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Synthesis and antimicrobial activity of new diazoimidazole derivatives containing an *N*-acylpyrrolidine ring

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Abstract

A series of 4-diazoimidazole-5-carboxamides bearing in position 2 lipophilic substituents was synthesized and their antimicrobial activity was evaluated in vitro against pathogenic Gram-positive, Gram-negative bacteria and fungi. Some compounds presented antifungal activity, particularly two derivatives (**1g** and **1h**) showed good MIC values (10–50 μg/ml) against both moulds and yeasts.

Keywords: Diazoimidazoles; Antifungal activity; Synthesis; SAR

1. Introduction

Diazoazoles have various types of biological activity [1], probably due to their high reactivity towards amine, hydroxyl and sulfydryl groups. They have inhibitory action against several tumor cell lines and enzymes, and their antimicrobial activity has also been demonstrated [2].

In a previous work, we reported the synthesis and the antimicrobial activity of a series of 2-substituted-4-diazoimidazole-5-carboxamides, bearing different substituents at C-2 and on the 5-carboxamido moiety [3]. Among the synthesized compounds, a moderate antifungal activity against yeasts was observed for those compounds bearing small groups on the carboxamido moiety and no hydrophilic substituent at the 2 position. The obtained results prompted us to continue our research in order to achieve additional data for a structure–activity relationship. Therefore, a series of 16 new diazoimidazoles **1a**–**p** has been prepared. These compounds have a pyrrolidine ring in the carboxamido moiety and in C-2 position either an alkyl of different length and steric hindrance, or a phenyl or a substituted-phenyl substituent. Our aim was to evaluate the effect of the increased lipophilicity on the biological activity.

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2. Chemistry

All the compounds **1a**–**p** were synthesized by standard procedures as reported in Scheme 1. The thioimidate salts **3a**–**p** were prepared from the appropriate nitriles **2a**–**p**, commercially available, and benzylmercaptan in anhydrous ether saturated with dry hydrochloric acid. The reaction of **3a**–**p** with the amide **6** according to Rose and Ainsworth [4] afforded the 2-substituted-4-aminoimidazoles **7a**–**p**. Compound **6** was prepared by reduction with amalgamated aluminum of ethyl cyanoglyoxylate-2-oxime **4** to amine **5** [5] and subsequent reaction with pyrrolidine, at 0 °C. Finally, diazotization of the amino derivatives **7a**–**p** with sodium nitrite in dilute hydrochloric acid followed by neutralization of the acid solution gave the desired 4-diazoimidazoles **1a**–**p**.

3. Microbiology

All the compounds **1a**–**p** were evaluated in vitro, for antimicrobial activity, by the agar dilution method [6] against representative human pathogenic fungi (*Candida albicans*, *Candida lypolitica*, *Cryptococcus neoformans*, *Aspergillus niger*), Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Proteus mirabilis*) bacteria. The minimal

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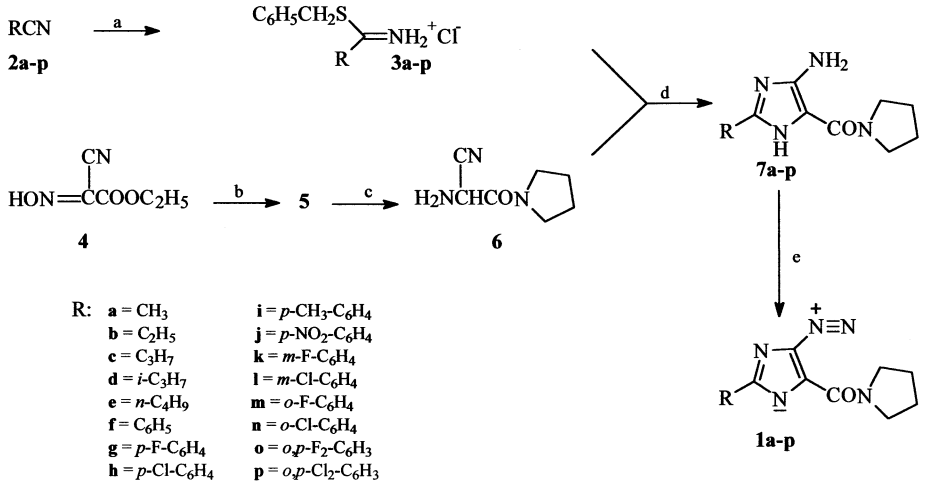
inhibitory concentrations (MICs, μg/ml) of the synthesized compounds **1a**–**p** investigated against yeasts (*C. albicans*, *C. lypolitica*, *C. neoformans*) and a filamentous fungus (*A. niger*) are presented in Table 1. The corresponding data of the previously published unsubstituted derivative **1** [3], amphotericin B and fluconazole are also shown. All the compounds showed a MIC value >100 μg/ml against bacteria, with the exception of compound **1j**, which exhibited a MIC = 10 μg/ml against *S. aureus*.

