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ORIGINAL ARTICLE

Coloration Technology

Synergistic anti-bacterial activity of imidazole-based azo dyes with polyvinylpyrrolidone-coated silver nanoparticles

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Abstract

The high demand for novel antimicrobial textiles by the medical, healthcare, hygiene, sportswear, personal protective equipment and filtration sectors has promoted the growth of functional textiles. However, the efficacy of antimicrobial agents against different pathogens is a considerable challenge because of the distinctive mechanisms of action and resistance. The development of novel synergistic antimicrobial dyes may offer numerous opportunities to enhance antimicrobial effectiveness. In this work, a novel imidazole-based azo dye with a *p*-methoxyphenyl group in the *N*-1 substituent of the imidazole ring (AzoIz-a), and corresponding amidrazone precursor (AmIz-a), were combined with polyvinylpyrrolidone-coated silver nanoparticles (AgNPs). The molecules, alone and combined with the AgNPs, were characterised by ultraviolet-visible spectrophotometry and zeta potential. Their synergistic effect was assessed against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The results were compared with a previously reported imidazole-based azo dye and precursor containing a methyl group in the imidazole ring (AmIz-b and AzoIz-b). The results showed interesting antimicrobial properties of the novel AzoIz-a molecules when combined with a very small concentration of AgNPs. The combination showed an additive effect for *S. aureus* and a synergistic effect for *E. coli* and *P. aeruginosa*. Considering the synergistic results, the effective concentration of the AzoIz-a decreased from more than 128 to 16-32 $\mu g.mL^{-1}$ by the addition of a small concentration of AgNPs (0.6-1.3 $\mu g.mL^{-1}$), which displayed comparable

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results with the AzoIz-b molecule. Thus, the application of these conjugates in textiles may lead to highly coloured materials with remarkable anti-bacterial properties, which warrant further exploration.

1 | INTRODUCTION

Antimicrobial textiles are functional materials that can kill microorganisms or inhibit their growth, and are therefore extremely useful for preventing the degradation of textile materials, avoiding cross-contamination, or even for treating microbial infections.[1–3] Antimicrobial agents applied to textile materials should present low toxicity to consumers, be effective against a large spectrum of pathogens and be selective only to unwanted microorganisms.[2] The high demand for novel antimicrobial textiles by the medical, healthcare, hygiene, sportswear, personal protective equipment and filtration sectors has substantially increased the interest in the development of functional textiles.[4–6]

Antimicrobial compounds and their safety have been the subject of constant research. Different types of antimicrobial textiles have been developed based on organometallics, phenols, triclosan, quaternary ammonium compounds, inorganic nanoparticles and antibiotics.[7–9] Moreover, functional dyes, which can have an antimicrobial character, have been used as a strategy to combine both functional finishes and conventional textile dyeing into one process.[10] However, the efficacy against different pathogens is a considerable challenge because of the distinctive mechanisms of action and resistance. The development of novel synergistic antimicrobial agents may thus offer numerous opportunities to enhance antimicrobial effectiveness, such as boosting the activity of individual agents, reducing dosages, minimising toxicity and amplifying the activity spectrum.[11] Synergistic approaches combine at least two substances that result in a superior efficacy when compared with that of individual agents.[12,13]

Azobenzene molecules are the most used scaffold for the development of coloured materials, and the ones containing a heterocycle, in the place of one or both benzene rings, present good tinctorial strength and brightness of shades.[14,15] Furthermore, non-commercial azobenzenes holding heteroaromatic moieties can also lead to interesting antimicrobial properties.[16] In particular, the imidazole ring was shown to have antimicrobial, analgesic and anti-inflammatory properties.[17] Recently, a novel class of imidazole-based azo dyes (AzoIz) were reported by our research group, presenting distinctive colours. These compounds showed effective antimicrobial properties against infectious yeasts but low anti-bacterial activity.[18,19]

