Synthesis and Antimicrobial Activity of New Substituted Fused 1,2,4-Triazole Derivatives

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A number of new substituted 1,2,4-triazole, 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole and 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazine derivatives were synthesized and tested for their antimicrobial activity against *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* species (Actinomycetes). The synthesized compounds displayed different degrees of antimicrobial activities or inhibitory actions.

Key words: 1,2,4-Triazoles, 1,2,4-Triazolo[3,4-*b*]1,3,4-thiadiazoles, 1,2,4-Triazolo[3,4-*b*]1,3,4-thiadiazines

Introduction

Many 1,2,4-triazoles have been reported to possess antibacterial, antifungal, antiviral, anti-inflammatory, anticonvulsant, antidepressant, antitubercular, antihypertensive, analgesic, hypoglycemic, herbicidal, and sedative properties. (Palaska *et al*., 2002; Amir and Shikha, 2004; Demirbas *et al*., 2004; Colanceska-Ragenovic *et al*., 2001; Labanauskas *et al.*, 2004; Al-Soud *et al*., 2004, Jones *et al*., 1965; Unangst *et al*., 1992; Mullican *et al*., 1993; Shams El-Dine and Hazzaa, 1974; Stillings *et al.*, 1986; Kane *et al*., 1988; George *et al*., 1971; Gall *et al*., 1978) 1,3,4-Thiadiazoles exhibit a broad spectrum of biological activities, possibly due to the presence of the toxophoric NCS moiety (Omar and Aboul Wafa, 1986). They find applications as antibacterial, antitumour, and anti-inflammatory agents, pesticides, herbicides, dyes, lubricants, and analytical reagents (Kurtzer, 1965; Foroumadi *et al*., 2001; Awad and El Ashry, 1998; Varvarasou *et al*., 1998; Holla *et al*., 2002). On the other hand, 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole derivatives obtained by fusing the biolabile 1,2,4-triazole and 1,3,4-thiadiazole rings as well as their dihydro analogues have been shown to possess antimicrobial (Swamy *et al*., 2006), antibacterial (Karabasanagouda *et al*., 2007), anti-inflammatory (Vinod *et al*., 2007; Birsen *et al*., 2007), antifungal, CNS-depressant, antiviral, hypocholesteremic, analgesic, anthelmintic, and herbicidal activities (Zhang and Sun, 1998). Ribavirin, fluconazole and cefazolin are antiviral, antifungal and antibacterial drugs which contain 1,2,4-triazole and 1,3,4-thiadiazole rings. In view of the above facts and as continuation of our programme of identification of new candidates that may be valuable in designing new, potent, selective, and less toxic antimicrobial agents (Abdel-Rahman *et al.*, 2008; El-Sayed *et al.*, 2008, 2009) we report in the present work the synthesis and antimicrobial activity of new substituted 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole derivatives.

Experimental

General

Melting points were determined using a Büchi apparatus. IR spectra (KBr) were recorded with a Bruker-Vector22 instrument (Bruker, Bremen, Germany). ^{1}H NMR spectra were recorded with a Varian Gemini spectrometer at 300 MHz and 200 MHz with TMS as internal standard. Chemical shifts are reported in δ scale (ppm) relative to TMS as a standard, and the coupling constants (J values) are given in Hz. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F_{245}. EI-mass spectra were recorded with a HP D5988 A 1000 MHz instrument (Hewlett-Packard, Palo Alto, CA, USA).

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Sample preparation

Each of the test compounds and standards was dissolved in 12.5% DMSO, at concentrations of 500 μg/mL. Further dilutions of the compounds and standards in the test medium were prepared at the required quantities.

Culture of microorganisms

Bacteria strains were supplied by Botany Department, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt, namely *Bacillus subtilis* (ATCC 6633) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative), and *Streptomyces* species (Actinomycetes). The bacterial strains were maintained on MHA (Mueller-Hinton agar, 17.5 g casein hydrolysate, 1.5 g soluble starch, 1000 mL beef extract) medium (Oxoid Chemical Co., UK) for 24 h at 37 °C. The medium was molten on a water bath, inoculated with 0.5 mL of a culture of the specific microorganism and poured into sterile Petri dishes to form a layer of about 3–4 mm thickness. The layer was allowed to cool and harden. With the aid of a cork-borer, cups of about 10 mm diameter were produced (Jorgensen *et al.*, 1999).