4. Experimental

Melting points were recorded in a Electrothermal open capillary apparatus and are uncorrected. Elemental analysis was performed for compounds **1a**–**p** and the results were within $\pm 0.4\%$ of the theoretical values. Infrared spectra (IR) in Nujol were recorded in a Perkin–Elmer 683 or a Nicolet Avatar 320 spectrophotometer (ν_{max} in cm^{-1}). ^1H and ^{13}C NMR spectra were registered in a Varian VXR 300 spectrometer, peak positions are given in parts per million (δ) relative to the standard chemical shift of the solvent. Coupling constants are reported in Hertz. Merck silica gel 60 (230–400 mesh) was used for column chromatography. Thin-layer chromatography (Merck silica gel 60 F_{254} analytical plates) was used to monitor reactions. Ether (Et_2O) and tetrahydrofuran (THF) were dried by distillation under nitrogen from sodium metal. The term 'dried' refers to the use of anhydrous sodium sulfate.



Scheme 1. Reagents: (a) benzylmercaptan/HCl; (b) amalgamated aluminum; (c) pyrrolidine; (d) EtOH, reflux; (e) $NaNO_2$/HCl.

Table 1
Antifungal activity in vitro of compounds **1a**–**p** (MIC [a]; μg/ml)

Comp.	R	*Candida albicans*	*Candida lypolitica*	*Cryptococcus neoformans*	*Aspergillus niger*
1 [b]	H	100	100	50	200
1a	CH_3	200	40	50	100
1b	C_2H_5	200	50	50	200
1c	n-C_3H_7	200	100	50	>200
1d	i-C_3H_7	>200	100	100	>200
1e	n-C_4H_9	200	100	50	>200
1f	C_6H_5	100	100	50	>200
1g	p-F–C_6H_4	50	10	10	25
1h	p-Cl–C_6H_4	25	25	25	25
1i	p-CH_3–C_6H_4	25	25	50	100
1j	p-NO_2–C_6H_4	100	50	50	200
1k	m-F–C_6H_4	100	100	100	100
1l	m-Cl–C_6H_4	200	100	100	>200
1m	o-F–C_6H_4	>200	200	200	>200
1n	o-Cl–C_6H_4	50	50	50	50
1o	o,p-F_2–C_6H_3	200	200	200	>200
1p	o,p-Cl_2–C_6H_3	100	50	50	100
Amphotericin B		2.5	20	10	20
Fluconazole		2.5	20	5	50

[a] Minimum inhibitory concentration.
[b] Ref. [3].

4.1. General procedure for preparation of thioimidic acid benzyl ester hydrochlorides (**3a–p**)

A stirred solution of the appropriate nitrile derivative **2a–p** (20 mmol) and benzylmercaptan (4.1 g, 33 mmol) in anhydrous ether (10 ml) or THF (15 ml) (**3j**) was saturated with dry hydrogen chloride and stirring was continued at room temperature (r.t.) for 24 h (**3a–l**) or 5 days (**3m–p**). Anhydrous ether was added to the solution until precipitation began. The white precipitate was collected and washed with dry ether, giving the thioimidate salts **3a–p**, pure enough for use in the next stage.

