Synthesis, crystal structure study, and evaluation of antimicrobial and antibiofilm activities of a novel dihydroisoquinoline-derived

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Highlights

-Synthesis and characterization studies of three dihydroisoquinoline imine, oxaziridine and nitrone.

-Structure of compound **4** was established by Single X-ray crystallography.

- In vitro antimicrobial and antibiofilm screening were studied.

Synthesis, Crystal Structure Study, and Evaluation of Antimicrobial and Antibiofilm Activities of a novel Dihydroisoquinoline-derived

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Abstract.

Models of dihydroisoquinoline imine **2**, oxaziridine **3** and nitrone **4** were synthesized and characterized through the mass spectrum, elemental analysis and NMR spectral studies. The crystal structure of compound **4** was determined from single crystal X-ray diffraction data.

All these compounds were evaluated for their antimicrobial and antibiofilm activities, represented here with MICs values and % anti-adherence activity. Antimicrobial activity showed MICs values ranged between 0.31 to more than 2.5 mg/ml and Anti-adherence activity reached more than 75% especially with nitrone compound **4** when applied at a concentration of 0.312 mg/ml.

Keywords: Imine, oxaziridine, nitrone, antimicrobial activity, anti-biofilm activity.

1. Introduction

Since their discovery by Emmons in 1956 [1], oxaziridines have gained increasing attention [2]. In particular, the oxaziridine is of interest because of an inherently weak N–O bond due to the strained ring that makes the molecule unusually highly reactive. Oxaziridines have increasingly been employed as oxygen or nitrogen donors in organic synthesis, depending on their structural electronic features and the nature of the nucleophile. [3,4] They are extensively employed in asymmetric syntheses [5] and as precursors of oxaziridinium salts [6]. Also, they possess basic properties that enhance isomerization and hydrolysis reactions in

acid media. The isomerization of oxaziridines has been reported to lead to nitrones [7], which are useful and versatile intermediates for a wide array of organic syntheses.

A plethora of methods exist for obtaining oxaziridines: the photoisomerisation of nitrones [8], the electrophilic amination of carbonyl compounds [9], the oxidation of imines with several oxidizing agents such as buffered Oxone [10], UHP/maleic hydride system [11], molecular oxygen in the presence of transition-metal complexes [12] and a nitrile /hydrogen peroxide system have been used for this purpose [13]. The most common for preparation involves the oxidation of an imine to afford the corresponding oxaziridine, using a peroxy acids, particularly meta-chloroperbenzoic acid (mCPBA) [10, 14, 15].

Oxaziridines have attracted considerable attention due to various biological properties such as anti-tumor,[16] anti-malarial,[17] and antifungal[18] and as analogues of penicillin.[19] Recently, dihydroisoquinoline oxaziridines have been shown to have potent hypolipidemic effect in high-fat diet-fed rats.[20] Furthermore, they are widely used as reagents and intermediates in the preparation of biologically active molecules [21-23]

Nitrones were first used to trap free radicals in chemical systems and then subsequently in biochemical systems. More recently, several nitrones, including alpha-phenyl-tertbutylnitrone (PBN), have been shown to have potent biological activity in many experimental animal models. Many diseases of aging, including stroke, cancer development, Parkinson disease, and Alzheimer disease, are known to have enhanced levels of free radicals and oxidative stress [24].

Nitrones are easily prepared by several methods well documented in the primary literature. The most used includes condensation reactions between carbonyl compounds [25, 26] and oxidation of amines, imines, or hydroxylamines [27, 28]. But the most effective method remains isomerization of oxaziridines [29, 30] which are interesting heterocyclic compounds containing oxygen, nitrogen, and carbon atoms in a three-member ring.

The aim of this study was the synthesis of imine **2**, oxaziridine **3** and nitrone **4**. The newly synthesized compounds were also evaluated for their antimicrobial and Anti-Biofilm activities in vitro.

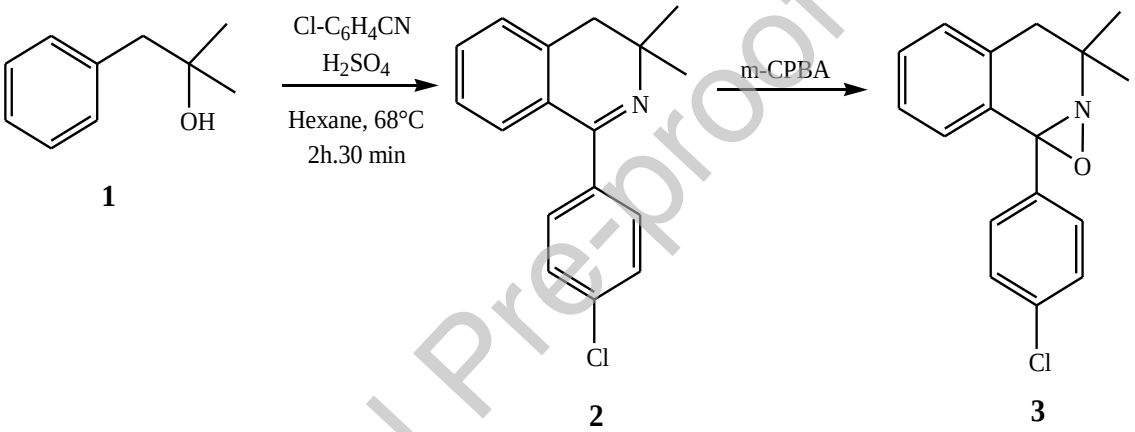
A suitable crystal of nitrone **4** was obtained by recrystallization and the molecular structure was confirmed by single X-ray analysis.

