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Article

Anti-*Sporothrix brasiliensis* activity of different pyrazinoic acid prodrugs: a repurposing evaluation

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From drug repurposing studies, this work aimed to evaluate the activity of different pyrazinoic acid (POA) derivatives against *Sporothrix brasiliensis*. The POA esters were prepared and characterized as previously reported by classical esterification reactions, with good to excellent yields. *Sporothrix brasiliensis* isolates from cats (*n*=6) and standard strains of *S. brasiliensis* and *S. schenckii* were used to assess the antifungal activity of the POA derivatives through broth microdilution assay (CLSI M38-A2). Among the tested compounds, molecules **3** and **4** showed fungistatic and fungicidal activities against all *Sporothrix* spp. strains, and the obtained MIC and MFC values ranged from 2.12 to 4.24 mg/mL and from 1.29 to 5.15 mg/mL, respectively. Compound **2** and **5** were active as *in vitro* inhibitors of fungal growth, but showed weak fungicidal activity, while molecules **1** and POA itself were inactive. The results suggest the activity of POA derivatives against *Sporothrix* spp. may be dependent on the lipophilicity. In addition, the antifungal susceptibility of the isolates to itraconazole was performed, showing that two *Sporothrix* isolates from cats were itraconazole-resistant. Compounds **3** and **4** were also active against these itraconazole-resistant isolates, indicating a possible alternative route to the standard mode of action of itraconazole.

Keywords: Sporotrichosis. *Sporothrix schenckii* complex. Itraconazole. Antifungal resistance. Prodrugs. Pyrazinoic acid derivatives.

INTRODUCTION

Among the zoonotic diseases with importance in human and veterinary medicine, sporotrichosis should be highlighted due to the increasing reports in both human and animals. This infection is clinically characterized by subcutaneous nodules, ulcers and crusts, which can spread to systemic organs (Madrid *et al*., 2012). This mycosis is caused by agents of *Sporothrix schenckii* complex (Marimon *et al*., 2007) through the cutaneous inoculation by contaminated plant material (De Araújo *et al*., 2015) or by bite and scratch of animals, especially sick cats (Madrid *et al*., 2012; Montenegro *et al*., 2014; Rodrigues *et al*., 2014a). In Brazil, *S. brasiliensis* is prevalent in feline outbreaks and is considered one of the most virulent species (Montenegro *et al*., 2014; Rodrigues *et al*., 2014a).

Recently, the emergence of antifungal resistance has made the therapeutic control of the disease in animals more difficult (Rodrigues *et al*., 2014a; Borba-Santos *et al*., 2015; Waller *et al*., 2016), and has encouraged the search for new molecules with antifungal potential. With this regard, drug repurposing (or also called repositioning) is an approach that is frequently under consideration to find novel therapeutic options to diseases in the urgent state. The drug repurposing approach is based on study known drugs that are already approved for some disease or condition to treat another. Classical drug discovery and development pipeline is a slow and expensive process to find a new chemical entity (NCE), which includes the clinical trials, registry and several other processes to reach the patients. A large number of these NCEs never reach the

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clinical trials, and some other does not get the approval after that (Corsello *et al*., 2017).

In this panorama, repositioning is advantageous since the data from the NCE is already available from the approved drug. This is the main reason why this approach can speed up the translation of the active agent from the bench to the patient (Ashburn, Thor, 2004). The most famous example of successful repositioning of a drug is certainly the discovery of sildenafil (Viagra) to erectile dysfunction, which was before evaluated as a cardiovascular agent.

Pyrazinoic acid (POA) is a widely known antimycobacterial agent. It is proposed that POA is the active form of pyrazinamide (PZA), a first-class antimycobacterial drug used in human tuberculosis (Figure 1). The mechanism of action of both PZA and POA is not totally understood, but it depends firstly on the activation of PZA by a *pyrazinamidase* (PZAse) (Zhang *et al*., 2003). PZA also demonstrated to inhibit the fatty acid synthase I (FASI) in the NADPH binding site by competition (Zimhony *et al*., 2000). POA itself shows poor antimycobacterial activity due to its poor penetration into mycobacteria, and thus POA esters with better penetration were reported as options to circumvent this issue.

