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Synthesis, Characterization, Antioxidant and Anti-fungal Activities of a New 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane

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Abstract

This study introduces a new compound, 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane, which is synthesized via a multicomponent condensation reaction involving 4-toluidine, isopropylamine, and paraformaldehyde in the presence of ethanol. The structures of this compound have been determined using several analytical techniques: TLC, UV, IR, ^{1}H NMR, and ^{13}C NMR. Based on two different methods, its antioxidant activity was measured by phenanthroline and ABTS tests and showed excellent activity. This novel molecule was tested against fungal strains by the disk diffusion method, and the results obtained were high.

Keywords: Synthesis;Antioxidant;Antifungal;1,3,5 triazinane.

INTRODUCTION

In contemporary times, heterocycles play a crucial role in synthetic organic chemistry, particularly those that include nitrogen. This is because of their vast therapeutic and pharmacological capabilities, as shown by several studies[1–6]. One of the essential nitrogen-containing heterocycles is 1,3,5-triazinanes and their derivatives. These compounds are very intriguing due to their substantial biological worth such as,antimicrobial activity[7], antitubercular[8],antibiofilm agents[9], insecticidal activity[10],antifungal activity[11] and use in several synthetic applications, including their employment as efficient reagents in various cycloaddition and aminomethylation

reactions[12], protecting groups[13], polymer chemistry[14], and metal ligands in symmetric catalysts for organic synthesis[15]. As a result, considerable attention has been given to developing highly efficient new 1,3,5-trazinanes derivatives[16,17].

MATERIALS AND METHODS

Synthesis

Paraformaldehyde(37%, 7 ml) was added to a stirred solution of 4-toluidine (20 mmole, 2.14 g) and isoprolylamine (10 mmole, 0.59 g, 0.86 ml) with 10 ml of ethanol. The mixture was stirred for 4 hours at room temperature. The verification of the reaction's completion was confirmed by the use of thin-layer chromatography (TLC). The resulting solution was evaporated on a rotary evaporator to dryness. The residual material was subjected to recrystallization with hexane. Yield: 82.85% (2.56g), M.p = 138°C,R_f ($CHCl_3$ 100%) = 0.41

IR (ν, cm^{-1}):3049.5(C-H, Ar), 2981-2849(C-H), 1599-1500 (C=C), 1283(C-N), 766 (C-H, Ar).

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm):6.96 – 6.82 (m, 10H, Ar), 5.04 (s, 2H, Ar-N-CH_2-N-Ar), 4.83 – 4.79 (m, 1H, CH_3-CH-CH_3), 4.67 (s, 4H, Ar-N-CH_2-N-CH_2-N-Ar), 2.17 (s, 6H, Ar-CH_3), 1.18 (s, 6H, CH_3-CH-CH_3).

^{13}CNMR (101 MHz, $CDCl_3$, δ ppm):146.38 (2C, N-C_{Ar}), 129.71 (2C, C_{Ar} -Me), 119.04- 118.01 (8C, CH=C), 82.92 (C, Ar-N-CH_2-N-Ar), 69.91 (C, CH_3-CH-CH_3), 69.53 (2C, Ar-N-CH_2-N-CH_2-N-Ar), 20.53 (4C, Ar-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 ($\mu g ml^{-1}$).

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 an absorption of 0.5.

Anti-fungal activity

The antifungal activity was evaluated against *fusarium oxysporum*, tomato phytopathogenic fungi, using the disc diffusion method[20].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-di-p-tolyl-1,3,5-triazinane(diluted in DMSO)at different concentrations (3, 1.5 and 0.75mg 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 closedand 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

Triazinanesuch as 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinanewas preparedfrom the reaction of isopropylamine and 4-toluidine with paraformaldehyde (Figure 1).

