Development and Evaluation of Imidazole Derivatives Hl1 And Hl2 for Antibacterial and Cytotoxic Activities

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Abstract

HL1 and HL2 imidazole derivatives were synthesized, described, and physiologically assessed in this work. Fisher and Sigma Aldrich analytical solvents were used for synthesis. Thin-layer and silica gel column chromatography purified the substance. MICs were obtained by assessing drugs' antibacterial efficacy against reference strains using broth microdilution. Staphylococcus aureus, MRSA, E. coli, P. aeruginosa, and Acinetobacter baumannii were these pathogens. NMR, FTIR, and XRD were used to characterize the structure. The MTS assay was used to assess HFF-1 cell viability for 24 and 48 hours. Controls included DMEM and Triton X-100. Statistical analysis employed triplicate experiments. The careful reproducibility methods spotlight synthesized imidazole derivatives' antibacterial and cytotoxic properties.

Keywords: *Imidazole derivatives; antibacterial; cytotoxicity; cell viability; drug.*

I. Introduction

One of the biggest problems in world health right now is the rise of bacteria that are resistant to antibiotics. Many traditional antibiotics are no longer effective against pathogens like (MRSA). The need for new antimicrobials with different modes of action to successfully fight against resistant strains has grown in response to this concerning trend. Because of its wide range of biological actions, such as antibacterial, antifungal, antiviral, and anticancer characteristics, imidazole derivatives have garnered a lot of attention among the many classes of heterocyclic compounds.

An extremely useful scaffold in pharmaceutical chemistry is imidazole, a ring with five carbon atoms and two nitrogen atoms. It has pharmacological promise due to its unusual molecular structure, which enables it to bind to enzymes, DNA, and cellular membranes, among other biological targets. The antibacterial effects of imidazole derivatives have been thoroughly investigated. Several medications that have been licensed for clinical use contain the imidazole molecule, including clotrimazole and metronidazole. Still, more imidazole-based drugs with better efficacy and less toxicity are required, despite the encouraging signs.

Improving the biological activity and selectivity of imidazole rings by adding different functional groups has been a prominent method in recent years. Lipophilicity, electronic dispersion, and bacterial target binding capabilities are all profoundly affected by imidazole nucleus substituents. To further improve antibacterial efficacy and decrease resistance development, hybrid compounds including imidazole and other biologically active moieties have been

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synthesized. This novel strategy optimizes biological activity by integrating aromatic functionality with the imidazole core; examples of this include the HL1 and HL2 derivatives, which contain naphthalen-2-ol structures and substituted phenyl groups.

The synthetic method involves purifying the product using silica gel column chromatography after refluxing it in glacial acetic acid with ammonium acetate under an argon environment. An array of spectroscopic methods, including X-ray diffraction, (NMR), and (FTIR), was employed to validate the structural identities of HL1 and HL2. Essential for linking structure with biological activity, these investigations provide light on the compounds' crystalline form, molecular architecture, and existence of functional groups.

To guarantee the safety of novel chemicals for possible therapeutic usage, it is essential to evaluate their cytotoxicity in addition to their antibacterial activity. Hence, the MTS test was used to study the antiproliferative effects of HL1 and HL2 on human skin fibroblast (HFF-1) cells. The survivability of compounds can be assessed at different doses and time periods using this colorimetric assay, which assesses cellular metabolic activity. It is crucial for medication development to incorporate cytotoxicity studies because they give a complete picture of the trade-off between antibacterial efficiency and possible harmful effects on normal human cells.

In order to address two essential features of drug discovery—the effectiveness against target pathogens and the safety for host tissues—the dual focus on antibacterial and cytotoxic properties is being pursued. The goal of this research is to add to the current effort to find new antimicrobial drugs that can overcome resistance and have low cytotoxicity. We will accomplish this by synthesizing and evaluating HL1 and HL2. In order to improve treatment profiles, it is important to combine structural characterization with biological evaluation to obtain useful data.

In addition, the study's methodology guarantees reproducibility and comparability with current literature by utilizing proven spectroscopic techniques and defined biological experiments. This methodological rigor lends credence to the results and bolsters HL1 and HL2's prospects as lead molecules for future pharmacological research.

