Synthesis and Antimicrobial Activity of a New Class of Azoimidazoles

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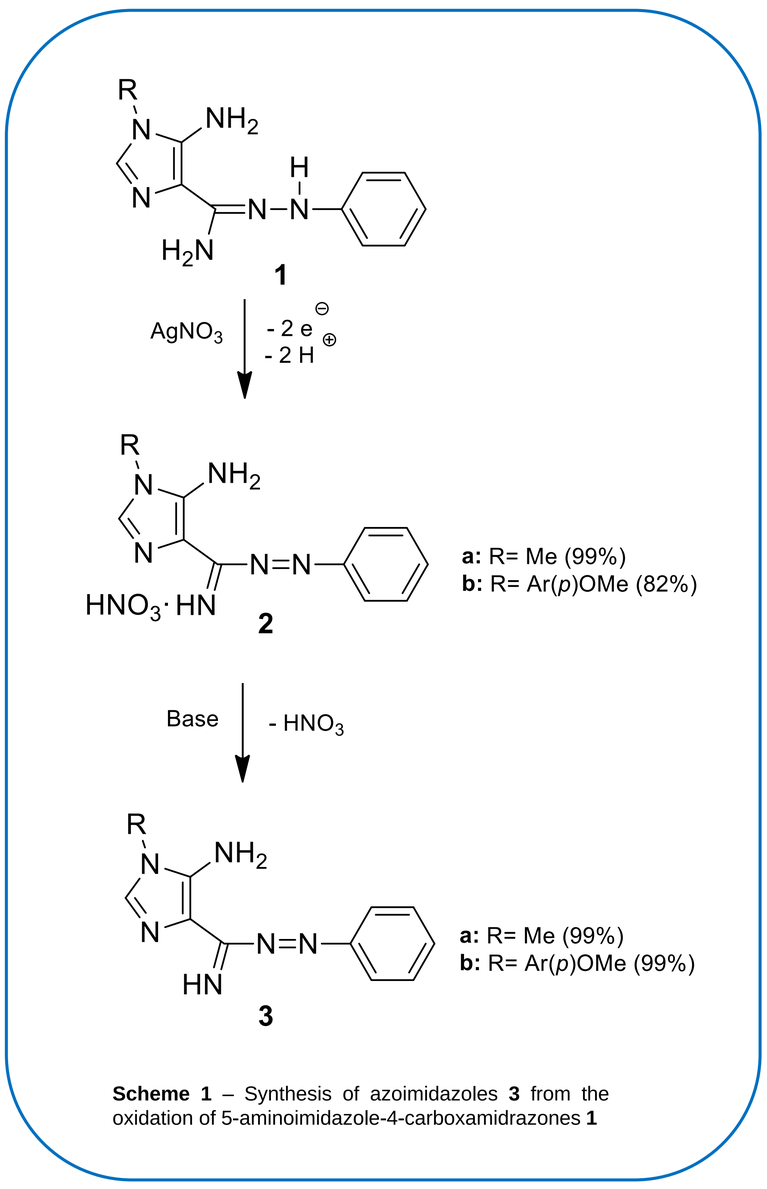
INTRODUCTION

The emergence of infectious diseases caused by new pathogens or multidrug-resistant (MDR) strains has been a global health threat over the last decades.[1] These infections are among the most severe healthcare problems and have been associated to several deaths and heavy economic burden per year.[2,3] The imidazole ring is present in several natural and synthetic molecules with biological activity namely on effective antimicrobial agents, which make it a vital anchor for the development of new therapeutic molecules in this field.[4] Furthermore, amidrazones are known for their high reactivity, thus being useful intermediates for the synthesis of compounds with a wide range of biological activities, including antimicrobial. The amidrazone derivatives have been applied in different subjects of chemistry, specifically in the synthesis of azo molecules.[5]

In a previous work, novel imidazole-based 5-aminoimidazole-4-carboxamidrazones were prepared and exhibited potent antimicrobial activity against *C. krusei* and *C. albicans*.[6] Further biological studies to elucidate the action mechanism revealed an interesting relationship between the antimicrobial activity and total intracellular ROS production by the yeasts.[7] Here, we present results obtained from its electrochemical and chemical oxidation, as well the antimicrobial activity of the oxidized products.

SYNTHESIS

Studies on the reactivity of amidrazones led us to find that the oxidation of amidrazones **1** in the presence of silver nitrate gave rise to azoimidazoles in the form of HNO_3 salts (**2**). The neutralization of these products generated azoimidazoles **3**.



Scheme 1 – Synthesis of azoimidazoles **3** from the oxidation of 5-aminoimidazole-4-carboxamidrazones **1**

ELECTROCHEMICAL CARACTERIZATION

Cyclic voltammograms (CV) were obtained from -0.3 to 0.3 V in a three-electrode cell using an Autolab PGSTAT 30 (Eco-Chemie) controlled by GPES 4.9 software. The working electrode was a glassy carbon electrode, a Pt wire and an Ag/AgCl (KCl 1M) were used as counter and reference electrode, respectively. Tetrabutylammonium tetrafluoroborate (TBAB) was used as supporting electrolyte.