2. Results and Discussion:

2.1. Synthesis:

In this study, we have developed a simple and efficient synthetic method of dihydroisoquinoline oxaziridine and nitrone from the corresponding oxaziridines. The oxaziridines presented in this paper were synthesized starting from the commercial tertiary alcohol **1** (Scheme 1).

The imine **2** was obtained by acid catalyzed reaction of 2-methyl-1-phenylpropan-2-ol **1** by Ritter-type procedure with 4-chlorobenzonitrile. The peracid oxidation of imine **2** lead quickly to oxaziridine **3** in good yields (Scheme 1).



1 **2** **3**

Scheme 1. Synthesis of oxaziridine **3**

Furthermore, mass spectrum showed a [M+H]+ peak at m/z 286,09831 corresponding to its molecular formula, $C_{17}H_{16}NClO$.

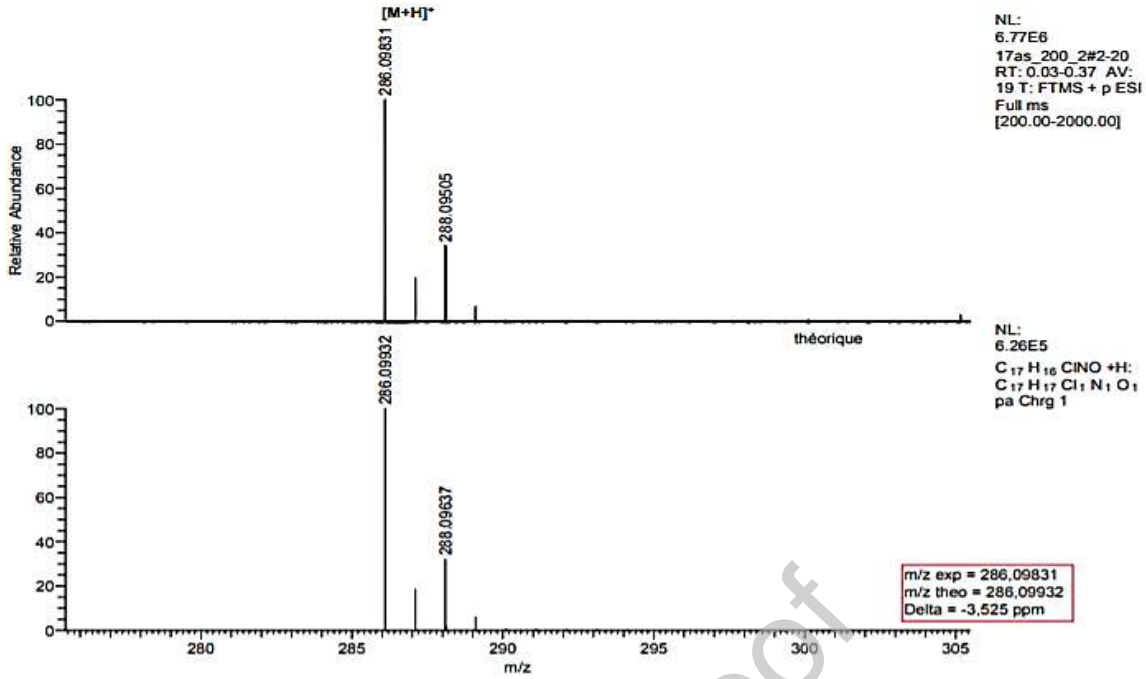
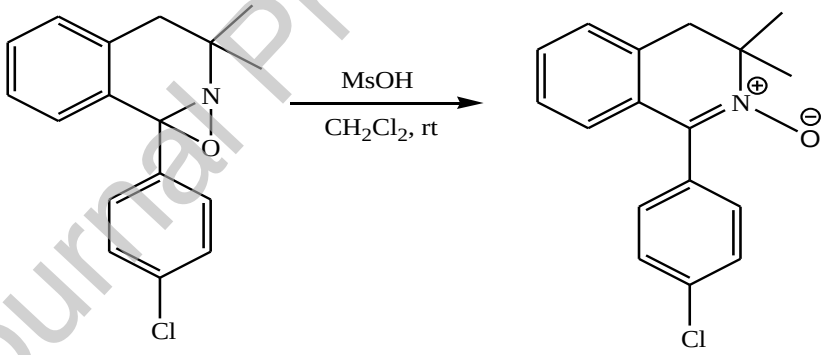


Figure 1 : FT-MS Spectrum of oxaziridine **3**.

