

Proceedings

# Synthesis and Preliminary Antibacterial Evaluation of A 2,4,5-Tri(hetero)arylimidazole Derivative <sup>†</sup>

Nuna L. P. Ramos <sup>1</sup>, Rui P. S. Oliveira <sup>2</sup>, Susana P. G. Costa <sup>1</sup> and M. Manuela M. Raposo <sup>1,\*</sup>

<sup>1</sup> Centre of Chemistry, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; nuna.ramos15@gmail.com (N.L.P.R.); spc@quimica.uminho.pt (S.P.G.C.)

<sup>2</sup> Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; Centre of Biological Engineering (CEB), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal; ruipto@bio.uminho.pt

\* Correspondence: mfox@quimica.uminho.pt

<sup>†</sup> Presented at the 24th International Electronic Conference on Synthetic Organic Chemistry, 15 November–15 December 2020; Available online: <https://ecsoc-24.sciforum.net/>.

Published: date

**Abstract:** The imidazole ring is a planar heterocycle whose derivatives are applied in several scientific areas, such as medicinal, materials and supramolecular chemistry. The presence of the imidazole ring in these structures is the key to the development of new drugs, since it is ubiquitous in naturally occurring biological structures. Therefore, over the past few decades, several imidazole derivatives have been synthesized and occupy a unique position in the field of medicinal chemistry due to their diverse biological activities. In order to continue the work developed by the research group, we report the synthesis of 2,4,5-tri(hetero)arylimidazole derivatives and their characterization by <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) and UV-Vis absorption spectroscopies. As a complement to the characterization of the synthesized 2,4,5-tri(hetero)arylimidazole derivatives, a screening for antibacterial activity showed inhibition of *Staphylococcus aureus* proliferation, suggesting antibacterial activity. Therefore, these new compounds have the potential for the development of new drugs.

**Keywords:** Imidazole; synthesis; antibacterial activity; Gram-positive bacteria

## 1. Introduction

In recent years, heterocyclic drugs have been introduced to the market aiming for the treatment of various diseases. The size and type of structures, together with the various substituents that can be introduced in these rings, contribute to the definition of their physical-chemical properties, as well as their potential biological activity [1].

The imidazole ring is part of a group of the most prominent heterocycles, since it is present in several natural products, such as histamine, purine, nitrogenous bases of DNA and histidine. Due to their characteristics, imidazole derivatives exhibit a wide spectrum of pharmacological and biological activities, being properly researched and studied by the pharmaceutical industries for the development of new drugs [1–3].

Imidazole derivatives are polar and ionizable aromatic compounds that are used to optimize parameters of solubility and bioavailability of existing molecules. In addition, the versatile structure of imidazole ring allows binding to numerous enzymes and receptors, at the biological level, through various weak interactions, leading to a wide range of biological activities. Thus, these derivatives play an extremely important role in medicinal chemistry, urging chemists to synthesize a diverse

range of imidazole derivatives as possible chemotherapeutic agents due to their antibacterial, antifungal, anti-inflammatory, antiviral, anticancer, antihistaminic and antituberculous biological activities [1–5].

*Staphylococcus aureus* is a widely distributed Gram-positive opportunistic pathogen that has been becoming progressively resistant against the antibiotics usually prescribed for infections. This microorganism is responsible for causing infections in the skin and mucous membranes of the human being and may penetrate the bloodstream through wounds or even through direct or indirect contact with a contaminated object [6–8]. So, resistant strains, such as methicillin-resistant *S. aureus*, represent a major health problem in hospital environments and even in public health. Therefore, the search for new drugs is of utmost importance to overcome resistance of pathogenic strains.

In this work we report the synthesis and spectroscopic characterization of an imidazole derivative substituted at positions 2, 4 and 5 of the imidazole ring, as well as the study of its potential antibacterial activity against *S. aureus*.

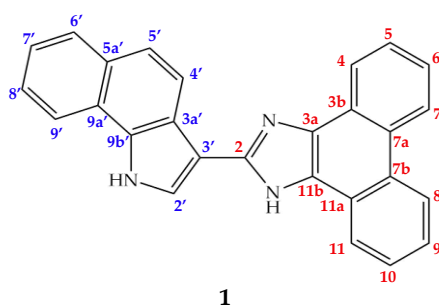
## 2. Materials and Methods

Commercial reagents were supplied by Sigma-Aldrich, Acros and Fluka and were used as received. Thin layer chromatography (TLC) was performed on silica gel 60 plates with fluorescence indicator F254 (Macherey-Nagel). The  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance spectra were recorded using a Bruker Avance III device at 400 MHz and 100.6 MHz, respectively, using the solvent peak as an internal reference. The assignment of the  $^1\text{H}$  and  $^{13}\text{C}$  signals was performed using two-dimensional heteronuclear correlation techniques, using as solvent  $\text{DMSO-}d_6$  with a 99.9% deuteration degree, containing 0.1% *v/v* tetramethylsilane from Sigma-Aldrich. The UV-visible absorption spectra was obtained using a Shimadzu UV/2501PC spectrophotometer.

