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ORIGINAL ARTICLE

Synthesis, Characterization, Antioxidant, Anti-fungal, and Antibacterial Activities of a New 1-Isopropyl-3,5-diphenyl-1,3,5-triazinane

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ABSTRACT: In this work, we present the green synthesis of a novel of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane, It is made without a solvent, The multicomponent condensation reaction between isopropylamine, aniline, and formaline. The structures of this compound have been characterized by TLC, UV, IR, ^{1}H NMR and ^{13}C NMR. The antioxidant activity showed good antioxidant activity in ABTS, Phenanthroline. The antimicrobial activity of this novel molecule was tested against four bacterial and fungal strains using the disk diffusion method. The obtained results exhibited a high level of antibacterial and antifungal activity.

INTRODUCTION

A big chunk (about 60%) of small drug molecules that have been cleared by the Food and Drug Administration (FDA) are made up of compounds with nitrogen heterocycles [1]. A lot of new nitrogen heterocycles have been made so that they can be used in material and biological studies [2-10]. The triazinanes are concerned with a large range of six-membered ring compounds which three nitrogen atoms in 1,3 and 5 positions [11].

The resistance to antibiotics is a big issue in both hospitals and everyday life [12]. Because many bacteria are becoming more and more resistant to antibiotics, there is an urgent need for new substances that work in different ways to treat bacterial infections [13]. They are still having trouble meeting the demand for new medicines [14]. Hexahydrotriazines are very good at killing different types of bacteria because they have a CN group and a halogen atom that serve as pharmacophore [15, 16]. 1,3-bis-butyl-5-(4-iodophenyl)-1,3,5-triazinane is one example [17].

MATERIALS AND METHODS

Synthesis

A mixture of aniline (20 mmole, 1.86 g, 1.83 ml),

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isoprolylamine (10 mmole, 0.59 g, 0.86 ml) and excess of formaline (37 %, 7 ml) without solvent. Energy and a catalyst. This reaction mixture was stirred at ambient temperature for 4h. It demonstrated that the reaction was complete by TLC. The residue was recrystallized with hexane. Yield: 69.8 % (1.93g), M.p = 82°C, R_f ($CHCl_3$100 %) = 0.41

IR (ν, cm^{-1}): 3082(C-H, Ar), 2951-2852(C-H), 1584.4 - 1499.4 (C=C), 1277.8(C-N), 759.0 (C-H, Ar).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.19 – 6.78 (m, 10H, Ar), 4.66 (s, 2H, Ar-N-CH_2-N-Ar), 4.30 (s, 4H, Ar-N-CH_2-N-CH_2-N-Ar), 3.25- 3.01 (m, 1H, CH_3-CH-CH_3), 1.18 (s, 6H, CH_3-CH-CH_3).

^{13}C NMR (101 MHz, $CDCl_3$, δ ppm): 149.38- 148.77 (2C, N-C_{Ar}), 129.33- 129.29- 121.03- 120.73- 117.79- 117.64 (10C, CH=C), 69.12 (C, Ar-N-CH_2-N-Ar), 68.63 (2C,Ar-N-CH_2-N-CH_2-N-Ar), 48.79 (C, CH_3-CH-CH_3), 20.80 (2C, CH_3-CH-CH_3).

Antioxidant activities

Antioxidant activity by ABTS

We tested the $ABTS^{\bullet+}$ scavenging activity using the method from Re et al. [18], with a few small changes. To make the $ABTS^{\bullet+}$ solution, 7 mM ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) were mixed with water. After that, this mix was kept at room temperature for 16 hours in the dark. After adding water to the $ABTS^{\bullet+}$ solution, the absorption at 734 nm was found to be 0.708±0.025. So, 40 µl of sample solution in methanol at different strengths and 160 µl of $ABTS^{\bullet+}$ solution came next. After that, the microplate was left to sit for 10 minutes before the absorbance at 734 nm was measured. Three copies of each test were done on all the materials. Two different types of antioxidants, BHA (butylated hydroxyanisol) and BHT (butylated hydroxytoluene), were used to compare the activities. The following method was used to figure out the percentage blockage of all samples:

% of ABTS radicals that are scavenged = [Acontrol – A sample / Acontrol] x 100

Where: Acontrol and Asample are the absorbance values of the standard sample and the sample itself, as read by a microplate reader.

