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Synthesis and antimicrobial activity of 1,3-*bis*-butyl-5-(4-iodophenyl)-1,3,5-triazacyclohexane

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ABSTRACT

This work describes the synthesis, structural characterization and antibacterial activities of 1,3,5-triazacyclohexane type of new compound 1,3-*bis*-butyl-5-(4-iodophenyl)-1,3,5-triazacyclohexane. The new triazacyclohexanes (R3TAC) with mixed aryl and alkyl *N*-substituents are synthesized by the reaction of a 1:2 mixture of 4-iodoaniline and *n*-butylamine with formalin. The synthesized compounds were characterized by spectral analysis IR, ^{1}H NMR and ^{13}C NMR. The compound was screened for it's antibacterial activity against Gram-positive and Gram-negative bacteria by the diffusion method on agar medium.

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1. Introduction

The formation of 1,3,5-triazacyclohexane from primary amines and formaldehyde has been known for more than one hundred years [1]. The different triazines were synthesized in the laboratory according to the procedure described elsewhere [2]. 1,3,5-Triazacyclohexanes can be employed as ligands for complexes used as catalyst in the polymerization and trimerization of olefines [3]. Further, the interest in triazacyclohexanes as ligand seems to growing rapidly [4-8].

Antibiotic resistance is a major problem in hospitals as well as in community settings [9]. Considering the ever growing antibiotic resistance developed by many bacteria, there is an immense need for new compounds with new mode of actions, for treatment of bacterial infections [10]. The need for new antibiotics continues to be a still standing challenge [11]. 1,3,5-Triazacyclohexanes containing halides exhibit high biological activity since they contain CN group and halogen atom as pharmacophore. 1,3,5-Triazacyclohexane showed an activity against the strains of microorganisms used [12].

2. Experimental

2.1. Instrumentation

Purity of the compounds was checked by thin layer chromatography (TLC) using CH_2Cl_2: petroleum ether (4:1, *v*:*v*). IR spectra were prepared on the Mattson Galaxy series FT-IR 5000 spectrophotometer using KBr discs. NMR spectra were recorded on Bruker spectrophotometer ARX 500 (500 MHz for proton and 100.62 MHz for carbon). The chemical shifts (δ) are expressed in parts per million (ppm). Tetramethylsilane (TMS) is used as internal reference. The spectra are recorded in deuterated chloroform $CDCl_3$ is used as solvent ($CHCl_3$: δ 7.26 ppm, $CDCl_3$: δ 77.0 ppm).

2.2. Synthesis

Formaline (37%, 3 mmol, 10 mL) was added dropwise to a stirred solution of 4-iodophenylamine (10 mmol) and *n*-butylamine (20 mmol) with in 3 mL of ethanol.

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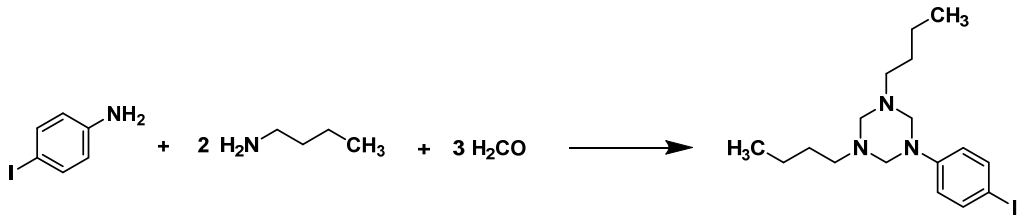
Table 1. Antibacterial activity of gentamicin expressed as the diameter of the inhibition zone in mm in the disk sensitivity assay.

Bacterial strains	Gentamicin
E. coli	31 mm
Resistent S. aureus	28 mm
S. aureus	30 mm

Table 2. Antibacterial activity of 1,3-*bis*-butyl-5-(4-iodophenyl)-1,3,5-triazacyclohexane expressed as the diameter of the inhibition zone in mm in the disk sensitivity assay.

The microbial strains	Concentrations (mg/L)		
	500	1000	2000
E. coli	24 mm	22 mm	20 mm
Resistent S. aureus	24 mm	22 mm	23 mm
S. aureus	10 mm	08 mm	09 mm



Scheme 1

The mixture was stirred for 7 hours at 20 °C. The resulting solution was evaporated on a rotary evaporator to dryness. Yield: 89%. Boiling point: 172-174 °C. R_f (Dichloromethane: petroleum ether, 3:1, *v*:*v*): 0.84. IR (KBr, ν, cm^{-1}): 3085-3060 (Ar-H), 2956-2859 (CH_3, CH_2), 1230-1030 (C-N), 817 (C-I), 750 (Ar-H). ^1H NMR (500 MHz, $CDCl_3$, δ, ppm): 0.90 (t, 6H, CH_3), 1.30 (m, 4H, CH_2), 1.42 (m, 4H, CH_2), 2.40 (t, 4H, CH_2), 3.39 (s, 2H, C_4H_9-N-CH_2-N-C_4H_9), 4.05 (s, 4H, C_4H_9-N-CH_2-N-Ar), 6.71 (d, 2H, Ar), 7.46 (d, 2H, Ar). ^{13}C NMR (125.76 MHz, $CDCl_3$, δ, ppm): 13.88 (CH_3-CH_2), 20.26 (CH_3-CH_2), 29.57 (C_2H_5-CH_2), 51.83 (C_3H_7-CH_2-N), 70.52 (C_4H_9-N-CH_2-N-C_4H_9), 74.51 (C_4H_9-N-CH_2-N-Ar), 82.62 (C-I), 118.79 (CH=C-), 137.62 (CH=C-), 149.67 (N-C=).

