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Synthesis, characterization and antimicrobial activity of a new 1,3-bis(4-fluorophenyl)-5-butyl-1,3,5-triazacyclohexane

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Abstract

In this paper, a new hexahydrotriazine derivative which have the structure 1,3-bis(4-fluorophenyl)-5-butyl-1,3,5-triazacyclohexane was synthesized by the condensation reaction of a 1 :2 :1 ratio, 4-fluoroaniline and butylamine with formalin in a basic solution. The synthesized compound was characterized by elementary analysis and spectroscopic methods IR, UV, mass spectroscopy, ^{1}H and ^{13}C NMR.

The new triazacyclohexane has been screened for its antimicrobial activity against Gram positive and Gram negative bacteria by the diffusion on agar medium.

Keywords: triazacyclohexanes; formalin; butylamine; antimicrobial activity; condensation.

1. Introduction

Synthesis of nitrogen compounds has been known for a long time [1], different Miller, E. C. Warner, J. Am. Chem. Soc. 54 (1932) 3698-3706triazacyclohexanes were synthesized in the Laboratory according to the procedure described by Miller et al [2]. Hexahydrotriazines are used in a variety of ways in industrial chemistry [3] and they are subject to some structural researchers in structural chemistry [4-5] triazacyclohexane and its derivatives can contribute in the synthesis of organometallic in homogenous catalysis [6-7] which can employed as ligand for new complexes used as catalyst in polymerization and trimerisation reaction of olefins [7-8], they can be used as inhibitors for anti corrosion activity [9].

Hexahydrotriazines exhibit high biological activity since they contain CN group and halogen F atom as pharmacophore [10] which are the subject of several investigations [11].

More recent biological activity studies that have studied the biological activity of triazacyclohexanes show that it is a new feature of our compounds as mentioned in the previous researchers work*: E. coli* is not sensitive against 1.3-bis(2-bromophenyl)-5-isopropyl-1.3.5-triazinanewhile *Resistent S.* aureus and *S. aureus* are sensitive against this compound [12] but *Salmonella Typhi, Bacillus Subtilus* and *Escherchia coli* are sensitive against 1,3-bis(4-bromophenyl)-5-ethyl-1,35-triazacyclohexane[10].

In the present work, the new unsymmetrically substituted 1,3,5-triazacyclohexane was synthesized through the condensation of 4-fluoroaniline and butylamine with formalin in a basic solution. Structure of triazacyclohexane derivative was established by spectroscopic FTIR, UV, mass spectroscopy, ^{1}H NMR and ^{13}C NMR and elementary analysis.

2. Experimental Part

2.1. Materials

4-fluoroaniline (98%), n-butylamine (98%), potassium hydroxide, formaline (37%), hexane and dichloromethane

2.2. Synthesis

2.2.1. Synthesis of 1,3-bis(4-fluorophenyl)-5-butyl-1,3,5-triazacyclohexane:

A mixture of 4- fluoroaniline and butylamine (2:1, v:v) ratio were stirred overnight at ambient temperature with water(10 cm^3), potassium hydroxide (1.18 g, 30 mmol) and an excess of formalin (6 mL). The resulting oily phase was extracted with CH_2Cl_2, dried with $MgSO_4$ and evaporated to dryness [4-8]. The solid obtained was recristallized from 50% n-hexane and 50% dichoromethane to afford 1,3- bis(4-flourophenyl)-5-butyl-1,3,5-Triazacyclohexane, Purity of the compound was confirmed by TLC (Rf= 0,57, CH_2Cl_2 100 %), this compound is stable at room temperature and obtained in high yield 83% [10-12,13].

The final obtained result was transparent plates. m.p=110°C.

2.3. Measurements

Melting point was determined with (Bransted/ Electrothermal) apparatus and are uncorrected. Analytical thin layer chromatography TLC were performed on Kieslgel 60 F_{254} (Merck) layer using CH_2Cl_2 as an eluent. NMR spectra were recorded on bruker spectrophotometer ARX 500 MHz for proton and 100,62 MHz for carbon. The chemical shifts are expressed in part per million (ppm).Tetramethylsilane (TMS) is used as internal reference, the spectra are recorded in deuterated chloroform $CDCl_3$.The infrared spectrum was recorded in KBr pellet on Shimadzu FT-IR 8201 PC (4000-400 cm^{-1}) spectrophotometer. The UV spectrum was recorded on Shimadzu spectrophotometer (200-1100 nm).
The MS analysis was carried out with a 3200 QTRAP mass spectrometer (Applied Biosystems SCIEX) equipped with an assisted atmospheric pressure ionization (API) source pneumatically. The sample was ionized in positive electrospray mode under the following conditions :Electrospray voltage (ISV): 5500 V; orifice voltage (OR): 20 V; Nebulization gas pressure (air):10 psi. The mass spectrum (MS) was obtained with a quadrupole analyzer.