The results were given as an IC_{50} number, which is the quantity needed to stop the reaction 50% of the time (µg ml^{-1}).

Antioxidant activity by phenanthroline

The test was done according to the steps outlined by Szydlowska-Czerniak et al [19]. To make the reaction mixture, 30 µl of o-phenanthroline (0.5% in methanol), 50 µl of $FeCl_3$ (0.2%), 110 µl of methanol, and 10 µl of the sample solution in methanol at different amounts were mixed. Subsequently, the mixture was kept for 20 minutes at 30°C before measuring the absorbance at 510 nm. The finding was given as the $A_{0.5}$ number, which means that the concentration gave absorption of 0.5.

Antibacterial activity

The antibacterial activity of the synthesized 1-isopropyl-3,5-diphenyl-1,3,5-triazinanewas carried outagainst four bacterial strains, which included two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 10876) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), using the agar well diffusion assay [20, 21].

For each strain, a swab is taken and several colonies are spread over the agar previously poured into the plates using the inoculation technique. The plates are then incubated in an oven at 37°C for 18 hours to revive the bacteria. Bacterial suspensions of the above strains were prepared by placing a few bacterial colonies in sterile physiological water at a concentration of 0.5 McF. A portion of each inoculum was used to inoculate Petri dishes containing Muller Hinton agar using the swabbing technique. A 6 mm sterile steel borer was employed to punch holes of 6 mm, and a volume of 35 µl of the compound solution at different concentrations (8, 4, 2, 1, and 0.5 mg l^{-1}) was introduced into the well. DMSO was the negative control; amoxicillin, vancomycin, and ciprofloxacin were the positive controls. The Petri dishes were then incubated at 37°C for 24 hours.

The results were expressed by measuring the diameter of the inhibition zones around the wells.

Anti-fungal activity

The antifungal activity was evaluated against *fusarium oxysporum*, tomato phytopathogenic fungi, using the disc diffusion method [22]. This assay was carried out in 90-mm petri plates containing 20 ml of potato dextrose agar mixed with an aliquot of synthesized 1-isopropyl-3,5-diphenyl-1,3,5-triazinane (diluted in DMSO) at different concentrations (3, 1.5 and 0.75 mg ml^{-1}). Firstly, a 6 mm diameter disc containing the pathogenic fungi was prepared and positioned in the center of the Petri dishes. These Petri dishes were well closed and incubated in an oven at 25°C for 6 days. The percentage of inhibition was calculated by the following formula:

$$I\% = ((C - T) / C) \times 100$$

Where: C represents the diameter of the control. T represents the diameter of inhibition observed in the sample.

RESULTS AND DISCUSSION

Synthesis

Triazinane such as 1-isopropyl-3,5-diphenyl-1,3,5-triazinane was preparedfrom the reaction of isopropylamine and aniline with formalin (Figure 1).

The mechanism of reaction by making Schiff bases (Figure 2).

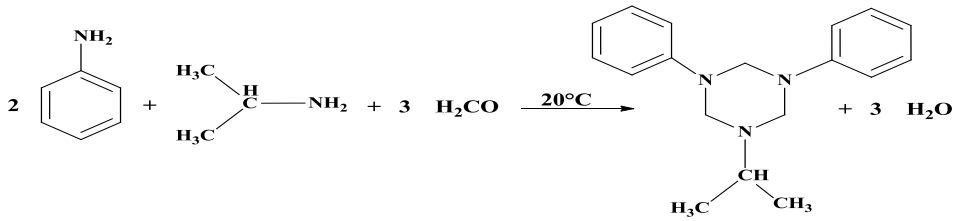

Figure1. 1-isopropyl-3,5-diphenyl-1,3,5-triazinane.

