Novel Halochromic and Antimicrobial Azopyrimidine Dyes: Synthesis, Colorimetric Studies and Biological Assays

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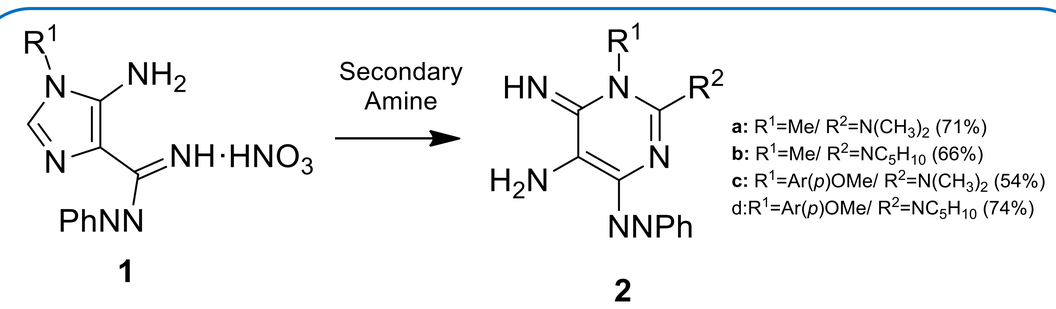
INTRODUCTION

Halochromism is the occurrence of a color change induced by a pH alteration. The main commercial classes of halochromic dyes are phthalides, triarylmethanes and fluorans, but other minor classes exist including styryl, merocyanines, indophenols and azobenzenes.[1a] The success of azobenzene dyes is due to their structural diversity, high molar extinction coefficient, and fastness properties in relation to light and washing fastness.[1b] Replacing one or both aryls with a heteroaryl offers broader structural diversity.[1c] In addition, the presence of basic sites, as well as H-bonding interactions, further affects the azo chromophore, leading to very different spectral properties. The synthesis of azopyrimidines and azopurines has long been known, and similarity to DNA nucleobases makes them promising candidates for applications in photopharmacology and biocompatible real-time information transmission.

In a recent work, a novel method for the synthesis of a new class of azo-pyrimidines with both halochromic and antimicrobial properties has been developed to obtain compounds with an unusual pattern of substituents in the heteroaryl unity. The new synthetic approach starts from imidazole derivatives that are easily obtained from accessible commercial reagents.[2]

SYNTHESIS

Preliminary results had suggested that azoimidazoles **1** easily evolve in the presence of amines, leading to the *in situ* formation of highly coloured products. These results motivated us to study the reactions of these imidazole-based precursors in the presence of secondary amines. A very fast reaction occurred as after neutralization of the azoimidazole intermediates **1** their basic forms promptly reacted with secondary amines through a novel and unexpected rearrangement. Deep coloured products were obtained, which were identified as azopyrimidines **2**.



Scheme 1 – Synthesis of azopyrimidines **2**

BIOLOGICAL ASSAYS

The antifungal activity of the azopyrimidines **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 (**Tables 1 – 3**).

Table 1 – Antifungal activity (MIC and MLC) of the azopyrimidines **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**	32 (64)	16 (32)	16 (32)	32 (64)	32 (64)	4 (4)	2 (2)
	2b	16 (32)	16 (32)	16 (32)	16 (32)	16 (32)	4 (4)	2 (2)
	2c	32 (64)	32 (64)	16 (64)	64 (128)	64 (128)	4 (4)	2 (4)
	2d	16 (32)	16 (32)	16 (32)	32 (64)	32 (64)	4 (4)	2 (2)

Table 2 – Antifungal activity (MIC and MLC) of the azopyrimidines **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**	128 (256)	64 (256)	64 (128)	32 (64)	128 (128)	16 (32)	32 (32)	32 (64)
	2b	64 (256)	64 (256)	64 (64)	32 (64)	64 (128)	16 (32)	16 (16)	32 (64)
	2c	128 (>256)	128 (>256)	128 (256)	64 (128)	256 (>256)	64 (64)	32 (32)	64 (128)
	2d	128 (>256)	128 (>256)	64 (128)	32 (64)	128 (>256)	32 (32)	32 (32)	64 (128)

Table 3 – Antibacterial activity (MIC and MLC) of the azopyrimidines **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)	32 (256)
	2b	256 (256)	32 (128)
	2c	>256 (>256)	32 (≥256)
	2d	>256 (>256)	32 (≥256)

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

COLORIMETRIC STUDIES

Colorimetric studies have been performed by UV-Vis spectroscopy at variable pH values and in different solvents, revealing interesting halochromic properties.