Nanomaterials may offer innovative routes to address the development of advanced products by improving some materials' properties or by promoting new ones. Inorganic nanoparticles have been widely studied because of their unique characteristics: the small size and high surface-to-volume ratio, ability to act at the subcellular level, surface adaptability and multifunctionality.[11,20] Silver nanoparticles (AgNPs) are renowned antimicrobial agents against a wide range of microorganisms, including bacteria, fungi and viruses with multiple mechanisms of action.[21,22] However, their application is limited because of concerns about the potential hazard they present to the environment and human health. Their properties and corresponding toxicity profile are complex and may undergo numerous dose-, size- and time-dependent mechanisms. For these reasons, the safe-by-design concept gains critical importance once it foresees the risk assessment in the early stages of development.[23,24] Synergistic antimicrobial studies were performed combining AzoIz with a methyl group in the *N*-1 substituent of the imidazole ring (AzoIz-b) and AgNPs, and the results obtained showed improved activity against the tested bacteria (*Staphylococcus aureus* and *Escherichia coli*).[19]

Because the structure of the compounds plays a critical role in determining their antimicrobial activity, the synergistic activity of another AzoIz, which was previously developed and presents a *p*-methoxyphenyl substituent in the *N*-1 position of the imidazole unit (AzoIz-a), was investigated in this work. This N^1-aryl azoimidazole (AzoIz-a) and the corresponding amidrazone precursor (AmIz-a) were prepared and characterised by proton nuclear magnetic resonance (^{1}H NMR) and Fourier Transform–infrared (FTIR) spectroscopy. Then, these AmIz-a and AzoIz-a compounds were combined with polyvinylpyrrolidone-coated AgNPs and synergistic and colorimetric studies were performed. The results were compared with data from the N^1-methyl azoimidazole (AzoIz-b) analogue previously studied. The colour of the compounds, alone and combined with AgNPs, was evaluated by ultraviolet–visible (UV–vis) spectrophotometry in an aqueous solution. The effect of the molecules' substituents on the stability of the AgNPs' dispersion was assessed by zeta potential. The synergistic effect of the conjugates was assessed against *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*. All the results suggest that the application of these AzoIz dyes in textiles may not only lead to coloured materials, but that their conjugation with a low concentration of AgNPs can also produce materials with remarkable properties, thus warranting further exploration in future research.

2 | MATERIALS AND METHODS

2.1 | Materials

Commercial polyvinylpyrrolidone-coated AgNPs (20-30 nm) were obtained from SkySpring Nanomaterials Inc. All the other reagents were purchased from Sigma-Aldrich without any purification. The reactions were followed by thin-layer chromatography using Macherey-Nagel aluminium sheets UV254.

2.2 | Synthesis of the AmIz compounds

5-amino-4-(cyanoformimidoyl)-1*H*-imidazoles were the initial compounds used in this work and were obtained from commercial reagents, including diaminomaleonitrile, triethyl orthoformate and *p*-anisidine or methylamine, according to a previous method developed by Alves et al.[25] Then the corresponding amidrazones were prepared according to a method previously developed by the current research group.[18,26]

2.3 | Synthesis of the AzoIz compounds (AzoIz-a and AzoIz-b)

The 5-amino-*N*′-phenyl-1*H*-imidazole-4-carbohydrazonamides were used to prepare azoimidazoles, as described in previous work by the current research group.[18] Briefly, silver nitrate (3.0 molar equiv.) was dissolved in acetonitrile (2.5 mL) in a 50 mL flask. Then, the AmIz were suspended in an acetonitrile/ethanol mixture (80:20) (10 mL), and the suspension was added to the silver nitrate solution under magnetic stirring at room temperature. A dark red suspension was formed immediately and, after 15 minutes, the solid was filtered and washed with acetonitrile and ethyl ether. The metallic silver formed during the synthesis was removed by centrifugation.

2.4 | AgNPs' redispersion, and preparation of the dispersions containing the AgNPs and the AmIz or AzoIz compounds

First, AgNPs were redispersed in water with a concentration of 10 μg.mL^{-1} using optimised conditions (ultrasonic bath of 130 W for 30 minutes at 40 Hz and an ultrasound tip of 500 W for 30 minutes at 20 Hz).[27] Then, water solutions containing 256 μg.mL^{-1} of each organic compound (AmIz and AzoIz) were prepared. To the solutions containing AmIz compounds, nitric acid (2.0 molar equiv.) was added to improve their solubility in water by protonating the imino group in the C-5 position of the imidazole ring.[18,19] The AgNPs' dispersion and organic compound solutions were mixed (1:1) (1.5 mL) and the dispersion was kept in an ultrasound bath for 10 minutes.