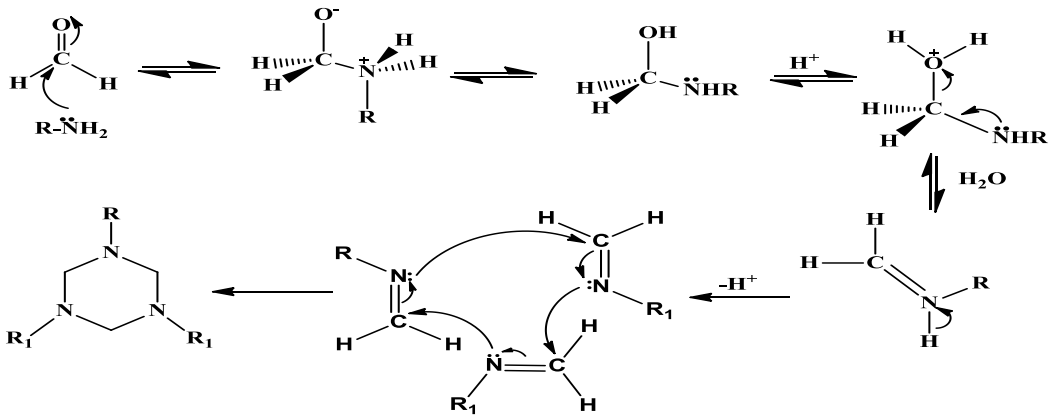

Figure 2. FTIR Study of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane.

There is a faint stretching band at 3082 cm^{-1} for the C-H aromatic structure. Four strong bands between 2951 and 2852 cm^{-1} are caused by the C-H stretching vibration of asymmetric and symmetric CH and CH_3 groups. Finally, there are two strong bands at 1593.4 and 1499.4 cm^{-1}. The stretching of the non-localized double bonds (C=C) within the aryl group is characterized by a medium-strong band at 1277.8 cm^{-1}. This band is caused by the symmetric deformation of three C-H methyl groups (CH_3) in the fingerprint region. Additionally, there is a strong band at 758 cm^{-1} which is due to the out-of-plane deformation of the C-H aromatic structure (Figure 3).

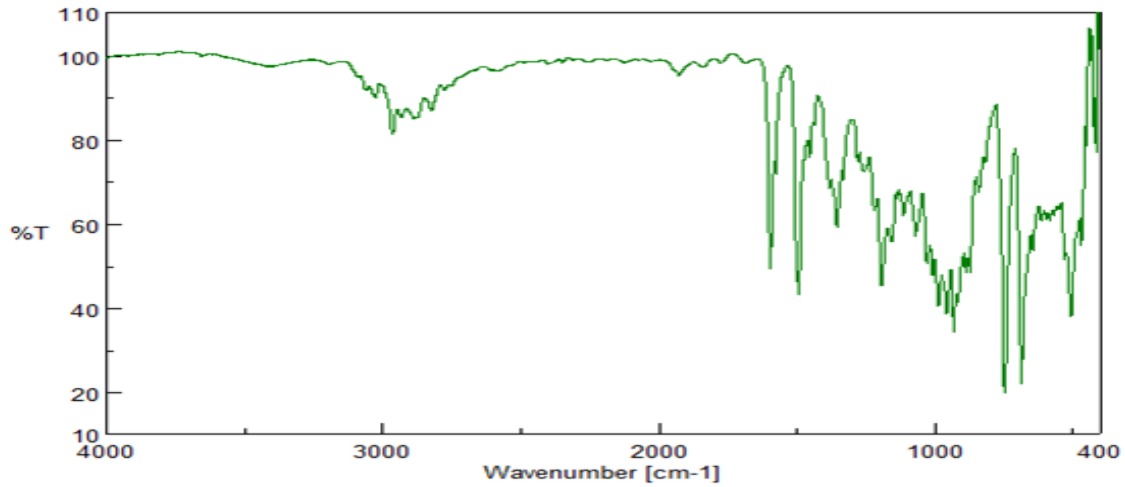

Figure 3. FTIR of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane.