Herein, we study the isomerization reaction of oxaziridine **3** (Scheme 2) with 3 equivalents of methanesulfonic acid (CH_3SO_3H) lead to nitrone 4 with goods yields.



3 **4**

Entry	AH	Oxaziridine	Time[b]	Yield of nitrone[b] (%) (conversion)[c]
1	3 eq	**3**	5 min	76 (100)
2	4 eq	**3**	>1min	78 (100)

Scheme2. Synthesis of nitrone **4**

Next, mass spectrum showed a [M+H]+ peak at m/z 286,09843 corresponding to its molecular formula, $C_{17}H_{16}NClO$.

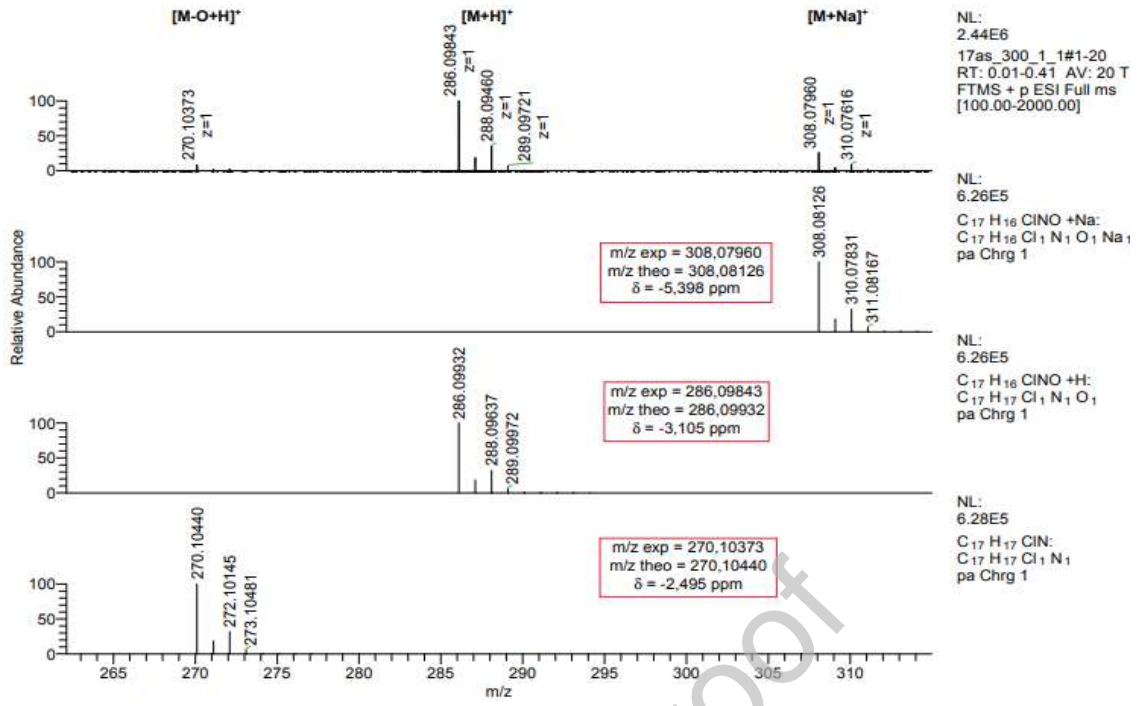


Figure 2 : FT-MS Spectrum of nitrone **4**.

The structures of compounds were confirmed by nuclear magnetic resonance (^{1} H NMR, ^{13}C NMR) and mass spectrometry. The NMR spectroscopy of all synthesized compounds was recorded using $CDCl_3$ as a solvent, their NMR spectra indicated characteristic signals. In the ^{1}H NMR spectra of imine **2**, the peak of gem-dimethyl corresponding to the 6 protons was observed as a singlet at 1.27 ppm and the two equivalent neighboring protons of $–CH_2$ group appeared as a singlet, while in the ^{1}H NMR spectra of oxaziridine **3**, these 6 protons appeared as a two-singlet state at 1.13 and 1.56 ppm and the equivalent protons neighboring of the $–CH_2$ group appeared as a doublet signal. Respectively, in the spectra ^{13}C NMR of compound **2**, the two carbons of gem-dimethyl were identified as one peak, but in the spectra ^{13}C NMR of compound **3** were observed as two peaks that suggest the influence of N-O bond.

2.2. Crystal structure of 4:

Crystals of nitrone **4** were obtained by recrystallization from hexane. The molecular structure of compound **4** is shown in Figure 3.

The synthesized compound **4** with formula $\mathbf{C_{17}H_{16}ClNO}$ crystallize in the triclinic symmetry, with the centrosymmetric space group $P\bar{1}$ and has the unit cell dimensions: a = 8.8119(9) Å, b = 9.3804(9) Å, c = 10.0799(10) Å and α= 113.355(3)°,β = 103.999(3)° and γ = 98.895(3)°.

The crystal structure of **4** is built from only the organic nitrone molecules interconnected between themselves *via* weak intermolecular interactions of Van Der Waals type. Such connectivity defines the supramolecular network of the structure (Figure 4).