3a: yield 90%; m.p. 158–159 °C (lit. [7]: 153–155 °C); ^{1}H NMR (DMSO-d_6): δ 2.66 (3H, s, CH_3), 4.67 (2H, s, CH_2), 7.37–7.40 (5H, m, ArH), 12.47 (1H, brs, NH). **3b** [4]: yield 88%; m.p. 116–118 °C; ^{1}H NMR (DMSO-d_6): δ 1.23 (3H, t, $J = 8$ Hz, CH_3), 2.87 (2H, q, $J = 8$ Hz, CH_3CH_2), 4.68 (2H, s, SCH_2Ph), 7.28–7.51 (5H, m, ArH), 12.50 (1H, brs, NH). **3c** [4]: yield 97%; m.p. 135–136 °C; ^{1}H NMR (DMSO-d_6): δ 0.89 (3H, t, $J = 8$ Hz, CH_3), 1.68 (2H, hex, $J = 8$ Hz, $CH_3CH_2CH_2$), 2.80 (2H, t, $J = 8$ Hz, $CH_3CH_2CH_2$), 4.66 (2H, s, SCH_2Ph), 7.35–7.49 (5H, m, ArH), 12.39 (1H, brs, NH). **3d**: yield 54%; m.p. 164–165 °C (lit. [8]: 165–166 °C (dec.)); ^{1}H NMR (DMSO-d_6): δ 1.28 (6H, d, $J = 6.9$ Hz, $2 \times CH_3$), 3.19 (1H, hept, $J = 6.9$ Hz, CH), 4.65 (2H, s, CH_2), 7.38–7.51 (5H, m, ArH), 12.35 (1H, brs, NH). **3e** [4]: yield 99%; m.p. 140–141 °C; ^{1}H NMR (DMSO-d_6): δ 0.87 (3H, t, $J = 7.4$ Hz, CH_3), 1.31 (2H, hex, $J = 7.6$ Hz, $CH_3CH_2CH_2$), 1.63 (2H, p, $J = 7.6$ Hz, $CH_3CH_2CH_2$), 2.84 (2H, t, $J = 7.6$ Hz, $CH_3CH_2CH_2CH_2$), 4.67 (2H, s, SCH_2Ph), 7.35–7.50 (5H, m, ArH), 12.45 (1H, brs, NH). **3f**: yield 88%; m.p. 174–176 °C (lit. [9]: 172–174 °C (dec.)); ^{1}H NMR (DMSO-d_6): δ 4.86 (2H, s, CH_2), 7.37–7.46 (3H, m, ArH), 7.55–7.66 (4H, m, ArH), 7.80 (1H, t, ArH), 7.94 (2H, d, ArH), 12.39 (1H, brs, NH). **3g** [10]: yield 59%; m.p. 180–182 °C; ^{1}H NMR (DMSO-d_6): δ 4.85 (2H, s, CH_2), 7.39–7.57 (7H, m, ArH), 8.03–8.08 (2H, m, ArH). **3h**: yield 29%; m.p. 179–180 °C (lit. [9]: 180–181 °C); ^{1}H NMR (DMSO-d_6): δ 4.81 (2H, s, CH_2), 7.39 (3H, dd, ArH), 7.53 (2H, dd, ArH), 7.69 (2H, d, ArH), 7.95 (2H, d, ArH). **3i**: yield 70%; m.p. 168–170 °C; ^{1}H NMR (DMSO-d_6): δ 2.41 (3H, s, CH_3), 4.81 (2H, s, CH_2), 7.38–7.46 (5H, m, ArH), 7.54 (2H, d, ArH), 7.84 (2H, d, ArH). **3j**: yield 65%; m.p. 164–165 °C (lit. [9]: 166–170 °C (dec.)); ^{1}H NMR (DMSO-d_6): δ 4.85 (2H, s, CH_2), 7.35–7.48 (3H, m, ArH), 7.58 (2H, d, ArH), 8.15 (2H, d, ArH), 8.35 (2H, d, ArH). **3k** [10]: yield 78%; m.p. 159–160 °C; ^{1}H NMR (DMSO-d_6): δ 4.85 (2H, s, CH_2), 7.29–7.41 (3H, m, ArH), 7.55 (2H, dd, ArH), 7.63–7.86 (4H, m, ArH). **3l** [10]: yield 59%; m.p. 154–155 °C; ^{1}H NMR (DMSO-d_6): δ 4.82 (2H, s, CH_2), 7.30–7.50 (5H, m, ArH), 7.85–7.90 (3H, m, ArH), 8.00 (1H, s, ArH). **3m** [10]: yield 31%; m.p. 170 °C; ^{1}H NMR (DMSO-d_6): δ 4.76 (2H, s, CH_2), 7.35–7.50 (7H, m, ArH), 7.69–7.77 (2H, m, ArH). **3n** [10]: yield 5%; m.p. 170 °C; ^{1}H NMR (DMSO-d_6): δ 4.75 (2H, s, CH_2), 7.38–7.51 (5H, m, ArH), 7.70 (2H, d, ArH), 7.93 (2H, d, ArH). **3o**: yield 46%; m.p. 159–160 °C; ^{1}H NMR (DMSO-d_6): δ 4.73 (2H, s, CH_2), 7.33–7.60 (7H, m, ArH), 7.79–7.84 (1H, m, ArH). **3p**: yield 7%; m.p. 160 °C; ^{1}H NMR (DMSO-d_6): δ 4.59 (2H, s, CH_2), 7.30–7.43 (6H, m, ArH), 7.61 (1H, s, ArH), 7.86 (1H, d, ArH).

4.2. 2-Amino-3-oxo-3-(1-pyrrolidinyl)-propionitrile (**6**)

Ethyl cyanoglyoxylate-2-oxime (**4**) (1.42 g, 10 mmol) was reduced with amalgamated aluminum to ethyl aminocyanoacetate (**5**). To a solution of the crude amine **5** in ether (20 ml), cooled to 0 °C, was added dropwise pyrrolidine (1.42 g, 20 mmol). The solution was stirred for 15 min, the solvent evaporated and the oily residue was purified on a silica gel column eluting with 8:2 chloroform–methanol, to give the amide **6** (35%). Oil; IR: 3360–3280 (NH_2), 2220 (CN), 1650 (C=O); ^{1}H NMR (DMSO-d_6): δ 1.75–1.92 (4H, m, pyrrolidine), 2.58 (2H, brs, NH_2, exch. D_2O), 3.25–3.40 (2H, m, pyrrolidine), 3.55–3.66 (2H, m, pyrrolidine), 4.77 (1H, s, CH); ^{13}C NMR (DMSO-d_6): δ 23.74, 25.60, 45.85, 45.96, 46.31, 119.41, 163.71.