^{1}H NMR Study of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane

The spectrum of 1H NMR exhibits the group methyl protons resonating as a doublet at δ 1.18 ppm, whereas the protons of the CH next to the CH_3 group are seen as a multiplet centered at δ 3.00-3.25 ppm. The two distinct CH_2 groups are seen as singlets at δ 4.30 ppm (alkyl-N-CH_2-N-Ar) and δ 4.66 ppm (Ar-N-CH_2-N-Ar) with an intensity ratio of 2:1, indicating the presence of an asymmetric hexadrotriazine derivative. Ultimately, the protons of the aromatic system manifest as a multiplet within the range of δ 6.78 and 7.19 ppm. (Figure 4).

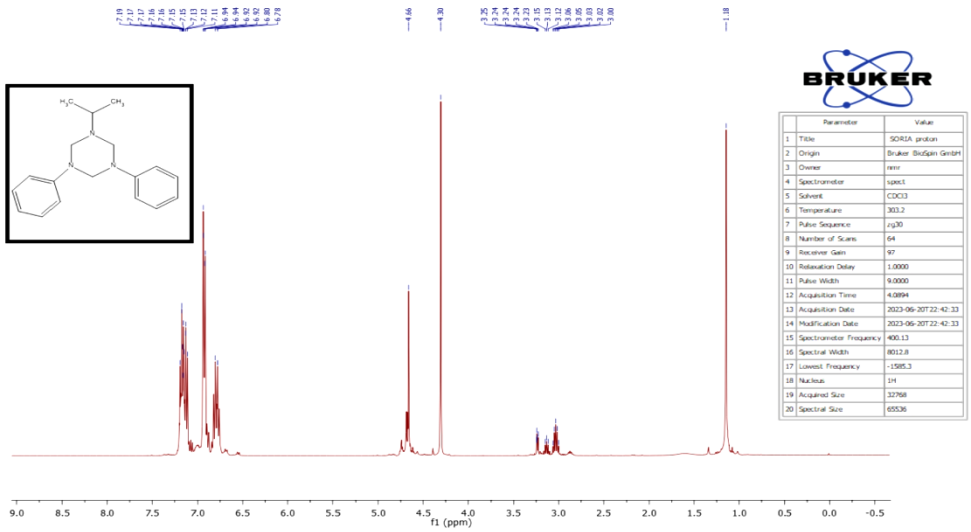

Figure 4. ^{1}H NMR of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane.

^{13}C NMR Study of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane

The ^{13}C NMR spectrum shows that the atoms of carbon of the group methyl are seen at a chemical shift of δ 20.80. Additionally, the carbon atom of the CH group, which is next to both CH_3 and -N-CH_2, is observed at a chemical shift of δ 48.79 ppm. The carbon atoms of the triazinane cycle are seen at chemical shifts of δ 68.63

(C_4H_9-N-CH_2-Ar) and 69.12 ppm (Ar-N-CH_2-N-Ar), while the remaining carbon atoms of the aryl groups are observed at chemical shifts of δ 129.33-117.36 ppm (-HC=C-N) and δ 149.38-148.77 ppm (N=C-). The largest peak at δ 77.41 ppm originates from the solvent. (Figure 5)