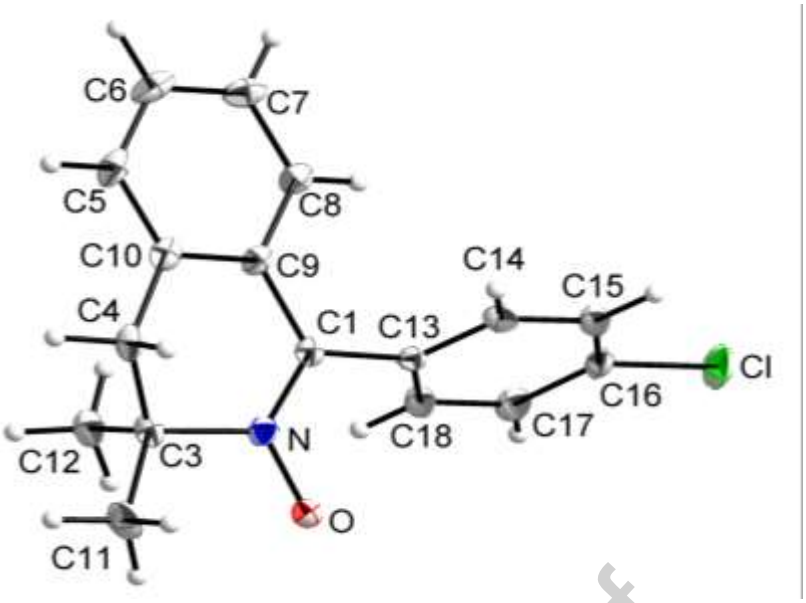


Figure 3. Molecular structure of the compound 1-(4-chlorophényl) -3, 3-diméthyl -3,4-dihydroisoquinoléine-2-oxide **4** with atom numbering.

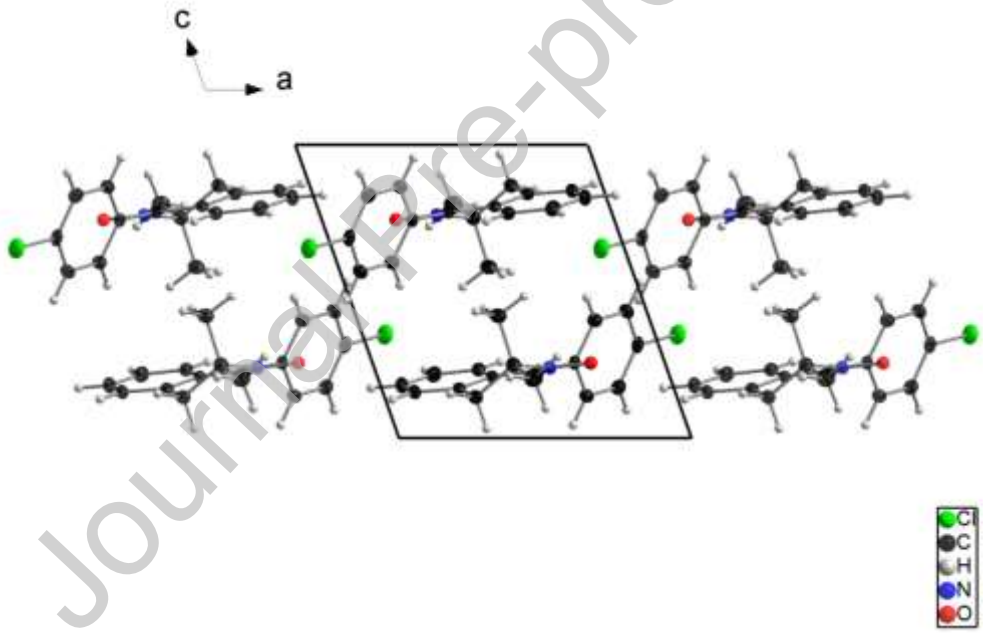


Figure 4: Projection of the structure of $C_{17}H_{16}ClNO$ along the crystallographic b axis, showing its supramolecular structure (For clarity, intermolecular weak interactions are omitted).

In the molecular structure of nitrone **4**, the tetrahydroisoquinoline unit is substituted by a chloro phenyl ring attached to C1, and two methyl groups attached to C3; the one is pseudo-equatorial and the second is pseudo-axial (Figure 3).

The dihedral angle between the plane containing the chloro phenyl attached to C1 and the plane formed by the atoms of the phenyl ring coordinated at C1 and C4 is equal to 73.575° (Figure 5).

Figure 5. Projection on planes axes.

2.3. Hirshfeld surface analysis:

Hirshfeld surface analysis was performed to more explain intermolecular interaction in crystal packing. The Hirshfeld analysis results are shown in figure 6. In all potential molecular contacts, H···H contacts constitutes the most important interactions in the crystal, contributing 49.4 % of the overall intermolecular interactions. The second most significant intermolecular interaction is H···C and H···Cl, resulting in 24.2 % and 14.9 % of the sum of intermolecular interactions, respectively. The intermolecular O···H interactions appear also with the proportion of 8.8 %. In addition, C···Cl, H···N and Cl···Cl interactions exist with a negligible contributing to the total intermolecular interactions. Finally, we see that the crystal structure is stabilized by a variety of interactions.