2.4. Antimicrobial Assays

The in vitro antimicrobial activity of 1,3-bis(4-fluorophenyl)-5-butyl-1,3,5-triazacyclohexane was determined by the wells diffusion method [10,14].

For these assays, cultures of the following microorganisms were used: *Staphylococcus aureus* is a facultative anaerobic, gram-positive coccal (round) bacterium also known as "golden staph" usually acts as a commensal bacterium it is a common cause of skin infections[15] , *Escherichia Coli* is an intestinal bacterium (gram negative) of mammals, very common in humans, Escherichia coli can be pathogenic resulting in gastroenteritis [16].

Pseudomonas aeruginosa, is a Gram-negative bacterium of the genus Pseudomonas. It can, under certain conditions, be pathogenic. Very resistant, more and more often responsible for nosocomial infections, it is one of the most difficult bacteria to treat clinically [17].

2.4.1. The culture media

For the study of the sensitivity of bacteria used for antimicrobial tests, Mueller Hinton agar is used and for the maintenance and isolation of bacterial strains, L'agar nutritive is used [11].

2.4.2. Preparation of pre-culture

In Petri dishes containing nutrient agar, the bacterial strains tested were cultured. After 18 h incubation at 37 °C, the preparation of the bacterial suspensions was made for each microorganism in 10 mL of sterile physiologic serum and with optical density of 1Mc Farland [12].

2.4.3. Sensivity test (Diffusion on agar medium method)

To develop the sensitivity test, different concentrations of the compound are obtained in DMSO (0.5, 1, 2, 4 and 8 mg/mL). The concentrations used in screening were chosen after determining the MICs of each compound. L'agar is poured, then, the inoculated plates were incubated for 24h at 37°C. Negative controls were prepared using DMSO employed for dissolving the tests compound. Gentamicin was used as standard for antimicrobial activity. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that the standard. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm) [10,12].

3. Results and discussion

The condensation of 4-fluoroaniline and butylamine with formalin afforded an unsymmetrically substituted triazacyclohexane [4,7].

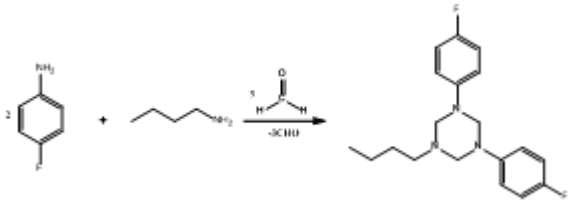

Figure 1. Synthesis of 1,3-bis(4-fluorophenyl)-5-butyl-1,3,5-triazacyclohexane

This product is stable at room temperature and obtained in high yield 83%. Recrystallization of the obtained solid product from dichloromethane and n-hexane gave transparent plates.

The mechanism of this condensation involves the attack of nucleophilic species with formaldehyde to form an imine which trimerizes to give 1,3,5-triazacyclohexane[6]

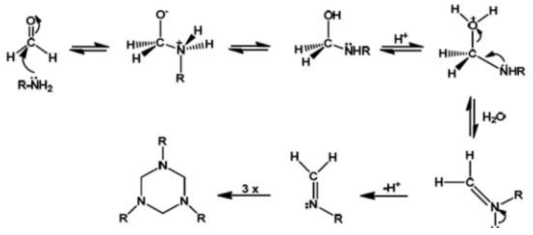

Figure 2. Reaction mechanism of the synthesis of triazacyclohexanes[7]

3.1. FTIR study of 1,3-bis(4-fluorophenyl)-5-buyl-1,35-triazacyclohexane:

The characterization of title compound has been explained by FTIR, UV/Vis, mass spectroscopy ^{1}H and ^{13}C NMR and elementary analysis. The infrared spectrum reveals a weak stretching band at 3080 cm^{-1} of C-H aryl system, four strong bands in 3000-2850 cm^{-1} range due to C-H stretching vibration of asymmetric and symmetric of CH_2 and CH_3 functional groups, at 1598 and 1496 cm^{-1} two strong bands, characterize the stretching non localized double bonds (C=C) of the aryl groups. A strong bond at 1461 cm^{-1} due to in plane symmetric deformation of the C-H band of the methylene group CH_2, a medium strong band at 1370 cm^{-1} due to symmetric deformation of three C-H methyl group CH_3, a strong sharp band at 1200 cm^{-1} resulting from the stretching vibration of C-F bond, in the deformation zone(finger print region), a strong band at 758 cm^{-1} due to out of the plane deformation C-H aryl system.