2.3. Antibacterial assays

2.3.1. Bacterial strains tested: Germs tested to detect antimicrobial activity of compounds

Escherichia coli, (also known as *E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms); *Staphylococcus aureus* is a Gram-positive *coccal* bacterium that is a member of the *Firmicutes*, and is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as Methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine.

2.3.2. The culture media

The nutrient agar for the isolation and maintenance of bacterial strains and the Mueller Hinton agar for the study of the susceptibility of bacteria used for antimicrobial tests.

2.3.3. Preparation of pre-cultures

Bacterial strains tested were grown in petri dishes containing nutrient agar. After 18 h incubation at 37 °C, bacterial suspensions with an optical density of 1 McFarland were prepared for each microorganism in 10 mL of sterile physiological saline.

2.3.4. Sensitivity test (Diffusion on agar medium method)

Based on the method described by NCCLS (1997), different concentrations of compound are obtained in DMSO (500, 1000 and 2000 mg/L). The appropriate agar is poured into Petri dishes of 90 mm diameter and inoculated with a freshly prepared pure bacterial suspension. A sterile Whatman paper disc is soaked with 20 µL of each dilution and gentamicin disk (30 µg) - antibiotic aminoglycoside active against a variety of bacteria-used as a positive control. All the discs are deposited on the surface of seeded agar, the whole is incubated for 24 hours at 37 °C. Upon application of the discs, the extracts and the antibiotic diffuse uniformly and after 24 hours of incubation, the presence of a circular zone of inhibition is sought.

3. Results and discussion

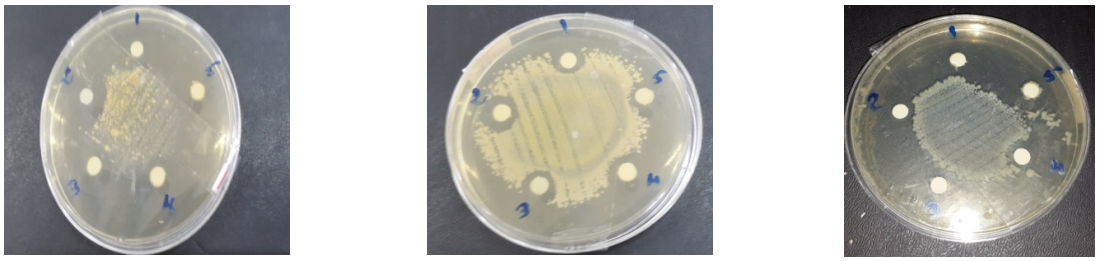
3.1. Synthesis

The unsymmetrically substituted triazcyclohexanes 1,3-*bis*-butyl-5-(4-iodophenyl)-1,3,5-triazacyclohexane were prepared from the condensation reaction of 4-iodophenylamine and *n*-butylamine with formaldehyde in high yield (89%) (Scheme 1). This compound is stable at room temperature. Unsymmetrically substituted products were obtained predominantly over the symmetrical triazacyclohexanes - much more than expected for statistical formation of triazacyclohexanes. This indicates higher stability for mixed substituents [13-15].

3.2. Biological activity

For the assessment of the antibacterial potential of the extracts studied, we choose to test them against many bacterial species, because each one has a particular cellular structures and metabolism [16]. The sensitivity to different strains has been classified by the diameter of the inhibition zone is as follows: Diameter less than 8 mm: not sensitive; Diameter of 9-14 mm: sensitive; Diameter 15-19 mm: very sensitive; Diameter greater than 20 mm: extremely sensitive.

Results presented in the Table 1 and 2 showed that *Staphylococcus aureus* is sensitive against 1,3-*bis*-butyl-5-(4-



Resistent *S. aureus* *S. aureus* *E. coli.*

Figure 1. Antibacterial activity of 1,3-bis-butyl-5-(4-iodophenyl)-1,3,5-triazacyclohexane in the disk sensitivity assay.

iodophenyl)-1,3,5-triazacyclohexane, while resistant *Staphylococcus aureus* and *Escherichia coli* are extremely sensitive against our compound. Our compound showed similar activity to that of the positive control, which has a broad spectrum of inhibition against a variety of bacteria (Figure 1).

In summary, the new unsymmetrically substituted triazacyclohexanes 1,3-*bis*-butyl-5-(4-iodophenyl)-1,3,5-triaza cyclohexane was prepared from two amines and formaldehyde. FT-IR and NMR analyses of the compounds was reported and the solid-state structures of some compound. The compound was targeted for their antibacterial activity against Gram-positive and Gram-negative bacteria by the diffusion method on agar medium text paragraph.

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