2.5 | Characterisation of the molecules or AgNPs alone and respective combinations

The organic compounds were characterised by ^{1}H NMR and FTIR spectroscopy. The ^{1}H NMR spectroscopy was performed at room temperature on a Bruker Avance 3400 spectrometer (^{1}H: 400 MHz). The data were reported by chemical shifts (ppm) and multiplicity (s: singlet, brs: broad singlet, d: doublet, t: triplet, dd: doublet of doublets, or m: multiplet). The IR spectra were obtained using potassium bromide cells on a Shimadzu IR-Affinity 1 FTIR spectrophotometer with an attenuated total reflectance accessory (ATR-Dia/KRS-5).

A UV–vis spectrophotometer (Shimadzu, UV-1800) was used to measure the absorbance spectra of the compounds before and after the mixing with AgNPs in water with a standard cuvette (1 cm-wide quartz cells).

The zeta potential of the AgNPs alone and combined with the organic molecules in the dispersion was measured using a Zeta Sizer-Nano (Malvern Instruments, Malvern, UK). Data were collected after 30 scans at 25 ± 1°C and measured in a moderate electrolytic concentrated solution. The presented values were obtained by averaging the measurements of three samples.

2.6 | Antimicrobial evaluation and synergism testing

The antimicrobial properties were assessed by determining the minimal inhibitory concentration (MIC) based on the methodology described by Wiegand et al.[28] The evaluated microorganisms were *S. aureus* American Type Culture Collection (ATCC) 6538, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Briefly, a preinoculum of each bacterium was prepared using tryptic soy broth for *S. aureus* and *E. coli* or nutrient broth for *P. aeruginosa*. The preinocula were incubated overnight at 37°C with a shaking speed of 120 rpm. Each one of the synthesised compounds and corresponding combined dispersions were serially diluted in water (Table 1). Subsequently, each dispersion was diluted 50% (v/v) with the inoculum of each bacterium comprising 1 × 10^7 CFU.mL^{-1} in its

TABLE 1 Concentrations of the initial solution (IS) and corresponding dilutions (D1-D6) (μg.mL^{-1}) of the amidrazone precursors (AmIz), azoimidazoles (AzoIz) and silver nanoparticles (AgNPs) that were used for synergistic studies and calculation of the minimal inhibitory concentration.

	Concentration (μg.mL^{-1})						
	IS	D1	D2	D3	D4	D5	D6
AgNPs	10	5	2.5	1.3	0.6	0.3	0.2
AmIz or AzoIz	256	128	64	32	16	8	4

corresponding fresh culture medium in flat-bottom 96-well plates. In the positive control samples, the bacteria suspensions were diluted in water without any compounds. The optical density (OD; wavelength: 600 nm) of each bacterium suspension was immediately measured using a 96-well plates microplate reader. Afterwards, the 96-well plates were incubated for ca. 20 hours at a shaking speed of 120 rpm and 37°C. The OD was determined, and the MIC was defined visually using a magnifying glass. The MIC was attributed to the lower dilution without turbidity. Furthermore, the MIC was confirmed using Equation 1. Each dilution was replicated twice.

$$OD = OD_f - OD_i, \quad (1)$$

where OD corresponds to the optical density of each sample, OD_i to the initial OD, and OD_f to the OD measured after 20 hours of incubation.

The synergistic effects were determined by calculating the fractional inhibitory concentration index (FICI) following Equation 2. The FICI value was interpreted as synergistic (≤ 0.5), additive (> 0.5 and ≤ 1.0), indifferent (> 1.0 and ≤ 4.0) or antagonistic (> 4.0).[29]

$$FICI = \frac{\text{MIC of agent A in combination}}{\text{MIC of the agent A alone}} + \frac{\text{MIC of agent B in combination}}{\text{MIC of the agent B alone}} \quad (2)$$