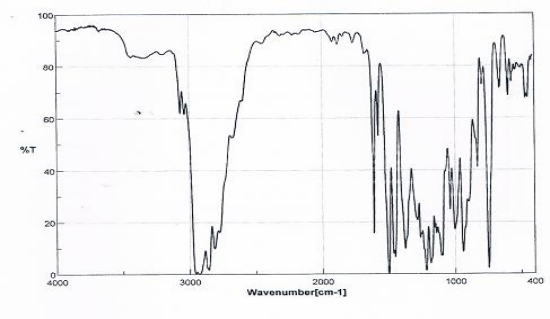

Figure 3. FTIR spectrum of 1.3-bis(4-fluorophenyl)-5-butyl-1.3.5-triazacyclohexane

3.2. ^{1}H NMR study of 1,3-bis(4-fluorophenyl)-5-buyl-1,3,5-triazacyclohexane:

The ^{1}H NMR spectrum shows protons of the methyl group resonate as triplet at δ 0.90 ppm, the two protons of CH_2 adjacent to CH_3 group appear as multiplet centered at δ 1.31 ppm. At δ 1.44 ppm appear as triplet the two protons of CH_2 group adjacent to both of CH_2-N and C_3H_5 groups (C_2H_5-C<u>H</u>$_2$-CH_2-N-). The triplet centered at δ 2.57 ppm shows the resonance of the protons of two CH_2 of the alkyl attached to the nitrogen (C_3H_7-C<u>H</u>$_2$-N-). The two various pics of methylene groups appear as singlets at respectively δ 4.22 ppm (alkyl-N-C<u>H</u>$_2$-N-Ar) and δ 4.67 ppm (Ar-N-C<u>H</u>$_2$-N-Ar) in a ratio of intensity 2:1 indicate asymmetric hexadrotriazine derivative. Finally, the protons of the aromatic system appear as multiplet in δ 6.92 and 7.00 ppm range.

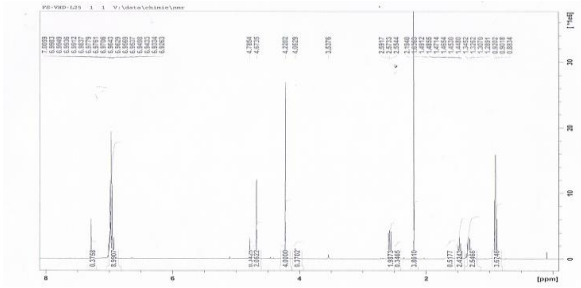

Figure 4. ^{1}H NMR spectrum of 1,3-bis(4-fluorophenyl)-5-butyl-1,3,5-triazacyclohexane

3.3. ^{13}C NMR study of 1,3-bis(4-fluorophenyl)-5-buyl-1,3,5-triazacyclohexane:

The ^{13}C NMR spectrum shows the carbons atoms of methyl group appear at δ 14.32, the carbon of the two CH_2 adjacent to both CH_3 and CH_2-N- appear at respectively δ 20.94 and δ 30.19 ppm, At δ 52.35 ppm appear the carbon atom of the alkyl attached to the nitrogen (C_3H_7-<u>C</u>H_2-N-). The carbons atoms of hexahydrotriazine cycle appears respectively at δ 70.69 (C_4H_9-N-<u>C</u>H_2-Ar) and 72.43 ppm (Ar-N-<u>C</u>H_2-N-Ar), the other carbon atoms of the aryl groups appear at δ 115.9 ppm (-H<u>C</u>=C-N), δ 119.83 ppm (H<u>C</u>=C-F) and δ 146.22 ppm (N=<u>C</u>-) and the carbons of aryl ring which has a fluorine atoms appear at δ 156.85 ppm (<u>C</u>-F). The biggest signal at δ 77.42 ppm is from the solvent.

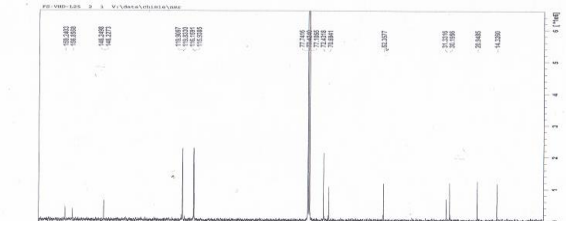

Figure 5. ^{13}C NMR spectrum of 1.3- bis(4-fluorophenyl)-5-butyl-1.3.5-triazacyclohexane

3.4. UV study of 1,3-bis(4-fluorophenyl)-5-buyl-1,3,5-triazacyclohexane:

The ultra violet spectrum of 1.3- bis(4-fluorophenyl)-5-butyl-1.3.5-triazacyclohexane Shows a weak signal at 280 nm characteristic the $n \rightarrow \pi^*$ transition.