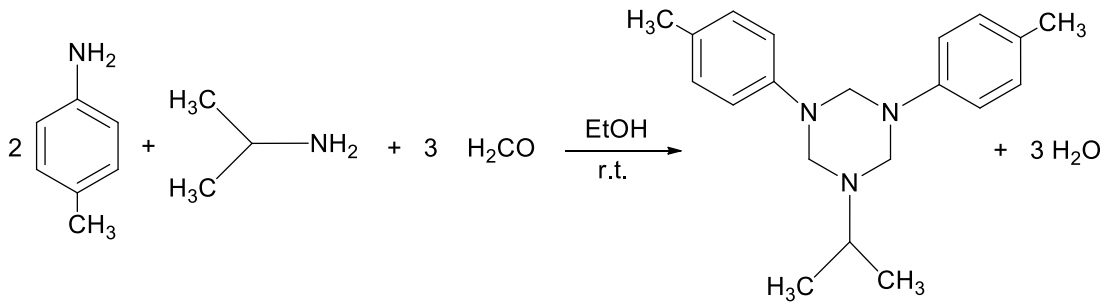

Figure1. Synthesis of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane

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

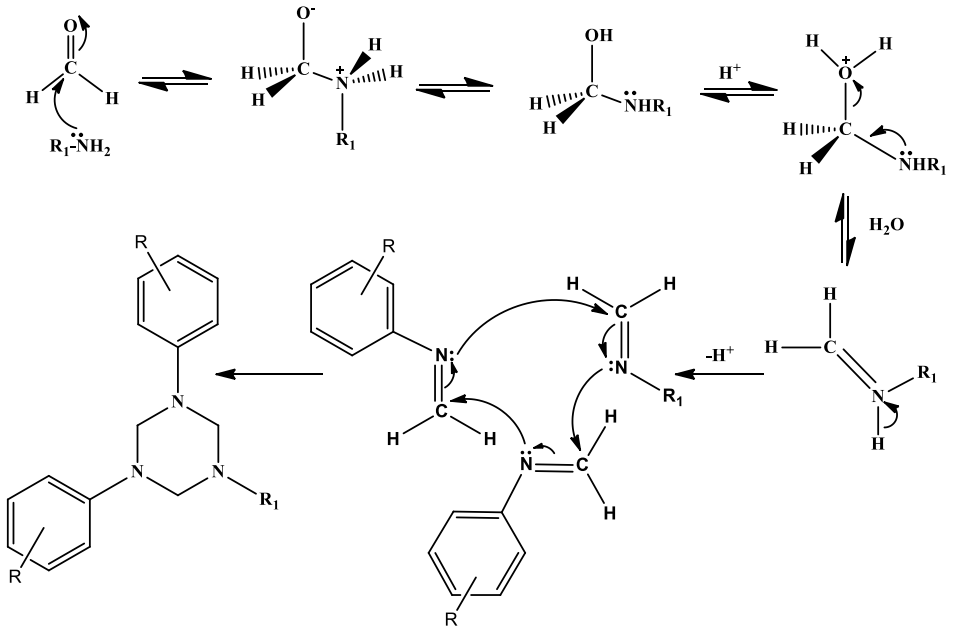

Figure2. Mechanism of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane.

FTIR Study of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane

There is a faint stretching band at 3049.5 cm^{-1} for the C-H aromatic ring. Three strong bands between 2849 and 2981 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 1500 and 1599 cm^{-1}.The stretching of the non-localized double bonds (C=C) within the aryl group is characterized by a medium-strong band at 1283 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 766 cm^{-1} which is due to the out-of-plane deformation of the C-H aromatic ring.