3 | RESULTS AND DISCUSSION

5-amino-*N*′-phenyl-1*H*-imidazole-4-carbohydrazonamides (AmIz) and the corresponding oxidised structures, the azo molecules (AzoIz), were previously synthesised by our research group using a mild and simple method that starts from the accessible 5-amino-4-cyanoformimidoyl imidazoles. Based on the susceptibility of the amidrazone precursors to oxidation, which was revealed by the emergence of coloured solids and solutions when they were exposed to the air, these amidrazones were fully oxidised using silver nitrate. This reaction was immediately completed, and the azo molecules (AzoIz) obtained showed a strong red colour. These compounds (AzoIz) presented interesting halochromic properties and antimicrobial activity against *Candida krusei*, *Candida albicans* and *Cryptococcus neoformans*, and also against biofilm formation. However, their activities against filamentous fungi (*Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Microsporum gypseum*) and bacteria (*S. aureus, E. coli* and *P. aeruginosa*) were very weak when compared with the activity on yeasts.[18,26,30,31] When the dyeing properties of some examples of this novel class of dyes were tested, the results showed a special affinity for proteic fibres and the dyed textiles exhibited halochromic properties.[32] Moreover, synergistic antimicrobial tests combining AmIz and AzoIz with a methyl group in the *N*-1 substituent of the imidazole ring (AmIz-b and AzoIz-b) and AgNPs showed improvement in the activity against *S. aureus* and *E. coli*.[19] In this work, the synergistic activity of another AzoIz previously developed, with a *p*-methoxyphenyl substituent in the *N*-1 position of the imidazole unit (AzoIz-a), was investigated. To start, the compounds with a methyl (R = Me) or *p*-methoxyphenyl (R = Ar(*p*)OMe) substituent in the *N*-1 position of the imidazole ring were synthesised. Then, the conjugation of these compounds (AmIz and AzoIz) with AgNPs was studied to obtain dyes with a higher coloration strength and a wider spectrum of antimicrobial action for the development of functional textiles (Figure 1).

The AmIz and AzoIz compounds were characterised by FTIR spectroscopy, ^{1}H NMR and UV–vis spectrophotometry to confirm the structures and verify the purity of the isolated products. The FTIR spectra showed the stretching modes of the phenyl and *p*-methoxyphenyl groups (at ca. 1608 and 1211 cm^{-1}).[33] In the same region (ca. 1554 cm^{-1}), typical peaks attributed to C=C stretching and C=N stretching from the imidazole ring were found.[34] In addition, the evident appearance of two bands in the region of C=N bonds (at 1678 and 1639 cm^{-1} for AzoIz-a; and at 1670 and 1639 cm^{-1} for AzoIz-b) supported the conversion of the hydrazone to azo group (Figure 2).

In the ^{1}H NMR, it was possible to detect the shift of the proton signal of the imidazole 2-*H* proton in the AmIz structure, observed at δ 7.10-7.13 ppm, to δ 7.88-8.02 ppm in the AzoIz. All the other proton signals also shifted to higher chemical shifts because of the withdrawing effects of the positive charge on the imidazole ring (Figure 3A).

The emergence of the red colour in the AzoIz molecules was also analysed by UV–vis spectrophotometry.

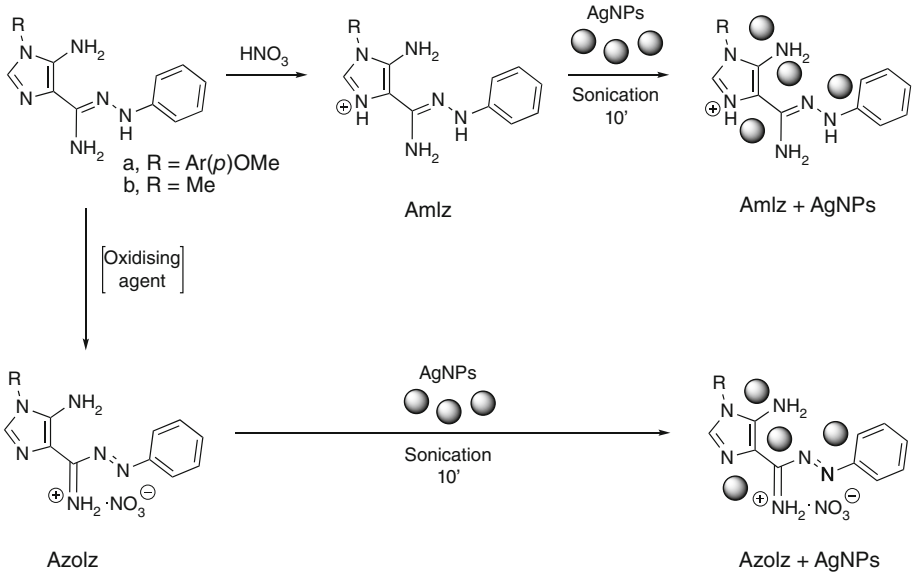

FIGURE 1 Schematics of the methodology used to obtain the organic compounds (amidrazones [AmIz] and azoimidazoles [AzoIz]) conjugated with silver nanoparticles (AgNPs).