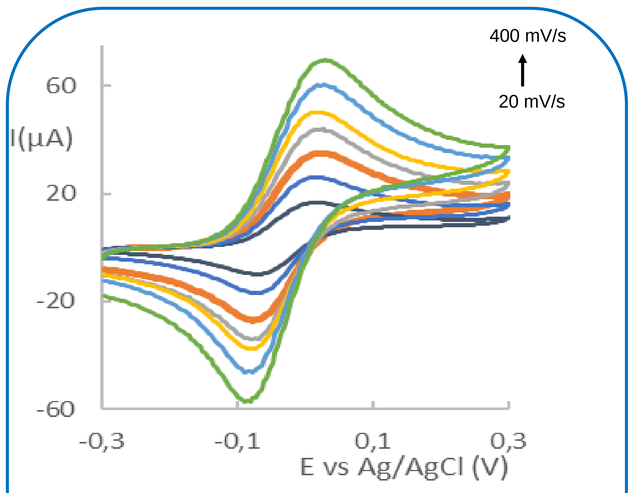


Figure 1 – CV obtained from 2.00 mM of **1** in acetonitrile containing TBAB (0.1 M) using a glassy carbon working electrode, at different scan rates 20-400 mV/s.

Table 1 – Anodic (Epa) and cathodic (Epc) peak potential, peak separation (ΔEp), anodic peak potential minus half-wave potential (Epa-$E_{1/2}$) and anodic and cathodic peak current ratios (Ipa/Ipc). Values obtained from CV at 100 mV/s scan rate.

Epa (mV)	-Epc (mV)	ΔEp (mV)	Epa-$E_{1/2}$ (mV)	|Ipa/Ipc|
13	69	82	63	0.92

Effect of scan rate (*v*) on potential and current

- Epa and Epc do not shift with the increase of *v.*
- ΔEp and Epa-$E_{1/2}$ values are consistent with a reversible one-electron process.
- Ipa/Ipc are always approximately 1.
- Direct proportionality between Ipa and Ipc with *v*.

All diagnostic criteria indicate a reversible one-electron process.[8]

ANTIMICROBIAL TESTS

The antifungal activity of the azoimidazoles in the form of HNO_3 salts (**2**) was evaluated against yeasts (*Candida* and *Cryptococcus* strains) and against filamentous fungi (*Aspergillus*, *Fusarium*, *Scedosporium*, *Mucor* and dermatophyte strains). The antibacterial activity of these compounds was also evaluated against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria.

Table 2 – Antifungal activity (MIC and MLC) of the azoimidazoles **2** against yeasts (*Candida* and *Cryptococcus* strains).

		Candida albicans ATCC 10231	*Candida albicans* DSY294 (S)	*Candida albicans* DSY296 (R)	*Candida glabrata* DSY562 (S)	*Candida glabrata* DSY565 (R)	*Candida krusei* ATCC 6258	*Cryptococcus neoformans* CECT1078
MIC[a] (MLC[b]) μg/mL	**2a**	64 (128)	32 (128)	16 (128)	64 (128)	64 (128)	4 (4)	2 (4)
	2b	64 (256)	32 (256)	32 (256)	64 (256)	64 (256)	4 (4)	4 (4)

Table 3 – Antifungal activity (MIC and MLC) of the azoimidazoles **2** against filamentous fungi: *Aspergillus*, *Fusarium*, *Scedosporium*, *Mucor* and dermatophyte strains.

		Aspergillus fumigatus ATCC 204305	*Aspergillus niger* ATCC 16404	*Fusarium solani* FF125	*Scedosporium* spp.	*Mucor* spp.	*Trichophyton rubrum* FF5	*Trichophyton mentagrophytes* FF7	*Nannizzia gypsea* FF3
MIC[a] (MLC[b]) μg/mL	**2a**	256 (>256)	256 (>256)	256 (>256)	256 (256)	>256 (>256)	64 (128)	64 (64)	128 (256)
	2b	256 (>256)	256 (>256)	256 (>256)	256 (>256)	256 (>256)	64 (128)	32 (64)	128 (≥256)

Table 4 – Antibacterial activity (MIC and MLC) of the azoimidazoles **2** against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria.

		Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC 25923
MIC[a] (MLC[b]) μg/mL	**2a**	>256 (>256)	128 (>256)
	2b	>256 (>256)	64 (≥256)

a) MIC-minimum inhibitory concentration; **b)** MLC-minimum lethal concentration.
(S) -Fluconazole susceptible strain; **(R)** -Fluconazole resistant strain

CONCLUSIONS

- Amidrazones **1** were easily oxidized with silver nitrate to give azoimidazoles **3** in excellent - very good yields.
- Cyclic voltammogram of **1** obtained in the -0.3 to 0.3 V potential range showed a reversible peak involving an electron at an extremely low oxidation potential, which proves the susceptibility of **1** to oxidation reactions.
- Azoimidazoles **2** exhibited good—moderate activity against *Candida*, *Cryptococcus* and dermatophyte strains.
- On the contrary, activity against other filamentous fungi and bacteria decreased significantly.

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