Agar diffusion technique

The antibacterial activities of the synthesized compounds were tested against *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* species (Actinomycetes) using MH medium. A stock solution of each synthesized compound (500 μg/mL) in DMSO was prepared, and graded quantities of the test compounds were incorporated in a specified quantity of sterilized liquid MH medium. Different concentrations of the test compounds in DMF were placed separately in cups in the agar medium. All plates were incubated at 37 ºC overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined as the intercept of the graphe of logarithmic concentrations versus diameters of the inhibition zones (Janssen *et al.*, 1987; Greenwood, 2000).

Results and Discussion

Chemistry

Potassium 2-[2-(naphthalen-1-ylmethoxy)-acetyl]hydrazinecarbodithioate (**1**) was synthesized from the corresponding acid hydrazide following a reported procedure (Mathew *et al.*, 2006). Heating of **1** with hydrazine hydrate at 100 °C afforded the 1,2,4-triazole derivative **2** after acidification of the reaction mixture with HCl (Fig. 1). The 1,2,4-triazole derivative **2** was used as a key starting material for the synthesis of fused 1,2,4-triazole derivatives. Thus, reaction of **2** with phenacyl bromide in ethanol at reflux temperature gave 3-[(naphthalen-1-yloxy)methyl]-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*]1,3,4-thiadiazine (**3**) in 82% yield.

The IR spectrum of **2** showed an absorption band at 3421 cm^{-1} corresponding to the NH_2 group in addition to the disappearance of the charataristic band of the C=O group in the starting potassium salt **1**. The ^{1}H NMR spectrum revealed the presence of a CH_2 signal as a singlet at δ 4.85 ppm, a NH_2 signal at δ 5.15 ppm in addition to signals of the aromatic protons at δ 6.87–7.81 ppm and of NH as a singlet at δ 12.87 ppm. The ^{1}H NMR spectrum of **3** showed two CH_2 signals at δ 4.18 and 4.98 ppm for the SCH_2 and OCH_2 groups, respectively, in addition to the aromatic protons at δ 6.89–7.83 ppm.

Condensation of **2** with 3,4,5-trimethoxy benzaldehyde and *p*-bromobenzaldehyde in ethanol in the presence of piperdine at reflux temperature afforded the corresponding arylidine derivatives **4a** and **4b**, respectively (Fig. 1). The ^{1}H NMR spectrum of **4b** as a representative example showed the CH_2 signal as a singlet at δ 4.84 ppm, the singlet at δ 6.88 ppm, corresponding to N=C*H*, in addition to the signals of the aromatic protons at δ 6.85–7.82 ppm and of NH as a singlet at δ 12.86 ppm.

When the 1,2,4-triazole derivative **2** was allowed to react with carbon disulfide in ethanol in the presence of KOH at reflux temperature, the 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole derivative **5** was obtained in 81% yield (Fig. 2). The ^{1}H NMR spectrum of **5** showed a CH_2 signal as a singlet at δ 4.88 ppm in addition to the signals of the aromatic protons at δ 6.86–7.85 ppm and of NH at δ 12.84 ppm as a singlet. Its mass spectrum revealed the molecular ion peak at *m/z* 315 ([M^+], 55%) corresponding to the molecular formula $C_{14}H_{10}N_4OS_2$ which was in agreement with the assigned structure.