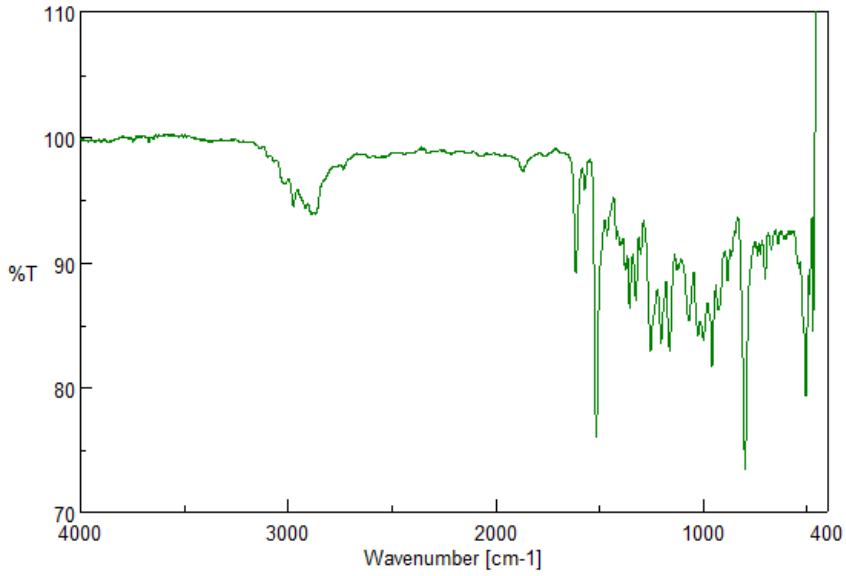

Figure 3. FTIR of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane.

^{1} H NMR Study of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane

The spectrum of 1H NMR exhibits the group methyl protons resonating as a doublet between δ 1.18- 2.17 ppm, whereas the protons of the CH next to the CH_3 group are seen as a multiplet centered at δ 4.79- 4.83 ppm. The two distinct CH_2 groups are seen as singlets at δ 4.67 ppm (alkyl-N-CH_2-N-Ar) and δ 5.04 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 6.82 and 6.96 ppm.

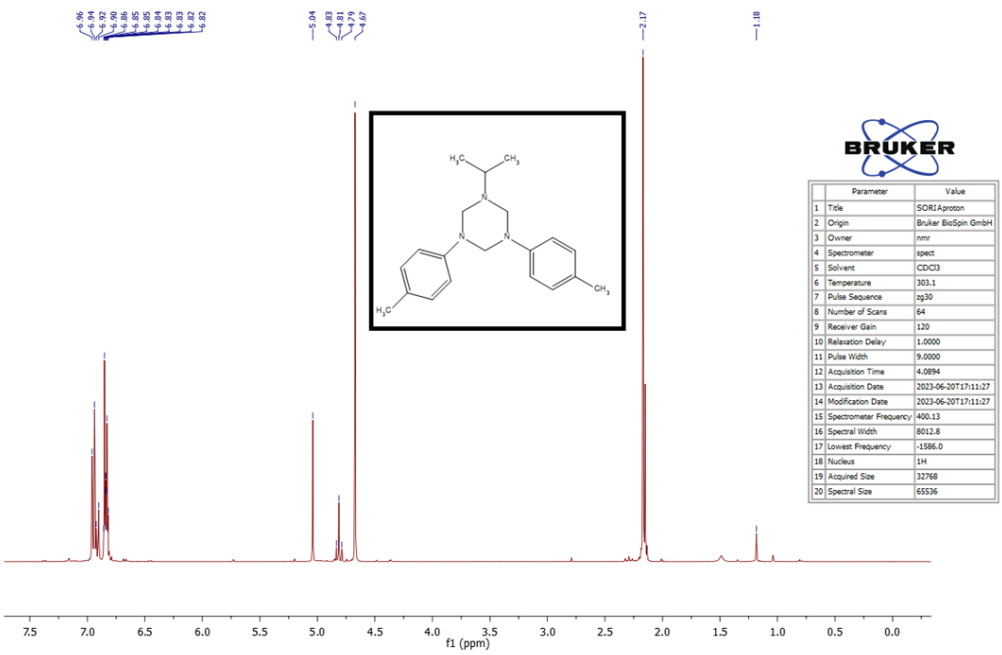

Figure 4.[1] H NMR of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane.

^{13}C NMR Study of 1-isopropyl-3,5-di-p-tolyl-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 20.53 ppm. 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 69.91 ppm. The carbon atoms of the triazinane cycle are seen at chemical shifts of δ 69.53 ppm (C_4H_9-N-CH_2-Ar) and 82.92 ppm (Ar-N-CH_2-N-Ar), while the remaining carbon atoms of the aryl

groups are observed at chemical shifts of δ 118.01-129.71 ppm (-HC=C-N) and δ 146.38 ppm (N=C-). The largest peak at δ 77.41 ppm originates from the solvent.