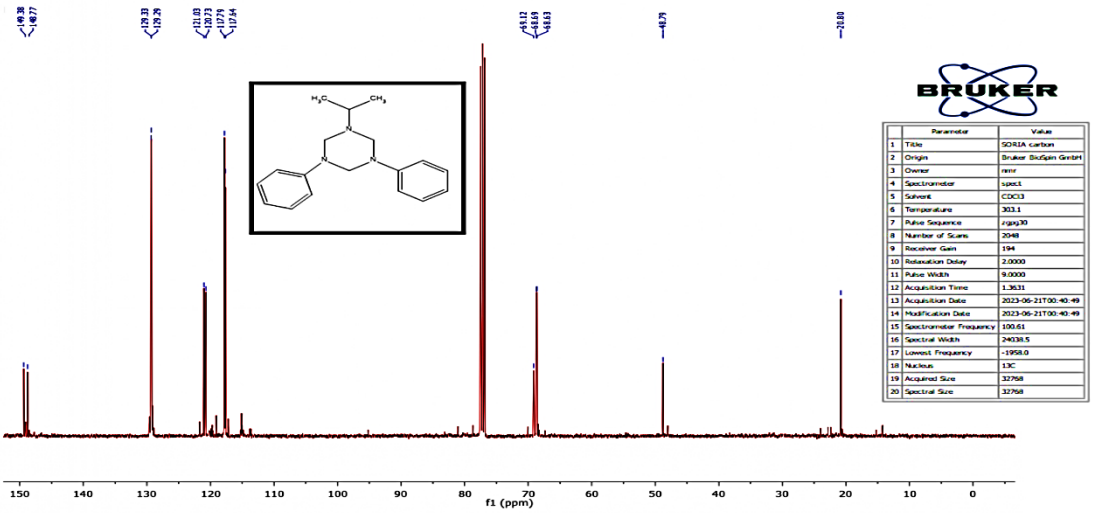

Figure 5. ^{13}C NMR of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane.

UV Study of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane

The UV spectra of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane.Exhibits a low-intensity signal at 242 nm, which is indicative of the n → π* transition. (Figure 6)

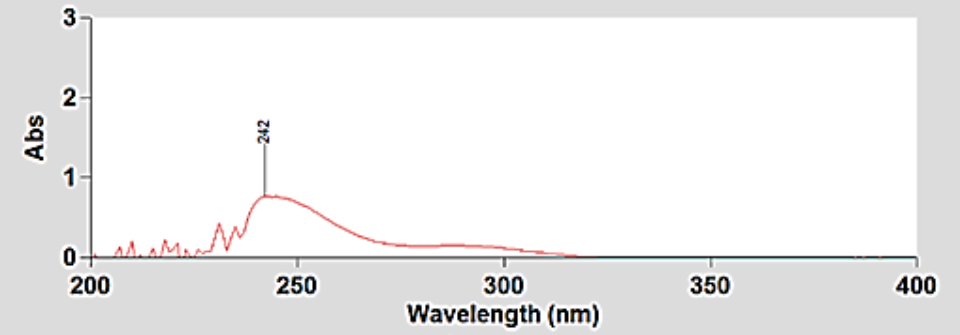

Figure 6. UV of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane.

Antioxidant activities

The 1-isopropyl-3,5-diphenyl-1,3,5-triazinane molecule has demonstrated exceptional antioxidant properties, indeed, the ability to inhibit ABTS radical was very interesting, the IC_{50} value for 1-isopropyl-3,5-diphenyl-1,3,5-triazinanewas low and in the same order of magnitude as the IC_{50} values of both standard compounds. Additionally, 1-isopropyl-3,5-diphenyl-1,3,5-triazinane capacity to convert Fe^{3+} to Fe^{2+} was intriguing, as it achieved a reduction in absorbance to $A_{0.5}$ at remarkably low concentrations of 14.73±0.91 µg ml^{-1}, confirming its heightened antioxidant potential. (Table 1).

Table 1. Determination of the antioxidant activity of 1-isopropyl-3,5-diphenyl-1,3,5-triazinaneby ABTS and phenanthroline assays.

