Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 2, 2013

Research Article

EFFICIENT CATALYTIC SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF 1,4-*BIS*(6-SUBSTITUTED-7-(2-ARYLHYDRAZONO)-7*H*-[1,2,4]TRIAZOLO[3,4-B][1,3,4]THIADIAZIN-3-YL)BENZENE DERIVATIVES USING CHITOSAN

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Received: 02 July 2012, Revised and Accepted: 25 Dec 2012

ABSTRACT

The reaction of 5, 5'-(1, 4-phenylene)*bis*(4-amino-3-mercapto-4*H*-1,2,4-triazole) **1** with hydrazonoyl halides **2A,B** gave a new series of 1,4-*bis*(6-substituted-7-(2-arylhydrazono)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene derivatives **4a-j**. An alternate method to synthesize **4a-j** was described. The structures of new compounds **4a-j** were established on the basis of their elemental analysis and IR, ^{1}H NMR, [13]C NMR and mass spectral data. All the title compounds were evaluated for their *in vitro* antimicrobial activity. All the compounds exhibited moderate to significant antibacterial and antifungal activities.

Keywords: Triazolo[3,4-b][1,3,4]thiadiazines, Hydrazonoyl halides, and Antimicrobial activities .

INTRODUCTION

1,2,4-Triazole system is an important starting material in the synthesis of biologically active heterocycles, which constitute an important class of organic compounds with diverse biological activities, including antiparasitic, analgesic, antibacterial and anti-inflammatory activities.[1-3] Moreover, the 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines are an important class of heterocycles in organic chemistry because its biological activities like, antimicrobial[1-6], anti-inflammatory[10,11], antiviral[12], anticancer[13], antitumor[14,15], antitubercular[16] activities, analgesic agents[17] and potent chemotype for the selective inhibition of PDE4[18,19], and many more. The purpose of this study is to find out novel 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives which might have potent antimicrobial activity.

MATERIAL AND METHODS

All melting points were measured on Electro thermal IA 9000 series digital melting point apparatus. The IR spectra were recorded in potassium bromide discs on a Pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometer. The NMR Spectra were recorded at 270 MHz on a Varian Mercury VX-300 NMR spectrometer. ^{1}H NMR (300 MHz) was run in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. Mass Spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyses and the biological evaluation of the products were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. All reactions were followed by TLC (Silica gel, Aluminum Sheets 60 F254, Merck). 5, 5'-(1, 4-phenylene)bis(4-amino-3-mercapto-4*H*-1,2,4-triazole) **1**[20] and hydrazonoyl halides **2A,B**[21,21] were prepared as reported in the literature.

Reactions of 5, 5'-(1, 4-phenylene)bis(4-amino-3-mercapto-4*H*-1,2,4-triazole)with hydrazonoyl halides : Equimolar amounts of **1** (0.306 g, 1mmol) and *N*-aryl-2-oxoalkanehydrazonoyl halides **2A,B** (2mmol) in dioxane (20 mL) containing chitosan (0.1 g) were heated under reflux for 6h. The hot solution was filtered to remove chitosan. The solvent was evaporated and the residue formed was collected by filtration and recrystallized from dimethylformamide to afford **4a-j**.

1,4-*Bis*(6-methyl-7-(2-phenylhydrazono)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene (4a) 72% yield, m. p. 286 °C; IR (KBr) *ν* 3176 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) *δ* 2.43 (s, 6H, 2CH$_3$), 7.21-8.17 (m, 14H, Ar-H), 10.61 (s, 2H, 2NH),[13]C NMR(DMSO-d_6)*δ* 13.4, 117.7, 121.2, 124.1, 124.8, 128.4, 136.1, 138.4, 146.4, 153.8, 156.5 ppm; MS, *m/z* (%) 590 (M$^+$, 18), 149 (40), 64 (100); Anal. Calcd for $C_{28}H_{22}N_{12}S_2$ (590.15): C, 56.93; H, 3.75; N, 28.46. Found: C, 56.73; H, 3.63; N, 28.23%.