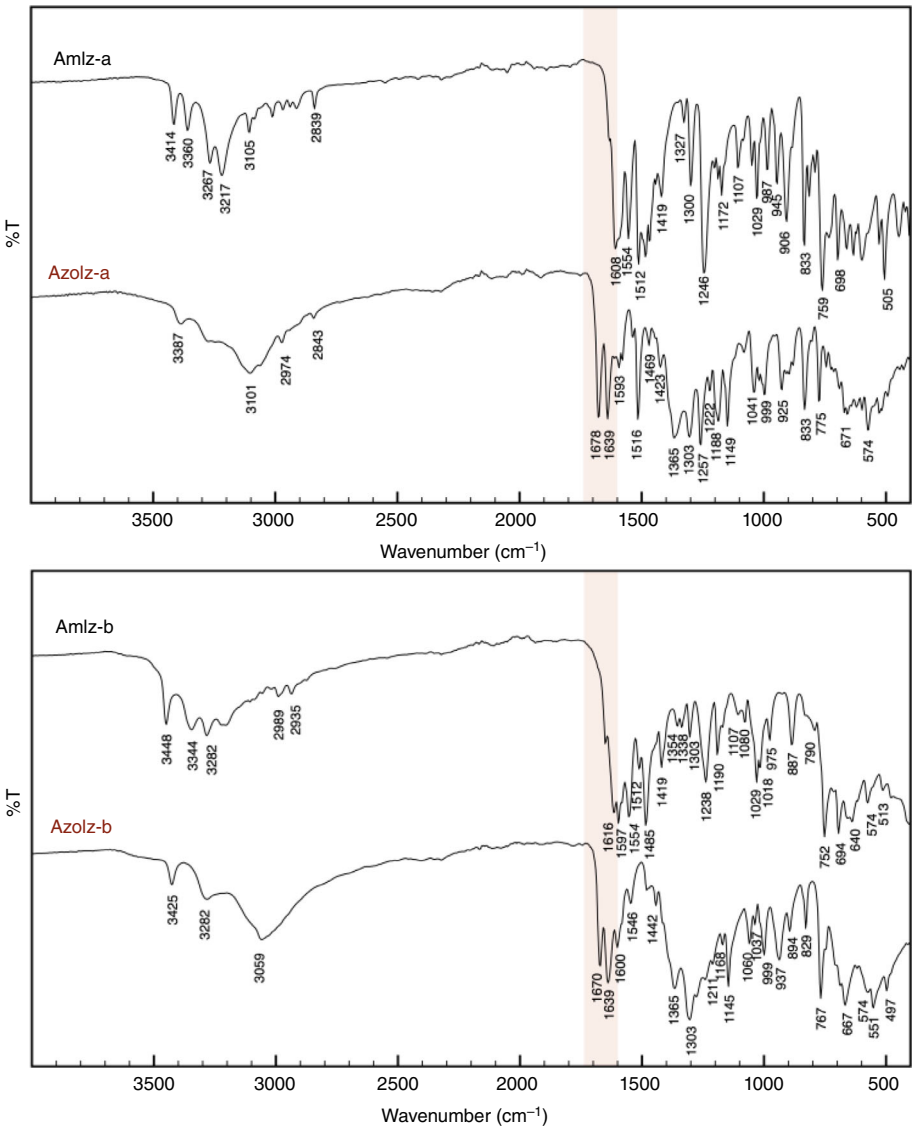

FIGURE 2 FTIR spectra of the compounds AmIz and AzoIz with a *p*-methoxyphenyl (AmIz-a and AzoIz-a) or methyl (AmIz-b and AzoIz-b) group in the imidazole ring. AmIz, amidrazones; AzoIz, azoimidazoles; FTIR, Fourier Transform–infrared.