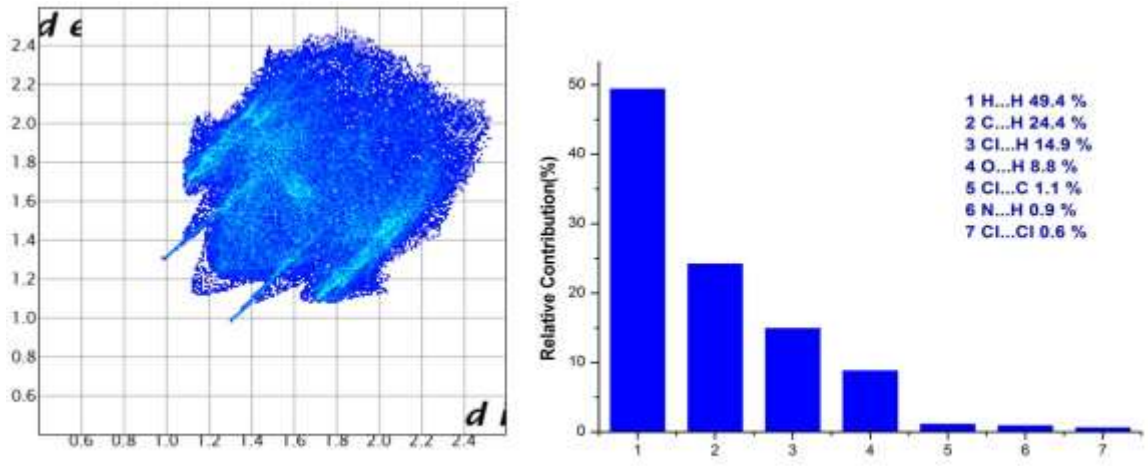


Figure 6. Hirshfeld surface analysis of $C_{17}H_{16}ClNO$

2.4. Biological Studies:

2.4.1. Antimicrobial activity:

Antibacterial activities represented with MICs values of the newly synthesized molecules are grouped in the following table1 :

Table 1: Results of the minimum inhibitory concentrations (MICs) obtained with the dihydroisoquinoline molecules **2**, **3** and **4** against the reference bacterial strains tested and the yeast *Candida albicans* (ND: not determined).

	(2)	**(3)**	**(4)**	**Chloramphenicol**
Microbial strains	**MIC (mg/ml)**	**MIC (mg/ml)**	**MIC (mg/ml)**	**MIC (mg/ml)**
Listeria monocytogenes	>2.5	>2.5	[0.625-0.31]	[0.312-0.156]
Micrococcus luteus	>2.5	[1.25-2.5]	[0.625-1.25]	[0.312-0.156]
Bacillus subtilis	>2.5	>2.5	[1.25-2.5]	[0.156-0.312]
Enterococcus faecium	>2.5	[1.25-2.5]	[1.25-2.5]	[0.312-0.156]
Escherichia coli	>2.5	[1.25-2.5]	[1.25-2.5]	[0.156-0.078]
Staphylococcus aureus	>2.5	>2.5	>2.5	[0.156-0.078]
Candida albicans	[0.625-1.25]	[0.625-0.31]	[0.625-0.31]	ND

Antibacterial activity revealed that oxaziridine **3** and nitrone **4** showed pronounced activities compared to imine **2**. In fact, MIC values exceeded 2.5 mg/ml with compound **2** for all tested bacteria, whereas with **3** and **4** MIC values ranged between 0.31 to 2.5mg/ml. Yeast strain (*Candida albicans*) was sensitive to the three tested compounds, but the activity remains more pronounced with **3** and **4** with MIC values ranged between 0.31 to 0.625 mg/ml. Previous works have demonstrated antibacterial activities of newly synthetized oxaziridines with MICs values ranged between 20 to 40 mg/[31]. However other synthetized oxaziridines showed prominent activities. In fact, a series of aromatic nitrones were tested for their antimicrobial activities. MICs value.s were ranged between 50µg/ml to more than 600 µg/ml and it has been concluded that the activity of nitrones is depending on the substituent on the aryl group of the nitrone [32].

2.4.2. Antiadhesive activity:

Results of antiadhesive activity are grouped in figure **7**. Tested concentrations varied between 0.312 to 0.019 mg/ml. Strain *Staphylococcus epidermidis* S61 was used as biofilm-forming model.