1,4-*Bis*(6-methyl-7-(2-p-tolylhydrazono)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene (4b) 74% yield, m. p. 297 °C; IR (KBr) *ν* 3175 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) *δ* 2.28 (s, 6H, 2CH$_3$), 2.42 (s, 6H, 2CH$_3$), 7.21-8.10 (m, 12H, Ar-H), 10.60 (s, 2H, 2NH) ppm; MS, *m/z* (%) 618 (M$^+$, 26), 416 (76), 64 (100); Anal. Calcd for $C_{30}H_{26}N_{12}S_2$(618.18): C, 58.23; H, 4.24; N, 27.17. Found: C, 58.02; H, 4.11; N, 27.01%.

1,4-*Bis*(7-(2-(4-chlorophenyl)hydrazono)-6-methyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)benzene(4c). 71% yield, m. p. 311 °C; IR (KBr) *ν* 3167 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) *δ* 2.45 (s, 6H, 2CH$_3$), 7.24-8.19 (m, 12H, Ar-H), 10.64 (s, 2H, 2NH) ppm; MS, *m/z* (%) 660(M$^+$+2, 31), 658 (M$^+$, 100), 164 (34), 64 (80); Anal. Calcd for $C_{28}H_{20}Cl_2N_{12}S_2$(658.08): C, 50.99; H, 3.06; N, 25.48. Found: C, 50.68; H, 3.01; N, 25.23%.

1,4-*Bis*(7-(2-(4-methoxyphenyl)hydrazono)-6-methyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)benzene (4d) 67% yield, m. p. 288°C; IR (KBr) *ν* 3178 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6)*δ* 2.44 (s, 6H, 2CH$_3$), 3.22 (s, 6H, 2CH$_3$), 7.18-8.15 (m, 12H, Ar-H), 10.57 (s, 2H, 2NH) ppm; MS, *m/z* (%) 650 (M$^+$, 64), 249 (44), 64 (100); Anal. Calcd for $C_{30}H_{26}N_{12}O_2S_2$ (650.17): C, 55.37; H, 4.03; N, 25.83. Found: C, 55.12; H, 3.89; N, 25.72%.

1,4-*Bis*(6-methyl-7-(2-(4-nitrophenyl)hydrazono)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)benzene(4e) 75% yield, m. p. 342 °C; IR (KBr) *ν* 3176 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) *δ* 2.46 (s, 6H, 2CH$_3$), 7.24-8.33 (m, 12H, Ar-H), 10.68 (s, 2H, 2NH)ppm; MS, *m/z* (%) 680 (M$^+$, 100), 306 (30), 194 (53); Anal. Calcd for $C_{28}H_{20}N_{14}O_4S_2$ (680.12): C, 49.41; H, 2.96; N, 28.81. Found: C, 49.32; H, 2.84; N, 28.67%.

1,4-*Bis*(6-phenyl-7-(2-phenylhydrazono)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene(4f) 68% yield, m. p. 321 °C; IR (KBr) *ν* 3166 (NH) cm^{-1}; (DMSO-d_6) *δ* 6.84-8.24 (m, 24H, Ar-H), 10.62 (s, 2H, 2NH) ppm;[13]C NMR(DMSO-d_6) *δ* 117.4, 122.5, 123.3,124.5, 125.4, 128.7, 129.1, 129.9, 131.9, 137.2, 137.8, 144.4, 152.2, 155.9 ppm; MS, *m/z* (%) 714 (M$^+$, 100), 232 (36), 77 (70); Anal. Calcd for $C_{38}H_{26}N_{12}S_2$ (714.18): C, 63.85; H, 3.67; N, 23.51. Found: C, 63.76; H, 3.56; N, 23.44%.

1,4-*Bis*(6-phenyl-7-(2-p-tolylhydrazono)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene (4g) 70% yield, m. p.328 °C; IR (KBr) *ν* 3168 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) *δ* 2.28(s, 6H, 2CH$_3$), 6.88-8.18 (m, 22H, Ar-H), 10.62 (s, 2H, 2NH) ppm ; MS, *m/z* (%) 744 (M$^+$+2, 10), 742 (M$^+$, 48), 160 (42), 77 (100); Anal. Calcd for $C_{40}H_{30}N_{12}S_2$ (742.22): C, 64.67; H, 4.07; N, 22.63. Found: C, 64.55; H, 4.01; N, 22.34%.

