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Synthesis and antibacterial evaluation of new heterocyclic system: [1,2,4]triazolo[3′,4′:6,1]pyridazino[4,3-*e*][1,3,4]thiadiazine

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Abstract

A series of 1,2,4-triazolo[3′,4′:6,1]pyridazino[4,3-*e*][1,3,4] thiadiazines have been synthesized by intermolecular cyclization of a hydrazide derivative with various triethyl orthoesters. The antibacterial activity of various derivatives of this new heterocyclic system was evaluated.

Keywords: heterocyclization; phenylisothiocyanate; triazolopyridazinothiadiazine; triethylorthoester.

Introduction

Fused 1,2,4-triazoles express antifungal (El-Hawash et al., 2006), bactericidal (Brown and Iwai, 1979), anxiolytic (Tarzia et al., 1988), and herbicidal (Peignier et al., 1991) activities, and can be applied as antidepressants (Sarges et al., 1990). By contrast, pyridazinothiadiazines are of interest as potential inhibitors of cyclic nucleotide phosphodiesterase (Naka and Furukawa, 1979), dyestuff (Elliot, 1977), and precursors to herbicides (Nissan Chemical Industries, 1981). Therefore, the synthesis of triazolopyridazinothiadiazines could be interesting from pharmacological and synthetic point of views. Several methods for the preparation of triazoles have been reported in the literature, and most of them are based on heterocyclic hydrazones and hydrazides as precursors (Bower and Doyle, 1957; Gibson, 1963; Pollak and Tisler, 1966; Crljenak et al., 1983; Bourgeois et al., 1993; Moreau et al., 1994; Sadana et al., 2003; Kumar et al., 2004). A review of the literature also showed that there is no report for the synthesis of fused triazolopyridazinothiadiazine systems.

As part of our ongoing studies dealing with the synthesis of new biologically active heterocyclic compounds (Bakavoli et al., 2007, 2008, 2009, 2010a,b, 2011a,b,c), we now describe the synthesis of the novel heterocyclic system [1,2,4]triazolo[3′,4′:6,1]pyridazino[4,3-*e*][1,3,4] thiadiazine. The antibacterial activities of the synthesized compounds against Gram-positive and Gram-negative bacteria are also reported.

Results and discussion

Chemistry

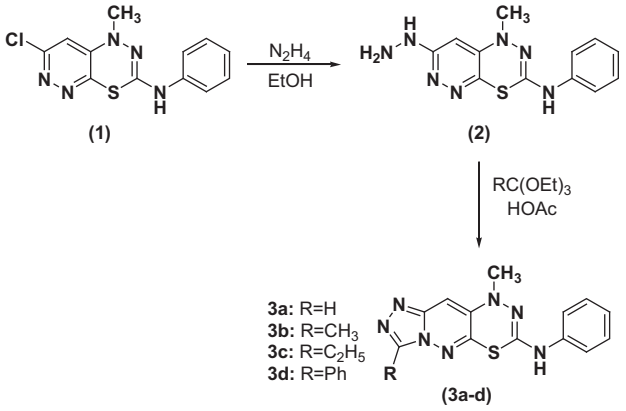
4-Bromo-3,6-dichloropyridazine, which was quantitatively prepared as previously reported (Alazawe and Elvidge, 1974), was treated with methylhydrazine in dry chloroform at room temperature to yield 3,6-dichloro-4-(1-methylhydrazino) pyridazine (Bakavoli et al., 2007). Treatment of the resulting compound with phenylisothiocyanate in the presence of triethylamine in boiling acetonitrile afforded the 3-anilino-7-chloro-1-methyl-1*H*-pyridazino[4,3-*e*][1,3,4]thiadiazine **(1)**. The reaction of compound **1** with hydrazine hydrate in refluxing ethanol gave 3-anilino-7-hydrazino-1-methyl-1*H*-pyridazino[4,3-*e*][1,3,4]thiadiazine **(2)**. This product subsequently underwent cyclocondensation with triethyl orthoesters in acetic acid under reflux conditions to give the substituted [1,2,4]triazolo[3′,4′:6,1]pyridazino[4,3-*e*][1,3,4] thiadiazine **3a–d** (Scheme 1).

All compounds **3a–d** were characterized by physical, spectral, and analytical data. For example, the ^{1}H NMR spectrum of **3b** does not show the NH_2 and NH signals of hydrazine moiety of compound **2** at δ 4.31 and 7.83 ppm, but instead exhibits a sharp signal at δ 2.54 belonging to the methyl group of the triazole ring indicating the ring closure. The IR spectrum also does not show the NH_2 and NH vibration bands of hydrazine substituent at ν 3310, 3300, and 3290 cm^{-1} of the hydrazino derivative **2**. The presence of the molecular ion peak of compound **3b** in its mass spectrum at *m/z* 311 and satisfactory microanalytical data fully support the given structure.