II. Review of Literature

Al-Ghamdi, Huda et al., (2024) Imidazole-derived chemicals may treat several harmful bacteria. Antimicrobial resistance is rising, and novel antimicrobial drugs are few. To address this, our study synthesized new imidazole compounds for antibacterial testing Results indicated potential possibilities. Chemical identification of imidazole derivatives was done using FTIR and NMR. Data showed that HL2, an imidazole derivative, had good cell survival after 24 and 48 hours. These chemicals hindered the development of the examined microorganisms. This study suggests using imidazole derivatives as antimicrobials.

S. A., Gaz. (2020) Public health is threatened by drug-resistant microorganisms, which are making antibiotics less effective. Thus, developing new antibacterial drugs is crucial. The authors planned to include relevant research produced after Rani et al.'s review article to demonstrate imidazole derivatives' chemical structures and antibacterial action. Over 150 compounds in 100 scientific journals were examined. Important material has been carefully selected and structured in tables and graphs. Further screening of imidazole derivatives may reveal broad-spectrum antibacterial compounds those fight resistant illnesses.

Abdulhameed, Safaa et al., (2020) Oxazole and thioxoimidazolidin were made using a simple method. The penultimate stage was acetyl chloride-triethylamine reaction (S 25, S 26). Triethylamine was made from S 32 and S 33. Molecular docking, FTIR, 1HNMR, and GC MASS spectra characterized derivatives. Several microorganisms were used to assess its antibacterial properties, including S. aureus, S. epidermitis, E. coli, and Klebsiella pneumoniae. Arg121 and Tyr356 are two amino acids that bind five approved NSAIDs. All of the manufactured compounds that docked well with the COX-2 active site had H-bond interactions. Arg121 and Tyr356 form H-bonds with diclofenac, lumiracoxib, and tolfenamic acid.

Zhang, Ling et al., (2014) A simple approach produced oxazole and thioxoimidazolidin. Thiosemicarbazide produced hydrochloric acid derivatives, whereas Benzene sulphonyl chloride produced chloro ethyl acetate derivatives. Finally, acetyl chloride and triethylamine interacted. manufactured triethylamine. FTIR, 1HNMR, GC MASS, and molecular docking studied derivatives. Antibacterial effectiveness was tested using S. aureus, S. epidermitis, E. coli, and Klebsiella pneumoniae. Five allowed NSAIDs bind Arg121 and Tyr356. All synthesized COX-2 active site docking compounds showed H-bond interactions. Tolfenamic acid, lumiracoxib, and diclofenac H-bond at Arg121 and Tyr356.

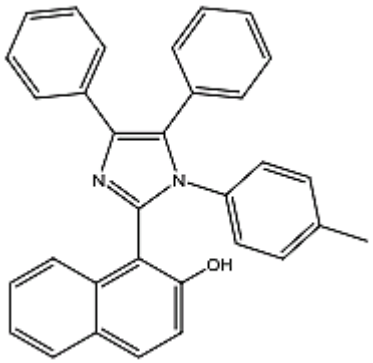
III. Materials and Methods

Materials

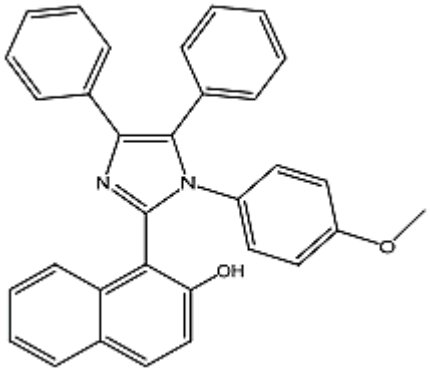
Sigma Aldrich and Fisher Scientific India offered synthetic analytical solvents. (TLC) on aluminum silica gel F254 with iodine indicated the purity of the final imidazole compounds separated them. The chemicals came from Sigma Aldrich India (Bengaluru, Karnataka), came from a licensed Indian pharmaceutical provider. (MHA) and broth (MHB) from Sigma Aldrich India were made per manufacturer's instructions.

Synthesized Imidazole Derivatives Antibacterial (HL1 and HL2)

Once the reaction mixture had been exposed for the specified amount of time, it was cooled and mixed with 20 mL of water. The resulting solid was then filtered off. Schemes 1 and 2 were followed to get the pure HL1 and HL2 products by washing the obtained precipitate with water and 10% acetic acid (4 × 5 mL).



Scheme 1: Synthetic Structure of HL1



Scheme 2: Chemical Illustration of HL2

NMR Spectroscopic Characterization

The 13CNMR scans were recorded using 500 MHz 2K spectrometers and the 1HNMR 32 scans were recorded using Bruker Avance 600 MHz spectrometers. Coupling constants were given in hertz (Hz) and chemical changes in parts per million (ppm).