Acidic medium => Bathochromic and hypochromic shifts;

Basic medium: => Hypsochromic and hyperchromic shifts.

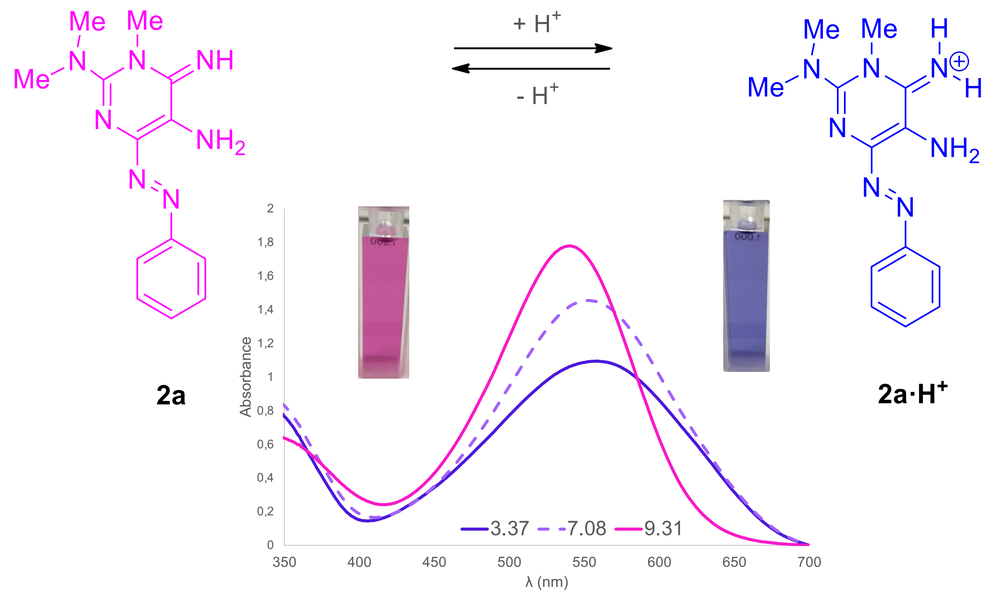


Figure 1 – Proposed structures of compound **2a** at different pH values according to ^{1}H NMR data (DMSO-d_6); UV-Vis absorption spectra of compound **2a** at different pH values.

Solvent and substituents effects:

- The values of maximum wavelength absorption vary between 551 nm (**2c**, ethanol) and 563 nm (**2b**, tetrahydrofuran);
- Dichloromethane causes a hyperchromic shift;
- Tetrahydrofuran causes a hypochromic shift;
- Aromatic groups in R^1 cause a hyperchromic shift.

Table 4 – Maximum wavelength values and molar absorptivity coefficient for compounds **2** in different solvents.

	Ethanol		Dichloromethane		Acetonitrile		Tetrahydrofuran	
	λ_{max}	ε	λ_{max}	ε	λ_{max}	ε	λ_{max}	ε
2a	552	(16.4±0.4)x10^3	556	(24.1±0.9)x10^3	558	(22.0±0.8)x10^3	560	(2.17±0.06)x10^3
2b	552	(9.5±0.2)x10^3	557	(22±1)x10^3	558	(16.2±0.5)x10^3	563	(15.1±0.2)x10^3
2c	551	(17.3±0.6)x10^3	554	(33±1)x10^3	555	(26.4±0.3)x10^3	560	(7.9±0.4)x10^3
2d	553	(16.9±0.2)x10^3	556	(26.9±0.8)x10^3	557	(25.2±0.3)x10^3	562	(25±1)x10^3

CONCLUSIONS

- Azopyrimidines **2** were easily obtained in moderate – good yields from azoimidazoles **1** through an unexpected rearrangement.
- Azopyrimidines **2** are interesting halochromic dyes, showing vibrant magenta color in basic medium and deep blue color in acidic medium.
- Azopyrimidines **2** also exhibit good—moderate activity against *Candida*, *Cryptococcus*, dermatophyte and *Staphylococcus aureus* strains.
- On the contrary, activity against other filamentous fungi and *Escherichia coli* was lower.

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