArH), 9.15 (bs, 2H, NH); $^{13}C$ NMR ($CDCl_3$): δ 8.9, 41.9, 117.3, 121.5, 125.5, 127.5, 130.9, 133.6, 141.7, 151.7, 152.0, 156.4; Anal. Calcd for $C_{23}H_{20}N_8O_2S_2$: C, 54.75; H, 3.99; N, 22.21. Found: C, 54.70; H, 3.90; N, 22.16. MS: *m/z* 505 ($M^+$ + 1).

**General procedure for the synthesis of bis[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines 7a–j:** A mixture of compound **6** (5.00 g, 0.01 mol) and corresponding phenacyl bromide (0.02 mol) in 20 mL of absolute ethanol, was refluxed for 6 h. The reaction mixture was concentrated and cooled to room temperature, and the remaining solvent was removed under reduced pressure, later 25 mL of diethyl ether was added and the reaction mixture was left at 0 °C for overnight. The precipitated solid was filtered off; the crude product thus obtained was purified by column chromatography, on silica gel with hexane-ethyl acetate as eluent, to afford pure compounds **7a–j**.

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis[(6-phenyl-7,8a-dihydro-1*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)methanone] (7a)**. Yield 72%; mp 182–184 °C; IR (KBr) ν 3031, 1624, 1590, 1457, 751 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.39 (s, 6H, $CH_3$), 3.98 (s, 4H, $CH_2$-S), 4.05 (s, 2H, $CH_2$), 7.40–7.60 (m, 12H, ArH), 7.80 (s, 2H, ArH), 8.12 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 9.0, 34.3, 43.7, 116.1, 117.3, 124.6, 129.0, 130.4, 130.9, 136.4, 145.0, 146.3, 157.3, 158.2, 172.0; Anal.

Calcd for $C_{39}H_{28}N_8O_2S_2$: C, 66.46; H, 4.00; N, 1590. Found: C, 66.400; H, 3.95; N, 15.84. MS: *m/z* 704 ($M^+$).

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis {[6-(4-methylphenyl)-7,8a-dihydro-1*H*-[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazin-3-yl]methanone} (7b)**. Yield 62%; mp 190–192 °C; IR (KBr) ν 3032, 1610, 1591, 1530, 1032, 756 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.31 (s, 6H, $CH_3$), 2.39 (s, 6H, $CH_3$), 3.98 (s, 4H, $CH_2$-S), 4.17 (s, 2H, $CH_2$), 7.20 (d, *J* = 7.6 Hz, 4H, ArH), 7.51 (s, 2H, ArH), 7.67 (s, 2H, ArH), 7.87 (d, *J* = 7.6 Hz, 4H, ArH), 8.92 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 8.9, 22.1, 32.2, 43.7, 117.3, 124.2, 128.2, 129.0, 130.3, 130.9, 134.5, 140.4, 141.6, 143.2, 152.9, 153.2, 163.0; Anal. Calcd for $C_{41}H_{32}N_8O_2S_2$: C, 67.19; H, 4.40; N, 15.29. Found: C, 67.12; H, 4.33; N, 15.25. MS: *m/z* 732 ($M^+$).

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis {[6-(4-methoxyphenyl)-7,8a-dihydro-1*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methanone} (7c).** Yield 70%; mp 211–213 °C; IR (KBr) ν 3035, 1620, 1596, 1535, 1070, 1030, 760 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.39 (s, 6H, $CH_3$), 3.67 (s, 6H, $OCH_3$), 3.98 (s, 4H, $CH_2$-S), 4.17 (s, 2H, $CH_2$), 6.86 (d, *J* = 8.2 Hz, 4H, ArH), 7.20 (d, *J* = 8.2 Hz, 4H, ArH), 7.50 (s, 2H, ArH), 7.67 (s, 2H, ArH), 8.92 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 8.4, 32.1, 43.3, 48.2, 113.7, 117.8, 124.7, 125.4, 130.2, 133.3, 134.3, 141.9, 143.7, 152.9, 153.2, 160.7, 163.2; Anal. Calcd for $C_{41}H_{32}N_8O_4S_2$: C, 64.38; H, 4.22; N, 14.65. Found: C, 64.32; H, 4.20; N, 14.69. MS: *m/z* 764 ($M^+$).