FTIR Spectroscopic Characterization

ThermoScientific's Nicolet iS10 FTIR instrument recorded the spectra. Synthesised imidazole derivatives were done on KBr discs at 4000–500 cm-1.

XRD Spectral Evaluation

The solid-state of imidazole derivatives was studied using a Rigaku Miniflex 300/600 with Cu Ka radiation. A glass slide was used to mount the specimen, and it was scanned at a speed of 5°/minute from 20.2° to 60° using an electrical current of 40 kV and 15 mA.

Assessment of Cell Viability

This research employed ATCC SCRC-1041 for human skin fibroblasts (HFF-1). HFF-1 cell line, FBS, antibiotic solution, and high glucose Dulbecco's Modified Eagle Medium (DMEM) were purchased from Sigma-Aldrich. The compounds were tested for antiproliferative activity using the Promega MTS assay. To prepare a 10,000 µg/mL stock solution, the HL1 and HL2 imidazole derivatives were dissolved in 10% w/v DMSO and then added to new cell culture media. Afterward, eight dilutions were tested from 5000 to 39 µg/mL. proliferation assay. To summarize, 1.5 x 10^3 HFF-1 cells per well were put in 96-well plates. Cultured cells were incubated at 37 ◦C overnight. After adherent, cells were cultured at 37 ◦C for 24 and 48 hours before introducing HL1 and HL2 at different dosages, as previously reported. Negative and positive controls were DMEM and 0.2% triton x-100, respectively. After 24 or 48 hours, cells were washed with sterile PBS (pH 7.4). Next, 100 µL of full DMEM medium and 20 µL of MTS reagent were added to each well. After that, a cell culture incubator kept the cells alive for three hours. The 492 nm absorbance was measured using a Cytation 3 microplate reader. The proportion of cellular viability was calculated using this equation:

$$\text{Cellular Viability (\%)} = (S - T)/(H - T) \times 100$$

S, T, and H indicate the cells' absorbance of the examined compounds, positive control, and negative control, respectively.

Evaluation of Antibacterial Activity

Imidazole derivatives (HL1 and HL2), vancomycin, and ciprofloxacin as experimental controls were assessed for their (MIC) using broth microdilution following the modified CLSI reference procedure. Vancomycin was dissolved in distilled water, while imidazole derivatives and ciprofloxacin were prepared in 10% DMSO. The derivatives were diluted in MHB using twofold serial dilution, starting at 5000 µg/mL and proceeding to 2.44 µg/mL. Antibiotics were diluted from 40 to 0.02 µg/mL before being added to 96-well plates. Inoculums were created from pure bacterial colonies with a cell density of 1.5 × 10^1 CFU/mL, following the 0.5 McFarland standard. Finally, 100 µL of solution was added to each well, resulting in 1.5 × 105 CFU/mL cell density. The inoculation wells included bacteria as a growth control, whereas the uninoculated wells contained solely MHB as a negative control. The 96-well plates were incubated overnight at 37 ◦C with constant 160 RPM shaking. At 600 nm UV absorbance, a PowerWave XS2 plate reader assessed bacterial growth suppression. The lowest concentration without observable growth or turbidity was the (MIC).