4.3. General procedure for preparation of 2-substituted 4-amino-5-(1-pyrrolidinyl)carbonylimidazoles (**7a–p**)

A solution of compound **6** (1.53 g, 10 mmol) in ethanol (5 ml) was treated portionwise with the appropriate crude thioimidic acid benzyl ester hydrochloride **3a–p** (10 mmol), and the mixture was heated under reflux for 15 min and then cooled. The solvent was evaporated in vacuo, the residue was purified by flash chromatography eluting with chloroform containing 1–5% methanol, giving 4-aminoimidazole derivatives **7a–p**·HCl, which were crystallized from a suitable solvent.

7a: yield 21%; m.p. 199–200 °C (MeOH–Et_2O); ^{1}H NMR (DMSO-d_6): δ 1.76 (4H, m, pyrrolidine), 2.10 (3H, s, CH_3), 3.47 (2H, m, pyrrolidine), 3.85 (2H, m, pyrrolidine), 5.71 (2H, s, NH_2, exch. D_2O), 11.16 (1H, brs, NH, exch. D_2O). **7b**: yield 32%; m.p. 190–192 °C (EtOH–Et_2O); ^{1}H NMR (DMSO-d_6): δ 1.12 (3H, t, $J = 7.7$ Hz, CH_3), 1.76 (4H, m, pyrrolidine), 2.44 (2H, q, $J = 7.7$ Hz, CH_2), 3.47 (2H, m, pyrrolidine), 3.84 (2H, m, pyrrolidine), 5.67 (2H, s, NH_2, exch. D_2O), 11.18 (1H, brs, NH, exch. D_2O). **7c**: yield 35%; m.p. 175–176 °C (EtOH–Et_2O); ^{1}H NMR (DMSO-d_6): δ 0.89 (3H, t, $J = 7.3$ Hz, CH_3), 1.59 (2H, hex, $J = 7.3$ Hz, $CH_3CH_2CH_2$), 1.78 (4H, m, pyrrolidine), 2.41 (2H, t, $J = 7.3$ Hz, $CH_3CH_2CH_2$), 3.49 (2H, m, pyrrolidine), 3.89 (2H, m, pyrrolidine), 5.68 (2H, s, NH_2, exch.

