Scientific paper

Synthesis and Antimicrobial Activity of Bis[4-methoxy-3-(6-aryl-*7H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl) phenyl]methanes and Bis[(triazolo[3,4-*b*]thiadiazepin-3-yl) phenyl]methanes

Avula Srinivas*

Department of Chemistry, Vaagdevi Degree & PG College, Kishanpura, Warangal, Telangana, India 506001

** Corresponding author: E-mail: asvas1978@gmail.com*

Received: 14-11-2015

Abstract

A series of novel bis[4-methoxy-3-(6-aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)phenyl]methanes and bis[(triazolo[3,4-*b*]thiadiazepin-3-yl)phenyl]methanes (**5a**–**e** and **6a**–**e**) has been synthesized and characterized by IR, ^{1}H and ^{13}C NMR, MS and elemental analysis. All the newly synthesized compounds were screened for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klobsinella aerogenes* and *Chromobacterium violaceum* and antifungal activity against *Candida albicans*, *Aspergillus fumigatus*, *Trichophyton rubrum* and *Trichophyton mentagrophytes*. Compounds **5b**, **5d**, **5e**, **6b**, **6c** and **6e** exhibited potent activity against the tested bacteria and fungi, and emerged as potential molecules for further development.

Keywords: Bis[4-methoxy-3-(6-aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)phenyl]methanes, bis[(triazolo [3,4-*b*]thiadiazepin-3-yl)phenyl]methanes, organic synthesis, antibacterial activity, antifungal activity.

1. Introduction

Heterocyclic compounds represent one of the most active clasess of compounds posessing 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 antidepresant.[11] It is interesting to use 1,2,4-triazole derivatives as precusors 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 activities.[16–21] In addition, it was reported that triazole fused with a six-membered ring system is also found to possess diverse applications in the field of medicine.[22–25] The commonly known systems are triazolo-pyridines,[26] triazolo-pyridazines,[27] triazolo-pyrimidines,[28] triazolo-triazines,[29] triazolo-pyrazines,[30] triazolo- triazenes,[31] a few monomeric triazolo-thiadiazine,[31] and triazolo-thiadiazepines,[32] although there are not many triazoles fused to thiadiazines and thiadiazepines, there is a number of them that are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities.[33–37]

In recent years attention has been increasingly paid to the synthesis of bis-heterocyclic compounds which exhibit various biological activities,[38–41] including antibacterial, fungicidal, tuberculostatic and plant-growth regulating properties. Further development during the recent years[42] indicates that the bis-heterocyclic compounds display much better antibacterial activity than the monomeric counterparts.

Owing to the immense importance and varied bioactivities exhibited by triazolo-thiadiazines and thiadiazepines and in continuation of our work on biologically active heterocycles,[43–51] we were stimulated to integrate thiadiazines moieties in a triazole framework, since these systems possess well documented antimicrobial activity. In this connection, some bis-heterocyclic compounds such as bis-triazolo thiadiazines and triazolo-thiadiazepines have been synthesized and evaluated for their antibacterial and antifungal activity. For the synthesis of target com-

pounds, 4-amino-1, 2, 4-triazol-3-thione is used as an intermediate because the amino and mercapto groups are appropriate nucleophile centers for the synthesis of fused heterocyclic compounds.

2. Results and Discussion

5,5'-Methylenebis(2-hydroxybenzoic acid) (**2**), required for the synthesis of the title compounds, was prepared according to the procedure described in the literature.[52] Compound **2** on reaction with methyl 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 **4** showed two absorption bands in the region of 3335–3235 and 2596 cm^{-1} assigned to NH_2 and SH groups, two absorption bands at1554 and 1512 cm^{-1} attributable to C=N vibrations, providing a strong evidence for the formation of a triazole ring. ^1H NMR spectrum of **4** 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 of **4** 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 a variety of phenacylbromides and 1,3-diaryl-2-propanone derivatives in ethanol at reflux produced bis[4-methoxy-3-(6-aryl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)phenyl]methanes **5a**–**e** and bis[4-methoxy-3-(6,8-diaryl)-7,8-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepin-3-yl)phenyl]methanes **6a**–**e** in 65–82% yield (Scheme 1). The elemental analyses, IR, ^1H and ^{13}C NMR and MS spectral data are consistent with the assigned structures. In the IR spectra of compounds **5a**–**e** and **6a**–**e** the absence of absorption bands due to –SH and $–NH_2$ stretching frequencies of parent compound **4** revealed the fusing between compound **4** and various phenacylbromides and 1,3-diaryl-2-propanones. 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 the triazole ring. In the ^1H NMR spectra of compounds **5a**–**e** and **6a**–**e** 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}CNMR spectra the signals of triazolo-thiadiazole ring were observed at δ 158.7, 149.0 and 138.2 ppm, respectively. The other signals were observed at the expected chemicals hifts with appropriate integrals. Elemental analyses are also consistent with the structures proposed for compounds **5a**–**e** and **6a**–**e**.