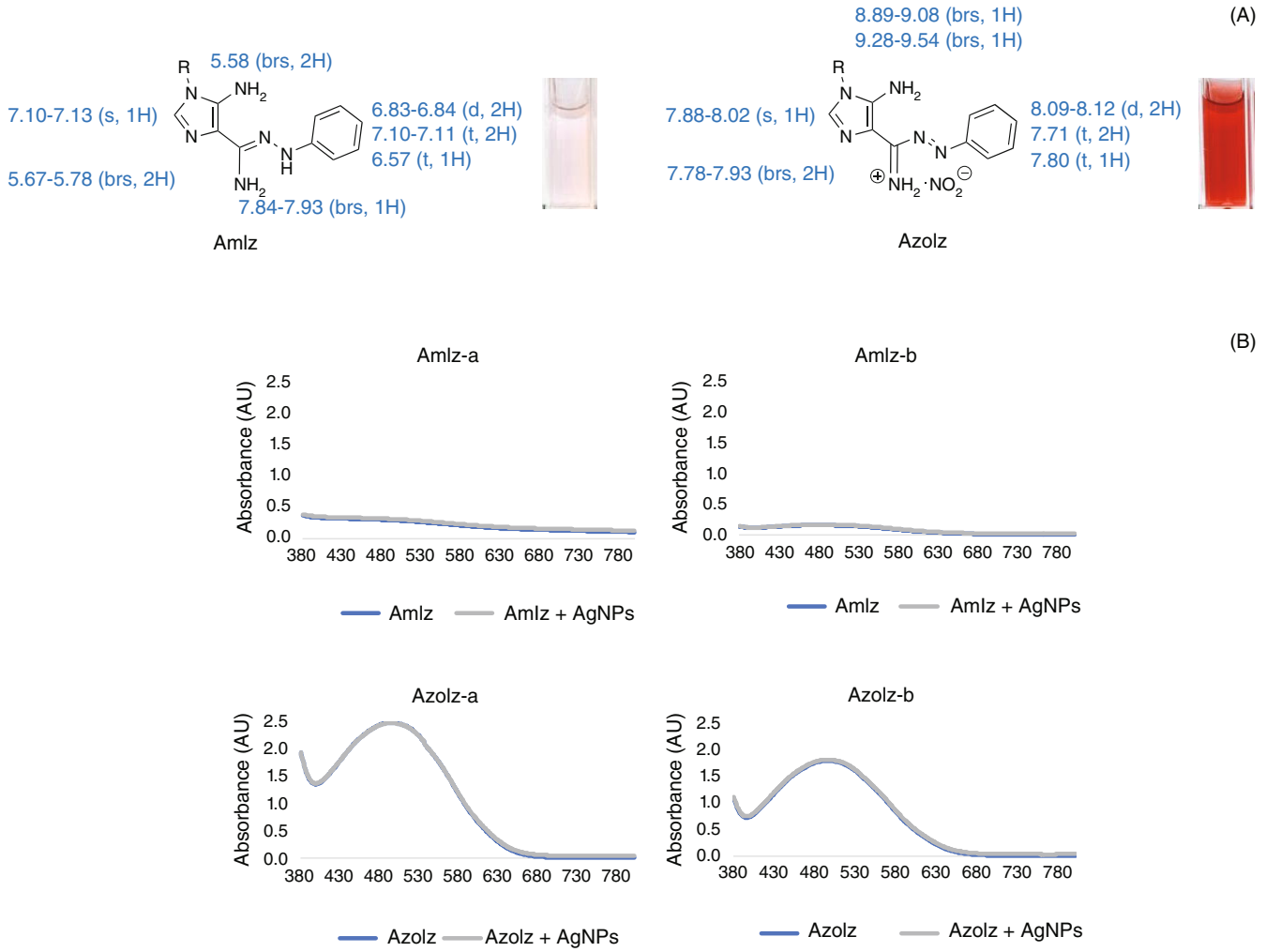

FIGURE 3 A, ^{1}H NMR data of the amidrazones (AmIz) and azoimidazoles (AzoIz) molecules and the emergence of red colour in the AzoIz water solutions; B, UV–vis spectra of the AmIz and AzoIz molecules alone (128 μg.mL^{-1}) and mixed with AgNPs (5 μg.mL^{-1}). ^{1}H NMR, proton nuclear magnetic resonance; AgNPs, silver nanoparticles; brs, broad singlet; d, doublet; s, singlet; t, triplet; UV–vis, ultraviolet–visible.