Antibacterial evaluations

The tested microorganisms were Gram-positive and Gram-negative bacteria. The sensitivity of the selected microorganisms, *Staphylococcus aureus* PTCC 1074, *Bacillus subtilis* PTCC 1365, *Escherichia coli* HB101 BA 7601C and *Pseudomonas aeruginosa* PTCC 1431, to compounds **3a–d** was determined using *in vitro* cultures that were dissolved in DMSO, and the tests were carried out using the disk diffusion method (Reeves and White, 1983).

Mueller-Hinton agar media were sterilized (15 min at 121°C) and poured into the plates to a uniform depth of 5 mm to solidify. The microbial suspension (1.2×10^8 CFU/mL) (0.5 McFarland Nephelometery Standards) was streaked over the surface of media using a sterile cotton swab (15 min at 180°C) to ensure confluent growth of the organisms. The tested compounds were dissolved in DMSO and the solution



Scheme 1 Synthesis of compounds **3a–d**.

was diluted with ethanol to a concentration of 100–400 µg/mL. The discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper) were impregnated with the prepared solution of compounds **3a–d**. Specifically, 1 mL of the solution was added to each bottle containing 12 discs and the discs were placed on Muller-Hinton agar media previously inoculated with bacterial suspension. The inhibition zones as a criterion for antimicrobial activity were measured in millimeters at the end of an incubation period of 24 h at 37°C. The biological activity results of compounds and streptomycin as a reference bactericidal antibiotic are shown in Table 1. These results were obtained in triplicate and the values with differences >5% were discarded and the measurement repeated. It can be concluded that compounds **3a–d** are highly active against *Bacillus subtilis* and *Staphylococcus aureus* and less active against *Pseudomonas aeruginosa* and *Escherichia coli* Gram-negative bacteria.

Experimental

The ^{1}H NMR (100 MHz, DMSO-d_6) spectra were recorded on a Bruker AC 100 spectrometer. The IR spectra (KBr discs) were obtained on a 4300 Shimadzu spectrometer. The electron impact mass spectra were recorded on a Varian Mat CH-7 at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA1112 microanalyzer.

N-(7-Chloro-1-methyl-1*H*-pyridazino[4,3-*e*][1,3,4] thiadiazin-3-yl)-*N*-phenylamine (1)

To a magnetically stirred solution of 3,6-dichloro-4-(1-methylhydrazino)pyridazine (3 mmol, 0.58 g) and triethylamine (3.1 mmol, 0.4 mL) in acetonitrile (25 mL), phenylisothiocyanate (3 mmol, 0.87 g) was added under an atmosphere of nitrogen. The solution was then heated under reflux for 4 h and the progress of the reaction was monitored by thin layer chromatography (TLC) using $CHCl_3$/MeOH (9:1). After cooling the mixture to room temperature, the resulting solid was filtered off and crystallized from ethanol to give **1** as yellow needles: yield 70%; mp 235°C; ^{1}H NMR: δ 3.22 (s, 3H), 6.91 (s, 1H), 7.02–7.53 (m, 5H), 9.42 (br s, 1H, D_2O exchangeable); IR: ν 3340, 3245, 1636, 1596, 746 cm^{-1}; MS: *m/z* 291 (M^+), 293 (M^++2). Anal. Calcd. for $C_{12}H_{10}ClN_5S$: C, 49.40; H, 3.45; N, 24.00; S, 10.99. Found, C, 49.24; H, 3.41; N, 24.03; S, 11.01.

N-(7-Hydrazino-1-methyl-1*H*-pyridazino[4,3-*e*][1,3,4] thiadiazin-3-yl)-*N*-phenylamine (2)

A solution of compound **1** (1 mmol, 0.29 g) in ethanol (15 mL) was treated with hydrazine hydrate (3 mmol) and the mixture was heated under reflux for 5–6 h. After completion of the reaction, as monitored by silica gel TLC, the solvent was removed under reduced pressure and the resulting solid was washed with water and crystallized from ethanol: yield 60%; mp 210–211°C; ^{1}H NMR: δ 3.33 (s, 3H), 4.31 (s, 2H, D_2O exchangeable), 6.24 (s, 1H), 6.81–7.62 (m, 5H), 7.83 (s, 1H, D_2O exchangeable), 9.22 (s, 1H, D_2O exchangeable); IR: ν 3310, 3300, 3290, 1600, 1550 cm^{-1}; MS: *m/z* 287 (M^+). Anal. Calcd. for $C_{12}H_{13}N_7S$: C, 50.16; H, 4.56; N, 34.12; S, 11.16. Found, C, 50.04; H, 4.41; N, 34.03; S, 11.01.