3. Antibacterial Evaluation

All the newly synthesized compounds **5a**–**e** and **6a**–**e** were screened for their antibacterial activity against Gram-positive bacteria *viz. Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), and Gram-negative bacteria *viz. Klobsinella aerogenes* (MTCC 39) and *Chromobacterium violaceum* (MTCC2656) by disc diffusion method.[53] For the antibacterial assay, standard inoculums (1–2 × 107 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 zone of inhibition data are presented in Table 1. The antibacterial screening revealed that all the tested compounds **5a**–**e** showed moderate to good inhibition towards all the tested strains. Compounds **5b**, **5d**, **5e**, **6b**, **6c** and **6e** exhibited potent inhibitory activity compared to the standard drug at the tested concentrations.

4. Antifungal Evaluation

Compounds **5a**–**e** and **6a**–**e** were also evaluated for *in vitro* antifungal activity against four fungi *viz. Candida albicans* (ATCC 10231), *Aspergillus fumigates* (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 incubated at ted at 28 °C for two weeks. Spo-

res were washed three times with sterile distilled water and re-suspended in distilled water to obtain an initial inoculums size of 10^5 spores/mL. The zones of inhibition were determined and compared with the standard drug amphotericin B (Table 2). Results of antifungal activity showed that most of the new compounds, *i.e.* **5b**, **5d**, **5c**, **6b**, **6c** and **6e** were active with moderate to good activity.

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 F254 plates from Merck and were visualized either by exposure to UV light or dipping in 1% aqueous $KMnO_4$ solution. Silica gel chromatographic columns (60–120 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 FT IR 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 2M 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): δ 2.17 (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–e: A mixture of compound **4** (0.01 mol) and the corresponding phenacylbromide (0.02 mol) in absolute ethanol (20 mL), was refluxed for 6 h. The reaction mixture was concentrated and cooled to room temperature, and the remaining solvent was removed under reduced pressure, then diethyl ether (25 mL) 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 the pure compounds **5a**–**e**.

Bis[4-methoxy-3-(6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)phenyl]methane (5a): Yield 82 %; mp 230–232 °C; IR (KBr): ν_{max} 2926, 1614, 1592, 1441, 1068, 796 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.42 (s, 2H, Ar-H), 6.95–7.60 (m, 12H, Ar-H), 6.51 (d, *J* = 9.1 Hz, 2H, Ar-H), 4.22 (s, 2H, CH_2), 3.89 (s, 6H, OCH_3), 3.56 (s, 4H, CH_2-S); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 162.3, 156.6, 154.3, 143.7, 135.1, 132.7, 131.3, 130.7, 129.7, 128.4, 127.9, 123.7, 117.6, 56.1, 42.0, 31.6; Anal. Calcd for $C_{35}H_{28}N_8S_2$: C, 64.01; H, 4.30; N, 17.0. Found: C, 63.96; H, 4.33; N, 16.95. MS: *m/z* 656 (M^+).

Bis[4-methoxy-3-(6-(4-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)phenyl]methane (5b): Yield 79%; mp 211–213 °C; IR (KBr): ν_{max} 3035, 1620, 1596, 1535, 1070, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.42 (s, 2H, Ar-H), 7.37 (d, *J* = 9.1 Hz, 2H, Ar-H), 7.16 (d, *J* = 8.6 Hz, 4H, Ar-H), 6.92 (d, *J* = 8.6 Hz, 4H, Ar-H), 6.51 (d, *J* = 9.1 Hz, 2H, Ar-H), 4.02 (s, 2H, CH_2), 3.89 (s, 6H, OCH_3), 3.79 (s, 6H, OCH_3), 3.59 (s, 9H, CH_2-S); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 162.3, 158.6, 156.9, 154.1, 143.7, 135.0, 133.4, 132.6, 131.3, 127.1, 123.7, 117.6, 113.2, 56.1, 48.7, 42.0. Anal. Calcd for $C_{37}H_{32}N_8O_4S_2$: C, 62.00; H, 4.50; N, 15.63. Found: C, 62.04; H, 4.44; N, 15.58. MS: *m/z* 716 (M^+).