D_2O), 11.15 (1H, brs, NH, exch. D_2O). **7d**: yield 31%; m.p. 169–171 °C (EtOH–Et_2O); ^1H NMR (DMSO-d_6): δ 1.16 (6H, d, $J = 6.9$ Hz, $2 \times CH_3$), 1.78 (4H, m, pyrrolidine), 2.78 (1H, hept, $J = 6.9$ Hz, CH), 3.48 (2H, m, pyrrolidine), 3.90 (2H, m, pyrrolidine), 5.57 (2H, s, NH_2, exch. D_2O), 11.16 (1H, brs, NH, exch. D_2O). **7e**: yield 56%; m.p. 128–129 °C (EtOH–Et_2O); ^1H NMR (DMSO-d_6): δ 0.88 (3H, t, $J = 7.5$ Hz, CH_3), 1.29 (2H, hex, $J = 7.5$ Hz, $CH_3CH_2CH_2$), 1.56 (2H, p, $J = 7.4$ Hz, $CH_3CH_2CH_2$), 1.79 (4H, m, pyrrolidine), 2.45 (2H, t, $J = 7.4$ Hz, $CH_3CH_2CH_2CH_2$), 3.37 (2H, m, pyrrolidine), 3.61 (2H, m, pyrrolidine), 5.63 (2H, brs, NH_2, exch. D_2O), 11.2 (1H, brs, NH, exch. D_2O). **7f**: yield 21%; m.p. 217–218 °C (EtOH–Et_2O); ^1H NMR (DMSO-d_6): δ 1.80 (4H, m, pyrrolidine), 3.46 (2H, m, pyrrolidine), 4.08 (2H, m, pyrrolidine), 5.92 (2H, brs, NH_2, exch. D_2O), 7.31 (1H, t, ArH), 7.41 (2H, t, ArH), 7.84 (2H, d, ArH), 12.10 (1H, s, NH, exch. D_2O). **7g**: yield 10%; m.p. 200 °C (EtOH); ^1H NMR (DMSO-d_6): δ 1.78 (4H, m, pyrrolidine), 3.46 (2H, m, pyrrolidine), 4.06 (2H, m, pyrrolidine), 5.91 (2H, s, NH_2, exch. D_2O), 7.25 (2H, t, ArH), 7.85 (2H, t, ArH), 12.09 (1H, brs, NH, exch. D_2O). **7h**: yield 20%; m.p. 258–260 °C (MeOH); ^1H NMR (DMSO-d_6): δ 1.80 (4H, m, pyrrolidine), 3.42 (2H, m, pyrrolidine), 4.04 (2H, m, pyrrolidine), 5.94 (2H, s, NH_2, exch. D_2O), 7.45 (2H, d, $J = 8.6$ Hz, ArH), 7.82 (2H, d, $J = 8.6$ Hz, ArH), 12.14 (1H, brs, NH, exch. D_2O). **7i**: yield 25%; m.p. 243–245 °C (EtOH); ^1H NMR (DMSO-d_6): δ 1.81 (4H, m, pyrrolidine), 2.30 (3H, s, CH_3), 3.42 (2H, m, pyrrolidine), 4.05 (2H, m, pyrrolidine), 5.85 (2H, s, NH_2, exch. D_2O), 7.19 (2H, d, $J = 8$ Hz, ArH), 7.70 (2H, d, $J = 8$ Hz, ArH), 11.97 (1H, brs, NH, exch. D_2O). **7j**: yield 15%; m.p. 285–286 °C (EtOH); ^1H NMR (DMSO-d_6): δ 1.80 (4H, m, pyrrolidine), 3.40 (2H, m, pyrrolidine), 4.09 (2H, m, pyrrolidine), 6.18 (2H, s, NH_2, exch. D_2O), 8.02 (2H, d, $J = 9$ Hz, ArH), 8.26 (2H, d, $J = 9$ Hz, ArH), 12.5 (1H, brs, NH, exch. D_2O). **7k**: yield 15%; m.p. 131–133 °C (EtOH); ^1H NMR (DMSO-d_6): δ 1.77 (4H, m, pyrrolidine), 3.44 (2H, m, pyrrolidine), 4.02 (2H, m, pyrrolidine), 5.97 (2H, s, NH_2, exch. D_2O), 7.15 (1H, m, ArH), 7.40 (1H, m, ArH), 7.60 (2H, m, ArH), 12.15 (1H, brs, NH, exch. D_2O). **7l**: yield 16%; m.p. 188–190 °C (MeOH); ^1H NMR (DMSO-d_6): δ 1.85 (4H, m, pyrrolidine), 3.62 (2H, m, pyrrolidine), 4.12 (2H, m, pyrrolidine), 5.90 (2H, s, NH_2, exch. D_2O), 7.52 (2H, m, ArH), 7.95 (1H, d, ArH), 8.09 (1H, s, ArH), 12.1 (1H, brs, NH, exch. D_2O). **7m**: yield 20%; m.p. 84–85 °C (EtOH); ^1H NMR (DMSO-d_6): δ 1.85 (4H, m, pyrrolidine), 3.45 (2H, m, pyrrolidine), 4.03 (2H, m, pyrrolidine), 5.84 (2H, s, NH_2, exch. D_2O), 7.30 (3H, m, ArH), 7.95 (1H, t, ArH), 11.49 (1H, brs, NH, exch. D_2O). **7n**: yield 42%; m.p. 120–122 °C (EtOH); ^1H NMR (DMSO-d_6): δ 1.80 (4H, m, pyrrolidine), 3.43 (2H, m, pyrrolidine), 4.00 (2H, m, pyrrolidine), 5.94 (2H, s, NH_2, exch. D_2O), 7.50 (3H, m, ArH), 8.05 (1H, d, ArH), 11.6 (1H, brs, NH, exch. D_2O). **7o**: yield 15%; m.p. 149–150 °C (EtOH); ^1H NMR (DMSO-d_6): δ 1.81 (4H, m, pyrrolidine), 3.60 (2H, m, pyrrolidine), 4.02 (2H, m, pyrrolidine), 5.84 (2H, s, NH_2, exch. D_2O), 7.15 (2H, m, ArH), 7.95 (1H, m, ArH), 11.5 (1H, brs, NH, exch. D_2O). **7p**: yield 16%; m.p. 231–233 °C (EtOH); ^1H NMR (DMSO-d_6): δ 1.80 (4H, m, pyrrolidine), 3.41 (2H, m, pyrrolidine), 4.00 (2H, m, pyrrolidine), 5.93 (2H, s, NH_2, exch. D_2O), 7.46 (1H, d, ArH), 7.64 (1H, s, ArH), 7.81 (1H, d, ArH), 11.71 (1H, brs, NH, exch. D_2O).