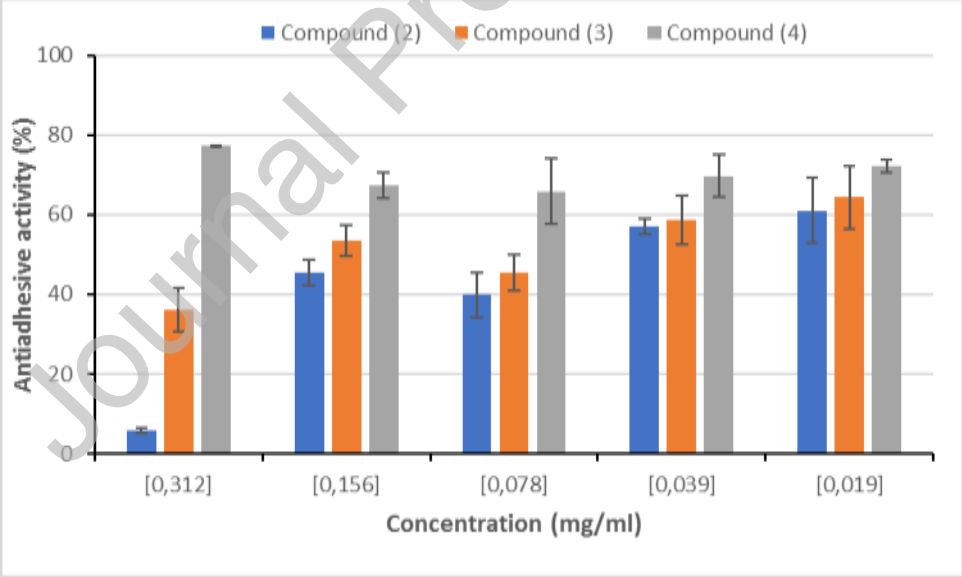


Figure 7: Antiadhesive activity of compounds **2**, **3** and **4**.

The structural modifications made in the molecules oxaziridine **3** and nitrone **4** were able to produce a significant improvement in activity, which could reach more than 75% in the molecule **4**. The anti-adhesion activity is correlated with the antibacterial activity as both

molecules **3** and **4** showed improvement in antimicrobial activity. To the best of our knowledge, this is the first report focusing on antiadhesive activities of such dihydroisoquinoline compounds. These newly synthetized compounds could have potential applications in medical fields to control bacterial adhesion in health care environments

3. Experimental Section:

3.1. Chemistry

3.1.1. General procedures.

Solvents were purified by standard procedures. Melting points (mp) were determined under a microscope with a Leitz Wet-zlair device and uncorrected. NMR spectra were recorded on an AC 400 Bruker spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C, chemical shifts (δ) were given in ppm and coupling constants (J) were given in hertz (Hz) and abbreviations for multiplicity are respectively called (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet triplet, m = multiplet).
All the reactions were monitored by TLC using commercial silica gel plates and visualization was accomplished by UV light.

3.1.2. Preparation of Imine 2.

To a cooled (0°C) solution (2 mL) of sulfuric acid, H_2SO_4 (95%) was added dropwise and under magnetic stirring, 1.25 eq of p-chlorobenzonitrile in 5 mL of hexane. Then, 500 mg (3.33 mmol) of tertiary alcohol **1** (commercial product) in 5 mL of hexane was added to the solution.

After returning to room temperature, the resulting mixture was stirred under reflux at 68°C for for 2.30 hours. Then, the solution is cooled at the room temperature and versed on ice-cold water under magnetic stirring. The solution is alkalized with ammonia. The organic layer was extracted with dichloromethane, washed with a saturated aqueous NaCl solution, dried over sodium sulfate, and filtered. The solvent was removed in vacuo and the obtained crude was purified by chromatography (silica gel, eluent dichloromethane/methanol 98:2). The purity of the products was checked by TLC.
mp: 112-115 °C; Yield (72%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.27 (s, 6H, $2CH_3$), 2.80 (2H, CH_2), 7.16 (m, 1H-Ar), 7.21 (m, 2H-Ar), 7.39 (m, 3H-Ar), 7.51 (m, 2H-Ar). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 27.56 ($2CH_3$), 38.84 (CH_2), 54.69 (C-$2CH_3$),

126.58, 127.67, 128.40, 130.26, 130.95, 135.10, 137.61, 137.68, 163.61. HRMS (ESI) [M + H]+ calc. For $C_{17}H_{17}ClN$ 270.1044; found 270.1034.

3.1.3. Preparation of Oxaziridine 3.

In small portions, a slight excess of m-chloroperbenzoic acid (1.5 eq of active oxygen), was added to a solution of imine **2** (200 mg) in 5 mL methanol under magnetic stirring at room temperature, (TLC control: dichloromethane: methanol 98:2). The solvent was evaporated and the residue obtained was taken up in dichloromethane. The solution was washed with a solution of sodium bicarbonate and then with a saturated aqueous solution of sodium chloride. The organic phase was dried on sodium sulfate, filtered, and concentrated.

mp: 147°C; Yield (85%) (72%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.13 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 2.55 (d, J = 15.4 Hz, 1H), 2.92 (d, J = 15.4 Hz, 1H), 7.02 (d, J = 7.1 Hz, 1H), 7.18 (m, 2H), 7.36 (m, 1H), 7.43 (m, 4H). ^{13}C NMR (CDCl3, 100MHz): δ 22.79, 28.72, 37.26, 56.84, 81.19, 126.40, 128.48, 128.71, 128.76, 129.56, 130.30, 130.61, 134.46, 135.42, 135.81. HR-MS (ESI) [M + H] + calc. For $C_{17}H_{17}ClNO$ 286.0993; found 286.0983.