Product	ABTS (IC_{50}(µg ml^{-1}))	Phenanthroline (($A_{0.5}$(µg ml^{-1}))
Compound	11.40±0.07	14.73±0.91
BHA*	4.34±0.31	9.30±0.07
BHT*	4.01±0.10	9.17±0.11

Values expressed are means ± SD of three measurements*standard compounds

Antibacterial activities

According to Table 2, 1-isopropyl-3,5-diphenyl-1,3,5-triazinane has demonstrated an impressive anti-bacterial effect against all tested bacterial strains. This effect was major against *Staphylococcus aureus*, where the diameter of the inhibition zone reaches 15 mm with 8 mg ml^{-1} of concentration. Not only that, but this result is more important than antibiotic (vancomycin) results against the same bacteria at the same concentration (8 mg ml^{-1}).

Table 2. Diameter of inhibition zone of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane against bacterial strains.

Compound	C (mg ml^{-1})	Inhibition zones (mm)			
		GRAM $^{-}$		GRAM $^{+}$	
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus cereus*
1-isopropyl-3,5-diphenyl-1,3,5-triazinane	8	9	8	15	9
	4	8	6	6	5
	2	4	5	4	4
	1	3	-	-	4
	0.5	-	-	-	4
Amoxicillin	8	17	-	-	-
Vancomycin	8	-	-	6	4
Ciprofloxacin	8	-	23	-	-

Antifungal activity

The antifungal activity was presented by the inhibition percentage of fungi growth. According to Figure 7. The molecule 1 – isopropyl - 3, 5-diphenyl - 1, 3, 5-triazinane showed an antifungal effect against *fusarium oxysporum*s pecies at all concentrations, but it was excellent at (3mg ml^{-1}), when the inhibitory percentage reaches 100%. This result candidates this molecule to be a conservator matter.

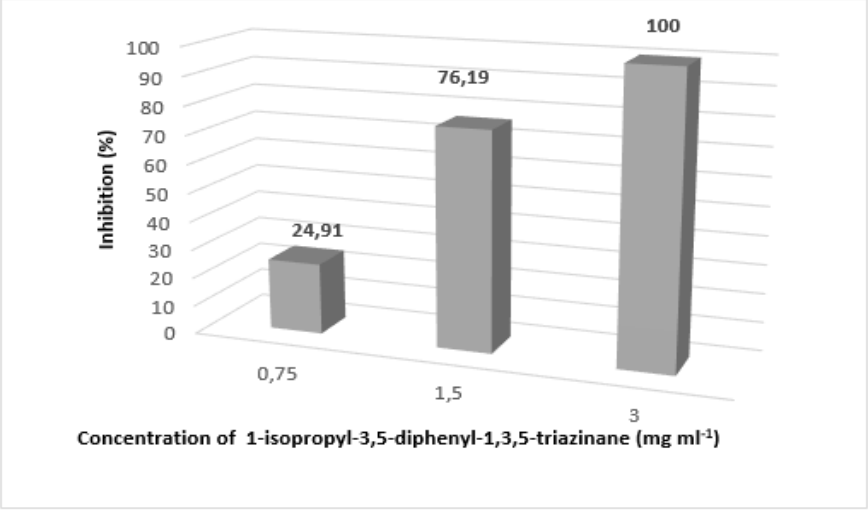

Figure 7. The antifungal ability of 1-isopropyl-3,5-diphenyl-1,3,5-triazinane against fungi ,*fusarium oxysporum.*

CONCLUSIONS

The new 1-isopropyl-3,5-diphenyl-1,3,5-triazinaneis synthesized in good yield and is characterized by UV, IR, ^{1}H NMR and ^{13}C NMR spectral data.

The newly synthesized compound is also screened for antioxidant, Anti-fungal, and antibacterial activities; it shows good activity.

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Conflict of interests

There are not conflicts of interest.

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