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Synthesis, spectral studies and antimicrobial activity of a new hexahydrotriazine compound

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Abstract

An unsymmetrical original structure of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-Triazacyclohexane was synthesized via a long one-step mixed condensation reaction of 2:1stoichiometric ratio, 2-bromoaniline and isopropylamine with formalin. The desired novel compoundwas obtained as colorless needles in good yield of 71%. The Characterization of the synthesized compound has been done by different spectroscopic methods like FT-IR, ^{1}H NMR ,^{13}C NMR, UV , and elementary analysis.

In this research work, we tested the antimicrobial activity of this new product where we used Gram-positive and Gram-negative bacteria by the diffusion method on agar medium.

Keywords: synthesis; amine; Hexahydrotriazines; antimicrobial activity; spectral techniques.

1. Introduction

Hexahydrotriazines are concerned with a large range of six-memberd ring compounds which contain three nitrogen atoms in 1,3 and 5 positions[1]. Throughout the years, a considerable attraction has been obtained to hexahydrotriazines[2]. Among various interests ,these kinds is used of products that exist in industrial chemistry[3]. For instance , N ,N', N''- trisubstituted 1,3,5- triazinanes can be used as reactants for the preparation of N-heterocyclic carbenes which served as substantial class of ligands in homognenous catalysis[4].They also can be used as inhibitors for anti-corrosion activity[5]. TAC can be employed as ligand for new complexes worked as catalyst in the polymerization and trimerization of olefins[6]. Antibiotic resistance is a major problem in hospitals as well as in community settings[7]. Considering the ever growing antibiotic resistance developed by many bacteria,there is an immense need for new compounds with new mode of actions, forthe treatment of bacterial infections[8]. The need for new antibiotics continues to be a still standing challenge[9]. for this reason, our scientific research team has focused on this aspect through the various researches that have been published recently in so many different articles. These articles have confirmed that triazacyclohexanes containing halides exhibit high biological activity against the strains of microorganisms used, since they contain CN group and halogen atom as pharmacophore[10, 11], As shown in some of the results obtained :*Staphylococcus aureus* is sensitive against 1,3-*bis*-butyl-5-(4-iodophenyl)-1,3,5-triazacyclohexane, while resistant *Staphylococcusaureus* and *Escherichia coli* are extremely sensitive against our compound[12], Staphylococcus *aureus* is sensitive against 1, 3-bis (4-iodophenyl)-5- (2-ethyl-1-hexyl)- 1, 3, 5- triazacyclohexane and 1, 3-bis (2-ethyl-1-hexyl)-5- (iodophenyl)- 1, 3, 5-triazacyclohexane, while resistant *Staphylococcus aureus* and *Escherichia coli* are extremely sensitive against our compounds[13]. From these results , we can say that antimicrobial activity is one of the most important applications that characterize these compounds.

In the present research work, the new unsymmetrically substituted 1,3,5-triazacyclohexane was synthesized and its structure was confirmed and characterized by using various spectral techniques like FT-IR, ^{1}H NMR and^{13}C NMR.This compound was targeted for their antibacterial activity against Gram-positive and Gram-negative bacteria by the diffusion method on agar medium.

2. Experimental Part

a. Materials

2-bromoaniline(98%), isopropylamine(98%), formaline (37%), ethanol (96%), hexane used for recrystallization

b. Synthesis

Synthesis of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-Triazacyclohexane :

An excess of formalin (37%, 5ml) was added dropwise to a mixture of (1:2) stoichiometric ratio of isopropylamine (10 mmol) and 2-bromoaniline(20 mmol,) which is dissolved in 25 ml of ethanol in a simple necked round bottomed flask and stirred for 12h (overnight) at 20 °C. The resulting

solution was evaporated in a rotary evaporator to dryness and the white residue was recristallized from n-hexane to afford 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-Triazacyclohexane , this compound is stable at room temperature and obtained in high yield 71% [14, 15]. The final obtained result was transparent needles.m.p. =119.5 °C

c. Measurements

Melting point was determined on a capillary melting point apparatus. The purity of the title compounds was screened by an analytical thin layer chromatography (TLC) conducted on percolated TLC plates (silica gel 60F254, Merk) visualized under UV light and using CH_2Cl_2: ethylique ether (9:1) as an eluent. ^1H and ^{13}C NMR spectra were recorded on ituvnmrs-vnmrs 500 MHz spectrometer at room temperature in $CDCl_3$ using TMS as internal reference and chemical shifts are expressed as δppm. The infrared spectra was recorded in KBr pellet on Shimadzu FT-IR 8201 PC (4000-400 cm^{-1}) spectrophotometer. The UV spectra was recorded on Shimadzu spectrophotometer (200-1100 nm).

d. *Antibacterial assays*

Bacterial strains tested: Germs tested to detectantimicrobial activity of compound

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); Staphylococcusaureus 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. aureussuch as Methicillin-resistant S.aureus (MRSA) is a worldwide problem in clinical medicine[12].

The culture media

The nutrient agar for the isolation and maintenance o fbacterial strains and the Mueller Hinton agar for the study of the susceptibility of bacteria used for antimicrobial tests[12, 13].