Bis[4-methoxy-3-(6-(4-hydroxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)phenyl]methane (5c): Yield 69%; mp 241–243 °C; IR (KBr): ν_{max} 3310, 3035, 1618, 1596, 1535, 1069, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.42 (s, 2H, Ar-H), 7.37 (d, *J* = 9.1Hz, 2H, Ar-H), 7.20 (d, *J* = 8.6 Hz, 4H, Ar-H), 6.67 (d, *J* = 8.6 Hz, 4H, Ar-H), 6.51 (d, *J* = 9.1 Hz, 2H, Ar-H), 4.02 (s, 2H, CH_2), 3.90 (s, 6H, OCH_3), 3.59 (s, 4H, CH_2-S); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 162.3, 161.4, 157.6, 154.3, 143.6, 136.1, 135.3, 132.6, 131.3, 125.0, 123.9, 117.6, 115.1,

56.2, 42.0; 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, 4.04; N, 16.30; MS: *m/z* 687 ($M^+ - 1$).

Bis[4-methoxy-3-(6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)phenyl]methane (5d): Yield 84%; mp 271–273 °C; IR (KBr): ν_{max} 3035, 1618, 1595, 1532, 1370, 1069, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.42 (s, 2H, Ar-H), 8.40 (d, *J* = 8.4 Hz, 4H, Ar-H), 7.82 (d, *J* = 8.4 Hz, 4H, Ar-H), 7.37 (d, *J* = 9.1 Hz, 2H, Ar-H), 6.51 (d, *J* = 9.1 Hz, 2H, Ar-H), 4.02 (s, 2H, CH_2), 3.90 (s, 6H, OCH_3), 3.61 (s, 4H, CH_2-S); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 162.2, 155.9, 154.1, 147.3, 143.6, 137.9, 135.1, 132.9, 132.1, 131.3, 129.4, 123.2, 117.6, 56.1, 42.0, 31.6; Anal. Calcd for $C_{35}H_{26}N_{10}O_6S_2$: C, 56.29; H, 3.51; N, 18.67. Found: C, 56.23; H, 3.49; N, 18.71; MS: *m/z* 746 (M^+).

Bis[4-methoxy-3-(6-(3,4-dichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-3-yl)phenyl]methane (5e): Yield 69%; mp 226–228 °C; IR (KBr): ν_{max} 3031, 1624, 1590, 1457, 1069, 750, 680 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.42 (s, 2H, Ar-H), 8.00 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.62 (s, 2H, Ar-H), 7.37–7.26 (m, 4H, Ar-H), 6.82 (d, *J* = 9.1 Hz, 2H, Ar-H), 4.00 (s, 2H, CH_2), 3.88 (s, 6H, OCH_3), 3.81 (s, 4H, CH_2-S); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 160.0, 157.0, 154.2, 143.7, 136.6, 135.1, 133.2, 132.0, 132.0, 131.3, 130.3, 129.6, 128.7, 123.8, 117.6, 62.0, 42.0, 33.0; Anal. Calcd for $C_{35}H_{24}Cl_4N_8O_2S_2$: C, 52.91; H, 3.04; N, 14.10. Found: C, 52.86; H, 3.00; N, 14.05; MS: *m/z* 794 (M^+).

General procedure for the synthesis of bis[4-methoxy-3-(6,8-diaryl)-7,8-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepin-3-yl)phenyl]methanes 6a–e: To a mixture of **4** (0.01 mol) and the corresponding 1,3-diaryl-2-propanone derivative (0.02 mol) in ethanol (50 mL) a few drops of glacial acetic acid was added and the reaction mixture refluxed for 5 h. At the end of the reaction, the ethanolic solution was concentrated to half of its volume under reduced pressure. The solid that separated from the concentrate was filtered and purified by column chromatography on silica gel with petrolether-ethyl acetate as eluent to afford the pure compounds **6a–e**.

Bis[4-methoxy-3-(6,8-diphenyl)-7,8-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepin-3-yl)phenyl]methane (6a): Yield 72 %; mp 210–212 °C; IR (KBr): ν_{max} 3065, 1619, 1595, 1335, 1070, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.32 (s, 2H, Ar-H), 7.69–7.66 (m, 6H, Ar-H), 7.50–7.35 (m, 12H, Ar-H), 7.24–7.16 (m, 4H, Ar-H), 6.69 (d, *J* = 9.1 Hz, 2H, Ar-H), 4.98 (dd, J_{XA} = 11.4, J_{XB} = 5.1 Hz, 2H, H_x), 4.00 (s, 2H, CH_2), 3.91 (s, 6H, OCH_3), 2.31 (dd, J_{AB} = 12.7, J_{AX} = 11.4 Hz, 2H, H_A), 2.13 (dd, J_{BX} = 5.1, J_{BA} = 12.7 Hz, 2H, H_B); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 170.0, 158.7, 152.9, 152.4, 144.0, 142.6, 133.7, 131.6, 130.5, 129.8, 129.0, 128.3, 127.7, 127.1, 126.4, 122.3, 118.1, 56.1, 55.3, 43.3, 42.0; Anal. Calcd for $C_{49}H_{40}N_8O_2S_2$: C, 70.31; H, 4.82; N, 13.39. Found: C, 70.26; H, 4.85; N, 13.32; MS: *m/z* 838 (M^+).