POA esters have shown good antimycobacterial activity *in vitro* against PZA-sensitive and resistant strains of *Mycobacterium tuberculosis* (Fernandes *et al*., 2014; Segretti *et al*., 2016). Furthermore, a repurposing study for PZA against *Leishmania* was reported (Mendez *et al*., 2009), showing the potential of these antimycobacterial compounds in affect other microorganisms, such as *Leishmania major*. However, none repurposing studies to POA derivatives were reported to date. Considering the repurposing strategy, the urgency of a novel therapeutic to sporotrichosis and the absence of literature information regarding the activity of POA derivatives against fungi, the aim of this study was to evaluate the activity of different POA derivatives against *Sporothrix brasiliensis*.

MATERIAL AND METHODS

Material

Chemical reagents for the preparation of the compounds were purchased from commercial sources in an adequate purity and used without prior treatment. ^{1}H and ^{13}C NMR spectra were recorded in a Bruker Ultrashield 300 spectrometer, operating at 300 MHz and 75 MHz, respectively, using $CDCl_3$ as a solvent with tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants (*J*) are reported in units of hertz (Hz), if applicable. The NMR data is in accordance with previous reports in the literature. Culture medium of Potato-dextrose agar and Sabouraud dextrose-agar (Kasvi, Liofilchem®, Italy) were used for fungal growth. For antifungal testing, the RPMI-1640 (Roswell Park Memorial Institute, Gibco®, Life Technologies Co., United States) was prepared as liquid medium with addition of MOPS [3-(N-morpholino propanesulfonic acid)] (Alamar Tecno-Científica Ltda, Brazil), and itraconazole was purchased from pharmaceutical industry (Sporanox®, Janssen-Pharmaceutica Ltda, Belgium).

Preparation of the molecules 1–4

In a round-bottom flask, 5 mmol of POA (0.620 g) were dissolved in 10 mL of appropriate alcohol (methanol, ethanol, butanol or 2-chloroethanol for compounds **1**-**4**, respectively), and 7 mmol of thionyl chloride (~0.5 mL) were added. The mixture was then heated to 60 °C and stirred overnight. The solvent was evaporated, and the oily residue was taken up in 10 mL of ethyl acetate and washed twice with 10 mL of saturated aqueous $NaHCO_3$, and 10 mL of brine. The organic phase was dried using anhydrous Na_2SO_4, and the solvent was evaporated. Methyl pyrazinoate (**1**), 80% yield. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ = ppm) 4.07 (s, 3H), 8.75 (dd, *J* = 2.4, 1.4 Hz, 1H), 8.80

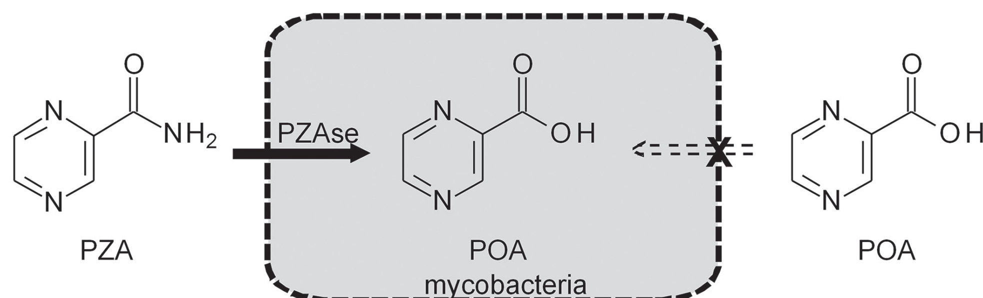

FIGURE 1 – Illustration of the mechanism of action of the pyrazinoic acid (POA) prodrugs, which POA itself cannot penetrate the mycobacterial membranes, whereas pyrazinamide (PZA) is activated to pyrazinoic acid (POA) inside mycobacteria.