General procedure for the preparation of [1,2,4] triazolo[3′,4′:6,1]pyridazino[4,3-*e*] [1,3,4]thiadiazines 3a–d

A solution of compound **2** (1 mmol, 0.28 g) in acetic acid (2 mL) was treated with a triethyl orthoester (1.1 mmol) and the mixture was heated under reflux for 5 h. After completion of the reaction, as monitored by silica gel TLC using $CHCl_3$/MeOH (9:1) as an eluent,

Table 1 Antibacterial data of the synthesized compounds **3a–d**.

Compound	Gram-negative bacteria		Gram-positive bacteria	
	Escherichia coli HB101 BA 7601C	*Pseudomonas aeruginosa* PTCC 1431	*Staphylococcus aureus* PTCC 1074	*Bacillus subtilis* PTCC 1365
3a	15[a] (-)[b] (400)[c]	13 (+) (500)	18 (++) (100)	15 (++) (200)
3b	17 (+) (400)	12 (+) (300)	18 (++) (100)	15 (++) (300)
3c	15 (-) (300)	12 (+) (300)	17 (++) (200)	14 (++) (200)
3d	14 (-) (400)	13 (+) (400)	17 (++) (100)	14 (++) (100)
Streptomycin (standard)	17	13	15	10

[a]Zones of inhibition in millimeters.

[b](++) Highly sensitive; (+) moderately sensitive; (-) slightly sensitive.

[c]Concentration in µg/mL and the maximum inhibition zone for each compound has been shown. Discs of each concentration were placed in triplicate in Muller-Hinton agar medium seeded with fresh bacteria separately and the average was reported.

the solvent was removed under reduced pressure. Water (5 mL) was added to the residue and the mixture was neutralized using 10% $NaHCO_3$ solution. The resulting solid was filtered off, washed with water, and crystallized from ethanol.

***N*-(1-Methyl-1*H*-[1,2,4]triazolo[3′,4′:6,1]pyridazino[4,3-*e*][1,3,4]thiadiazin-3-yl)-*N*-phenylamine (3a)** Yield 60%; mp 281–282°C; ^{1}H NMR: δ 3.25 (s, 3H), 6.91–7.62 (m, 5H), 9.21 (s, 1H), 9.52 (s, 1H, D_2O exchangeable), IR: ν 3290, 1605, 1550 cm^{-1}; MS: *m/z* 297 (M^+). Anal. Calcd. for $C_{13}H_{11}N_7S$: C, 52.51; H, 3.73; N, 32.97; S, 10.78. Found, C, 52.84; H, 3.81; N, 32.59; S, 10.53.

***N*-(1,7-Dimethyl-1*H*-[1,2,4]triazolo[3′,4′:6,1]pyridazino[4,3-*e*][1,3,4]thiadiazin-3-yl)-*N*-phenylamine (3b)** Yield 55%; mp 270–271°C; ^{1}H NMR: δ 2.54 (s, 3H), 3.27 (s, 3H), 6.91–7.63 (m, 5H), 9.41 (s, 1H, D_2O exchangeable); IR: ν 3310, 1605, 1553 cm^{-1}; MS: *m/z* 311 (M^+). Anal. Calcd. for $C_{14}H_{13}N_7S$: C, 54.00; H, 4.21; N, 31.49; S, 10.30. Found, C, 54.30; H, 4.28; N, 31.53; S, 10.25.

***N*-(7-Ethyl-1-methyl-1*H*-[1,2,4]triazolo[3′,4′:6,1]pyridazino[4,3-*e*][1,3,4]thiadiazin-3-yl)-*N*-phenylamine (3c)** Yield 70%; mp 250–251°C; ^{1}H NMR: δ 1.31 (t, *J*=7.4 Hz, 3H), 2.96 (q, *J*=7.4 Hz, 2H), 3.30 (s, 3H), 6.92–7.62 (m, 5H), 9.36 (s, 1H, D_2O exchangeable); IR: ν 3305, 1610, 1500 cm^{-1}; MS: *m/z* 325 (M^+). Anal. Calcd. for $C_{15}H_{15}N_7S$: C, 55.37; H, 4.65; N, 30.13; S, 9.85. Found, C, 55.11; H, 4.48; N, 29.93; S, 9.65.

***N*-(1-Methyl-7-phenyl-1*H*-[1,2,4]triazolo[3′,4′:6,1]pyridazino[4,3-*e*][1,3,4]thiadiazin-3-yl)-*N*-phenylamine (3d)** Yield 50%; mp 245–246°C; ^{1}H NMR: δ 3.26 (s, 3H), 6.89–8.11 (m, 10H), 9.43 (s, 1H, D_2O exchangeable); IR: ν 3310, 1605, 1550 cm^{-1}; MS: *m/z* 373 (M^+). Anal. Calcd. for $C_{19}H_{15}N_7S$: C, 61.11; H, 4.05; N, 26.26; S, 8.59. Found, C, 61.03; H, 4.01; N, 26.23; S, 8.55.

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