Bis[4-methoxy-3-(6-phenyl-8-(4-chlorophenyl)-7,8-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepin-3-yl)phenyl]methane (6b): Yield 76%; mp 223–225 °C; IR (KBr): ν_{max} 2943, 1621, 1599, 1513, 1034, 771, 694 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.50 (s, 2H, Ar-H), 7.90 (d, *J* = 8.3 Hz, 4H, Ar-H), 7.40–7.35 (m, 12H, Ar-H), 7.00 (d, *J* = 8.3 Hz, 4H, Ar-H), 6.90 (d, *J* = 9.1 Hz, 2H, Ar-H), 5.80 (dd, J_{XA} = 11.4, J_{XB} = 5.1 Hz, 2H, H_X), 3.95 (s, 2H, CH_2), 3.91 (s, 6H, OCH_3), 2.31 (dd, J_{AB} = 12.7, J_{AX} = 11.4 Hz, 2H, H_A), 2.14 (dd, J_{BX} = 5.1, J_{BA} = 12.7 Hz, 2H, H_B); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 170.0, 158.7, 152.4, 150.0, 144.0, 142.6, 137.1, 133.7, 131.6, 130.3, 129.8, 129.7, 129.0, 128.3, 127.7, 122.3, 118.1, 56.1, 55.3, 43.3, 42.0; Anal. Calcd for $C_{49}H_{38}Cl_2N_8O_2S_2$: C, 64.97; H, 4.23; N, 12.37. Found: C, 64.93; H, 4.19; N, 12.33; MS: *m/z* 906 (M^+).

Bis[4-methoxy-3-(6-phenyl-8-(4-methoxyphenyl)-7,8-dihydro[1,24]triazolo[3,4-*b*][1,3,4]thiadiazepin-3-yl)phenyl]methane (6c): Yield 81%; mp 241–243 °C. IR(KBr): ν_{max} 3061, 1620, 1596, 1534, 1070, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.32 (s, 2H, Ar-H), 7.50–7.35 (m, 12H, Ar-H), 7.11 (d, *J* = 8.4 Hz, 4H, Ar-H), 6.72 (d, *J* = 8.4 Hz, 4H, Ar-H), 6.69 (d, *J* = 9.1 Hz, 2H, Ar-H), 4.75 (dd, J_{XA} = 11.4, J_{XB} = 5.1 Hz, 2H, H_X), 4.00 (s, 2H, CH_2), 3.91 (s, 6H, OCH_3), 3.72 (s, 6H, OCH_3), 2.30 (dd, J_{AB} = 12.7, J_{AX} = 11.4 Hz, 2H, H_A), 2.14 (dd, J_{BX} = 5.1, J_{BA} = 12.7 Hz, 2H, H_B); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 170.1, 161.4, 153.4, 144.3, 143.7, 142.6, 133.7, 131.6, 129.8, 129.0, 128.4, 127.7, 122.3, 118.1, 114.5, 58.6, 56.7, 56.0, 55.2, 43.4, 42.0; Anal. Calcd for $C_{51}H_{44}N_8O_4S_2$: C, 68.28; H, 4.94; N, 12.49. Found: C, 68.25; H, 4.90; N, 12.53; MS: *m/z* 898 (M^+).