1,4-*Bis*(7-(2-(4-chlorophenyl)hydrazono)-6-phenyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)benzene (4h) 72% yield, m. p. 331°C; IR (KBr) *ν* 3168 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6) *δ* 6.88-8.18 (m, 22H, Ar-H), 10.66 (s, 2H, 2NH) ppm ; MS, *m/z* (%) 784 (M$^+$+2, 13), 782 (M$^+$, 43), 249 (58), 77 (100); Anal. Calcd for

$C_{38}H_{24}Cl_2N_{12}S_2$ (782.11): C, 58.24; H, 3.09; N, 21.45. Found: C, 58.12; H, 3.01; N, 21.21%.

1,4-*Bis*(7-(2-(4-methoxyphenyl)hydrazono)-6-phenyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)benzene (4i) 70% yield, m. p. 318 °C; IR (KBr) *ν* 3168 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) *δ* 2.29(s, 6H, $2CH_3$), 6.98-8.18 (m, 22H, Ar-H), 10.66 (s, 2H, 2NH) ppm ; MS, *m/z* (%) 775 (M^+, 100), 248 (71), 52 (70); Anal. Calcd for $C_{40}H_{30}N_{12}O_2S_2$ (774.21): C, 62.00; H, 3.90; N, 21.69. Found: C, 61.88; H, 3.76; N, 21.45%.

1,4-*Bis*(7-(2-(4-nitrophenyl)hydrazono)-6-phenyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)benzene (4j) 74% yield, m. p. 320°C; IR (KBr) *ν* 3168 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) *δ* 7.08-8.26 (m, 22H, Ar-H), 10.78 (s, 2H, 2NH) ppm ; MS, *m/z* (%) 804 (M^+, 30), 368 (100), 232 (56), 77 (83); Anal. Calcd for $C_{38}H_{24}N_{14}O_4S_2$ (804.15): C, 56.71; H, 3.01; N, 24.36. Found: C, 56.55; H, 2.93; N, 24.31%.

Synthesis of 3a:

To a solution of 5, 5'-(1, 4-phenylene)bis(4-amino-3-mercapto-4*H*-1,2,4-triazole) **1** (0.306g, 1mmol) and hydrazonoyl halides **2Aa** (2 mmol each) in dioxane (20 mL) was added chitosan (0.1 g). The reaction mixture was allowed to stir at room temperature for 2 h, then the solution was filtered to remove chitosan, diluted with water. The solid that precipitated was filtered off, dried and crystallized from dimethylformamide to give 5,5'-(1,4-phenylene)*bis*(4-amino-4*H*-1,2,4-triazole-5,3-diyl) *bis*(2-oxo-*N*'-phenylpropanehydrazonothioate) **3a**. Yield 70 %, pale yellow solid, mp. 262 °C; IR (KBr) *ν* 3396, 3176, 3091 (NH), 1731 (CO) cm^{-1}; ^1H NMR (DMSO-d_6) *δ* 2.32 (s, 6H, $2CH_3$), 4.92 (s, 4H, $2NH_2$), 7.12-8.08 (m, 14H, Ar-H), 10.60 (s, 2H, 2NH) ppm; MS, *m/z* (%) 626 (M^+, 53), 401 (58), 306 (69), 235 (68), 155 (100), 77 (80); Anal. Calcd for $C_{28}H_{26}N_{12}O_2S_2$(626.17): C, 53.66; H, 4.18; N, 26.82. Found: C, 53.41; H, 4.02; N, 26.64 %.

Cyclization of compound 3a

To a solution of the appropriate thio-hydrazonate (**3a)** (1 mmol) in dioxane (10 mL) was added chitosan (0.1g). The reaction mixture was refluxed for about 2 h during which the reactant **3** dissolved and a new product precipitated. The latter was collected by filtration and crystallized from ethanol to give the respective **4a** proved identical with that obtained above by method A.