Reaction of **5** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**6**) in acetone at room temperature afforded the thioglycoside derivative

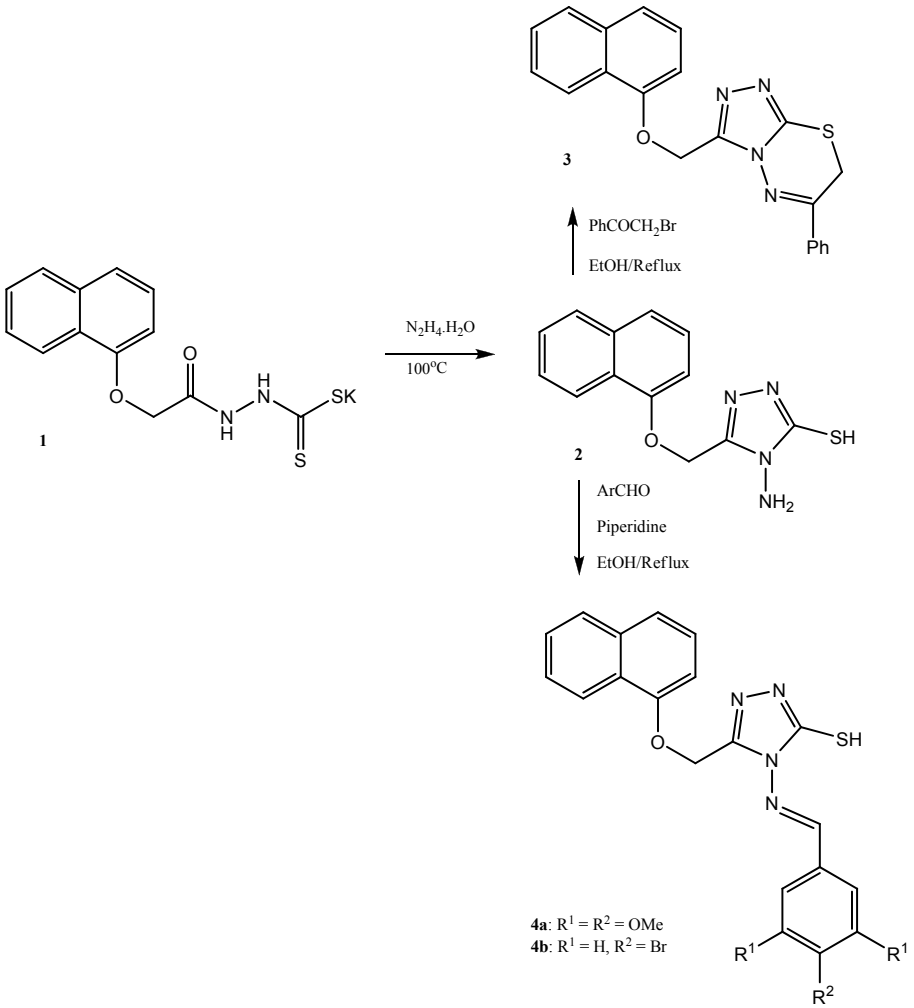

Fig. 1. Synthesis of new 1,2,4-triazole derivatives.

7 in 82% yield. Its ^{1}H NMR spectrum revealed the presence of the *O*-acetyl-methyl groups at δ 1.88–2.08 ppm, the signals of the sugar protons at δ 3.89–5.12 ppm and the anomeric proton as a doublet at δ 5.69 ppm with a coupling constant of 9.8 Hz indicating β-configuration of the thioglycosidic bond.

Deacetylation of **7** by methanolic ammonia solution at room temperature afforded the deprotected thioglycoside **8**. Its IR spectrum showed characteristic absorption bands at 3453–3472 cm^{-1} corresponding to the hydroxy groups. The ^{1}H NMR spectrum of **8** revealed the absence of the acetyl-methyl signals and instead signals corresponding to the sugar hydroxy groups appeared at δ 4.12–5.22 ppm.

Reaction of **5** with acrylonitrile in ethanol in the presence of triethyl amine at reflux temperature afforded 3-{3-[(naphthalen-2-yloxy)methyl]-6-thioxo-[1,2,4]triazolo[3,4-*b*]1,3,4-thiadiazol-5(6*H*)-yl}propanenitrile (**9**). The structure of **9** was confirmed by means of IR, ^{1}H NMR and mass spectra.