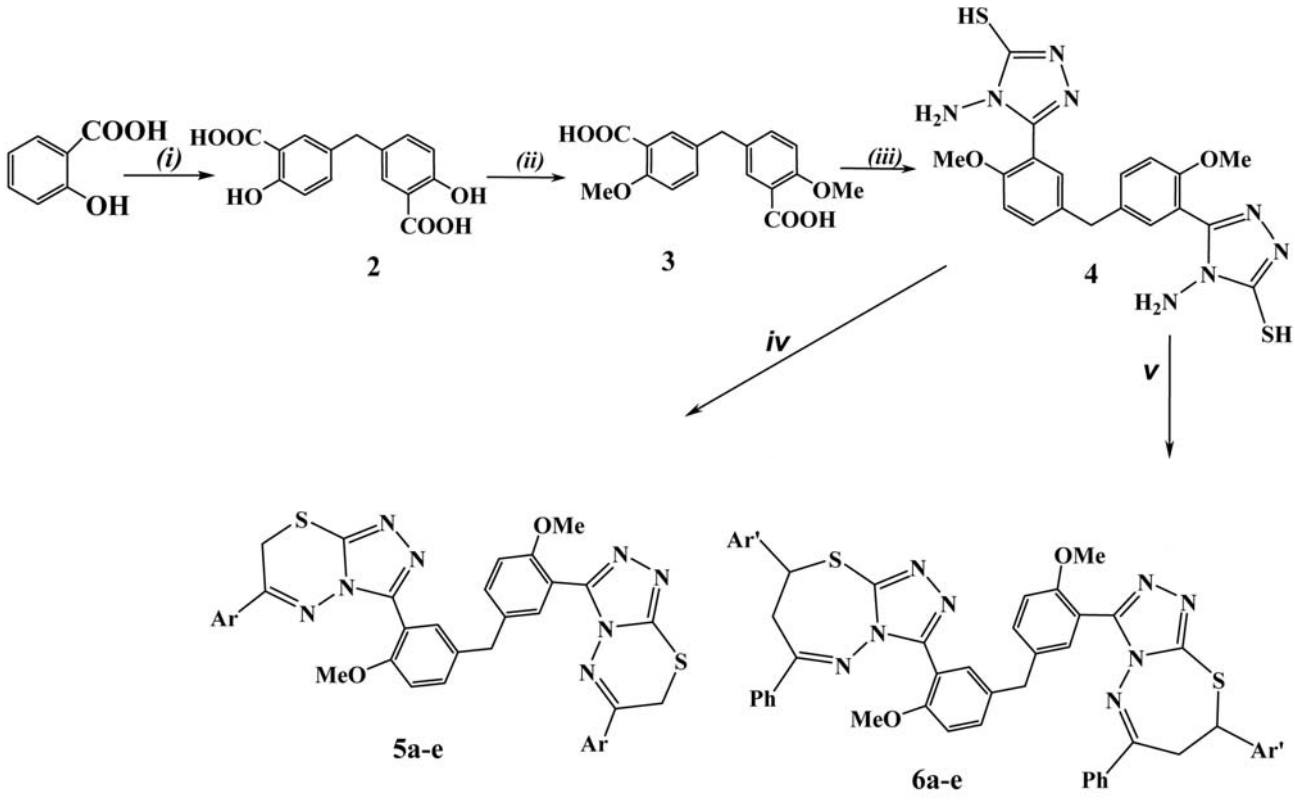
Bis[4-methoxy-3-(6-phenyl-8-(3-nitrophenyl)-7,8-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepin-3-yl)phenyl]methane (6d): Yield 69%; mp 238–240 °C; IR (KBr): ν_{max} 3059, 1618, 1595, 1530, 1370, 1068, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.32 (s, 2H, Ar-H), 8.47 (d, *J* = 7.7 Hz, 2H, Ar-H), 8.00 (s, 2H, Ar-H), 7.50–7.35 (m, 12H, Ar-H), 7.40–7.30 (m, 12H, Ar-H), 6.69 (d, *J* = 9.1 Hz, 2H, Ar-H), 4.75 (dd, J_{XA} = 11.4, J_{XB} = 5.1 Hz, 2H, H_X), 4.00 (s, 2H, CH_2), 3.91 (s, 6H, OCH_3), 2.30 (dd, J_{AB} = 12.7, J_{AX} = 11.4 Hz, 2H, H_A), 2.13 (dd, J_{BX} = 5.1, J_{BA} = 12.7 Hz, 2H, H_B); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 169.8, 157.9, 153.5, 147.9, 143.7, 142.4, 138.3, 134.4, 135.8, 131.6, 130.1, 129.8, 129.0, 128.3, 127.6, 125.0, 122.3, 121.4, 118.1, 56.1, 55.3, 43.3, 42.0; Anal. Calcd for $C_{49}H_{38}N_{10}O_6S_2$: C, 63.49; H, 4.13; N, 15.11. Found: C, 63.50; H, 4.06; N, 15.06; MS: *m/z* 928 (M^+).

Bis[4-methoxy-3-(6-phenyl-8-(3-hydroxy-4-methoxyphenyl)-7,8-dihydro[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazepin-3-yl)phenyl]methone (6e): Yield 74%; mp 249–251 °C; IR (KBr): ν_{max} 3438, 3031, 2925, 1621, 1590, 1513, 1275, 1024, 750 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.40 (s, 2H, Ar-H), 7.50–7.35 (m, 12H, Ar-H), 7.18 (d, J = 8.3 Hz, 2H, Ar-H), 7.11 (s, 2H, Ar-H), 6.69–6.58 (m, 4H, Ar-H), 4.76 (dd, J_{XA} = 11.4, J_{XB} = 5.1 Hz, 2H, H_X), 4.49 (s, 2H, OH), 4.00 (s, 2H, CH_2), 3.91 (s, 6H, OCH_3), 3.76 (s, 6H, OCH_3), 2.31 (dd, J_{AB} = 12.7, J_{AX} = 11.4 Hz, 2H, H_A), 2.13 (dd, J_{BX} = 5.1, J_{BA} = 12.7 Hz, 2H, H_B); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 169.8, 158.4, 152.7, 150.1, 147.6, 143.8, 142.1, 133.9, 131.6, 129.7, 129.2, 127.9, 127.2, 123.0, 122.3, 118.1, 117.0, 116.4, 112.1, 59.6, 56.1, 55.7, 43.3, 42.0; Anal. Calcd for $C_{51}H_{44}N_8O_6S_2$: C, 65.93; H, 4.77; N, 12.06. Found: C, 65.88; H, 4.81; N, 12.05; MS: *m/z* 930 (M^+ – 1).