Synthesis of 1,4-*bis*(6-substituted-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene (7A,B) To an equimolecular amounts (0.306 g, 1 mmol) of compound **1** and phenacyl bromide or chloroacetone (2 mmol) in dioxane, chitosan (0.1g) was added. The reaction mixture was refluxed with stirring for 6 h. The hot solution was filtered to remove chitosan. The reaction mixture was cooled; the precipitated solid was filtered, washed thoroughly with water, dried and recrystallized from ethanol to afford the title compounds **7A**, **B**.

1,4-*Bis*(6-methyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene (7A) 78% yield, m. p. 238 °C; IR (KBr) *ν* 1605 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) *δ* 2.39 (s, 6H, $2CH_3$), 4.09 (s, 4H, $2CH_2$), 3.91 (s, 2H, CH_2), 7.67-7.92 (m, 4H, Ar-H) ppm; MS, *m/z* (%) 382 (M^+, 100), 127 (56), 64 (78); Anal. Calcd for $C_{16}H_{14}N_8S_2$ (382.08): C, 50.25; H, 3.69; N, 29.30. Found: C, 50.25; H, 3.69; N, 29.30%.

1,4-*Bis*(6-phenyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene (7B) 80% yield, m. p. 266 °C; IR (KBr) *ν* 1597 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) *δ* 4.28 (s, 4H, $2CH_2$), 7.32-7.96 (m, 14H, Ar-H)ppm; MS, *m/z* (%) 506 (M^+, 54), 104 (70), 77 (100); Anal. Calcd for $C_{26}H_{18}N_8S_2$ (506.11): C, 61.64; H, 3.58; N, 22.12. Found: C, 61.48; H, 3.42; N, 22.01%.

Coupling of 1,4-*bis*(6-substituted-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene (7A,B): To a solution of compounds **7A,B** (1 mmol) in pyridine (10 mL) was added the diazonium chloride solution, [prepared as usual by diazotizing aniline (2 mmol) in hydrochloric acid (6 mL, 6 M) with sodium nitrite (0.14 g, 2 mmol) in 5 mL water] portion wise with stirring and cooling. After complete addition, the reaction mixture was left for 12 hrs. The precipitate formed was collected by filtration, washed with water, dried and then recrystallized from dimethylformamide to give pure **4a-j**.

Antimicrobial Screening

Antimicrobial activity was assessed by serial two-fold dilution technique. Ciprofloxacin was used as a standard drug for antibacterial activity and clotrimazole was used as a standard drug for antifungal activity. All the compounds were dissolved in dimethylsulfoxide to give a concentration of 10μg ml^{-1}. Two-fold dilutions of test and standard compounds were prepared in double strength nutrient broth I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi). The stock solution was serially diluted to give concentrations of 25–0.78 μg ml^{-1} in nutrient broth. The inoculum size was approximately 106 colony forming units (CFU/ml). The tubes were incubated at 37± 1 °C for 24 h (bacteria) and 25 °C for 7 days (A. niger). After that, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound.