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis {[6-(3-methoxyphenyl)-7,8a-dihydro-1*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methanone} (7d)**. Yield 69%; mp 205–207 °C; IR (KBr) ν 3034, 1617, 1592, 1541, 1070, 1032, 755 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.39 (s, 6H, $CH_3$), 3.67 (s, 6H, $OCH_3$), 3.98 (s, 4H, $CH_2$-S), 4.16 (s, 2H, $CH_2$), 7.30-7.40 (m, 8H, ArH), 7.51 (s, 2H, ArH), 7.66 (s, 2H, ArH), 8.92 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 8.9, 32.1, 43.4, 54.8, 116.3, 117.1, 118.0, 124.7, 125.9, 130.0, 130.8, 131.7, 134.2, 141.8, 143.9, 152.2, 153.3, 161.7, 163.6; Anal. Calcd for $C_{41}H_{32}N_8O_4S_2$: C, 64.38; H, 4.22; N, 14.65. Found: C, 64.33; H, 4.24; N, 14.60. MS: *m/z* 764 ($M^+$).

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis {[6-(4-chlorophenyl)-7,8a-dihydro-1*H*-[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazin-3-yl]methanone} (7e)**. Yield 70%; mp 177–179 °C; IR (KBr) ν 3030, 1610, 1591, 1547, 1030, 754, 686 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.38 (s, 6H, $CH_3$), 3.98 (s, 4H, $CH_2$-S), 4.19 (s, 2H, $CH_2$), 7.29 (d, *J* = 8.1 Hz, 4H, ArH), 7.49 (s, 2H, ArH), 7.67 (s, 2H, ArH), 7.92 (d, *J* = 8.1 Hz, 4H, ArH), 8.92 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 8.7, 32.5, 43.5, 117.2, 123.6, 127.7, 128.8, 130.9, 133.0, 134.3, 135.0, 141.8, 143.5, 152.2, 153.7, 163.5; Anal. Calcd for $C_{39}H_{26}Cl_2N_8O_2S_2$: C, 60.54; H, 3.39; N, 14.48. Found: C, 60.50; H, 3.33; N, 14.44. MS: *m/z* 774 ($M^+$).

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis {[6-(3,4-dichlorophenyl)-7,8a-dihydro-1*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methanone} (7f)**. Yield 70%; mp 184–186 °C; IR (KBr) ν 3035, 1618, 1590, 1532, 1030, 752, 680 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.38 (s, 6H, $CH_3$), 3.98 (s, 4H, $CH_2$-S), 4.18 (s, 2H, $CH_2$), 7.40–7.55 (m, 8H, ArH), 7.92 (d, *J* = 8.6 Hz, 2H, ArH), 8.92 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 8.5, 32.3, 43.7, 117.3, 124.0, 129.2, 130.4, 131.0, 132.0, 132.5, 132.9, 134.1, 134.7, 141.8, 143.7, 152.9, 153.2, 163.5; Anal. Calcd for $C_{39}H_{24}Cl_4N_8O_2S_2$: C, 55.59; H, 2.87; N, 13.30. Found: C, 55.53; H, 2.84; N, 13.26; MS: *m/z* 842 ($M^+$).

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis {[6-(4-bromophenyl)-7,8a-dihydro-1*H*-[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazin-3-yl]methanone} (7g)**. Yield 71%; mp 196–198 °C; IR (KBr) ν 3030, 1611, 1586, 1530, 1030, 758, 586 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.39 (s, 6H, $CH_3$), 3.98 (s, 4H, $CH_2$-S), 4.18 (s, 2H, $CH_2$), 7.50–7.60 (m, 8H, ArH), 7.90 (d, *J* = 8.0 Hz, 4H, ArH), 8.92 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 8.7, 32.5, 43.4, 117.2, 124.4, 125.0, 129.4, 130.9, 131.4, 133.3, 134.3, 141.9, 143.1, 152.8, 153.3, 163.2; Anal. Calcd for $C_{39}H_{26}Br_2N_8O_2S_2$: C, 54.30; H, 3.04; N, 12.99. Found: C, 54.24; H, 3.00; N, 12.94. MS: *m/z* 862 ($M^+$).