3.1.4. Preparation of Nitrone 4.

Methanesulfonic acid (50 mg, 0.52 mmol) was added to a solution of oxaziridine **3** (50 mg, 0.17mmol) in dichloromethane (6 ml) and the mixture was stirred at room temperature. A control of the reaction mixture by TLC (dichloromethane) indicated the disappearance of the oxaziridine. The solution was diluted with dichloromethane (25 ml) and washed with a solution of sodium bicarbonate. The organic phase was dried on sodium sulfate, filtered, and concentrated.

mp: 125°C; Yield (76%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.52 (s, 6H, 2-CH_3), 3.18 (s, 2H, CH_2), 6.83 (d, J = 7.8 Hz, 1H), 7.18 (m, 1H), 7.29 (m, 2H), 7.48 (m, 4H). ^{13}C NMR ($CDCl_3$, 100MHz): δ 24.63, 41.64, 67.23, 126.12, 127.06, 127.76, 128.51, 128.74, 130.11, 130.43, 130.96, 131.74, 134.77, 140.20. HR-MS (ESI) [M + Na] + calc. For $C_{17}H_{16}ClNO$ Na 308.0812; found 308.0796.

3.2. X-ray crystallography

A suitable crystal for X-ray diffraction single crystal experiment (colourless prism, dimensions= 0.450 x 0.400x 0.320 mm) was selected and mounted with a cryoloop on the goniometer head of a APEXII Kappa-CCD (Bruker-AXS) diffractometer equipped with a CCD-LDI-APEX2 detector, using Mo-Kα radiation (λ= 0.71073 A°, multilayer

monochromator) at T= 150(2) K. Crystal structure has been described in triclinic symmetry and P1 centric space group. Cell parameters have been refined as follows: a = 8.8119(9), b = 9.3804(9), c = 10.0799(10) Å, α = 113.355(3), , β = 103.999(3), γ = 98.895(3) °,V = 712.25(12) $Å^3$. Number of formula unit Z is equal to 2 and calculated density d and absorption coefficient μ values are 1.332 $g.cm^{-3}$ and 0.263 mm^{-1} respectively. Crystal structure was solved by dual-space algorithm using SHELXT program [33], and then refined with full-matrix least-squares methods based on F^2 (SHELXL program [34]). All non-Hydrogen atoms were refined with anisotropic atomic displacement parameters. H atoms were finally included in their calculated positions and treated as riding on their parent atom with constrained thermal parameters. A final refinement on F^2 with 3237 unique intensities and 183 parameters converged at $\omega R(F^2)$ = 0.1019 (R_F = 0.0384) for 2676 observed reflections with ($I > 2\sigma$). Crystallographic data are given in supplementary material.

3.3. Biological Testing

3.3.1. Antimicrobial activity: Determination of Minimum Inhibitory Concentration (MIC)

The antimicrobial activities of newly synthesized dihydroisoquinoline compounds **2**, **3** and **4** were investigated using micro-dilution method in 96-well plates as suggested by the National Committee for Clinical Laboratory Standards [35]. Assays were carried out with five bacterial strains *Listeria monocytogenes Micrococcus luteus Bacillus subtilis Enterococcus faecium*, *Escherichia coli* and *Staphylococcus aureus*, and one yeast strain which is *Candida albicans*. Each bacterium was grown in LB medium overnight at 30 °C. The culture was then adjusted to an optical density of 0.3-0.6 at a wavelength of 600 nm. The compounds were dissolved in dimethylsulfoxide (DMSO) to adequate concentrations and then filtered. A volume of 100 μl was added in each well of two columns of the plate with medium and successive two-fold dilution was prepared in plate to obtain final compound concentration of 2.5 mg/ml to 4.8 μg/ml. An aliquot (20 μl) of the bacterial/yeast dilution was added to each well and a final volume of 200 μl/well was adjusted with medium. Wells containing only LB medium with inoculum and these containing medium, inoculum and Ampicillin served as controls. Microplates were incubated at 30 °C for 18–24 h. After incubation, bacterial growth was evaluated by the MTT revelation. 20 μl of 3- (4, 5-dimethyl-thiazol-2-yl)- 2,5- diphenyl-tetrazolium bromide (MTT) at concentration of 0.5 mg/ml was prepared in water, filtered and added to each well. The plates were incubated for 20 min in the dark with stirring. The viable

microorganisms were detected by the change of yellow MTT color to purple. The MIC was defined as the lowest compound concentration that completely inhibited any visual growth of the microorganisms being tested. So, the MIC interval was taken from the first well devoid of bacterial growth and the first well showing growth. MICs values of chloramphenicol as control antibiotic were also determined in the same described conditions.