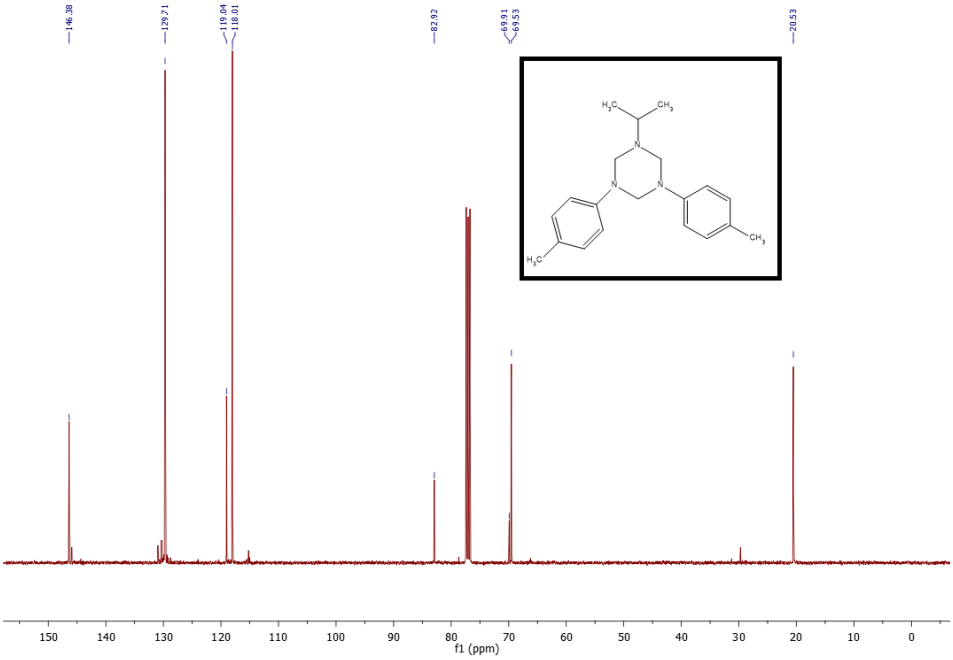

Figure 5.^{13}C NMR of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane.

UV Study of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane

The UV spectra of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane.Exhibits a low-intensity signal at 241.0778 nm, which is indicative of the n → π* transition.

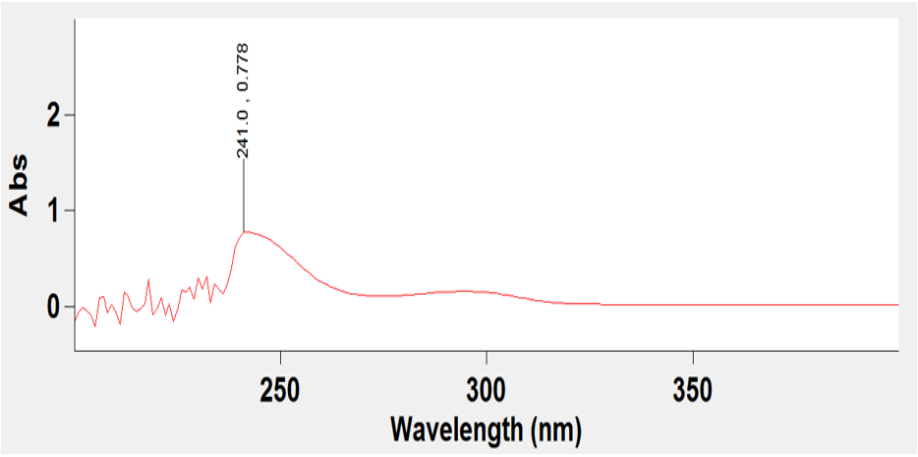

Figure 6. UV of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane.

Antioxidant activities

Table 1.Determination of the antioxidant activity of 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinaneby ABTS and

phenanthroline assays.