RESULTS AND DISCUSSION

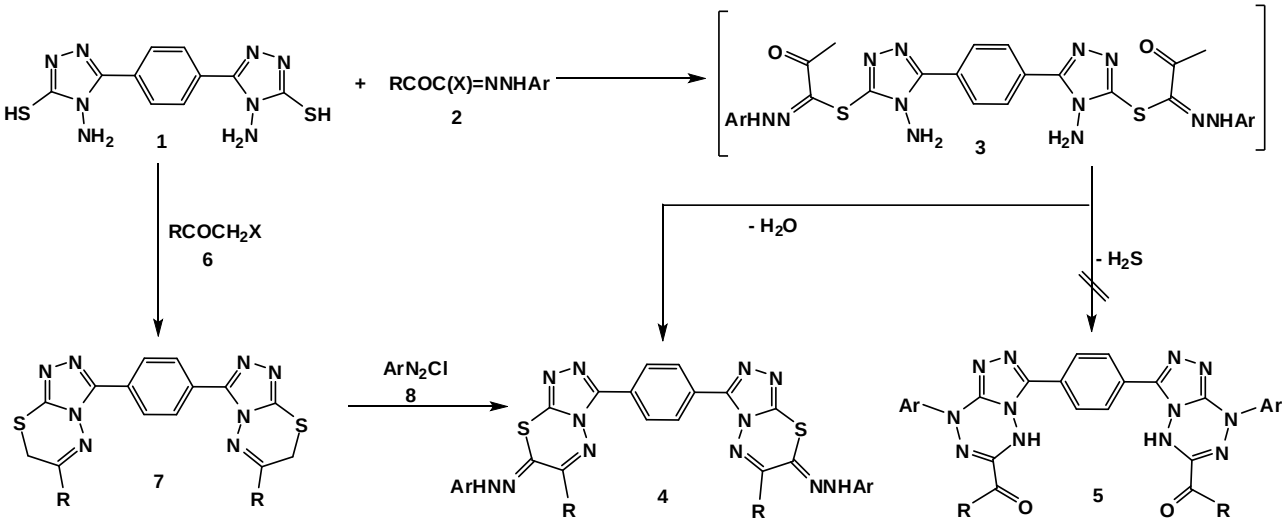
5, 5'-(1, 4-phenylene)*bis*(4-amino-3-mercapto-4*H*-1,2,4-triazole)**1** was prepared starting from diethylterphethalate with hydrazine to yield the *bis*-acid hydrazide. Treatment of the bis- acid hydrazide with carbon disulphide in ethanolic potassium hydroxide yielded the corresponding potassium dithiocarbazate, which was cyclized by heating with hydrazine to yield **1** in good yield.[20]

Reaction of 5, 5'-(1, 4-phenylene)*bis*(4-amino-3-mercapto-4*H*-1,2,4-triazole)**1** with two molar equivalents of each of hydrazonoyl halides **2A,B** in dioxane in the presence of chitosan under reflux gave in each case a single product proved to be the respective 1,4-*bis*(6-substituted-7-(2-arylhydrazono)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene derivatives **4a-j** not 1,4-*bis*(8-aryl-6-substituted-5,8-dihydro-[1,2,4]triazolo[4,3-b][1,2,4,5] tetrazin-3-yl)benzene **5a-j.**

The direct formation of **4** from **1** and **2** indicates that the initially formed bisthiohydrazonates **3** undergo *in situ* dehydrative cyclization under the employed reaction conditions to give **4** as end products (Scheme 1). The intermediacy of **3** was confirmed by their isolation and conversion into **4**. For example, reaction of **1** with **2Aa**, taken as a typical example of the series studied, in dioxane in the presence of chitosan at room temperature afforded **3a** in 70% yield. When the latter ester **3a** was refluxed in the above conditions for 2 h, it yielded the respective 1,4-*bis*(6-methyl-7-(2-phenylhydrazono)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)benzene **4a** in 81% yield (Scheme 1). The structure of the *bis*-thiohydrazonate ester **3a** was evidenced by its spectral (1H NMR, MS and IR) and elemental analysis data. For example, its mass spectrum showed the molecular ion peak at the expected *m/z* value 626.17 (53%). Also, like other aryl and heteroaryl thiohydrazonate esters, the mass spectrum of **3a** showed a characteristic peak at *m/z* 306 (69%) corresponding to the cationic fragment of **1**. The mass spectra of both aryl and heteroaryl thiohydrazonates were reported to be characterized by the presence of peaks assigned to the corresponding arenethiols and heteroarylthiols, respectively.[23,24]

