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In vitro ANTIBACTERIAL EVALUATION OF 1,2,4-TRIAZOLE COMPOUNDS CONTAINING PURINE MOIETY

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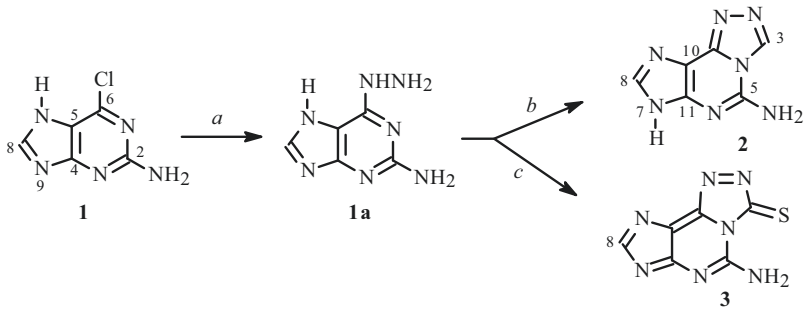
*9*H-*[1,2,4]Triazolo[4,3-g]purin-5-amine (**2**) was synthesized by reaction of 6-hydrazinyl-7*H-*purin-2-amine (**1a**) with formic acid under reflux conditions. Treating compound **1a** in ethanol with CS_2 in the presence of KOH yielded 5-amino-3*H-*[1,2,4]triazolo[4,3-g]purine-3-thione (**3**). The new synthesized compounds were characterized using IR, ^{1}H NMR, and elemental analysis. The synthesized compounds have been screened for antibacterial activity.*

Keywords: 6-hydrazinyl-7*H*-purin-2-amine, 9*H*-[1,2,4]triazolo[4,3-g]purin-5-amine, 5-amino-3*H*-[1,2,4]triazolo[4,3-g]-purine-3-thione, synthesis, antibacterial activity.

Triazoles are an important class of heterocyclic compounds that have received considerable attention for their biological activity such as antimicrobial [1], antifungal [2, 3], antioxidant [4], antiinflammatory [5], antitubercular [6], and anticancer activity [7]. Research articles that have been published during the last few years show that substituted 1,2,4-triazoles and their *N*-bridged heterocycles have received considerable attention during the last two decades as they are endowed with a variety of biological activities and have a wide range of therapeutic properties.

Considering this, we have synthesized 1,2,4-triazole compounds containing the purine moiety: 9*H*-[1,2,4]triazolo[4,3-g]-purin-5-amine (**2**) and 5-amino-3*H*-[1,2,4]triazolo[4,3-g]purine-3-thione (**3**). The synthesized compounds have been screened for *in vitro* antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* PR 23/07 by the cup plate method at concentrations of 1, 3, and 5 mg/mL (Table 1). DMF was used as solvent control.

2-Amino-6-chloropurine treated with hydrazine hydrate in ethanol under reflux conditions yielded 6-hydrazinyl-7*H*-purin-2-amine (**1a**). Compound **1a** is a useful starting material for synthesis of a variety of heterocycle–fused purines. Treating compound **1a** with HCOOH for 6 h yielded 9*H*-[1,2,4]triazolo[4,3-g] purin-5-amine (**2**). In addition, the reaction of 6-hydrazinyl-7*H*-purin-2-amine (**1a**) with CS_2 was a possible route to get the fused purino-1,2,4-triazole-3-thione ring (Scheme 1).



a. N_2H_4, EtOH; *b*. HCOOH; *c*. CS_2, KOH, EtOH

Scheme 1. The synthetic route for the preparation of compounds **2** and **3**.

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TABLE 1. Antibacterial Activity – Diameters of Growth Inhibition Zones of Compounds **2** and **3**, mm

Bacteria	**2**			**3**		
	Concentration, mg/mL					
	1	3	5	1	3	5
Escheria coli ATCC® 25922	–	4	4	3	4	6
Staphylococcus aureus ATCC® 25923	2	2	5	3	3	3
Klebsiella pneumoniae PR 23/07	4	5	6	–	–	3

TLC was used for monitoring of the reaction, the structures of products **2** and **3** were assessed by IR and NMR and elementaly analysis.