Table 1. Antibacterial activity of **5a–e** and **6a–e**

Compound	Zone of inhibition in mm[a]			
	B. subtilis	*S. aureus*	*K. aerogenes*	*C. violaceum*
5a	11	12	10	12
5b	22	24	25	26
5c	12	10	12	8
5d	24	24	22	26
5e	24	24	24	25
6a	8	10	11	6
6b	20	22	20	22
6c	20	21	22	22
6d	8	11	11	12
6e	24	22	25	22
Streptomycin	25	30	30	30

[a] Streptomycin (50 μg / disc) was used as positive reference and compounds **5a–e** and **6a–e** (50 μg / disc) were screened.



Scheme 1. Synthetic route to methylene bis[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles and bis[4-methoxy-3-(6,8-diaryl)-7,8-dihydro[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazepinyl)phenyl]methane

Compounds	a	b	c	d	e
5. Ar =	C_6H_5-	4-MeO-C_6H_4-	4-HO-C_6H_4-	4-NO_2-C_6H_4-	3,4-di-Cl-C_6H_3-
6. Ar' =	C_6H_5-	4-MeO-C_6H_4-	4-HO-C_6H_4-	4-NO_2-C_6H_4-	3,4-di-Cl-C_6H_3-

Reagents and conditions: (i) CH_2O, H_2SO_4, reflux; (ii) MeI, K_2CO_3, DMF, rt; (iii) (iii) Thiocarbohydrazide, heat; (iv) $PhCOCH_3$, EtOH, reflux. (v) PhCOCH=CHAr^l, reflux.

Table 2. Antifungal activity of **5a–e** and **6a–e**

Compound (μg/mL)[a]	Minimum inhibition concentration (MIC)			
	C. albicans	*A. fumigatus*	*T. rubrumT*	*. mentagropytes*
5a	10	12	14	16
5b	22	25	15	20
5c	10	10	12	10
5d	24	22	12	22
5e	26	20	22	23
6a	16	12	10	8
6b	25	22	20	23
6c	21	24	19	25
6d	12	10	10	14
6e	24	25	20	22
Streptomycin	30	30	25	25

[a] Amphotericin (100 μg / disc) was used as positive reference and compounds **5a–e** and **6a–e** (100 μg / disc) were screened.

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 and bis[(triazolo[3,4-*b*]thiadiazepin-3-yl)phenyl]methanes **5a–e** and **6a–e** has been synthesized and evaluated for their antimicrobial activity against various bacterial and fungal strains. The screened compounds **5b**, **5d**, **5e**, **6b**, **6c** and **6e** exhibited potent antimicrobial activity compared to standard drug at the tested concentrations. Most of the other compounds also showed appreciable activity against the tested bacteria and fungi, and emerged as potential molecules for further development.

7. Acknowledgements

The author is thankful to the Director, CSIR-Indian Institute of Chemical Technology, Hyderabad, India, for providing research facilities and CSIR-New Delhi for the Reseach Associate fellowship.

