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Original article

Syntheses, in vitro antibacterial and antifungal activities of a series of *N*-alkyl, 1,4-dithiines

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Abstract

A series of dithiines were synthesized by cyclization of 4-(alkylamino)-4-oxobutanoic acids under the action of $SOCl_2$. Their in vitro antibacterial and antifungal activities have been evaluated against reference strains and versus reference compounds. The so-called 'isoimides' **2a**, **2b** were totally inactive whereas some imides had low MICs for few bacteria and for few fungal microorganisms.

Keywords: Dithiine; Imide; Isoimide; Antimicrobial agents

1. Introduction

In previous works [1–3], we have investigated the syntheses and the biological properties of a series of maleimides and succinimides. In order to obtain other succinimides derivatives possessing antimicrobial and/or antifouling activities, we have studied the cyclization of 4-(alkylamino)-4-oxobutanoic acids **1**, easily obtained from aliphatic amines and succininic anhydride (Fig. 1). Surprisingly a very few papers are dealing with the synthesis and properties of dithiines.

2. Chemistry

In these experimental conditions, the corresponding succinimides were not obtained and it could be shown that, when the reaction was carried out in polar aprotic solvents (e.g. THF, dioxane), the 4,8-dithiine-indacene-1,3,5,7-tetraones **3** (diimides **3**) were produced via 3,7-bis-isoimino-4,8-dithiaindacene-1,5-diones **2** (diisoimides **2**). The diisomides **2** could be considered as the kinetically controlled compounds and may be isolated (as rose crystals), only when they had a low solubility in the reaction medium. Otherwise, an isomerization led to the green diimides **3**, which were always poorly soluble (Fig. 2).

This methodology has been reported (without experimental details) by Michaïlidis et al. [4], and three of these 1,4-dithiin-2,3,5,6-tetracarboxydiimides were used as synthons for Diels–Alder cycloadditions, by Hayakawa et al. [5]. Only one patent described a biological use of some 1,4-dithiin-2,3,5,6-tetracarboxydiimides as anthelmintics [6]. These poor data prompt us to synthesize a series of these compounds and evaluate their biological properties. Hence, we report herein, next to the synthesis, the in vitro antibacterial and antifungal properties of a series of dithiines **2** and **3**. One will observed that dithiin diimide **3b** was not obtained.

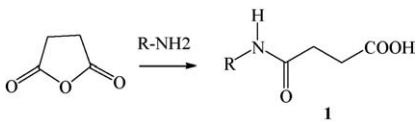

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Fig. 1. Syntheses of succinamic acids. Substituents: a: R = benzyl; b: R = cyclohexyl; c: R = isopropyl; d: R = propyl; e: R = butyl.

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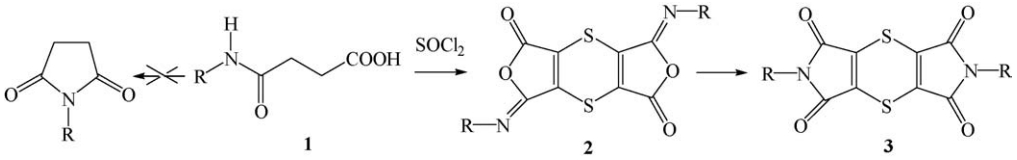

Fig. 2. Syntheses and structures of dithiin diisoimides (**2**) and diimides (**3**).

3. Microbiological evaluation

3.1. Antibacterial activities

The results of the screening tests of *N*-alkyl, 1,4-dithiines isoimides **2a,b** and *N*-alkyl, 1,4-dithiines **3a–e** were reported in Table 1 which showed the minimum inhibitory concentrations (MICs) of dithiines against two Gram-positive bacteria (*S. aureus* ATCC 25923, *E. faecalis* ATCC 29212) and two Gram negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853). *D*imethyl sulfoxide has no antibacterial effect at a concentration up to 512 μg ml^{-1}. The isoimides **2a** and **2b** are totally inactive versus the four strains. Conversely, the imides **3a** to **3e** have some activities against *S. aureus* and *E. faecalis* with MICs ranging from 2 to 16 μg ml^{-1}. Concerning *E. coli* and *P. aeruginosa* MICs of 128 μg ml^{-1} are considered as inactive. Nevertheless reference compounds (ampicillin, kanamycin, erythromycin and ofloxacin) are much more efficient with the exception of *P. aeruginosa* usually resistant to many antibiotics.