EXPERIMENTAL

General. All reactions were performed under an atmosphere of argon in oven-dried glassware. Anhydrous solvents for reactions were obtained by filtration through activated alumina or by storage over molecular sieves (4A). Thin-layer chromatography was performed on silicagel plates (Macherey–Nagel, 0.25 mm, UV254). Visualization was achieved either under UV light or by staining in dip solutions [vanillin (15 g), ethanol (250 mL), and concentrated H_2SO_4 (2.5 mL); or *p*-anisaldehyde (10 mL), concentrated H_2SO_4 (10 mL), concentrated acetic acid (2 mL), and ethanol (180 mL)] followed by heating with a heat gun. Melting points were determined with a Buechi apparatus. The infrared (IR) spectra were recorded as KBr pellets in a Bomem MB 100 mid FT-IR spectrophotometer. The nuclear magnetic resonance (NMR) spectra were recorded using a Varian EM 360 with tetramethylsilane as internal standard.

9*H*-[1,2,4]Triazolo[4,3-g]purin-5-amine (2). A mixture of 6-chloro-7*H*-purin-2-amine (2.9 mmol) and hydrazine hydrate (2.9 mmol) was heated under reflux for 15 min. The obtained crude product was filtered, allowed to cool, and then dissolved in a formic acid (15 mL) under reflux conditions for 6 h. After the reaction was completed and cooled to room temperature, the mixture was poured onto crushed ice (100 g) and 1 N NaOH solution. The crude white product was filtered off, washed with alcohol, and recrystallized from the mixture of acetone–DMF (*N*,*N*-dimethylformamide) (1:1).

Yield 87%, mp 298–300°C. IR (KBr, ν_{max}, cm^{-1}): 3400–3120 (N–H); 3080–3030 (C–H), 1612–1600 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.00–8.75 (2H, aromatic), 10.3 (N–H), 3.90 (NH_2). Elementary analysis: $C_6H_5N_7$: 175.06; calcd %C, 41.14; %H, 2.88; %N, 55.98; experimentally: %C, 40.92; %H, 2.94; %N, 55.62.

5-Amino-3*H*-[1,2,4]triazolo[4,3-g]purine-3-thione (3). A mixture of 6-chloro-7*H*-purin-2-amine (2.9 mmol) and hydrazine hydrate (2.9 mmol) was heated under reflux for 15 min. The obtained crude product **1a** was allowed to cool. After cooling, the precipitate was filtered off and dried. After 24 h, product **1a** was dissolved in 25 mL ethanol, an alcohol solution of KOH and 3.5 mL CS_2 were added, and the whole refluxed for 25 min. After cooling, the crude yellow to brown product was filtered off, washed with alcohol, and recrystallized from a mixture of methanol and DMF (1:1).

Yield 73%, mp > 300°C. IR (KBr, ν_{max}, cm^{-1}): 3360–3160 (N–H), 3010 (C–H), 1608–1600 (C=N), 1220 (C=S). ^{1}H NMR (DMSO-d_6, δ, ppm): 8.65 (1H, H-8), 4.20 (NH_2). Elementary analysis: $C_6H_3N_7S$: 205.02. Experimentally: %C 35.33; %H 1.42; %N 47.52. calcd: %C 35.12; %H 1.47; %N 47.78.

Antibacterial Activity. Compounds **2** and **3** were screened for their antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pseumoniae* by the disc diffusion method of Kirby–Bayer at three concentrations (1, 3, and 5 mg/mL) in DMF. These solutions were added to each filter disc, and the plates were incubated at 37°C and examined for zone of inhibition around each disc after 48 h. The results are summarized in Table 1.

The antibacterial activity of the synthesized compounds was found to be mild to moderate.

According to preliminary antibacterial screening by the paper disc method, compound **2** was found to be active against all the bacterial strains except in a concentration 1 mg/mL in *Escherichia coli*. The antibacterial activity of compound **2** is stronger in *Staphylococcus aureus* and *Klebsiella pneumoniae* PR 23/07. Results show that the antibacterial activity of compound **3** in *Escherichia coli* is moderate, while the antibacterial activity of compound **3** is stronger in *Staphylococcus aureus* (at concentrations of 1 and 3 mg/mL and did not change with increase in concentration) and weaker in *Klebsiella pneumoniae* at the same concentration.

1,2,4-Triazole compounds containing the purine moiety were synthesized and evaluated for *in vitro* antibacterial activity. The biological evaluation of these purino-triazole derivatives generally showed mild to moderate activity against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pseumoniae.*

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