Product	ABTS (IC_{50}(µg ml^{-1}))	Phenanthroline (($A_{0.5}$(µg ml^{-1}))
Compound	10.06±1.12	6.24±0.66
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

The 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinanemolecule has demonstrated exceptional antioxidant activities; indeed, the ability to convert Fe^{3+} to Fe^{2+} was exciting, the $A_{0.5}$ value (6.24±0.66 µg ml^{-1}) for this molecule was low and in the same order of magnitude as the $A_{0.5}$ values of both standard compounds BHA and BHT (9.30 ± 0.07 and 9.17 ± 0.11 µg ml^{-1}, respectively). Additionally, 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinanecapacity to inhibit ABTS radical was intriguing, as it achieved a reduction in absorbance to IC_{50} at deficient concentrations of 10.06±1.12 µg ml^{-1}, demonstrating its strong antioxidant capability.

Antifungal activity

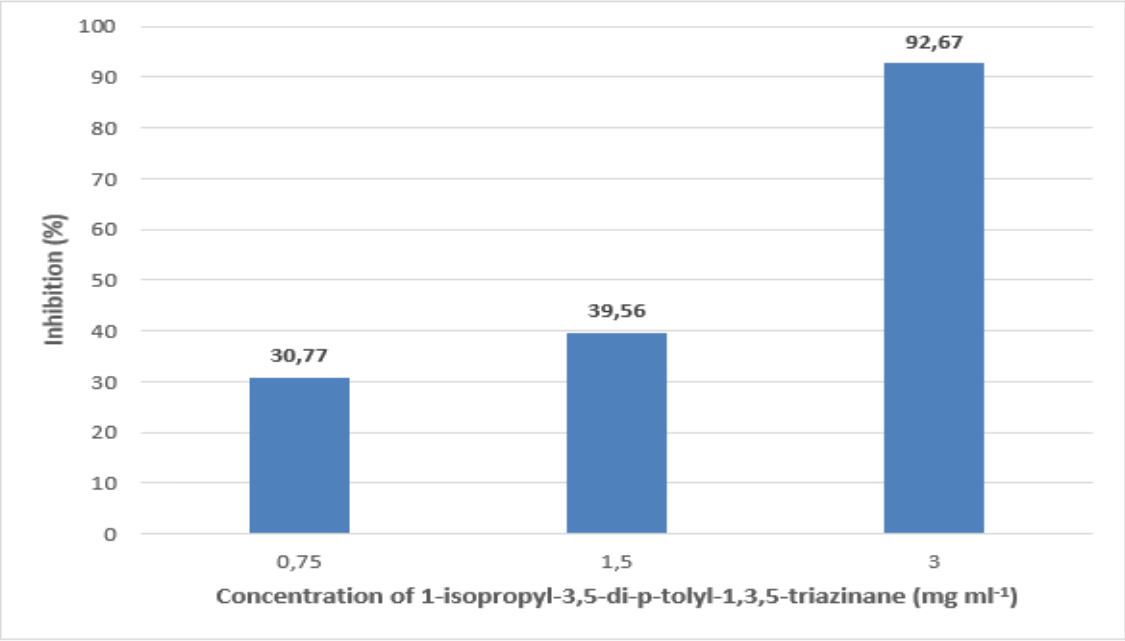

Figure 7. Theantifungalability of1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane againstfungi, fusarium oxysporum.

The antifungal activity of the synthesized molecule was tested against fungi, *Fusarium oxysporum* by the disc diffusion method. The growth zone of each fungus was measured in cm, and the antifungal activity was measured as percentage inhibition (%), according to **Figure 07**. The compound showed an antifungal effect against *fusarium oxysporum* species at all concentrations. The inhibitory percentage started from 30.77% to92.67 %, which was excellent at (3 mg ml^{-1}). This result indicates that this compound is a potential candidate for becoming a preservative substance.

CONCLUSIONS

The newly synthesized 1-isopropyl-3,5-di-p-tolyl-1,3,5-triazinane is obtained in a good yield and is characterized by UV, IR, ^{1}H NMR, and ^{13}C NMR spectroscopic techniques. The recently synthesized compound is also evaluated for its antioxidant and anti-fungal properties; it exhibits significant efficacy.