3.3.2. Antibiofilm: Antiadhesive activity

In this test, 96-well flat bottom plates were used for biofilm cultures [36]. The biofilm-forming strain, *S. epidermidis* S61, was grown overnight in TSB medium at 30 °C and diluted with the same fresh medium supplemented with 2.25% (w/v) glucose as previously reported by Jardak et al., 2017. Briefly, One-hundred microliter of the bacterial culture dilution was added into each well to obtain a final OD600 nm of 0.1. Then, 100 µL of each compound dissolved in TSB with DMSO, at various concentrations, was added into wells to reach final concentrations from 0.312mg/ml to 0.019 mg/ml. Wells containing only TSB/DMSO medium supplemented with 2.25% glucose and bacterial suspension were served as controls. Plates were incubated for 24 h at 30 °C under static conditions. After incubation, the wells were emptied into a mini container by inverting the plates. Each well was gently washed twice with 250 µL of sterile phosphate buffered saline (PBS 1 X, pH 7.2) in order to remove the planktonic cells [37]. After washing, plates were dried at 60 °C for 60 min in a thermostatically controlled oven. Then, wells were stained with 150 µL of crystal violet (0.2%) freshly prepared in 20% (v/v) ethanol for 15 min at room temperature. After staining incubation, crystal violet was removed, and excess dye was washed three times with sterile water. Finally, 200 µL of glacial acetic acid (33%, v/v) was added to each well and plates were incubated for 1 h at room temperature. The optical density (OD) was measured at 570 nm using a Varioskan Flash microplate reader (Thermo Scientific). The percentage of the adhesion inhibition was calculated by the following formula:

% adhesion inhibition= [(OD (growth control) – OD (treatment)/ OD (growth control) x 100]

4. Conclusion

We have successfully synthesized and characterized of three compounds imine **2**, oxaziridine **3** and nitrone **4** and the crystal structure of the novel nitrone **4** was determined. Furthermore, the antimicrobial study of these compounds revealed that the titled compounds **2-4** possess

variable MIC values against a number of microorganisms. Among them, oxaziridine **3** and nitrone **4** showed pronounced antibacterial activities compared to imine **2**.

Besides, the synthesized compounds **2-4** were tested for their Antiadhesive activity. Results showed that the tested nitrone **4** exhibited a good Antiadhesive activity.

The new confirmed crystal structure of compound **4** can be considered interesting for further modification and help relate structure to activity trends, with the perspective to become a new member as an antimicrobial and Antiadhesive activity agent for the development of new active compounds.

Supplementary material

Supplementary information file containing data tables of the crystal structure of compound **4**, spectral (^{1}H and ^{13}C) NMR of compounds **(1-4)** are available and

Crystallographic data for the structure **4** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC (2122367).

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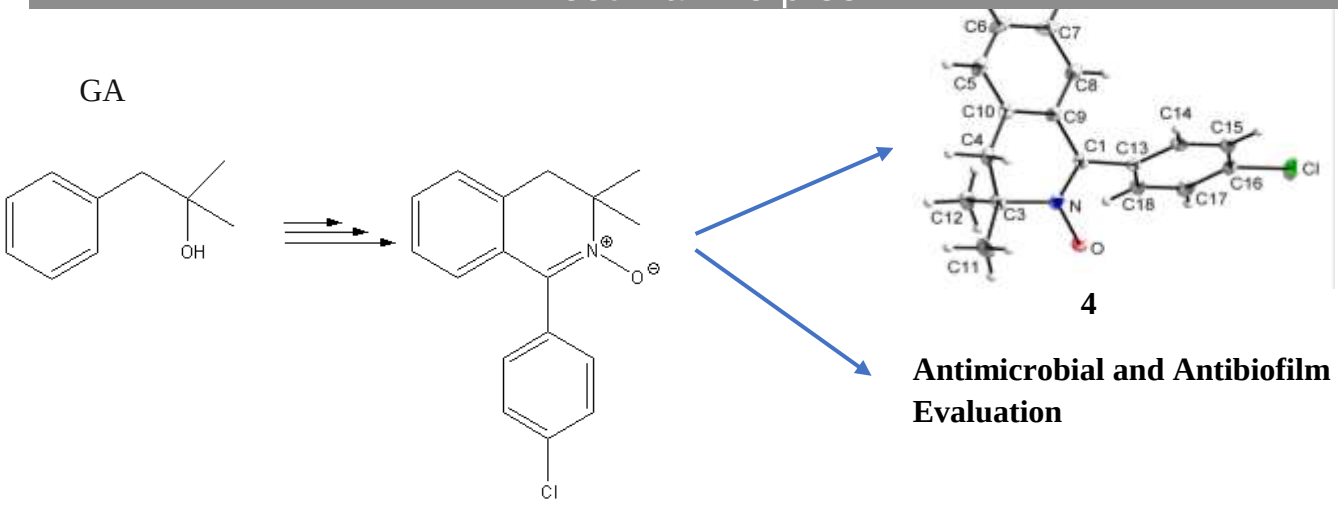

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Antimicrobial and Antibiofilm Evaluation

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.