(d, J = 2.4 Hz, 1H), 9.35 (d, J = 1.4 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz, δ = ppm) 53.2, 143.4, 144.4, 146.3, 147.8, 164.4. Ethyl pyrazinoate (**2**), 77% yield. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ = ppm) 1.47 (t, J = 7.1 Hz, 3H), 4.53 (q, J = 7.1 Hz, 2H), 8.74 (dd, J = 2.4, 1.4 Hz, 1H), 8.78 (d, J = 2.4 Hz, 1H), 9.33 (d, J = 1.4 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz, δ = ppm) 14.3, 62.5, 143.7, 144.5, 146.4, 147.7, 164.0. Butyl pyrazinoate (**3**), 60% yield. ^{1}H-NMR (CDCl3, 300 MHz, δ = ppm) 0.99 (t, J = 7.4 Hz, 3H), 1.49 (sext, J = 7.4 Hz, 2H), 1.83 (quint, J = 7.1 Hz, 2H), 8.75 (dd, J = 2.4, 1.4 Hz, 1H), 8.78 (d, J = 2.4 Hz, 1H), 9.33 (d, J = 1.4 Hz, 1H). ^{13}C-NMR (CDCl3, 75 MHz, δ = ppm) 13.8, 19.2, 30.6, 66.3, 143.6, 144.5, 146.3, 147.6, 164.0. 2-Chloroethyl pyrazinoate (**4**), 85% yield. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ = ppm) 3.95 (t, J = 5.6 Hz, 2H), 4.75 (t, J = 5.6 Hz, 2H), 8.82 (dd, J = 2.4, 1.4 Hz, 1H), 8.87 (d, J = 2.4 Hz, 1H), 9.34 (d, J = 1.4 Hz, 1H). ^{13}C-NMR ($CDCl_3$, 75 MHz, δ = ppm) 41.2, 65.5, 143.1, 144.6, 146.4, 148.0, 163.5.

Preparation of the molecule 5

In a round-bottom flask, 12 mmol of POA (1.488 g) were dissolved in 10 mL of acetonitrile, when 5 mmol of 1,2-dibromoethane (0.940 g) and 10 mmol of triethylamine (1.100 g) were added. The reaction mixture was stirred under reflux for 12 h, and the solvent was then evaporated. The residue was taken up in 10 mL of ethyl acetate, and washed twice with 10 mL of aqueous $NaHCO_3$, and 10 mL of distilled water. The organic layer was dried using anhydrous Na_2SO_4, and the solvent was evaporated. The crude product was purified using flash column chromatography, using a hexane-ethyl acetate gradient as eluent. 2-(Pyrazinoyloxy)-ethyl pyrazinoate (**5**), 40% yield. ^{1}H-NMR ($CDCl_3$, 300 MHz, δ = ppm) 4.85 (s, 4H), 8.74 (dd, J = 2.4, 1.5 Hz, 2H), 8.78 (d, J = 2.4 Hz, 2H), 9.32 (d, J = 1.5 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 75 MHz, δ = ppm) 63.6, 143.0, 144.5, 146.5, 148.0, 163.8.

Anti-*Sporothrix* spp. activity

For *in vitro* tests, *S. brasiliensis* clinical isolates (*n*=6) obtained from cats with sporotrichosis in Pelotas/RS (codes S68, S120, S141, S144 and S146) and São Lourenço do Sul/RS (code S119), which both cities are located in southern Brazil, were used, as well as standard strains from human sporotrichosis by *S. brasiliensis* (Ss 177, also identified as IPEC 16919 from *Instituto de Pesquisa Clínica Evandro Chagas,* FIOCRUZ, Brazil) and *S. schenckii* sensu stricto (Ss 126), totaling eight fungal isolates. All isolates were molecularly identified at *Laboratório de Micologia Médica Molecular* (Federal University of São Paulo, Brazil) by PCR-restriction fragment length polymorphism analysis (Rodrigues, de Hoog, de Camargo, 2014b). The *in vitro* antifungal test was performed using the broth microdilution method, according to the CLSI M38-A2 standard protocol (NCCLS, 2008).