The ^1H NMR spectrum of **3a** revealed two broad signals at *δ* 4.92 (4H) and 10.60 (2H) assignable to the hydrazino NH_2 and hydrazono NH protons, respectively. Both signals disappeared upon exchange with deuterium oxide. Its IR spectrum showed in addition to the NH bands at 3396 and 3091 cm^{-1}, carbonyl bands at 1731 cm^{-1}. The structure of the isolated products **4a-j** was elucidated on the basis of their spectral (MS, IR and 1H NMR) and elemental analysis data in addition to their alternate synthesis outlined below. For example, both their IR spectra revealed the absence of the amino group and they showed NH absorption in the region 3160-3190 cm^{-1}. Also, their ^1H NMR spectra revealed the absence of the signal of NH_2 protons which is present in the spectrum of **1**, but they exhibit a hydrazone NH proton signal in the region *δ* 10.1-10.8[25,26] which is extinguished by addition of deuterium oxide. Their mass spectra revealed their molecular ion peaks at the expected *m/z* values (see Experimental Section).

Alternative synthesis of **4a-j** can be shown at depicted in (Scheme 1). Thus reaction of bis-thiole **1** with chloroacetone **6A** and phenacyl bromide **6B** gave triazolo[3,4-b][1,3,4] thiadiazines **7A,B**. Coupling of **7A,B** with diazotized aniline in pyridine afforded the corresponding hydrazone derivative **4a-j**, similar in all respects (m.p.; mixed m.p., and IR) to that obtained from **1** and **2A,B**.



2, 6 : A; R = CH_3, X = Cl
B; R = Ph, X = Br

7 : A; R = CH_3, B; R = Ph

3 and 4	R \ Ar
a	CH_3 \ Ph
b	CH_3 \ 4-$CH_3C_6H_4$
c	CH_3 \ 4-ClC_6H_4
d	CH_3 \ 4-$OCH_3C_6H_4$
e	CH_3 \ 4-$NO_2C_6H_4$
f	Ph \ Ph
g	Ph \ 4-$CH_3C_6H_4$
h	Ph \ 4-ClC_6H_4
i	Ph \ 4-$OCH_3C_6H_4$
j	Ph \ 4-$NO_2C_6H_4$

Scheme 1

Antimicrobial Activity

The synthesized compounds were evaluated for their in vitro antimicrobial activity against two Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis), two Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa) and two fungal strains (Candida albicans and Aspergillus niger) by cup-plate method.[27] All the synthesized compounds (**4a–j**) showed significant antimicrobial activity, against bacterial strain, E. coli (MIC 12.5–1.56 μg ml^{-1}), P. aeruginosa (MIC 12.5–1.56 μg ml^{-1}), S. aureus (MIC 6.25–1.56 μg ml^{-1}) and B. subtilis (MIC 6.25–1.56 μg ml^{-1}) as compared to the standard drug ciprofloxacin and against fungal strain, C. albicans (MIC 6.25–1.56 μg ml^{-1}) and A. niger (12.5–1.56 μg ml^{-1}) as compared to the standard drug clotrimazole (Table 1).

Table 1: Antibacterial and antifungal activities of the tested compounds (4a-j)

Sample no.	Minimum inhibitory concentration (μg / ml sample)					
	Gram-negative		Gram-positive		Fungi	
	E. coli	P. aeruginosa	S. aureus	B. subtilis	C. albicans	A. niger
4a	6.25	6.25	6.25	3.12	3.15	6.25
4b	6.25	3.12	3.12	1.56	3.15	1.56
4c	3.12	12.5	3.12	6.25	1.56	3.12
4d	12.5	6.25	6.25	1.56	3.12	6.25
4f	6.25	3.12	1.56	6.25	3.12	1.56
4g	6.25	1.56	6.25	3.12	3.12	6.25
4h	3.12	6.25	3.12	1.56	1.56	3.12
4i	12.5	6.25	3.12	3.12	6.25	3.12
4j	6.25	12.5	6.25	6.25	1.56	1.56
Ciprofluxacin	0.01	0.25	0.15	0.12	--	--
Clotrimazole	--	--	--	--	0.10	0.30

CONCLUSIONS

In conclusion, a new series of 1,4-*bis*(6-substituted-7-(2-arylhydrazono)-7*H*-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazin-3-yl)benzene derivatives **4a-j** were synthesized and assayed for their *in vitro* antimicrobial activity. All the title compounds exhibited moderate to significant antibacterial and antifungal activities.

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