Reaction of **9** with sodium azide in DMF and in the presence of ammonium chloride at 100 °C gave the tetrazole derivative **10**. The ^{1}H NMR spectra of **9** and **10** showed signals of the two CH_2 groups, each as a triplet at δ 3.92–4.18 ppm, and

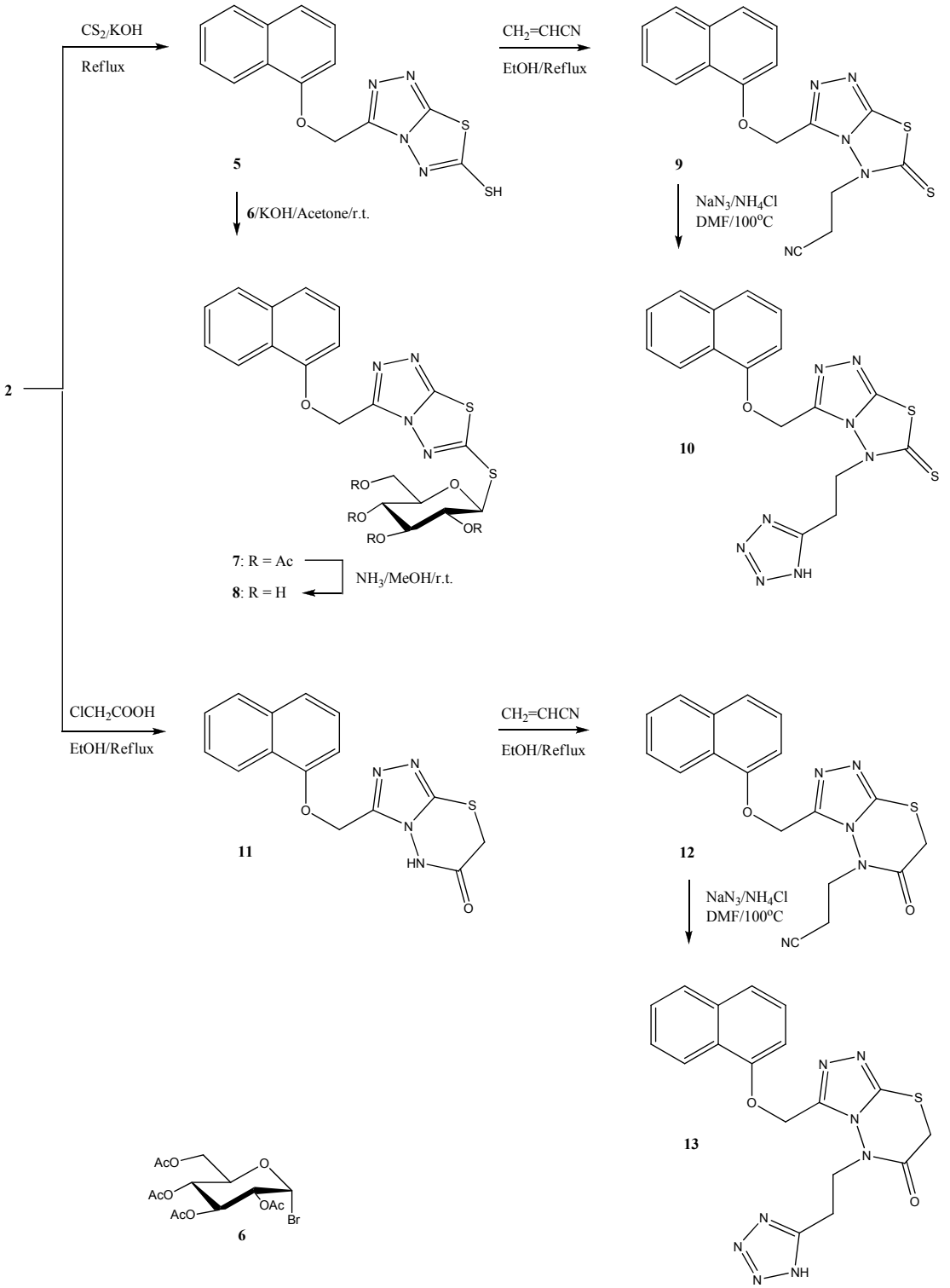

Fig. 2. Synthesis of new fused 1,2,4-triazole derivatives.

of the other two CH_2 groups, each as a singlet at δ 4.85 and 5.07 ppm, in addition to the signals of the aromatic protons at δ 6.87–7.89 ppm (Fig. 2).