*4.4. General procedure for preparation of 2-substituted 4-diazo-5-(1-pyrrolidinyl)carbonylimidazoles (**1a–p**)*

A stirred solution of sodium nitrite (0.18 g, 2.6 mmol) in water (3 ml) was cooled in an ice bath and treated dropwise with a solution of the appropriate amine (1.9 mmol) in dilute hydrochloric acid (4 ml). The solution was stirred at 0 °C for 30 min, then at r.t. for 1 h. The aqueous solution was extracted with dichloromethane (3×20 ml), the organic layer was washed with saturated aqueous sodium hydrogen carbonate (2×10 ml), dried and evaporated to afford an oily residue which was purified by column chromatography eluting with 8:2 AcOEt–petroleum ether and crystallized.

1a: yield 22%; m.p. 52–53 °C (EtOH–Et_2O); IR: 2122 (CN_2^+), 1610 (C=O); ^1H NMR (DMSO-d_6): δ 1.86 (4H, m, pyrrolidine), 2.36 (3H, s, CH_3), 3.49 (2H, t, pyrrolidine), 3.93 (2H, t, pyrrolidine); ^{13}C NMR (DMSO-d_6): δ 18.09, 23.60, 26.40, 47.06, 48.65, 102.72, 158.31, 158.49, 159.22. **1b**: yield 35%; m.p. 28–30 °C (EtOH–Et_2O); IR: 2125 (CN_2^+), 1610 (C=O); ^1H NMR (DMSO-d_6): δ 1.22 (3H, t, $J = 7.5$ Hz, CH_3), 1.85 (4H, m, pyrrolidine), 2.70 (2H, q, $J = 7.5$ Hz, CH_2), 3.47 (2H, t, pyrrolidine), 3.93 (2H, t, pyrrolidine); ^{13}C NMR (DMSO-d_6): δ 12.77, 23.60, 25.20, 26.41, 47.03, 48.63, 102.43, 158.21, 158.53, 164.06. **1c**: yield 74%; m.p. 48–50 °C (EtOH–Et_2O); IR: 2130 (CN_2^+), 1610 (C=O); ^1H NMR (DMSO-d_6): δ 0.91 (3H, t, $J = 7.4$ Hz, CH_3), 1.70 (2H, hex, $J = 7.4$ Hz, $CH_3CH_2CH_2$), 1.85 (4H, m, pyrrolidine), 2.64 (2H, t, $J = 7.4$ Hz, $CH_3CH_2CH_2$), 3.46 (2H, t, pyrrolidine), 3.92 (2H, t, pyrrolidine); ^{13}C NMR (DMSO-d_6): δ 13.79, 21.24, 23.26, 26.07, 33.43, 46.67, 48.29, 102.33, 157.81, 158.18, 162.68. **1d**: yield 33%; oil; IR: 2125 (CN_2^+), 1615 (C=O); ^1H NMR (DMSO-d_6): δ 1.25 (6H, d, $J = 6.9$ Hz, $2 \times CH_3$), 1.86 (4H, m, pyrrolidine), 3.00 (1H, hept, $J = 6.9$ Hz, CH), 3.48 (2H, t, pyrrolidine), 3.95 (2H, t, pyrrolidine); ^{13}C NMR (DMSO-d_6): δ 21.49, 23.15, 25.95, 30.68, 46.56, 48.16, 101.96, 157.62, 158.17, 167.25. **1e**: yield 40%; oil; IR: 2122 (CN_2^+), 1610 (C=O); ^1H NMR (DMSO-d_6): δ 0.88 (3H, t, $J = 7.3$ Hz, CH_3), 1.33 (2H, hex, $J = 7.3$ Hz, $CH_3CH_2CH_2$), 1.66 (2H, p, $J = 7.3$ Hz,