The UV–vis spectra showed the appearance of an intense absorbance band (at ca. 493 nm) in these structures. The solution of the compounds varied from uncoloured (AmIz) to red (AzoIz) (Figure 3A). After mixing with AgNPs, any colour change was detected by UV–vis spectrophotometry, indicating that the AgNPs did not interfere with the chromophores of these molecules. However, zeta potential measurements indicated interactions between the AzoIz molecules and nanoparticles, because the zeta potential values changed significantly. The AgNPs presented a zeta potential of −14.60 mV that increased to values ranging from −3.76 to 16.6 mV. Because those dispersions with zeta potential values greater than +30 mV or of less than −30 mV are considered stable, only the compound AzoIz-b was shown to improve the stability of the AgNPs.[35] This can be attributed to the lower steric hindrance and higher water solubility of the methyl group compared with the *p*-methoxyphenyl group. Moreover, electrostatic interactions between the molecule and polyvinylpyrrolidone in AgNPs may occur when the amino group is protonated under acidic conditions. Using the other compounds, the zeta potential obtained was shown to promote some agglomeration of the AgNPs (Table 2).

In general, the AmIz or AzoIz compounds and AgNPs were conjugated in concentrations lower than their individual MICs and the mixtures were tested against *S. aureus*, *E. coli* and *P. aeruginosa*. The MICs of organic compounds ranged from 64 to more than 128 μg.mL^{-1}, and the MICs of the AgNPs alone were reported to vary from 1250 to 4000 μg.mL^{-1} (Table 3).[36] The combinations were obtained in the ratio 1:1 by ultrasound mixing a dispersion of AgNPs (10 μg.mL^{-1}) and organic compound solutions (256 μg.mL^{-1}). Afterwards, the mixtures were

TABLE 2 Zeta potential of AgNPs alone (5 μg.mL^{-1}) and mixed with the AmIz or AzoIz molecules (128 μg.mL^{-1}).

	AgNPs	AmIz-a + AgNPs	AmIz-b + AgNPs	AzoIz-a + AgNPs	AzoIz-b + AgNPs
Zeta potential (mV)	−14.60 ± 0.32	2.97 ± 0.55	−2.58 ± 0.42	−3.76 ± 0.23	16.6 ± 0.62

Abbreviations: AgNPs, silver nanoparticles; AmIz, amidrazones; AzoIz, azoimidazoles.

TABLE 3 Minimum inhibitory concentrations (MICs, μg.mL^{-1}) of the amidrazone precursors (AmIz), corresponding azoimidazoles (AzoIz) and silver nanoparticles (AgNPs).

	MIC (μg.mL^{-1})				
	AmIz-a	AmIz-b	AzoIz-a	AzoIz-b	AgNPs
Staphylococcus aureus	>128	>128	64	>128	4000
Escherichia coli	>128	>128	>128	>128	4000
Pseudomonas aeruginosa	>128	>128	>128	>128	1250

TABLE 4 Anti-bacterial activity of the amidrazones (AmIz) or azoimidazoles (AzoIz) and silver nanoparticles (AgNPs) combinations that presented a positive result (μg.mL^{-1}), corresponding FICI and interpretation (S: FICI ≤ 0.5; or A (ad: 0.5 < FICI ≤ 1.0).

Molecule	Bacteria	Molecule	AgNPs	FICI	Interpretation
AzoIz-a	*Staphylococcus aureus*	64.0	2.5	1.00	A
	Escherichia coli	32.0	1.3	0.25	S
	Pseudomonas aeruginosa	16.0	0.6	0.13	S
AzoIz-b	*Staphylococcus aureus*	64.0	2.5	0.50	S
	Escherichia coli	32.0	1.3	0.25	S
	Pseudomonas aeruginosa	4.0	0.2	0.03	S

Abbreviations: A, additive; FICI, fractional inhibitory concentration index; S, synergistic.

serially diluted and mixed with the microorganisms to test their antimicrobial activity. Those mixtures that completely inhibited the microorganisms' growth were assessed visually (by turbidity) and by measuring their OD.