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis {[6-(4-nitrophenyl)-7,8a-dihydro-1*H*-[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazin-3-yl]methanone} (7h)**. Yield 70%; mp 220–222 °C; IR (KBr) ν 3035, 1618, 1595, 1532, 1370, 1030, 755 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.39 (s, 6H, $CH_3$), 3.98 (s, 4H, $CH_2$-S), 4.17 (s, 2H, $CH_2$), 7.51 (s, 2H, ArH), 7.68 (s, 2H, ArH), 7.90 (d, *J* = 8.3 Hz, 4H, ArH), 8.27 (d, *J* = 8.3 Hz, 4H, ArH), 8.92 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 8.4, 32.2, 43.6, 117.3, 124.0, 126.4, 130.9, 133.4, 134.4, 135.5, 141.6, 143.7, 149.0, 152.4, 153.5, 163.1; Anal. Calcd for $C_{39}H_{26}N_{10}O_6S_2$: C, 58.94; H, 3.30; N, 17.62. Found: C, 58.91; H, 3.22; N, 17.56. MS: *m/z* 794 ($M^+$).

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis {[6-(3-nitrophenyl)-7,8a-dihydro-1*H*-[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazin-3-yl]methanone} (7i)**. Yield 66%; mp 232–234 °C; IR (KBr) ν 3035, 1614, 1590, 1520, 1370, 1030, 757 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.39 (s, 6H, $CH_3$), 3.98 (s, 4H, $CH_2$-S), 4.18 (s, 2H, $CH_2$), 7.50 (s, 2H, ArH), 7.67 (s, 2H, ArH), 7.90-8.00 (m, 8H, ArH), 8.92 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 8.5, 32.3, 43.7, 117.3, 124.0, 126.8, 127.9, 128.0, 129.8, 130.4, 134.1, 136.4, 141.8, 143.7, 148.3, 152.9, 153.2, 163.5; Anal. Calcd for $C_{39}H_{26}N_{10}O_6S_2$: C, 58.94; H, 3.30; N, 17.62. Found: C, 58.92; H, 3.24; N, 17.56. MS: *m/z* 794 ($M^+$).

**[5,5'-Methylenebis(3-methylbenzofuran-7,5-diyl)]bis{[6-(4-hydroxyphenyl)-7,8a-dihydro-1*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl]methanone} (7j)**. Yield 70%; mp 189–191 °C; IR (KBr) ν 3310, 3035, 1618, 1596, 1030, 754 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$): δ 2.39 (s, 6H, $CH_3$), 3.98 (s, 4H, $CH_2$-S), 4.17 (s, 2H, $CH_2$), 5.11 (s, 2H, OH), 6.81 (d, *J* = 8.2 Hz, 4H, ArH), 7.20 (d, *J* = 8.2 Hz, 4H, ArH), 7.50 (s, 2H, ArH), 7.67 (s, 2H, ArH), 8.92 (s, 2H, ArH); $^{13}C$ NMR (DMSO-$d_6$): δ 8.3, 32.3, 43.5, 114.5, 117.4, 123.9, 126.0, 130.1, 134.7, 135.0, 141.5, 143.3, 152.7, 153.2, 159.8, 163.2; Anal. Calcd for $C_{39}H_{28}N_8O_4S_2$: C, 63.57; H, 3.83; N, 15.21. Found: C, 63.54; H, 3.79; N, 15.16. MS: *m/z* 736 ($M^+$).

## 5. Conclusions

A series of novel bis[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazines **7a–j** has been synthesized by the reaction of [5,5'-methylenebis(3-methylbenzofuran-7,5-diyl)]bis[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methanone] (**6**) and a variety of phenacyl bromides and evaluated for their antimicrobial activity against various bacterial and fungal strains. Some of these compounds exhibit excellent antibacterial and antifungal activity and can be classified as antimicrobial agents.

## 6. Acknowledgements

The authors are grateful to the Director, Indian Institute of Chemical Technology, Hyderabad, India, for providing NMR and Mass spectral data. Financial assistance from the UGC SAP (Phase-I)-DRS Programme, New Delhi, India, is greatly acknowledged.