$CH_3CH_2CH_2$), 1.85 (4H, m, pyrrolidine), 2.67 (2H, t, $J = 7.3$ Hz, $CH_3CH_2CH_2CH_2$), 3.47 (2H, t, pyrrolidine), 3.93 (2H, t, pyrrolidine); ^{13}C NMR (DMSO-d_6): δ 14.07, 22.12, 23.50, 26.31, 30.27, 31.33, 46.93, 48.53, 102.56, 157.92, 158.43, 163.04. **1f**: yield 43%; m.p. 137–138 °C (EtOH–Et_2O); IR: 2130 (CN_2^+), 1600 (C=O); ^1H NMR ($CDCl_3$): δ 1.90 (4H, m, pyrrolidine), 3.54 (2H, t, pyrrolidine), 4.08 (2H, t, pyrrolidine), 7.38 (3H, m, ArH), 8.12 (2H, d, ArH); ^{13}C NMR ($CDCl_3$): δ 23.16, 26.01, 46.55, 48.19, 103.87, 126.67, 128.21, 129.02, 132.92, 157.92, 158.28, 158.44. **1g**: yield 30%; m.p. 128–130 °C (EtOH–Et_2O); IR: 2125 (CN_2^+), 1600 (C=O); ^1H NMR ($CDCl_3$): δ 1.91 (4H, m, pyrrolidine), 3.55 (2H, t, pyrrolidine), 4.09 (2H, t, pyrrolidine), 7.06 (2H, t, ArH), 8.10 (2H, t, ArH); ^{13}C NMR ($CDCl_3$): δ 23.07, 25.92, 46.43, 48.10, 103.87, 114.82, 115.10, 128.53, 128.64, 129.20, 129.23, 157.49, 157.83, 158.57. **1h**: yield 50%; m.p. 135–137 °C (EtOH–Et_2O); IR: 2130 (CN_2^+), 1600 (C=O); ^1H NMR ($CDCl_3$): δ 1.91 (4H, m, pyrrolidine), 3.54 (2H, t, pyrrolidine), 4.09 (2H, t, pyrrolidine), 7.41 (2H, d, $J = 8.6$ Hz, ArH), 8.08 (2H, d, $J = 8.6$ Hz, ArH); ^{13}C NMR ($CDCl_3$): δ 23.07, 25.92, 46.47, 48.05, 104.16, 128.04, 128.35, 131.70, 134.12, 156.89, 157.76, 158.19. **1i**: yield 45%; m.p. 153–154 °C (MeOH); IR: 2120 (CN_2^+), 1608 (C=O); ^1H NMR ($CDCl_3$): δ 1.90 (4H, m, pyrrolidine), 2.33 (3H, s, CH_3), 3.52 (2H, t, pyrrolidine), 4.06 (2H, t, pyrrolidine), 7.17 (2H, d, $J = 7.9$ Hz, ArH), 7.97 (2H, d, $J = 7.9$ Hz, ArH); ^{13}C NMR ($CDCl_3$): δ 20.94, 23.08, 25.95, 46.45, 48.08, 103.56, 126.57, 128.81, 130.24, 138.72, 157.83, 158.28, 158.48. **1j**: yield 32%; m.p. 158–160 °C (dec.) (EtOH–Et_2O); IR: 2130 (CN_2^+), 1605 (C=O); ^1H NMR ($CDCl_3$): δ 1.74 (4H, m, pyrrolidine), 3.39 (2H, t, pyrrolidine), 3.92 (2H, t, pyrrolidine), 8.02 (4H, m, ArH); ^{13}C NMR ($CDCl_3$): δ 22.96, 25.81, 46.39, 48.06, 102.62, 123.16, 127.04, 138.39, 147.28, 156.26, 157.59, 158.09. **1k**: yield 28%; m.p. 121–123 °C (EtOH–Et_2O); IR: 2120 (CN_2^+), 1620 (C=O); ^1H NMR ($CDCl_3$): δ 1.99 (4H, m, pyrrolidine), 3.68 (2H, t, pyrrolidine), 4.21 (2H, t, pyrrolidine), 7.09 (1H, t, ArH), 7.40 (1H, q, ArH), 7.93 (2H, m ArH); ^{13}C NMR ($CDCl_3$): δ 23.63, 26.47, 46.98, 48.72, 103.72, 114.26, 116.46, 122.71, 129.92, 130.14, 135.50, 157.92, 158.54, 158.89. **1l**: yield 18%; m.p. 132–134 °C (EtOH–Et_2O); IR: 2110 (CN_2^+), 1605 (C=O); ^1H NMR ($CDCl_3$): δ 1.93 (4H, m, pyrrolidine), 3.59 (2H, t, pyrrolidine), 4.13 (2H, t, pyrrolidine), 7.32 (2H, m, ArH), 7.72 (1H, d, ArH), 7.85 (1H, s, ArH); ^{13}C NMR ($CDCl_3$): δ 24.14, 26.99, 47.53, 49.25, 104.83, 125.73, 127.60, 129.81, 130.21, 131.99, 134.96, 158.10, 158.98, 159.48. **1m**: yield 37%; m.p. 94–95 °C (EtOH–Et_2O); IR: 2140 (CN_2^+), 1605 (C=O); ^1H NMR ($CDCl_3$): δ 1.90 (4H, m, pyrrolidine), 3.60 (2H, t, pyrrolidine), 4.13 (2H, t, pyrrolidine), 7.09–7.29 (3H, m, ArH), 8.12 (1H, t, ArH); ^{13}C NMR ($CDCl_3$): δ 23.65, 26.49, 47.01, 48.77, 104.29, 116.48, 116.92, 121.30, 124.08, 130.79, 131.08, 155.64, 158.22, 158.58. **1n**: yield 25%; m.p. 105–106 °C (EtOH–Et_2O); IR: 2140 (CN_2^+), 1600 (C=O); ^1H NMR ($CDCl_3$): δ 1.99 (4H, m, pyrrolidine), 3.68 (2H, t, pyrrolidine), 4.21 (2H, t, pyrrolidine), 7.41 (2H, d, ArH), 8.13 (2H, d, ArH); ^{13}C NMR ($CDCl_3$): δ 23.88, 26.73, 47.24, 48.97, 105.29, 128.67, 129.00, 129.30, 131.99, 135.66, 157.32, 158.77, 159.09. **1o**: yield 24%; oil; IR: 2120 (CN_2^+), 1600 (C=O); ^1H NMR ($CDCl_3$): δ 1.99 (4H, m, pyrrolidine), 3.68 (2H, t, pyrrolidine), 4.18 (2H, t, pyrrolidine), 6.94 (2H, m, ArH), 8.19 (1H, m, ArH); ^{13}C NMR ($CDCl_3$): δ 23.78, 26.63, 47.14, 48.86, 105.07, 111.50, 111.75, 118.00, 128.65, 132.22, 132.36, 155.62, 158.44, 158.63. **1p**: yield 50%; m.p. 90–92 °C (EtOH); IR: 2139 (CN_2^+), 1622 (C=O); ^1H NMR ($CDCl_3$): δ 1.91 (4H, m, pyrrolidine), 3.62 (2H, t, pyrrolidine), 4.11 (2H, t, pyrrolidine), 7.26 (1H, d, ArH), 7.45 (1H, s, ArH), 7.95 (1H, d, ArH); ^{13}C NMR ($CDCl_3$): δ 23.64, 26.49, 46.98, 48.77, 104.08, 127.00, 130.76, 131.79, 132.61, 133.50, 135.26, 156.69, 157.76, 158.47.