The synergistic studies using AmIz and AzoIz molecules were assessed by FICI calculation, which is a suitable indicator to represent the level of synergy between two antimicrobial agents, and it is calculated by dividing the MIC resultant from combination therapy by the MIC of the individual agent.[11,37] Analysing the FICI values obtained (Table 4), it was possible to detect some positive results. When AgNPs were combined with AmIz structures, it was not possible to obtain any additional antimicrobial effect against *S. aureus, E. coli* and *P. aeruginosa* in the tested concentrations. However, interesting additive and synergistic effects were found using the AzoIz molecules. Against *S. aureus*, an additive effect was detected with the AzoIz-a molecule, while a synergistic effect was obtained when the AzoIz-b was used. However, a higher concentration of both molecules was needed when compared with the Gram-negative bacteria (64 μg.mL^{-1} of AzoIz combined with 2.5 μg.mL^{-1} of AgNPs; Table 4). For Gram-negative bacteria (*E. coli* and *P. aeruginosa*), the effective concentration decreased from more than 128 μg.mL^{-1} to values ranging from 16 to 32 μg.mL^{-1} by the addition of a small concentration of AgNPs (from 0.6 to 1.3 μg.mL^{-1}) using the AzoIz-a molecule. In compound AzoIz-b, the concentration decreased from more than 128 to 4.0-32 μg.mL^{-1} by the addition of AgNPs (0.2-1.3 μg.mL^{-1}) (Table 4). Although good synergic effects were observed for both AzoIz compounds, the AzoIz-b molecule showed improved synergistic antimicrobial activity when compared with the AzoIz-a, which was supported by the zeta potential results. The molecule with a methyl group (AzoIz-b) showed improved stabilisation of the AgNPs in the dispersion and increased their electric charge at the surface of the particles, indicating a predominance of positively charged particles, which may explain the improved synergistic antimicrobial activity. Indeed, it was previously reported that a positive zeta potential enhances the antimicrobial activity of particles against both Gram-negative and Gram-positive bacteria.[38] Differences in their membranes promote distinctive interactions with the antimicrobial agents. *Staphylococcus aureus* displays a thicker peptidoglycan layer (ca. 80 nm thick) that is populated with teichoic and teichuronic acids.[39,40] The peptidoglycan wall consists of long glycan chains comprising repeating *N*-acetylglucosamine and *N*-acetylmuramic acid units, crosslinked by short peptide chains.[41] The Gram-negative bacteria possess a very thin (ca. 3-4 nm in thickness) outer layer composed of lipopolysaccharides.[42,43] Furthermore, the zeta potential of *E. coli* was

demonstrated to be considerably more negative than that of *S. aureus*, which may increase the affinity with positively charged antimicrobial agents.[43] Therefore, the higher activity against *E. coli* can be justified. These results are in agreement with those from the literature, as the negative charge of the membrane and cell-wall biomolecules may electrostatically interact with the positively charged combinations of AgNPs and molecules. Despite this, a higher surface charge of nanoparticles may promote a higher toxicity by also increasing the electrostatic interactions with cells and promoting the endocytic uptake.[44] Thus, the conjugation of AgNPs with AzoIz-a can be a promising strategy for decreased cytotoxicity of the developed nanoparticles, while ensuring that the antimicrobial properties are maintained.

4 | CONCLUSIONS

The synergic anti-bacterial effect of combining AzoIz-a molecules (R = *p*-methoxyphenyl group) with AgNPs was demonstrated and compared with the analogue AzoIz-b (R = methyl group), to evaluate the effect of different substituents in the *N*-1-position of the imidazole ring. The molecule with a *p*-methoxyphenyl group (AzoIz-a) showed an additive effect for *S. aureus* and a synergistic effect for *E. coli* and *P. aeruginosa*. Here, the zeta potential of the AgNPs increased from −14.60 to −2.58 mV. However, the combination of the AzoIz-b and AgNPs increased the zeta potential of the AgNPs by an even greater extent, as the values changed from −14.60 to 16.60 mV, leading to establishing more electrostatic interactions between the conjugates and the outer layers of the bacteria. Consequently, the increased positive charge in the combination of the AzoIz-b and AgNPs acted in synergy for both Gram-positive and Gram-negative bacteria. Although lower concentrations were found for Gram-negative bacteria (*E. coli* and *P. aeruginosa*), the less positive combination of the AzoIz-a and AgNPs only exhibited synergy for Gram-negative bacteria.

Based on these achievements, it was possible to conclude that higher antimicrobial activity against *E. coli* and *P. aeruginosa* was found by combining these novel molecules with AgNPs. Moreover, these results suggest that the antimicrobial effects of the conjugates may be adjusted by the surface charge of the conjugates, which might be tailored by the judicious design of the group attached to the *N*-1 substituent of the imidazole ring. Thus, the textile dyeability process, the cytotoxicity evaluation and corresponding antimicrobial tests can be a future object of analysis for the development of innovative functional textiles.

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