The fungal inoculum was prepared from colonies grown in mycelial phase on potato-dextrose agar at 27 °C for seven days. Saline solution (2 mL) with Tween 80 (1%) was added to the surface of each colony and the fungal content was scraped with a scalpel blade to collect filament cells and conidia. The fungal content was transferred and suspended in tubes containing sterile saline, and adjusted to 1.0 McFarland scale and, subsequently, in an ultraviolet (UV)-visible spectrophotometer (Spectrum Instruments Co.) with transmittance adjusted to 80-82% at the fixed wavelength of 530 nm. Suspensions were diluted in RPMI-1640 medium buffered with 2% glucose and MOPS at 1:50, v/v). The molecules were prepared in RPMI-1640 (1.6 mL) and DMSO (0.05 mL) to promote their dilution. From the initial concentration, ten serial dilutions were performed on RPMI-1640 medium buffered with MOPS directly on microplates to obtain a final concentration of 3.35–0.006 mg/mL (**1**); 3.06–0.006 mg/mL (**2**); 4.24–0.008 mg/mL (**3**); 5.15–0.01 mg/mL (**4**); 2.9–0.005 mg/mL (**5**) and 3.18–0.006 mg/mL (POA).

Fungal suspension (100 µl) was added in all tested wells, including in the positive control. Extract suspensions diluted in RPMI-1640 were used as negative control. Positive control consisted in RPMI-1640 with fungal suspension. For itraconazole preparation, the internal content was diluted using DMSO and prepared according to standards guidelines (NCCLS, 2008) to obtain a final concentration of 16 to 0.0313 µg/mL. Microplates were incubated at 35°C for 72 h to obtain the results of minimum inhibitory concentration (MIC). For minimum fungicidal concentration (MFC), 10 µl aliquots of the wells with no fungal growth were transferred to Petri dishes containing Sabouraud dextrose agar and incubated at 27 °C for 72 h to visualize fungal growth. All experiments were performed in duplicate.

LogP calculation

The estimated logP was calculated using the software Marvin version 14.8.11, using the calculation plugin implemented in the software. The calculation method is based on the publication of Viswanadhan *et al*. (1989), which is weighted by Klopman method and Physprop database data. Considered electrolyte concentrations (Na^+, K^+ and Cl^-) were 0.1 mol/L.

Statistical analysis

Analysis of variance and comparison of geometric means were performed by the Kruskal-Wallis test. Data were analyzed using the statistical software BioEstat® (version 5.3), and a P value of 0.05 was considered significant.

RESULTS

The five POA derivatives **1-5** (Figure 2) were prepared using the previously reported methodology from our group (Segretti *et al.,* 2016), with moderate to good yields. The spectroscopic evaluation led to data in accordance with the literature.

As can be seen in Table I, a variable antifungal activity among the POA and their derivatives **1-5** is noted, which the parent drug POA presented only weak or no anti-*Sporothrix* spp. activity. However, the esterification of the carboxylic acid led to improved activity. In this way, the compounds **3** and **4** can be highlighted due to fungistatic and fungicidal activities against 100% of *S. brasiliensis* isolated from cats (6/6) and *S. schenckii* standard strain from a human. For compound **3**, the antifungal activity values ranged from 2.12 to 1.06 mg/mL (MIC) and from 4.24 to 1.06 mg/mL (MFC), whereas for compound **4** ranged from 0.32 to 2.56 mg/mL (MIC) and from 1.29 to 5.15 mg/mL (MFC). However, one *S. brasiliensis* standard strain from human (Ss177) was not sensitive to none compound.