4.5. Antimicrobial assays

The minimal inhibitory concentrations (MICs) were determined by the two-fold agar dilution method [6]. Mueller–Hunton Agar (BBL) was used to test the antibacterial activity against: *S. aureus* ATCC 29213, *E. faecalis* ATCC 8043, *E. coli* ATCC 11105, *P. mirabilis* MB 81. Sabouraud Dextrose Agar (Difco) was used to evaluate the antimycotic activity against: *C. albicans* ATCC 10231, *C. lypolitica* CBS 6124, *C. neoformans* L 29, *A. niger* L 32. MB and L strains were obtained from our collection. All experiments were performed in triplicate, using inocula of 10^6 cfu (colony forming units)/ml of yeasts and bacteria, and 10^5 conidia/ml of mould. Fungal and bacteria plates were incubated at 28 °C for 48 h and at 37 °C for 24 h, respectively. Stock solutions of the tested compounds were prepared in *N*,*N*-dimethylformamide (DMF): as this solvent is inhibitory for some microrganisms, it is essential to reduce the final concentration in test media to a non-inhibitory level; a final concentration of less than 1% DMF was found to be satisfactory. In the assay conditions, all the tested compounds proved to be stable.

5. Results and discussion

The results reported in Table 1 show that the introduction in position 2 of alkyl substituents does not affect activity against *C. neoformans*, with the exception of **1d** (MIC = 100 μg/ml), while this substitution is detrimental in regard to the activity against *C. albicans*. The presence of a methyl group (**1a**) increases the activity against *C. lypolitica* and *A. niger*, while more lipophilic substituents do not influence activity against

C. lypolitica and cause loss of activity against *A. niger* (**1c**–**1e**). The introduction of a phenyl ring in position 2 of the imidazole moiety affected in a different way the activity: the unsubstituted phenyl group decreased the activity against *A. niger* (MIC > 200 μg/ml), a *p*-halogenophenyl group led to the most active derivatives: compound **1g** showed the best activity against *C. lypolitica* and *C. neoformans* (MIC = 10 μg/ml), compound **1h** exhibited the broadest antifungal spectrum (MIC = 25 μg/ml) against yeasts and moulds. Particularly, the efficacy of these products against *C. lypolitica*, *C. neoformans* and *A. niger* can be compared with that showed by amphotericin B and fluconazole, used as reference compounds for inhibitory activity against fungi.

The introduction in *para* position of the phenyl ring of substituents with different electronic and steric properties caused a decrease of activity against *A. niger* (**1i**) with regard to compound **1h** or a reduced susceptibility against both yeasts and moulds (**1j**). *meta*- (**1k**, **1l**) and *ortho*-substitution (**1m**, **1n**) or disubstitution (**1o**, **1p**) gave less active compounds.

The obtained results suggest that the introduction of a substituent in position 2 of the imidazole moiety with increasing lipophilicity does not significantly influence antifungal activity with the exception of a *para*-substituted phenyl ring which is important for improved activity and spectrum. The activity seems independent of the electron nature of the substituent (**1h** compared with **1i** or **1j**), *p*-chloro- and *p*-fluoro-phenyl groups impart the highest activity and the broadest spectrum. The Cl group on the phenyl ring results in a more suitable substituent, it retains some activity also in *ortho*- or *ortho*,*para*-positions.

Considering the antifungal activity of compounds **1g** and **1h**, particularly against the mould *A. niger*, further study will be devoted to the improvement of the biological profile.

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