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[12, 13].

Sensitivity test (Diffusion on agar medium method)

Based on the method described by NCCLS (1997), different concentrations of compound are obtained in DMSO (100, 250, 500, 750 and 1000 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[12, 13].

3. Results and discussion

An unsymmetrically substituted 1,3,5-hexahydrotriazine, 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-,Was obtained by a mixed condensation reaction of 2bromoaniline and isopropyl with formaldehyde (formalin) [16, 17].

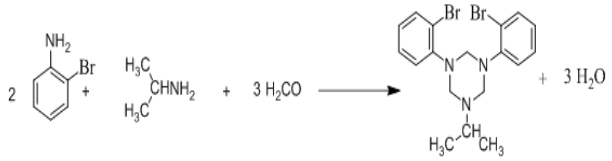

Figure 1: synthesis of1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane

This product is stable at room temperature and afforded in high yield (71%). Recrystallized from n-hexane gave transparent needles.The synthesis mechanism is probably passes through imines, which trimerizes to give the title compound[15].

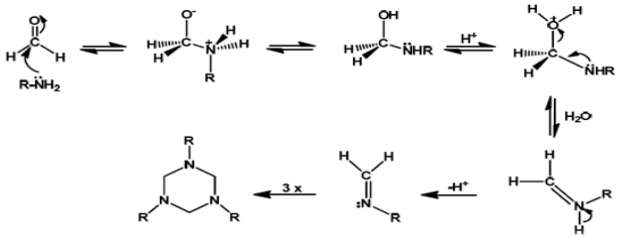

Figure 2. Reaction mechanism of the synthesis of a Hexahydrotriazines

FT-IR study of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane:

The characterization of the title compound has been interpreted by FT-IR, ^1H-NMR and ^{13}C-NMR . The infrared spectrum reveals a strong sharp band at 518cm^{-1} resulting from the stretching vibration of C-Br band. Two absorption bands at 1586 and 1470 cm^{-1} are shown by the six-membered aromatic system (νC=C), another absorption band at 758cm^{-1} characteristic of the C-H out- of- plane vibration of the aromatic system. Moreover, these results correspond to the results recorded in the previous study of the infrared spectrum of 3,5-bis(4-chlorophenyl)-1- propyl-1.3.5-triazinane) in the same region , but with different frequencies(νC=C at 1492 and 1590 cm^{-1},C-H out- of-plane at 752cm^{-1}), While C-Cl band appears at 815cm^{-1} [15].

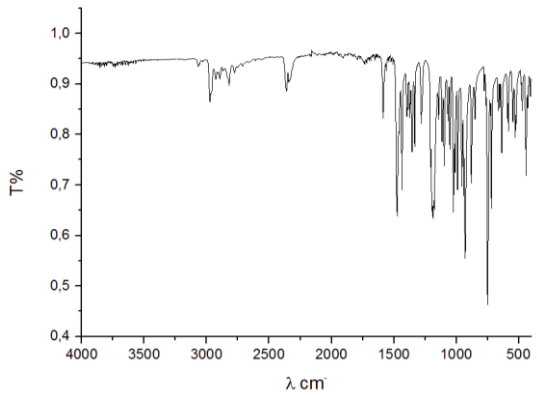

Figure 3. FTIR spectrum of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane[1]

H NMR study of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane:

The ^{1}H NMR spectrum of the title compound shows the six chemically and magnetically equivalent protons of two methyl groups adjacent to the methine group(CH)of the mono isopropyl group appear in the form of a doublet (signal of two peaks) at δ =1.09 ppm. The proton of the methin group (CH) adjacent to the nitrogen atom resonate as a multiplet at δ =3.21 ppm.The protons of the heterocyclic triazine appear as two singlets at δ= 4.35(Alkyl-N-C**H**$_2$-N-Aryl), and= 4.71 ppm (Ar-N-C**H**$_2$-N-Ar) as in 3,5-bis(4-chlorophenyl)-1- propyl-1.3.5-triazinane The protons of the heterocyclic triazine
appear in the same region , but with different frequencies(at δ= 4.25 and δ= 4.71 ppm) [15].

The protons of the aromatic system appear between δ= 6.87 and δ= 7.52 ppm as multiplets, while in the 3,5-bis (4-chlorophenyl) - 1-propyl-1, 3, 5-triazinane the protons of the aromatic system appear as a two doublets at 6.80 and 7.50 ppm [15], the two peaks at δ= 1.52 and δ= 2.19 represent the impurities.