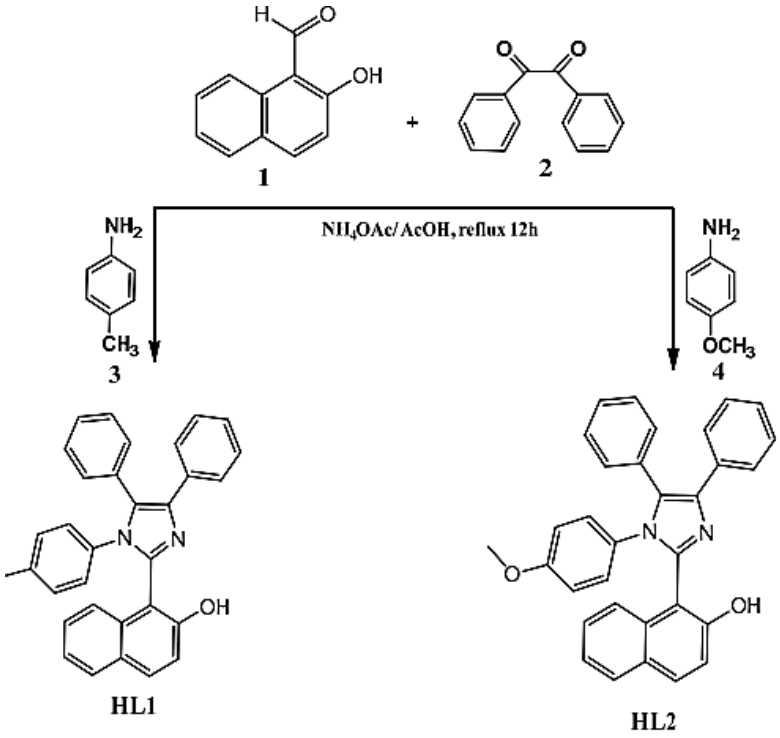
Statistical Analysis

The trials were done in triplicate and reported as the average plus or minus SD using GraphPad Prism v.10.

IV. Results and Discussion

Chemical Characterization of Imidazole Derivatives

Scheme 3 provides the techniques for making the new imidazole derivatives HL1 and HL2. The derivatives were made via the Debus-Radziszewski reaction. Benzil and 4-methylaniline or 4-methoxyaniline was produced by refluxing glacial acetic acid with 2-hydroxy-1-naphthaldehyde (1) and ammonium acetate. Imidazoles (HL1 and HL2). Both compounds' molecular structures were confirmed by IR and 1HNMR.



Scheme 3: Synthesis Pathway of Imidazole Compounds HL1 and HL2

The HL1 and HL2 infrared spectra revealed stretching bands at 1513-1589 cm−1 and 1602-1626 cm−1, corresponding to the functional groups -C=C- and -C=N-. The stretching frequency of 3040-3058 cm−1 suggests aromatic C-H stretch. Additional 1HNMR spectra reveal that the synthesized compounds include 20 aromatic protons at 6-8 ppm. The 13CNMR spectra show C=N at 143.42, 143.87 ppm, aromatic C at 109.67-137.67, and C-OH at 154.94, 154.95. This confirms target molecular shapes. This was confirmed by crystalline imidazole derivative researchers. Researchers mixed thiourea with methanol-dissolved imidazole or 2-methylimidazole in one study in another. Imidazole compounds containing 2-Hydroxy-1-naphthaldehyde should be more antimicrobial. This medication works against Staphylococcus aureus and E. coli, according to research. 2-hydroxy-1-naphthaldehyde was used to generate 2-amino-3-methylpyridine complexed with metal ions. Thus, HL1 and HL2 were examined for antibacterial and cytotoxic properties.

Results of Cell Viability Assessment

The HL1 and HL2 imidazole derivatives' effects on human dermal fibroblasts' metabolic activity were assessed using the MTS test after 24 and 48 hours of incubation. A cell viability experiment using HFF-1, a common cell line, was

used to evaluate these newly invented chemicals' safety. The sensitive HFF-1 cell line is susceptible to any cytotoxicity from the materials utilized. New drugs and chemicals are also tested for cytotoxicity using HFF-1 cells. At 24 and 48 hours following cell exposure, this examination examined how exposure length influenced cell viability.

Figure 1A shows the MTS test result after 24 hours of HL1 and HL2 incubation. Figure 1A shows that HFF-1 cells had substantial metabolic activity (≥80%) at low doses (39 and 78 µg/mL) after 24 hours of HL1 treatment. At doses over 313 µg/mL, cellular viability decreased significantly to less than 50%. In contrast, Figure 1A reveals significant cell viability in HFF-1 cells exposed to HL2 derivative for 24 hours at all dosages tested, from 5000 to 39 µg/mL.