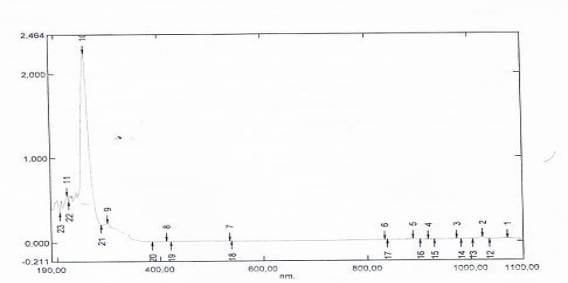

Figure 6. The ultra violet spectrum of 1,3- bis(4-fluorophenyl)-5-butyl-1,3,5-triazacyclohexane

3.5. *Elementary analysis of 1,3-bis(4-fluorophenyl)-5-buyl-1,3,5-triazacyclohexane*:

Elementary analysis of this compound ($C_{19}H_{23}F_2N_3$):

Anal. calcd. for $C_{19}H_{23}F_2N_3$: C, 69.29 ; H,6.25 ; N,12.52 ; F, 11.94 . Found: C, 69.18; H,6.22 ; N,12.42 ; F,11.57%. Molecular Weight : 331.40.

3.6. Mass of 1,3-bis(4-fluorophenyl)-5-buyl-1,3,5-triazacyclohexane

The signals observed during the positive electrospray ionization of the 1,3-bis(4-fluorophenyl)-5-buyl-1,3,5-triazacyclohexane sample make it possible to conclude that there exists in solution a 331 molar mass compound. Therefore it could be the compound whose structure has been proposed.

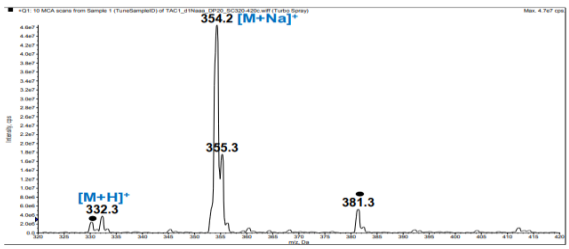

Figure 7. mass spectrum of1,3- bis(4-fluorophenyl)-5-butyl-1,3,5-triazacyclohexane

3.7. Biological Activity

In the present work, antimicrobial activity of new hexahydrotriazine compound against one Gram positive bacteria (*Staphylococcus aureus*) and two Gram negative bacteria (*E. coli, P. aeruginosa*) were tested using diffusion well technique. The sensivity to different stains has been classified by the diameter of the inhibition zone (in mm) including the disk diameter was measured for each treatment: Diameter less than 8 mm: not sensitive; Diameter of 9-14 nm: sensitive; Diameter 15-19 nm: very sensitive; Diameter greater than 20 nm: extremely sensitive [14].

Results presented in the table 2 showed that is *Staphylococcus aureus* and *E. coli* are very sensitive against 1,3-bis(4-fluorophenyl)-5-butyl-1,3,5-triazinane at a concentration of 8 mg /mL whereas *P. aeruginosa* is sensitive against our compound.

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	30.5
S. aureus	24.5
P. aeruginosa	26.5

Table 2. Antibacterial activity of 1.3- bis(4-fluorophenyl)-5-butyl-1.3.5-Triazacyclohexane as the diameter of the inhibition zone in mm in the disk sensitivity assay

The microbial strains	Concentrations				
	0.5mg /mL	1mg/mL	2mg/mL	4mg/mL	8mg/ml
E. coli	/	/	/	/	19.5
S. aureus	/	/	/	/	18
P. aeruginosa	/	/	/	/	9

4. Conclusion

In this study, new hexahydrotriazine derivative has been successfully synthesized by the condensation reaction of a mixture of 4-fluoroaniline and butylamine with formalin in a basic solution, the structure of this new compound was identified by spectral data FTIR, UV, mass spectroscopy ^{1}H NMR and ^{13}C NMR and elementary analysis. This compound was screened for their antibacterial activity: *Staphylococcus aureus* and *E. coli* are very sensitive against 1,3-bis(4-fluorophenyl)-5-butyl-1,3,5-triazinane at a concentration of 8 mg /mL while *P. aeruginosa* is sensitive against our compound.

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