8. References

1. A. T. Colak, F. Colak, N. Atar, A. Olgun, *Acta Chim. Slov.* **2010**, *57*, 212–221.
2. H. M. Gaber, I. S. A. Hafiz, K. M. ElSawy, 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. Abdel 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. E. C. Kohn, L. A. Liotta, U. S. Patent 637145; *Chem. Abstr.* **1991**, *115*, 248099 page.
8. A. J. Srivatava, S. Swarup, V. K. Saxena, *J. Indian Chem.Soc.* **1991**,*68,* 103–108.
9. R. H. G. Udupi, V. Suresh, S. R. Setty, A. R. Bhat, *J. Indian Chem. Soc.* **2000**, *77*, 303–308.
10. G. Turan-Zitouni, Z. A. Kaplancikli, K. Erol, F. S. Kiliç, *Il Farmaco* **1999**, *54*, 218–223. http://dx.doi.org/10.1016/S0014-827X(99)00016-6
11. J. M. Kane, M. W. Dudley, S. M. Sorensen, F. P. Miller, *J. Med. Chem*. **1988**, *31*, 1253–1258. http://dx.doi.org/10.1021/jm00401a031
12. G. Yao, S. Haque, L. Sha, G. Kumaravel, J. Wang, T. M. Engber, E. T. Whalley, P. R. Conlon, H. Chang, W. F. Kiesman, R. C. Petter, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 511–515. http://dx.doi.org/10.1016/j.bmcl.2004.11.062
13. C. B. Vu, P. Shields, B. Peng, G. Kumaravel, X. Jin, D. Phadke, J. Wang, T. Engber, E. Ayyub, R. C. Petter, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4835–4838. http://dx.doi.org/10.1016/j.bmcl.2004.07.048
14. A. K. Sadana, Y. Mirza, K. R. Aneja, O. Prakash, *Eur. J. Med. Chem*. **2003**, *38*, 533–536. http://dx.doi.org/10.1016/S0223-5234(03)00061-8
15. J. C. Bussolari, R. P. Panzica, *Bioorg. Med. Chem.***1999**, *7*, 2373–2379. http://dx.doi.org/10.1016/S0968-0896(99)00184-4
16. T. R. Hovsepian, E. R. Dilanian, A. P. Engoian, R. G. Melik Ohanjanian, *Khim. Get. Soed.* **2004**, *9*, 1377–1381.
17. A. Cansız, M. Koparır, A. Demirdağ, *Molecules* **2004**, *9*, 204–212. http://dx.doi.org/10.3390/90400204
18. L-X. Zhang, A-J. Zhang, X-X. Chen, X-X. Lei, X-Y. Nan, D -Y. Chen, Z-Y. Zhang, *Molecules* **2002**, *7*, 681–689. http://dx.doi.org/10.3390/70800681
19. A. A. F. Wasfy, *J. Chem. Res.* **2003**, *8*, 457–458. http://dx.doi.org/10.3184/030823403103174786
20. T. A. Abdallah, M. A. Darwish, H. M. Hassaneem, *Molecules* **2002**, *7*, 494–500. http://dx.doi.org/10.3390/70600494
21. K. Colanceska-Ragenovic, V. Dimova, V. Kakurinov, D. Gabor Molnar, A. Buzarovska, *Molecules* **2001**, *6*, 815–824. http://dx.doi.org/10.3390/61000815
22. H. Koike, N. Imanashi, Y. Natsume, S. Morooka, *Eur. J. Pharmacol., Mol. Pharmacol.* **1994**, *269,* 299–309.
23. Y. Tanabe, H. Yamamoto, M. Murakami, K. Yanagi, Y. Kubota, H. Okumura, Y. Sanimistu, G. Suzukamo, *J. Chem. Soc., Perkin Trans. I* **1995**, 935–947. http://dx.doi.org/10.1039/p19950000935
24. M. V. Diurno, O. Mazzoni, G. Correale, I. G. Monterry, A. Calignano, G. La Rana Bolognese *Il Farmaco,* **1999**, *54*, 579–583.
25. T. Previtera, M. G. Vigorita, M. Basile, F. Orsini, F. Benetollo, G. Bombieri, *Eur. J. Med. Chem.* **1994**, *29*,317–324. http://dx.doi.org/10.1016/0223-5234(94)90102-3