On the other hand, only 83.3% (5/6) of feline *S. brasiliensis* were sensitive to inhibitory activity of **2** (MIC from 3.06 to 0.77 mg/mL) and **5** (MIC from 2.9 to 0.05 mg/mL), but a weak fungicidal activity was noted in only two feline isolates for both molecules, which the values of MFC on 50% and 90% of overall isolates (MFC_{50} and MFC_{90}, respectively) were >3.06 mg/mL for **2** and >2.90 mg/mL for **5**. The remaining molecules were considered weakly active against *S. brasiliensis* ($MIC_{50\ and\ 90}$ and $MFC_{50\ and\ 90}$ were >3.34 mg/mL for molecule **1** and >3.18 mg/mL for POA).

Molecules **3** and **4** were the only active molecules on the overall microorganisms since the values of MIC_{90} and MFC_{90} were 2.12 mg/mL and 4.24 mg/mL (for **3**) and were 2.56 mg/mL and 5.15 mg/mL (for **4**). The remaining molecules did not present this activity, showing values higher than the maximum concentration tested. Considering the overall *Sporothrix* spp. tested, 75% (6/8) were sensitive to the antifungal choice for therapy in human and animals, itraconazole (Table II). The cut-off point was followed according to document M38-A2 (NCCLS, 2008), in which fungal isolates with MIC values lower than or equal to 4 µg/mL are considered sensitive. On the other hand, two feline isolates showed MIC values ranging from 8 to >16 µg/mL and were then considered itraconazole-resistant isolates.

DISCUSSION

POA is considered the active form of the tuberculostatic drug PZA, produced by PZA hydrolysis from a specific mycobacterial enzyme, PZAse (Zhang *et al*., 2003). PZA (and even POA) is especially active against the slow-growing intracellular *M. tuberculosis*, suggesting