Table 1
In vitro antibacterial activities of *N*-alkyl, 1,4-dithiines isoimides **2** and *N*-alkyl, 1,4-dithiines **3** compounds (MICs, μg ml^{-1})

Compound	*S. aureus* ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
2a	256	256	256	256
2b	256	256	256	256
3a	16	2	128	128
3c	4	4	128	128
3d	8	4	128	128
3e	8	4	128	128
Reference compounds				
Ampicillin	0.5	1	4	> 16
Kanamycin	2	32	2	> 16
Erythromycin	0.25	2	> 4	> 4
Ofloxacin	0.5	2	0.03	2

a: R = benzyl; b: R = cyclohexyl; c: R = isopropyl; d: R = propyl; e: R = butyl.

3.2. Antifungal activities

The antifungal activities of the compounds **2a,b** and **3a–e** (see Table 2) were determined according to the guidelines of the Clinical and Laboratory Standards Institute (formerly NCCLS) for yeasts and for filamentous fungi [7,8]. Fungal strains such as *Candida albicans*, *Candida parapsilosis*, *Aspergillus fumigatus* were used in the aim to evaluate interest of the investigational compounds against the species currently responsible for the majority of human mycoses. The test strains included also some yeast species of which a characteristic feature is the frequent resistance to major antifungals: *Candida krusei* has an intrinsic resistance to fluconazole, while majority of *Candida lusitaniae* strains presents high MIC to amphotericin B.

As observed for bacteria, the isoimides **2a** and **2b** are relatively inactive, whereas the isomide **3d** has some antifungal activity especially against *C. albicans* (< 0.1 μg ml^{-1}), and **3e** against *C. parapsilosis and C. krusei* (0.4 μg ml^{-1}). The product **3c** shows a low MIC particularly against *A. fumigatus* (0.1 μg ml^{-1}), and also against *C. albicans* (0.8 μg ml^{-1}). The compound **3a** shows a poorest spectrum of activity of *N*-alkyl, 1,4-dithiines molecules.

If we compare these results to the in vitro susceptibility of these fungal species to the reference antifungals mainly to

Table 2
In vitro antifungal activities of *N*-alkyl, 1,4-dithiines isoimides **2** and *N*-alkyl, 1,4-dithiines **3** compounds (MICs 50, μg ml^{-1})

Compound	*C. parapsilosis* ATCC 22019	*C. krusei* ATCC 26258	*C. albicans* ATCC 90028	*C. lusitaniae* ATCC 410086	*A. niger* ATCC 980483435	*A. fumigatus* wild strain [a]
	MIC50	MIC50	MIC50	MIC50	MIC50	MIC50
2a	> 51.2	> 12.8	> 51.2	> 51.2	> 51.2	> 25.6
2b	> 51.2	> 51.2	> 51.2	> 51.2	> 51.2	> 51.2
3a	6.4	3.2	3.2	3.2	> 51.2	> 51.2
3c	> 51.2	51.2	0.8	12.8	> 51.2	0.1
3d	3.2	1.6	< 0.1	6.4	3.2	1.6
3e	0.4	0.4	1.6	6.4	> 51.2	0.8
Reference compounds (MIC range)						
Amphotericin B	0.25–1	0.25–2	0.5–2	0.12–1	0.25–1	0.5–2
Fluconazole	2–8	16–64	0.25–1	0.25–1	–	–
Flucytosine	0.12–0.5	4–16	0.5–2	0.12–4	–	–
Itraconazole	–	–	–	–	0.5–12	0.12–1

a: R = benzyl; b: R = cyclohexyl; c: R = isopropyl; d: R = propyl; e: R = butyl.