For the study of antibacterial activity, a strain of bacteria *S. aureus* ATCC 6538 was used.

### 2.1. Synthesis and Spectroscopic Characterization of Imidazole Derivative 1

The 1*H*-benzo[*g*]indole-3-carbaldehyde (1 equiv.), 9,10-phenanthrenequinone (1 equiv.) and ammonium acetate (20 equiv.) were dissolved in glacial acetic acid (5 mL) and the reaction mixture was stirred and heated at reflux for 8 h. The reaction was monitored by TLC, using dichloromethane as eluent. Then, the reaction mixture was allowed to cool to room temperature and the compound precipitated, which was isolated by filtration and washed with petroleum ether (40–60 °C). It was obtained as a light green solid in 73 % yield (*m* = 0.111 g).



1

$^1\text{H}$  RMN (400 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  = 7.49 (dt, *J* = 8.0 e 1.2 Hz, 1H, H7'), 7.60–7.64 (m, 3H, H5 + H10 + H8'), 7.70–7.77 (m, 3H, H5' + H6 + H9), 8.02 (d, *J* = 8 Hz, 1H, H6'), 8.31 (d, *J* = 2.8 Hz, 1H, H2'), 8.43–8.48 (m, 2H, H9' + H4 ou H11), 8.71 (s largo, 1H, H4 ou H11), 8.85 (d, *J* = 8.4 Hz, 3H, H7 + H8 + H4'), 12.55 (s, 1H, NH indole), 13.17 (s, 1H, NH imidazole) ppm.

$^{13}\text{C}$  RMN (100.6 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  = 108.87 (C3a'), 120.72 (C9'), 120.84 (C5'), 121.41 (C3'), 121.65 (C4'), 121.84 (C4 + C11), 122.00 (C9a'), 123.09 (C2'), 123.87 (C7 + C8), 124.20 (C7'), 124.74 (C5 + C10), 125.66 (C8'), 126.98 (C6 + C9), 127.26 (C3a + C11b + C3b + C11a), 128.48 (C6'), 130.22 (C5'), 131.26 (C9b'), 147.34 (C2) ppm. The signals for carbons 7a and 7b were not visible, as already reported in the literature for similar fused systems.

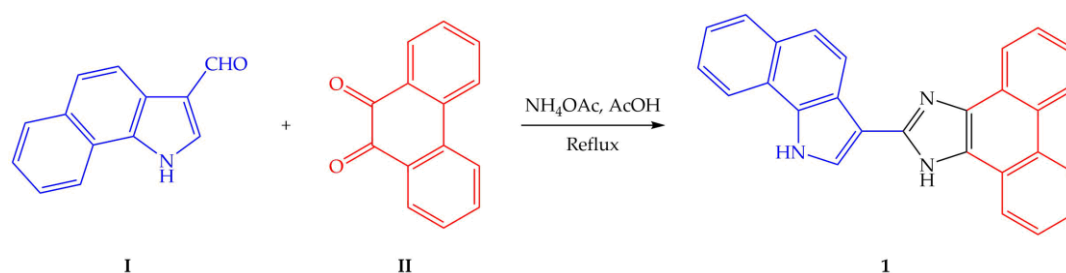
## 2.2. Antibacterial Activity of Imidazole Derivative 1

Cultures were prepared by selecting two morphologically similar colonies of *S. aureus* ATCC 6538 on LB agar (1% *w/v* tryptone, 0.5% *w/v* yeast extract, 1% *w/v* NaCl, 2% *w/v* agar) plate to inoculate LB liquid medium (LB without agar) and incubation for 4–6 h at 37 °C, 200 revolutions per minute. The turbidity of the culture was adjusted to 0.5 of the McFarland scale by diluting with fresh medium. For each Petri dish, top agar was prepared with 100  $\mu$ L cell suspension and 5 mL LB agar (with 1.5% of agar) at 50 °C. The mixture was poured on the plates (approximately 5 mL) containing 15 mL LB agar previously prepared and cooled down. Sterilized Whatman No. 1 filter paper, 6-mm diameter disks were placed onto the medium with the top agar after 15 min. Ten microliters of test solutions were applied to each of the corresponding disks and were allowed to diffuse at room temperature before incubation at 37 °C for 18 h. For the preparation of these solutions, 7.6 mg of the imidazole derivative **1** were weighed and dissolved in DMSO. This solution was diluted 500 and 1000 times (through serial dilutions), with concentrations of 3.0 and 1.5 mg/mL, respectively. Control disks were prepared by adding the same amount of each solvent mixture.