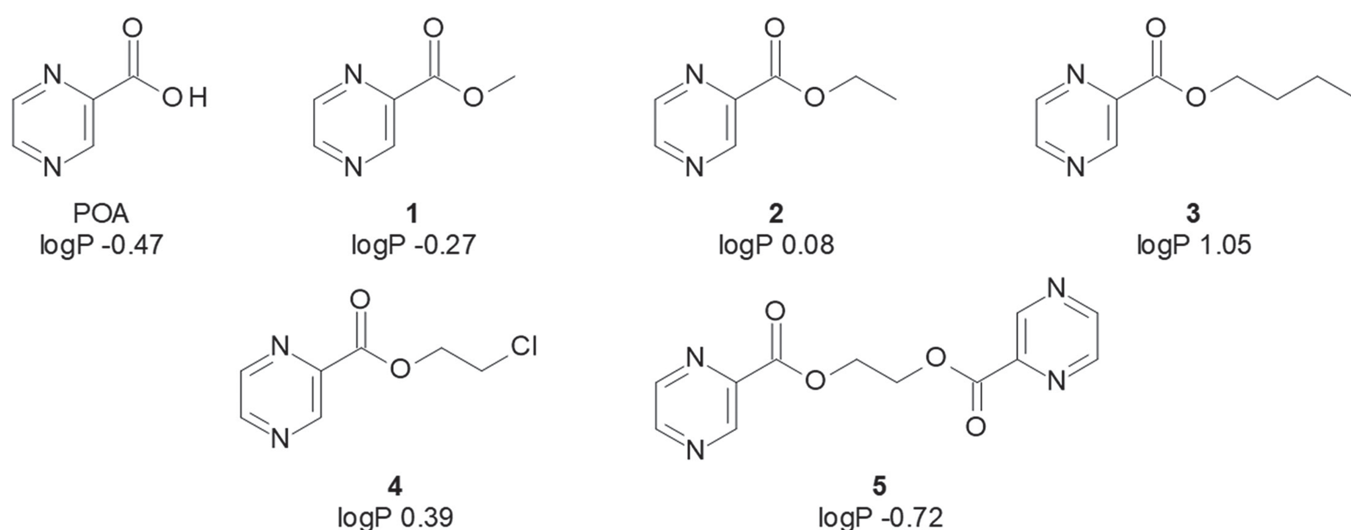

FIGURE 2 - Chemical structure of the pyrazinoic acid (POA) and their derivatives **1**-**5** evaluated in this work, and respective experimental values of partition coefficient (log P) as quantitative measure of the lipophilicity.

TABLE I - Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of different pyrazinoic acids (POA) molecules (mg/mL) to *Sporothrix brasiliensis* isolated from cats in Southern Brazil[a]

Code [b]	Host (Braz. State)	1		2		3		4		5		POA	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
S68	Feline (RS)	0.83	0.83	0.77	0.77	1.06	2.12	1.29	1.29	0.73	0.73	1.59	3.18
S119	Feline (RS)	1.67	3.34	0.77	0.77	1.06	1.06	0.32	2.56	0.05	0.73	n.i.	n.f.
S120	Feline (RS)	n.i.	n.f.	n.i.	n.f.	2.12	4.24	1.29	2.56	n.i.	n.f.	n.i.	n.f.
S141	Feline (RS)	n.i.	n.f.	3.06	n.f.	2.12	2.12	2.56	2.56	2.9	n.f.	n.i.	n.f.
S144	Feline (RS)	n.i.	n.f.	3.06	n.f.	2.12	2.12	1.29	2.56	2.9	n.f.	n.i.	n.f.
S146	Feline (RS)	0.83	3.34	1.53	n.f.	2.12	2.12	1.29	2.56	2.9	n.f.	n.i.	n.f.
Ss177 [c]	Human (RJ)	n.i.	n.f.	n.i.	n.f.	n.i.	n.f.	n.i.	n.f.	n.i.	n.f.	n.i.	n.f.
Ss126 [c]	Human (SP)	n.i.	n.f.	n.i.	n.f.	2.12	4.24	2.56	5.15	n.i.	n.f.	n.i.	n.f.
Overall [d]	50%	n.i.	n.f.	3.06	n.f.	2.12	2.12	1.29	2.56	2.9	n.f.	n.i.	n.f.
	90%	n.i.	n.f.	n.i.	n.f.	2.12	4.24	2.56	5.15	n.i.	n.f.	n.i.	n.f.

[a] Concentration range tested: 3.35-0.006 mg/mL (**1** – Methyl pyrazinoate); 3.06-0.006 mg/mL (**2** – Ethyl pyrazinoate); 4.24–0.008 mg/mL (**3** – Butyl pyrazinoate); 5.15-0.01 mg/mL (**4** – 2-Chloroethyl pirazynoate); 2.9-0.005 mg/mL (**5** – 2(Pyrazinoyloxy)-ethyl pyrazinoate); 3.18-0.006 mg/mL (POA – Pyrazinoic acid); [b] "S" strains are *Sporothrix brasiliensis* and belong to the culture collection of the Faculty of Veterinary, Federal University of Pelotas, UFPEL, RS, Brazil; [c] "Ss" strains belong to the culture collection of the Federal University of São Paulo (UNIFESP, SP, Brazil) – Ss177, *Sporothrix brasiliensis* standard strain (other codes: FMR 8314, *Facultat de Medicina i Ciències de la Salut*, Reus, Spain; IPEC 16968, *Instituto de Pesquisa Evandro Chagas*/FIOCRUZ, RJ, Brazil); Ss 126, *Sporothrix schenckii* standard strain; [d] 50%, MIC/MFC at which 50% of overall isolates were inhibited/killed; 90%, MIC/MFC at which 90% of overall isolates were inhibited/killed. n.i.: no inhibitory activity at the concentrations tested; n.f.: no fungicidal activity at the concentrations tested.