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Conflict of interests

there are no conflicts of interest

References

1. Basak, M. *et al.* A Review on the Reported Antibacterial Activity of 1,3,5-Triazine. *J. Pharm. Res. Int.***33**, 279–292 (2021).
2. Mahmoodi, N. O., Mohammadi Zeydi, M., Biazar, E. & Kazeminejad, Z. Synthesis of novel thiazolidine-4-one derivatives and their anticancer activity. *Phosphorus, Sulfur Silicon Relat. Elem.***192**, 344–350 (2017).
3. Adjissi, L. *et al.* Synthesis, characterization, DFT, antioxidant, antibacterial, pharmacokinetics and inhibition of SARS-CoV-2 main protease of some heterocyclic hydrazones. *J. Mol. Struct.***1270**, 134005 (2022).
4. Park, W. S. *et al.* Synthesis and biological evaluation of triazepane derivatives as DPP-IV inhibitors. *J.*

*Fluor. Chem.***130**, 1001–1010 (2009).

5. Pinkner, J. S. *et al.* Rationally designed small compounds inhibit pilus biogenesis in uropathogenic bacteria. *Proc. Natl. Acad. Sci. U. S. A.***103**, 17897–17902 (2006).

6. Khalaj, A. *et al.* Discovery of a novel nitroimidazolyl-oxazolidinone hybrid with potent anti Gram-positive activity: Synthesis and antibacterial evaluation. *Eur. J. Med. Chem.***46**, 65–70 (2011).

7. Lefrada, L. *et al.* Synthesis and antimicrobial activity of 1,3-bis-butyl-5-(4-iodophenyl)-1,3,5-triazacyclohexane. *Eur. J. Chem.***8**, 82–84 (2017).

8. Dandia, A., Jain, A. K. & Sharma, S. ChemInform Abstract: Task-Specific Ionic Liquid-Mediated Facile Synthesis of 1,3,5-Triaryltriazines by Cyclotrimerization of Imines and Their Biological Evaluation. *ChemInform***45**, 130839 (2014).

9. Ayrim, N. B. *et al.* Hexahydro-1,2,3-triazine Derivatives: Synthesis, Antimicrobial Evaluation, Antibiofilm Activity and Study of Molecular Docking Against Glucosamine-6-Phosphate. *Indones. J. Chem.***24**, 141–151 (2024).

10. Qin, Y. *et al.* Novel (E)-β-farnesene analogues containing 2-nitroiminohexahydro-1,3,5-triazine: Synthesis and biological activity evaluation. *Molecules***21**, 1–14 (2016).

11. Pareek, P. K., Mithlesh, Kriplani, P., Ravikant & Ojha, K. G. Rapid synthesis and biological activities of some new benzothiazol-2- ylhexahydro-s- triazine derivatives. *Phosphorus, Sulfur Silicon Relat. Elem.***185**, 1338–1345 (2010).

12. Liang, D., Xiao, W. J. & Chen, J. R. Recent Advances of 1,3,5-Triazinanes in Aminomethylation and Cycloaddition Reactions. *Synth.***52**, 2469–2482 (2020).

13. Knapp, S., Hale, J. J., Bastos, M. & Gibson, F. S. Amino protection using triazones. *Tetrahedron Lett.***31**, 2109–2112 (1990).

14. Gungor, F. S. & Kiskan, B. Tailoring polyvinyl alcohol with triazinanes and formaldehyde. *React. Funct. Polym.***124**, 115–120 (2018).

15. Latreche, S., Mousser, A., Kociok-Köhn, G., Köhn, R. D. & Schaper, F. Unsymmetrically substituted triazacyclohexanes and their chromium complexes. *Polyhedron***29**, 1399–1404 (2010).

16. Podrezova, A. G. *et al.* Three-component synthesis of bridged 1,3,5-triazinanes. *Tetrahedron***150**, 133750 (2024).

17. Rabouel, I. *et al.* Molecules25225475.Pdf. 1–17.

18. Re, R. *et al.* Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine* vol. 26 1231–1237 at (1999).

19. Szydłowska-Czerniak, A., Dianoczki, C., Recseg, K., Karlovits, G. & Szłyk, E. Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. *Talanta* vol. 76 899–905 at https://doi.org/10.1016/j.talanta.2008.04.055 (2008).

20. Campbell, B. Technical section. *The Annals of The Royal College of Surgeons of England* vol. 45 493–496 at https://doi.org/10.1308/rcsann.2013.95.7.532 (1966).