## 3. Results and Discussion

### 3.1. Synthesis and Spectroscopic Characterization of the Imidazole Derivative 1

Imidazole **1** was synthesized through a Radziszewski reaction, by reacting 1*H*-benzo[*g*]indole-3-carbaldehyde (I) with 9,10-phenanthrenequinone (II) in presence of ammonium acetate and acetic acid, as solvent and catalyst, during 8 h, giving the product as a light green solid with 73% yield (Scheme 1).



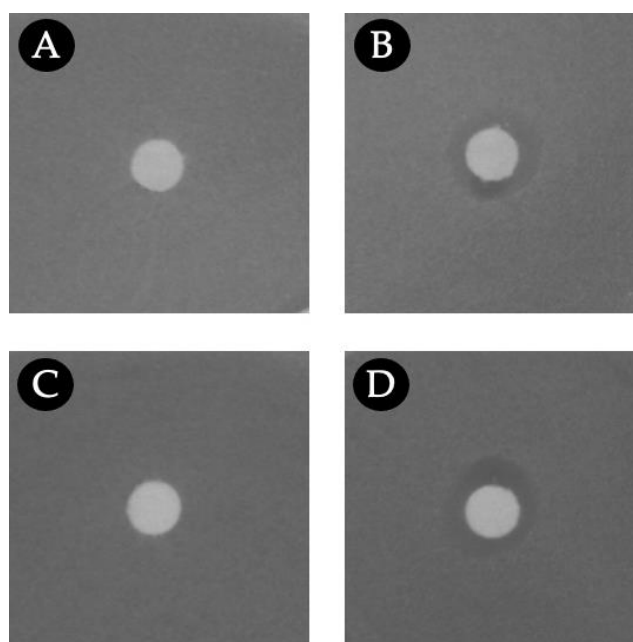
**Scheme 1.** Synthesis of imidazole derivative **1**.

This imidazole derivative was characterized by <sup>1</sup>H, <sup>13</sup>C and two-dimensional NMR. The characteristic signal for the NH proton of the imidazole ring was observed in the <sup>1</sup>H NMR spectrum. The planarity of the aromatic system attached at positions 4 and 5 of the imidazole ring influences the chemical shift of NH from imidazole. The fused aromatic system provides greater flatness and rigidity to the system. Thus, the electronic density on the NH of the imidazole ring in this compound will be lower, when compared to other systems that are not planar, leading to a higher chemical shift of this group. In addition, a correlation can be made between the electron donating properties of the  $\pi$ -conjugated system, linked to position 2 of the imidazole and the chemical shift of the NH of this heterocycle. Derivative **1** has in its structure benzoindole, which is an electron-rich heterocycle that can act as an auxiliary electron donor to the imidazole ring, leading to a lower chemical shift of the imidazole NH.

Imidazole derivative **1** was also characterized by UV-Vis absorption spectroscopy, with a  $1 \times 10^{-5}$  mol dm<sup>-3</sup> in acetonitrile solution. The compound showed an intense absorption band ( $\log \epsilon = 4.4$ ) at 315 nm.

### 3.2. Antibacterial Activity of Imidazole Derivative 1

The antibacterial effects of imidazole **1** against *S. aureus* are depicted in Figure 1. Unlike control disks, a clear inhibition zone is present around the test disks, which diameter is larger when higher concentration was applied (Table 1), suggesting a dose-response effect.



**Figure 1.** Antibacterial assay with disk diffusion method against *S. aureus*. Negative control (A) was prepared by adding 10 µL to the disk of a DMSO/H<sub>2</sub>O solution (0.1% v/v DMSO); (B) represents the assay with 1.5 µg of compound 1. Negative control (C) was prepared by adding 10 µL to the disk of a DMSO/H<sub>2</sub>O solution (0.5% v/v DMSO); (D) represents the assay with 3.0 µg of compound 1. These images are representative of at least three independent assays.