TABLE II - Susceptibility of *Sporothrix schenckii* complex isolates to itraconazole (μg/mL)

Sporothrix schenckii complex (*n*)	Host	Itraconazole (*n*)[a]		
		Sensitive	Resistant	Total
S. brasiliensis (6) b	Feline (RS/Brazil)	4	2	6
S. brasiliensis (1) [c]	Human (RJ/Brazil)	1	-	1
S. schenckii (1) [c]	Human (SP/Brazil)	1	-	1
Overall (8)	-	6	2	8

[a] Sensitivity (MIC ≤4 μg/mL) and resistance (MIC>4 μg/mL) defined according to the cut-off suggested by the M38-A2 (NCCLS, 2008); [b] Clinical isolates from feline sporotrichosis belong to the culture collection of the Faculty of Veterinary, Federal University of Pelotas, UFPEL, RS, Brazil; [c] Standard strain from human sporotrichosis belong to the culture collection of the Federal University of São Paulo (UNIFESP, SP, Brazil) – *Sporothrix brasiliensis* (Ss177, other codes: FMR 8314, *Facultat de Medicina i Ciències de la Salut*, Reus, Spain; IPEC 16919, *Instituto de Pesquisa Evandro Chagas*/FIOCRUZ, RJ, Brazil); *Sporothrix schenckii* (Ss 126).

that its action depends on some characteristics present in this subpopulation (Segretti *et al.,* 2016). Although POA is the responsible for most of the observed effects of PZA, it cannot pass the mycobacterial membranes and thus it only possesses weak or no antimycobacterial activity. To improve its efficacy independently from the PZAse activation step, POA esters have been prepared and evaluated as antimycobacterial agents, showing very improved efficacy than POA (Fernandes *et al*., 2014; Segretti *et al*., 2016). On the other hand, POA and its ester derivatives do not show considerable activity against other bacteria.

Considering that mycobacteria and fungi (Willcocks, Wren, 2014) present some similarities (such as slow-growing, the capacity of synthesizing threalose and inositol), we raised one question: Can POA and its derivatives be active against fungi? To answer this question in this work and taking advantage of the repositioning strategy (Ashburn and Thor, 2004; Corsello *et al*., 2017), we decided to explore the antifungal potential of these compounds against *Sporothrix* spp. clinical isolates from humans and animals with sporotrichosis. Considering that PZA can interfere in enzymes present in *Leishmania* that

have similar function than mycobacterial FASI (Mendez *et al*., 2009), by analogy it could also act in similar enzymes from fungi. To the best of our knowledge, this is the first report comprising the antifungal activity of POA and derivatives against this fungal microorganism.

The results showed that POA itself did not present important antifungal activity in the tested concentrations. This is probably due to a penetration issue through fungal cell membranes, which are considerably lipophilic. The ionization state of POA in the physiological pH difficult its penetration into the fungal cell, so the ester derivatives that do not ionize in this pH would improve the penetration and therefore the antifungal activity (Zhang *et al*., 2003; Fernandes *et al*., 2014). As can be noted in Table I, more lipophilic POA derivatives **1-4** have shown better antifungal activity. The more lipophilic (represented by logP values, Figure 2) the molecule, the higher antifungal activity. Compounds **3** (logP 1.05) and **4** (logP 0.39) showed the better anti-*Sporothrix* activity, exhibiting activity even in itraconazole-resistant isolates. The role of lipophilicity can be observed when comparing the activity of compounds **1** to **4**, being **3** > **4** > **2** > **1**.