When the triazole **2** was reacted with chloroacetic acid in ethanol at reflux temperature, the 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazine derivative **11** was obtained in 77% yield. The ^{1}H NMR spectrum of **11** showed the two CH_2 signals as singlets at δ 4.86 and 5.02 ppm for the SCH_2 and OCH_2 groups, respectively, in addition to the aromatic protons at δ 6.87–7.85 ppm and the NH signal at δ 11.36 ppm.

Reaction of **11** with acrylonitrile in ethanol in the presence of triethyl amine at reflux temperature afforded the *N*-substituted 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazine derivative **12**. Its IR spectrum showed a characteristic band at 2202 cm^{-1} for the CN group. The ^{1}H NMR spectrum showed the signals of two CH_2 groups, each as a triplet at δ 3.89 and 4.12 ppm, and of the other two CH_2 groups, each as a singlet at δ 4.84 and 5.05 ppm, in addition to the signals of the aromatic protons at δ 6.84–7.87 ppm.

When **12** was allowed to react with sodium azide in DMF in the presence of ammonium chloride at 100 °C, the tetrazole derivative **13** was afforded in 77% yield. Its ^{1}H NMR spectrum showed the signals of the four CH_2 groups at δ 3.96, 4.21, 4.86, and 5.12 ppm in addition to signals of the aromatic protons at δ 6.88–7.89 ppm. Its IR and mass spectra agreed with the assigned structure (Fig. 2).

Antimicrobial activity

The antimicrobial activity of the synthesized compounds was evaluated against three microorganisms; *Bacillus subtilis* (ATCC 6633) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative), and *Streptomyces* species (Actinomycetes). The values of minimal inhibitory concentrations (MICs) of the tested compounds are presented in Table I. The results of the antimicrobial activity test revealed that **5**, **11**, and **13** showed the highest activity against *B. subtilis* with MIC values of 75 μg/mL followed by compounds **2**, **4b**, and **8**. Compounds **4b** and **10** showed the highest inhibitory activity against *P. aeruginosa*, whereas **4b** and **5** were the most active among the series of tested compounds against *Streptomyces* species with MIC values of 75 μg/mL. The results also revealed that some compounds showed little or no activity against the microorganisms (Table I).

Table I. Minimum inhibitory concentration (MIC in μg/mL) of the title compounds. The negative control DMSO showed no activity.

Compound	*Bacillus subtilis* (Gram-positive)	*Pseudomonas aeruginosa* (Gram-negative)	*Streptomyces* species (Actinomycetes)
2	100	–[a]	125
3	125	250	250
4a	125	500	125
4b	100	75	75
5	75	–	75
7	125	250	100
8	100	250	–
9	250	–	500
10	250	75	500
11	75	100	125
12	–	–	–
13	75	250	100
Penicillin	31	46	33

[a] Totally inactive (MIC > 500 μg/mL).

From the structure-activity relationship it is clear that compounds with the 1,2,4-triazolo-1,3,4-thiadiazole moiety with a free thiol-thione group showed the highest activity against both *B. subtilis* and *Streptomyces* species. Furthermore, substitution at the *p*-position in the phenyl ring in **4b** increased its activity against the three microorganisms. It is also clear that the tetrazole-containing compounds revealed higher antimicrobial activity in comparison with the corresponding nitrile derivatives. On the other hand, the 1,2,4-triazolo-1,3,4-thiadiazine derivative **11** with a –CO–NH– group showed the highest activity of the tested 1,2,4-triazolo-1,3,4-thiadiazine derivatives. Moreover, the deacetylated thioglycoside **8** showed higher activity than the corresponding protected analogue **7**.

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