**Table 1.** Diameter of inhibition zones (mm) of imidazole derivative 1 against *S. aureus*. Values were calculated taking into account two and three independent replicas for 3.0 µg and 1.5 µg of compound, respectively.

Amount of Compound 1 (µg)	Diameter (mm)
1.5	8.67
3.0	9.25

This new compound is substituted at carbons 2, 4 and 5 of the imidazole ring. The antibiotics derived from imidazole known to date with clinical use, mostly have a substituent at the NH group of the imidazole in their structure. Thus, compound 1 introduces a novel substitution pattern with potential antibiotic activity to medicinal chemistry because imidazole derivatives of this type showing antibacterial activity against the bacterium *S. aureus* have not yet been reported in the literature. In addition, in antibiotic sensitivity tests with this microorganism, amounts between 10–30 µg of antibiotics are normally used [9], suggesting that this new compound might have higher activity than the usually prescribed antibiotics.

#### 4. Conclusions

Imidazole derivative 1 was synthesized in a good yield (73%) using a simple synthetic methodology and an easy purification procedure. The compound was characterized by the usual NMR and UV-Vis absorption spectroscopic techniques.

The synthesized compound showed antibacterial activity against *S. aureus* at low concentrations, and the diameter of the inhibition zone appears to depend on the concentration of the compound used. Imidazole derivative 1 presents a substitution on all carbons of the imidazole ring, revealing to be a novel molecule to use as a potential antibiotic against *S. aureus*.

**Funding:** This research was funded by the Foundation for Science and Technology (FCT) for financial support to CQ/UM (UID/QUI/00686/2020). Thanks are also due to Fundação para a Ciência e Tecnologia (Portugal) for financial support to the Portuguese NMR Network (PTNMR, Bruker Avance III 400-Univ. Minho).

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Hossain, M. A Review on Heterocyclic: Synthesis and Their Application in Medicinal Chemistry of Imidazole Moiety. *Sci. J. Chem.* **2018**, *6*, 83, doi:10.11648/j.sjc.20180605.12.
2. Zheng, X.; Ma, Z.; Zhang, D. Synthesis of Imidazole-Based Medicinal Molecules Utilizing the van Leusen Imidazole Synthesis. *Pharmaceuticals* **2020**, *13*, 37, doi:10.3390/ph13030037.
3. Brahmabhatt, H.; Molnar, M.; Pavić, V. Pyrazole Nucleus Fused Tri-Substituted Imidazole Derivatives as Antioxidant and Antibacterial Agents. *Karbala Int. J. Mod. Sci.* **2018**, *4*, 200–206, doi:10.1016/j.kijoms.2018.01.006.
4. Shalini, K.; Sharma, P.; Kumar, N. Imidazole and Its Biological Activities: A Review. *Chem. Sin* **2016**, *1*, 36–47.
5. Guda, R.; Kumar, G.; Korra, R.; Balaji, S.; Dayakar, G.; Palabindela, R.; Myadaraveni, P.; Yellu, N.R.; Kasula, M. EGFR, HER2 Target Based Molecular Docking Analysis, in Vitro Screening of 2, 4, 5-Trisubstituted Imidazole Derivatives as Potential Anti-Oxidant and Cytotoxic Agents. *J. Photochem. Photobiol. B Biol.* **2017**, *176*, 69–80, doi:10.1016/j.jphotobiol.2017.09.010.
6. Osés, S.M.; Pascual-Maté, A.; de la Fuente, D.; de Pablo, A.; Fernández-Muiño, M.A.; Sancho, M.T. Comparison of Methods to Determine Antibacterial Activity of Honeys against Staphylococcus Aureus. *NJAS Wageningen J. Life Sci.* **2016**, *78*, 29–33, doi:10.1016/j.njas.2015.12.005.
7. Lehar, S.M.; Pillow, T.; Xu, M.; Staben, L.; Kajihara, K.K.; Vandlen, R.; DePalatis, L.; Raab, H.; Hazenbos, W.L.; Hiroshi Morisaki, J.; et al. Novel Antibody-Antibiotic Conjugate Eliminates Intracellular *S. Aureus*. *Nature* **2015**, *527*, 323–328, doi:10.1038/nature16057.
8. Nasser, A.; Azimi, T.; Ostadmohammadi, S.; Ostadmohammadi, S. A Comprehensive Review of Bacterial Osteomyelitis with Emphasis on Staphylococcus Aureus. *Microb. Pathog.* **2020**, *148*, 104431, doi:10.1016/j.micpath.2020.104431.
9. Hudzicki, J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *Am. Soc. Microbiol.* **2009**.

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).