Interestingly, compound **5** is the more hydrophilic compound (logP –0.72) in the series, but it exhibited some anti-*Sporothrix* spp. activity. Compound **5** was designed as a double prodrug of POA, that after hydrolysis it will generate 2 moles-equivalent of POA instead of **1**. It is a strategy to increase the intracellular concentration of POA, duplicating its efficacy (Segretti *et al*., 2016). In counterpart, the presence of two POA moieties leads to increased hydrophilicity, which limits the penetration of **5** into the fungal cell. This can be the reason for the limited antifungal activity observed for this compound.

Although a more specific study must be done to determine the mechanism of POA derivatives' action in fungi, the results suggest that esterification is necessary to POA exert its action inside the fungal cell. Since POA itself also presented poor activity in *Sporothrix brasiliensis* as observed previously to mycobacteria (Fernandes *et al*., 2014; Segretti *et al*., 2016), it is possible that the esters must be hydrolyzed by cytoplasmic esterases, generating the active POA inside the fungal cell, in a similar way that was illustrated in Figure 1. However, it is also possible that the esters may exert fungistatic action *per se*, i.e. independently of the hydrolysis step.

Regarding the *S. brasiliensis* tested, the S68 and S119 isolates were sensitive to POA derivatives **1–5**; S141 and S144 were sensitive to **3** and **4** and both were inhibited by **2** and **5**. In standards strains, *S. schenckii* (Ss126) was only sensitive to **3** and **4**, and *S. brasiliensis* (Ss177) was not sensitive to any compounds tested. Although all these cited *Sporothrix* species were itraconazole-susceptible isolates, a difference in the profile of antifungal susceptibility was observed and probably is due to the virulence factors related to them, like melanin (Mario *et al*., 2016), and other factors still poorly understood. On the other hand, the itraconazole-resistant isolates recognized in S120 and S146 were sensitive to **3** and **4**, but only S146 was sensitive to **1** and was inhibited to **2** and **5**. The ability to resist to the action of itraconazole was also showed in *S. brasiliensis* isolated from feline and canine sporotrichosis (Waller *et al.,* 2017), as well as in other *Sporothrix* species, such as *S. albicans* and *S. luriei* from feline and canine cases, respectively (Oliveira *et al.,* 2011) and *S. globosa* from human sporotrichosis (Fischman Gompertz *et al*., 2016). In our study, the activity profile of POA derivatives in two itraconazole-resistant isolates (S120 and S146) shed light on the activity of these molecules. These isolates were only sensitive to inhibitory and fungicidal activities of **3** (MIC/MFC values 2.12–4.24 mg/mL) and **4** (MIC/MFC values 1.29–2.56 mg/mL) molecules, indicating a probable alternative route of these compounds from the mechanism of action of itraconazole.

The main mode of action of itraconazole is the inhibition of lanosterol 14-α-demethylase (Kelly *et al*., 1995) that is a key enzyme from the cytochrome P450 family in the ergosterol biosynthesis (Borgers, Van de Ven, 1989). Ergosterol is a membrane sterol important for fungal integrity and maintenance. As consequence, the inhibition of this enzyme leads to the inhibition of the fungal growth. The inhibition of ergosterol biosynthesis together with the increased permeability of the fungal cell membrane by ergosterol deficiency, lead to morphological changes like abnormalities at the plasma membrane, the cell wall and cytoplasmic vacuoles, with defective cell division, abortive hyphal outgrowth and loss of cell viability (Borgers, Van de Ven, 1989). The activity of compounds **1-5** seems to be independent of lanosterol 14-α-demethylase inhibition since their action occurs independently of the resistance. Maybe a similar role played in *M. tuberculosis* and *L. major* may be involved in this effect, however the mechanism of action for POA derivatives is still unknown.

This study demonstrates the potential of pyrazinoic acid and their derivatives as antifungal, which the most lipophilic molecules were active, including against itraconazole-resistant *S. brasiliensis*. The findings support potential usefulness of these molecules in the treatment of sporotrichosis and further studies should be undertaken to understand their mechanism of action and to evaluate their safe use.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding this manuscript.

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