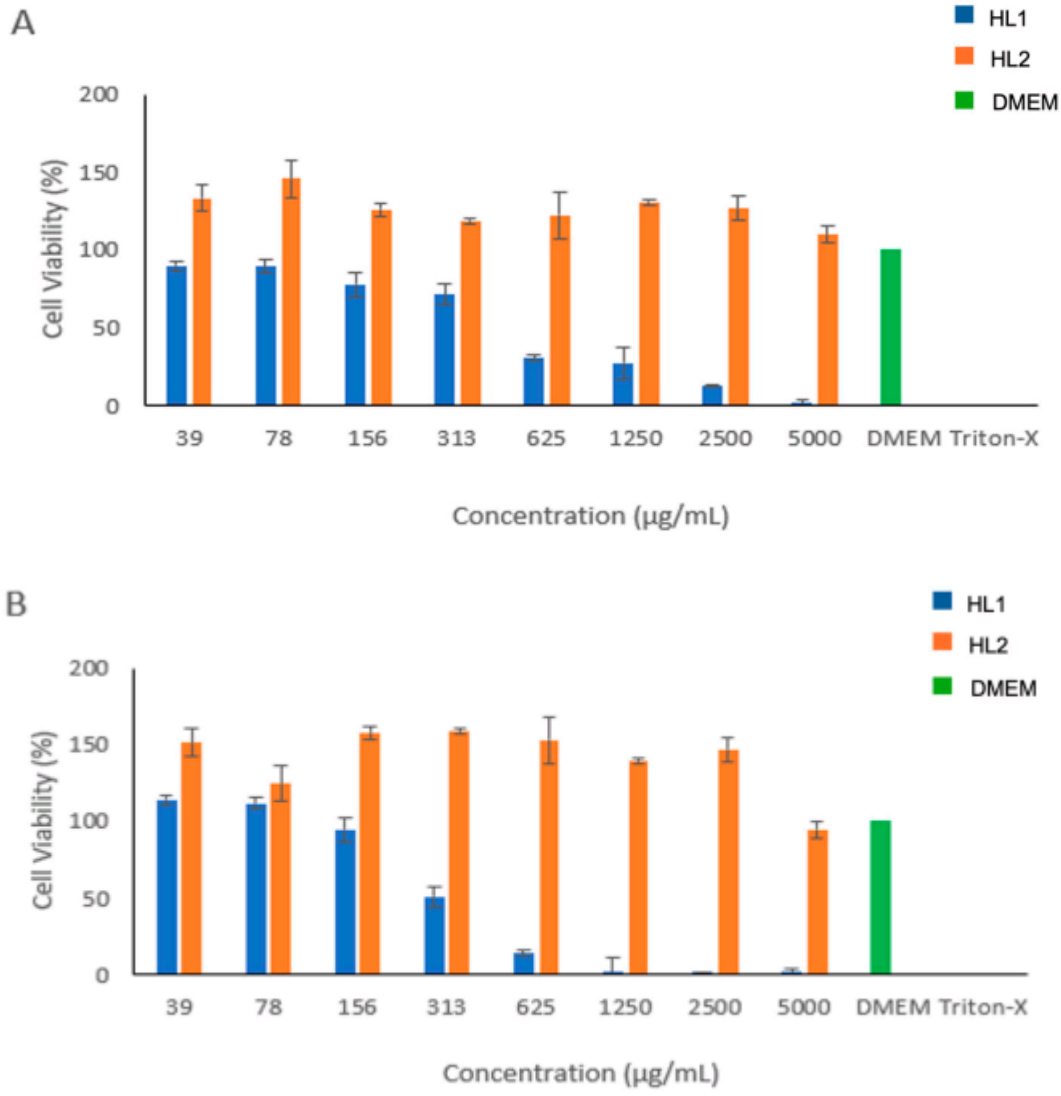


Figure 1: MTS assay showing cell viability (%) of HFF-1 cells treated with varying concentrations of HL1 and HL2 at 24 h (A) and 48 h (B).

Data are mean ± SD (n = 3). DMEM and 0.2% Triton X-100 were negative and positive controls. Figure 1B shows MTS data, and we evaluated HL1 and HL2 compounds in 48 hours with HFF-1 cells. High cell viability (≥80%) was seen at HL1 derivative concentrations of ≤156 µg/mL, but drastically decreased at dosages of at least 313 µg/mL. No change in cell viability was seen at any concentration (5000-39 µg/mL) when HL2 was administered (Figure 1B). Unprotected monosaccharide derivatives of 2-(4-aminophenyl)-1H-imidazo[4,5-f] phenanthroline were studied. Compared to cisplatin, these compounds showed substantial antiproliferative action against HeLa, PC3, MCF7, and HFF1. According to reports, imidazole derivatives with benzene sulfonamide, 4-

chloro and 3,4-dichloro substituents in the benzene ring, and 2-ethylthio and 3-ethyl groups in the imidazole ring can inhibit tumor growth in human malignant melanoma IGR39 and triple-negative breast cancer MDA-MB-231 cell lines. Additionally, nine piperazine-tagged imidazole derivatives were evaluated on MCF-7, PC3, Du145, HepG2, and HFF-1 cell lines. Two of the nine had the highest anticancer effects on HepG2 and MCF-7 cells, whereas HFF-1 cells were very faintly impacted. The produced HL2 imidazole derivative had no adverse effects on tested cells at concentrations up to 5000 µg/mL.