## 7. References

1. A. T. Çolak, F. Çolak, N. Atar, A. Olgun, *Acta Chim. Slov*. **2010**, *57*, 212–221.
2. H. M. Gaber, I. S. A. Hafiz, K. M. EISawy, S. M. Sherif, *Acta Chim. Slov*. **2010**, *57*, 230–243.
3. R. Rohini, P. M. Reddy, K. Shanker, V. Ravinder, *Acta Chim. Slov*. **2009**, *56*, 900–907.
4. E. R. Kotb, M. A. EI-Hashash, M. A. Salama, H. S. Kalf, N. A. M. A. Wahed, *Acta Chim. Slov*. **2009**, *56*, 908–919.
5. P. Štefanič Anderluh, G. Vilfan, A. Prezelj, U. Urleb, *Acta Chim. Slov*. **2009**, *56*, 669–673.
6. A. R. B. A. EI-Gazzar, H. N. Hafez, *Acta Chim. Slov*. **2008**, *55*, 359–371.
7. M. Palcut, L. Rábara, *Acta Chim. Slov*. **2009**, *56*, 845–851.
8. M. Abdel-Magid, M. Awas, M. Seada, K. Elmahdy, M. El-Sayed, *Acta Chim. Slov*. **2009**, *56*, 852–859.
9. S. G. Küçükgüzel, S. Rollas, H. Erdeniz, M. Kiraz, *Eur. J. Med. Chem.* **1999**, *34*, 153–160.
10. H. Yuksek, A. Demirbas, A. Ikizler, C. B. Johansson, C. Celik, A. A. Ikizler, *Arzn. Forsch. Drug Res*. **1997**, *47*, 405–409.
11. B. Tozkoparan, N. Gökhan, G. Aktay, E. Yeşilada, M. Ertan, *Eur. J. Med. Chem.* **2000**, *35*, 743–750.
12. A. A. Ikizler, E. Uzunali, A. Demirbas, *Indian J. Pharm. Sci.* **2000**, *5*, 289–294.
13. N. Demirbaş, R. Ugurluoglu, A. Demirbaş, *Bioorg. Med. Chem.* **2002**, *10*, 3717–3723.
14. G. Turan-Zitouni, M. Sývacý, F. S. Kiliç, K. Erol, *Eur. J. Med. Chem.* **2001**, *36*, 685–689.
15. A. Demirbas, C. B. Johansson, N. Duman, A. A. Ikizler, *Acta. Pol. Pharm. Drug Res.* **1996**, *53*, 117–122.
16. A. Ikizler, N. Demirbas, A. A. Ikizler, *J. Hetrocycl. Chem.* **1996**, *33*, 1765–1770.
17. F. Malbec, R. Milcent, P. Vicart, *J. Hetrocycl. Chem*. **1984**, *21*, 1769–1774.
18. B. S. Holla, K. N. Poojary, B. S. Rao, M. K. Shivananda, *Eur. J. Med. Chem*. **2002**, *37*, 511–517.
19. B. S. Holla, P. M. Akberali, M. K. Shivananda, *Il Farmaco*, **2001**, *56*, 919–923.
20. A. K. Sadana, Y. Mirza, K. R. Aneja, O. Prakash, *Eur. J. Med. Chem.* **2003**, *38*, 533–536.
21. J. C. Bussolari, R. P. Panzica, *Bioorg. Med. Chem.* **1999**, *7*, 2373–2379.
22. M. Koufaki, A. Detsi, E. Theodorou, C. Kiziridi, T. Calogeropoulou, A. Vassilopoulos, A. P. Kourounakis, E. Rekka, P. N. Kourounakis, C. Gaitanaki, P. Papazafiri, *Bioorg. Med. Chem.* **2004**, *12*, 4835–4841.
23. H. Srinivas, I. A. Ramakrishna, A. N. Arvind, *Tetrahedron Lett*. **2005**, *46*, 4585–4588.
24. J. L. Vennerstrom, C. K. Angerhofer, M. T. Maklet, J. A. Williams, *Antimicrob. Agents Chemother.* **1995**, *39*, 2742–2746.
25. B. Kunze, L. Pridzum, R. Jansen, E. Jurkiewicz, G. Hunsmann, G. Hofle, H. J. Reichenboch, *Antibiot*. **1993**, *46*, 1782–1786.
26. E. C. Kohn, L. A. Liotta, *US Potent,* **1991**, 637145.
27. A. J. Srivastava, S. Swarup, V. K. Saxena, *J. Indian. Chem. Soc*. **1981**, *68*, 103–108.
28. M. Kopp, J. C. Lancelot, P. Dallemagne, S. Rault, *J. Heterocycl. Chem*. **2001**, *38*, 1045–1050.
29. Z. A. Kaplancýklý, G. Turan-Zitouni, A. Özdemir, G. Revial, *Eur. J. Med. Chem.* **2008**, *43*, 155–159.
30. J. L. Vennerstrom, M. T. Maklet, C. K. Angerhofer, J. A. Williams, *Antimicrob. Agents Chemother.* **1995**, *39*, 2671–2676.
31. B. Kunze, R. Jansen, L. Pridzum, E. Jurkiewicz, G. Hunsmann, G. Hofle, H. J. Reichenboch, *Antibiot*. **1993**, *46*, 1752–1759.
32. D. D. Erol, M. D. Aytemir, N. Yuluð, *Eur. J. Med. Chem.* **1993**, *31*, 731–734.
33. C. Sanjeeva Reddy, L. Sanjeeva Rao, A. Nagaraj, *Acta Chim. Slov*. **2010**, *57*, 726–732.
34. C. Sanjeeva Reddy, D. C. Rao, V. Yakub, A. Nagaraj, *Acta Chim. Slov*. **2010**, *57*, 798–807.
35. A. Srinivas, A. Nagaraj, Ch. Sanjeeva Reddy, *Eur. J. Med. Chem*. **2010**, *45*, 2353–2358.