26. R. C. Sharma, D. Kumar, *J. Indian Chem. Soc.* **2000**, *77*, 492–496.
27. E. Piscapo, M. V. Diurno, R. Gagliardi, O. Mazzoni, *Boll. Soc. Ital. Biol. Sper*. **1989**, *65*, 853–859.
28. H. Ueno, T. Oe, I. Snehiro, S. Nakamura, US Patent 1997,5594116; *Chem. Abstr*. **1977**, *126*, 157507 page.
29. B. S. Holla, N. K. Poojary, B. S. Rao, M. K. Shivananda, *Eur. J. Med. Chem*. **2002**, *37*, 511–517. http://dx.doi.org/10.1016/S0223-5234(02)01358-2
30. B. S. Holla, P. M. Akberali, M. K. Shivananda, *Il Farmaco* **2001**, *56*, 919–927. http://dx.doi.org/10.1016/S0014-827X(01)01124-7
31. Z. A. Kaplancıklı, G. Turan-Zitouni, A. Özdemir, G. Revial, *Eur. J. Med. Chem*. **2005**, *43*, 155–159. http://dx.doi.org/10.1016/j.ejmech.2007.03.019
32. (a) M. Abbas, A. Hasson, *Chem. Pop.* **2003**, *57*, 267. (b) M. Gupta, S. Paul, R. Gupta, *Indian J. Chem.* **2009**, *48B*, 460.
33. J. L. Vennerstrom, M. T. Makler, C. K. Angerhofer, J. A. Williams, *Antimicrob. Agents Chemother.* **1995**, *39*, 2671–2677. http://dx.doi.org/10.1128/AAC.39.12.2671
34. M. Kritsanida, A. Mouroutsou, P. Marakos, N. Pouli, S. Papakonstantinou-Garoufalias, C. Pannecouque, M. Witvrouw, E. De Clercq, *Il Farmaco* **2002**, *57*, 253–257. http://dx.doi.org/10.1016/S0014-827X(01)01189-2
35. B. S. Holla, B. K.Sarojini, B. S. Rao, P. M. Akberali, N. S. Kumari, V. Shetty, *Farmaco* **2001**, *56*, 565–570. http://dx.doi.org/10.1016/S0014-827X(01)01094-1
36. F. Naser, M. Akbar, E. Sattar, B. F. Mohammad Ali, M. Hassan, *J. Chin. Chem. Soc*. **2009**, *56*, 1043–1047. http://dx.doi.org/10.1002/jccs.200900151
37. B. S. Holla, R. Gonsalves, S. Shenoy, *Il Farmaco* **1998**, *53*, 574–578. http://dx.doi.org/10.1016/S0014-827X(98)00068-8
38. H. K. Urman, O. Bulay, B. Clayson, P. Shubik, *Cancer Lett*. **1975**, *1*, 69–74. http://dx.doi.org/10.1016/S0304-3835(75)95362-8
39. D. Duksin, E. Katchalski, L. Sachs, *Proc. Natl. Acad. Sci.* **1970**, *67*, 185–192. http://dx.doi.org/10.1073/pnas.67.1.185
40. A. K. Field, A. A. Tyrell, G. P. Lampson, M. R. Hilleman, *Proc. Natl. Acad. Sci*. **1967**, *58*, 1004–1010. http://dx.doi.org/10.1073/pnas.58.3.1004
41. C. R. Lambert, M. Willheim, H. Streibel, F. Krodofter, P. Schmidt, *Experimentia* **1964**, *20*, 452–457. http://dx.doi.org/10.1007/BF02152146
42. S. Onca, M. Punar, H. Erakosy, *Chemotherapy* **2004**, *50*, 98–100. http://dx.doi.org/10.1159/000077810
43. A. Srinivas, A. Nagaraj, C. S. Reddy, *Eur. J. Med. Chem*. **2010**, *45*, 2353–2358. http://dx.doi.org/10.1016/j.ejmech.2010.02.014
44. C. S. Reddy, A. Srinivas, M. Sunitha, A. Nagaraj, *J. Heterocycl. Chem*. **2010**, *47*, 1303–130. http://dx.doi.org/10.1002/jhet.474
45. C. S. Reddy, A. Nagaraj, A. Srinivas, G. P. Reddy, *Indian J. Chem*. **2010**, *49B*, 617–622.
46. A. Srinivas, C. S. Reddy, A. Nagaraj, *Chem. Pharm. Bull.* **2009**, *57*, 685–693. http://dx.doi.org/10.1248/cpb.57.685
47. C. S. Reddy, A. Srinivas, A. Nagaraj, *J. Heterocycl. Chem.* **2009**, *46*, 497–502. http://dx.doi.org/10.1002/jhet.100
48. C. S. Reddy, A. Nagaraj, A. Srinivas, G. P. Reddy, *Indian J. Chem*. **2009**, *48B*, 248–254.
49. C. S. Reddy, A.Srinivas, A. Nagaraj, *J. Heterocyclic. Chem.* **2008**, *45*, 999–1003.
50. C. S. Reddy, A.Srinivas, A. Nagaraj, *J. Heterocyclic. Chem.* **2008**, *45*, 1121–1125.
51. C. S. Reddy, G. P. Reddy, A. Nagaraj, A. Srinivas, *Org. Commun*. **2008**, *1*, 84–94.
52. H. Clemenson, *J. Am. Chem. Soc.* **1911**, *33*, 737–742.
53. National Committee for Clinical Laboratory Standards (NCCLS). Standard methods for dilution antimicrobial susceptibility tests for bacteria, which grows aerobically. Nat.Comm. Lab. Stands. Villanova, 1982, pp. 242.

Povzetek

Pripravili smo novo serijo bis[4-metoksi-3-(6-aril-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]tiadiazin-3-il)fenil]metanov in bis[(triazolo[3,4-*b*]tiadiazepin-3-il)fenil]metanov (**5a–e** in **6a–e**) ter nove spojine karakterizirali z IR, ^{1}H in ^{13}C NMR, MS ter elementno analizo. Vsem novim spojinam smo določili tudi antibakterijsko aktivnost proti *Bacillus subtilis*, *Staphylococcus aureus*, *Klobsinella aerogenes* in *Chromobacterium violaceum* ter aktivnost proti glivam *Candida albicans*, *Aspergillus fumigatus*, *Trichophyton rubrum* in *Trichophyton mentagrophytes*. Spojine **5b**, **5d**, **5e**, **6b**, **6c** in **6e** so pokazale veliko aktivnost proti testiranim bakterijam in glivam ter so se izkazale za potencialne molekule za nadaljnji razvoj.