Results of Antibacterial Activity Evaluation

Staphylococcus aureus, MRSA, ATCC 29213, and Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, ATCC 1744, and ATCC 747 were tested for antibiotic efficacy. Bacteria were treated with imidazole derivatives at dosages from 5000 to 2.44 µg/mL. Vancomycin and ciprofloxacin were used as reference antibiotics at concentrations from 40 to 0.02 µg/mL. Table 1 shows the reference antibiotic and imidazole derivative MIC values against all investigated bacterial strains. The minimum inhibitory concentration (MIC) of HL1 against Gram-positive bacteria was 625 µg/mL for Staphylococcus aureus and 1250 µg/mL for MRSA. In contrast, HL2 inhibited both strains at 625 µg/mL. The minimum inhibitory concentration (MIC) of HL2 against Escherichia coli, Pseudomonas aeruginosa, and Acinetobacter baumannii reached 2500 µg/mL, surpassing Gram-positive bacteria. Additionally, HL1 had a higher minimum inhibitory concentration (MIC) than HL2 for Acinetobacter baumannii (1250 µg/mL) and Pseudomonas aeruginosa (5000 µg/mL). No inhibition was observed for Escherichia coli (>5000 µg/mL). Common drugs vancomycin and ciprofloxacin have MICs of 10-0.02 µg/mL against the tested bacterial strains. No inhibition of Pseudomonas aeruginosa or Acinetobacter baumannii was seen with vancomycin at 40 µg/mL.

Table 1: Minimum inhibitory concentration (MIC) values of imidazole derivatives presented as mean ± SD.

Bacterial Strains	**HL1 (µg/mL)**	**HL2 (µg/mL)**	**Vancomycin (µg/mL)**	**Ciprofloxacin (µg/mL)**
Staphylococcus aureus (ATCC 29213)	630 ± 0.05	620 ± 0.03	0.65 ± 0.01	0.15 ± 0.01
Staphylococcus aureus (MRSA; ATCC 43300)	1240 ± 0.03	635 ± 0.05	0.30 ± 0.01	0.17 ± 0.03
Escherichia coli (ATCC 25922)	>4900	2450 ± 0.04	9.5 ± 0.02	0.03 ± 0.01
Acinetobacter baumannii (ATCC 747)	1260 ± 0.08	2480 ± 0.06	>42	0.17 ± 0.01
Pseudomonas aeruginosa (ATCC 1744)	4950 ± 0.03	2550 ± 0.06	>41	0.60 ± 0.09

Some imidazole compounds have worked to inhibit bacterial growth by interfering with cell wall construction or protein production. Brahmbhat et al. examined Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Bacillus subtilis for 3-(2-4-diphenyl-1H-imidazole-z-y)-1H-pyrazole susceptibility. Researchers showed that the derivative outperformed conventional medicines against bacteria. In another study, Demchenko et al. found that 3-biphenyl-3H-imidazo[1,2-a]azepin-1-ium bromide derivatives defeated Cryptococcus neoformans and Staphylococcus aureus. Data indicates the MIC ranges from 4 to 8 µg/mL. Several imidazole compounds are widely used therapeutically. The antibiotic metronidazole treats anaerobic bacteria that cause vaginal and digestive tract infections. Another example is clotrimazole, which kills fungus and Gram-positive bacteria.

V. Conclusion

HL1 and HL2, two new imidazole derivatives, were synthesized and studied in this work, showing potential antibacterial and cytotoxic properties. Our NMR, FTIR, and XRD investigations validated the structural integrity and purity of both compounds, laying the groundwork for biological examination. The antibacterial experiments showed that HL1 and HL2 suppress a variety of clinically relevant bacterial strains, including multidrug-resistant infections like MRSA, suggesting they might be useful antimicrobials. Importantly, both compounds showed little toxicity at effective doses in human skin fibroblast (HFF-1) cells, indicating a good therapeutic index. This balance between antibacterial activity and minimal cytotoxicity is essential for preclinical research of these compounds. The findings demonstrate the utility of imidazole-based scaffolds in drug discovery, especially when modified to increase biological activity. Overall, HL1 and HL2 are potential prospects for novel antibiotics that help combat antibiotic resistance. To maximize their therapeutic potential and clinical usefulness, future research should examine their mechanism-of-action and in vivo effects.

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