36. C. Sanjeeva Reddy, D. C. Rao, V. Yakub, A. Nagaraj, *Chem. Pharm. Bull*. **2010**, *58*, 805–810.
37. C. Sanjeeva Reddy, L. Sanjeeva Rao, G. Rajesh Kumar, A. Nagaraj, *Chem. Pharm. Bull*. **2010**, *58*, 1328–1331.
38. C. Sanjeeva Reddy, M. Vani Devi, M. Sunitha, A. Nagaraj, *Chem. Pharm. Bull*. **2010**, *58*, 1622–1626.
39. E. Clemmensen, A. H. C. Heitman, *J. Am. Chem. Soc*. **1911**, *33*, 733–739.
40. H. W. Seely, D. P. J. Van, Microbes in action: A laboratory manual of microbiology. Taraporevala DB Sons & Co Pvt Ltd Bombay, **1975**, p. 55.
41. A. I. Barry, The antimicrobial suspectibility test, Principles of Practices, 4th Ed Educational low- Priced Books Scheme (ELBS) **1976**, p. 80.

## Povzetek

Z reakcijo med [5,5'-metilenbis(3-metilbenzofuran-7,5-diil)]bis[(4-amino-5-tiokso-4,5-dihidro-1*H*-1,2,4-triazol-3-il)metanonom] (**6**) in nekaterimi različnimi fenacil bromidi smo v etanolu pod pogoji refluksa (6 h) pripravili serijo novih bis[1,2,4]triazolo[3,4-*b*][1,3,4]tiadiazinov **7a**–**j**. Vse nove spojine smo *in vitro* testirali na določene seve bakterij, kot so *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae* in *Shigella flexneri*. Spojine **7a**, **7c** in **7g** so bile močno aktivne proti celotnim uporabljenim organizmom. Spojina
**7c** je pokazala aktivnost, ki je bila celo večja od aktivnosti standardne učinkovine neomicin in skoraj enaka aktivnosti streptomicina. Spojinam **7a**–**j** smo tudi preverili delovanje proti različnim glivam, kot so *Aspergillus niger*, *Candida albicans*, *Aspergillus flavus* in *Rhizopus oryzae*. Spojini z metoksifenilno skupino (**7d**) in z diklorofenilno skupino (**7f**) sta pokazali opazno aktivnost proti testiranim vrstam gliv.