[a] Fungal collection of the 'Centre Hospitalier Universitaire de Grenoble'; Service de Parasitologie–Mycologie B.P.217, Grenoble cedex 9 France.

amphotericin B, which remains the "gold standard" in mycology because of its wide spectrum of activity, all the other compounds can be considered as inactive.

However, we must note that the screening of antifungal activity only on the basis of in vitro techniques can be uncertain, as demonstrated these last years during the development of the new class of echinocandins. Indeed, the fungal cell is particularly complex, and according to the target of antifungal classes and their site of activity, MIC results can show wide variations between strains and species.

4. Conclusions

The reaction of thionyl chloride with succinamic acids appeared to be a useful method for the synthesis of *S*-containing heterocyclic compounds such as 1,4-dithiins, which were usually obtained with yields close to 50%. In the high majority of examples, the products crystallized spontaneously and the products did not require further purification.

Some dithiin diimides (compounds **3a**, **3c**, **3d** and **3e**) have a moderate antibacterial activity against Gram-positive organisms (*S. aureus* and *E. faecalis*), but *E. coli* and *P. aeruginosa* were resistant.

The antifungal activity of the new dithiins compounds **3c**, **3d** and **3e** against some yeasts and moulds of medical interest could open a new way of investigation for future. To confirm the potential value of these new compounds, a large number of strains should be tested for the species which appear sensitive to the products, especially *C. albicans*, *C. krusei* and *A. fumigatus*. Indeed there is a need for research of new antifungals to address infections due to an expanding number of opportunistic fungi.

Conversely, dithin diisoimides (compounds **2a** and **2b**) are totally inactive despites of their close structure similarities with the dithin diimides. The present study adds new data in the relationships of imides and their antimicrobial activities [1–3].

5. Experimental part

Melting points were taken on a Leitz 350 heated stage microscope and are not corrected. ^{1}H NMR spectra were recorded on a Bruker Avance DPX 400 and a WP80 DS instruments and were reported in ppm downfield from internal tetramethylsilane. Chemical shifts were reported in ppm (δ) relative to TMS. IR spectra were run on a Bruker IF 55 spectrometer. Elemental analyses were within ± 0.35 of the theoretical values. Analytical thin layer chromatography was performed on a plastic sheet (0.2 mm, silica gel 60 F_{254}, Merck). Silica gel 60 (70–230 mesh, Merck) was used for column chromatography.

*5.1. General procedure for the preparation of compounds **1***

Fifteen millimols of succinic anhydride were dissolved into 10 ml of dioxane and 15 mmol of the correspondent amine in 10 ml of dioxane was slowly added. The solution was warmed at 80 °C for 30 min. and the succinamic acid **1** crystallize by cooling. The white crystals were filtered off, dried and recrystallized from dioxane.

1a: m.p. 144 °C (85%). IR (KBr): *ν* CO 1691; 1642.
1b: m.p. 171 °C (90%). IR (KBr): *ν* CO 1697; 1642.
1C: m.p. 108 °C (90%). IR (KBr): *ν* CO 1731; 1641.
1d: m.p. 101 °C (85%). IR (KBr): *ν* CO 1414; 1642.
1e: m.p. 96 °C (85%). IR (KBr): ν CO 1695; 1650.

*5.2. General procedure for the preparation of compounds **2***

One gram of the succinamic acid was dissolved into 10 ml of ether and 13 ml of thionyl chloride was slowly added. The solution was allowed to r.t. for 6 h and the diisoimide was filtered off, washed with 2 × 5 ml of ether and recrystallized.

2a: Rose crystals, m.p.: 210 °C. IR: 1791, 1696 cm^{-1}. ^{1}H NMR ($CDCl_3$): 4.78 (s, 4H, CH_2); 7.31–7.38 (m, 10H, Ar).