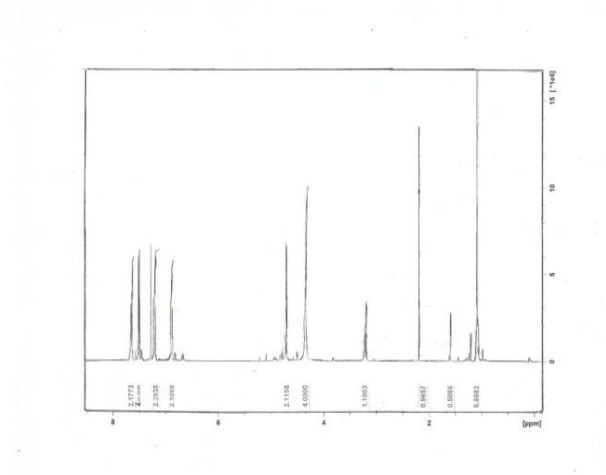

Figure4. ^{1}H NMR spectrum of1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane

^{13}C NMR study of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane

In the ^{13}C NMR spectra of the title compound, the carbon atoms of the two methyl group appear at δ =20.90 ppm, the carbon of methine group appears at δ = 50 ppm . The ^{13}C NMR spectrum displays two signals at δ= 69.80 and 71.42 ppm corresponding to two carbon atoms of the triazinane cycle as in 3,5-bis (4-chlorophenyl) - 1-propyl-1, 3, 5-triazinane[15]. The two carbon atoms of the triazinane cycle appear in the same region , but with different frequencies(at 68.63and 71.27 ppm)

Those of the aryl groups appear at δ=123.64, 125.11, 128.45, 133.86 and 148.47 ppm (in the same region as in 3,5-bis (4-chlorophenyl) - 1-propyl-1, 3, 5-triazinane but with different frequencies)[15], and the C-Br atom appears at δ =119.98 ppm while the C-Cl atom appears at 147.93 in 3,5-bis (4-chlorophenyl) - 1-propyl-1, 3, 5-triazinane [15]

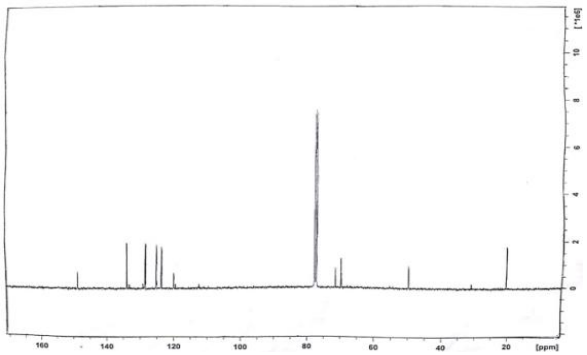

Figure5. ^{13}C NMR spectrum of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane

UV study of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane

The ultra-violet spectrum of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane
Shows a weak signal at 293 nm characteristic the n →π* transition. These results are different from the previous ultraviolet spectrum of 3,5-bis (4-chlorophenyl) -1-propyl-1,3,5-triazinane[15] due to the difference in the measurement method (the first new compound is recorded in the solid state by the reflection of UV rays , however the ultraviolet spectrum of the second compound is recorded in a solution state via absorption of UV rays.

Elementary analysis of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane
Elementary analysis of this compound **($C_{18}H_{21}Br_2N_3$)** shows the results of the percentage of elements as follows in the table1.

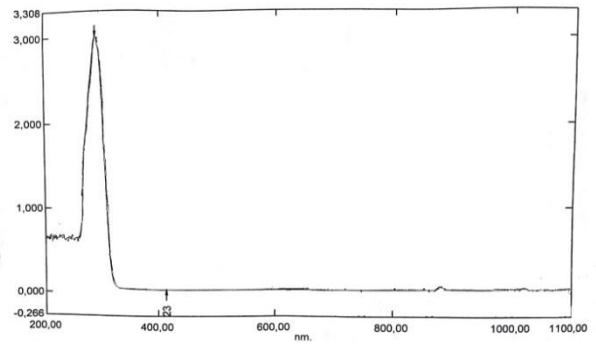

Figure 6. The ultra-violet spectrum of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazacyclohexane

Table 1: results of the elementary analysis:

results	% nitrogen	% carbon	hydrogen	sulphur
1	9.59	49.21	5.09	0.00
2	9.54	49.08	5.07	0.00
Average	9.57	49.15	5.08	0.00
theory	9.57	49.23	4.82	0.00

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 . 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 [12]. Results presented in the Table 2 showed that E. coli is not sensitive against 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-triazinane while Resistent S. aureus and S. aureus are sensitive against our compound.

Table 2. 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
Resistent S. aureus	28
S. aureus	30

Table 3 : Antibacterial activity of 1.3- bis(2-bromophenyl)-5-isopropyl-1.3.5-Triazacyclohexaneas expressed as the diameter of the inhibition zone in mm in the disk sensitivity assay.

The microbial strains	Concentrations				
	100	250	500	750	1000
E. coli	/	/	/	/	/
Resistent S. aureus	/	/	9	10	12
S. aureus	/	8	9	10	10

4. Conclusion

In this manuscript, we have described the synthesis of a new triazacyclohexanes (R3TAC) derivative by the condensation reaction of a 2:1 mixture of 2-bromoaniline and isopropylamine with formalin in ethanol . The synthesized compounds was obtained in good yield, was recrystalised with hexane. The structure of the synthesized compound was confirmed and characterized by using various spectral techniques like FT-IR, ^{1}H NMR,^{13}C NMR, UV and elementary analysis. The compound was targeted for their antibacterial activity against Gram-positive and Gram-negative bacteria by the diffusion method on agar medium.

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