2b: Rose crystals, m.p.: 228 °C. IR: 1789, 1774, 1701 cm^{-1}. ^{1}H NMR ($CDCl_3$): 1.23–2.13 (m, 20H, CH_2); 3.75–3.82 (m, 2H, CH).

*5.3. General procedure for the preparation of compounds **3***

By the same method used for the preparation of the compounds **2**, using dioxane as solvent.

3a: Green needles, m.p.: 224 °C. IR: 1712, 1697 cm^{-1}. ^{1}H NMR ($CDCl_3$): 4.64 (s, 4H, CH_2); 7.31–7.35 (m, 10H, Ar).

3c: Green needles, m.p.: 220 °C. IR: 1713, 1696 cm^{-1}. ^{1}H NMR (DMSO d_6): 1.35 (d, 12H, CH_3, J = 6.9 Hz); 4.23–4.30 (m, 2H, CH).

3d: Green needles, m.p.: 211 °C. IR: 1714, 1693 cm^{-1}. ^{1}H NMR (DMSO d_6): 0.88 (t, 6H, CH_3, J = 7.38 Hz); 1.48–1.61 (m, 4H, CH_2); 3.45 (t, 4H, CH_2, J = 7.09 Hz).

3e: Green needles, m.p.: 214 °C. IR: 1713, 1693 cm^{-1}. ^{1}H NMR (DMSO d_6): 0.91 (t, 6H, CH_3, J = 7.35 Hz); 1.26–1.35 (m, 4H, CH_2); 1.51–1.58 (m, 4H, CH_2);.3.49 (t, 4H, CH_2, J = 7.05 Hz).

6. Bacteriological assays

In vitro antimicrobial activities of the compounds were determined by the twofold broth dilution method in Mueller Hinton nutrient broth. The concentration of mother solutions was 1024 μg ml^{-1} (50/50 water–methyl sulfoxide). Minimal Inhibitory Concentration (MIC), was defined as the lowest drug concentration resulting in complete inhibition of growth after 18 h of incubation at 37 °C. Methyl sulfoxide has no antibacterial activity at a concentration up to 20% in water. The tested organisms were *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

7. Antifungal assays

Antifungal susceptibility testing was determined by the reference methods of the Clinical and Laboratory Standards Insti-

tute (formerly NCCLS). Broth microdilution methodologies: M27-A2 for yeasts and M38-A for filamentous fungi (7, 8). Briefly the test isolates included two quality control (QC) strains (*C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 26258*),* three yeast and mould reference strains (*C. albicans* ATCC 90028, *C. lusitaniae* ATCC 410086, *Aspergillus niger* ATCC 9804.83435) and one *A. fumigatus* clinical isolate, obtained from a proven invasive aspergillosis case at the University Hospital of Grenoble (France). Stock solutions of dithiines were prepared in *d*imethyl sulfoxide (water–methyl sulfoxide: 50/50), then they were diluted by the twofold broth dilution method in RPMI 1640 medium buffered to pH 7.0 with MOPS, as recommended by the NCCLS. The final solvent concentration was reduced to 1%, and at this concentration it had no antifungal effect. Regarding susceptibility testing, isolates were grown on Sabouraud medium at 35 °C for a period of 24–48 h for yeasts and 7 days for moulds. Final inoculum of yeast suspensions was 5×10^3 CFU ml^{-1} and suspensions of conidial spores of *Aspergillus* were adjusted to obtain an inoculum of 1×10^4 CFU ml^{-1}. The final ranges of drug concentrations tested were 0.1–51.2 μg ml^{-1} for all the dithiines compounds. Minimal inhibitory concentrations (MICs) were determined after 24 h of incubation of microdilution trays at 35 °C for *Candida* strains and 48 h at 30 °C for *Aspergillus* strains. MICs were determined spectrophotometrically at 405 nm, and defined as the lowest concentration of drug that produced 50% inhibition of growth relative to the drug-free growth control.

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