Communication

Synthesis and Identification of Pentathiepin-Based Inhibitors of *Sporothrix brasiliensis*

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Abstract: *Sporothrix brasiliensis* is the causative agent of zoonotic sporotrichosis in Brazil and is currently referred to as the most virulent species among those of clinical importance within the genus. Sporotrichosis is an emergent disease that has come to the forefront over two decades with a recent hot spot of sporotrichosis infection emerging in the state of Rio de Janeiro. The source of these infections is now at epidemic proportions with more than 4000 cases reported in Rio de Janeiro, Brazil, alone since 1998. We developed a focused library of a rare pentathiepin ring system and identified a potent substitution pattern that yielded compounds 21 and 22. These compounds were more potent than itraconazole which is the current standard of care for sporotrichosis.

Keywords: sporotrichosis; 1,2,3,4,5-pentathiepin; varacin; anti-fungal; sulfur heterocycle

1. Introduction

Sporotrichosis is an emergent disease that has come to the forefront over two decades after being first described in 1898 [1,2]. A recent hot spot of *Sporothrix* infection has emerged in the state of Rio de Janeiro, Brazil [3]. The source of these infections is now at hyperendemic proportions with more than 4000 cases reported in this region since 1998 [3–5].

*Sporothrix* species are usually non-pathogenic environmental fungi that are closely related to decaying wood, plants, and soil [6]. The majority of human and animal infections occur when the epidermis is damaged, allowing plant matter/soil, along with the fungus, to enter into the body [7]. The incidence of zoonotic infection is on the rise with feline transmission of *Sporothrix* which has been identified as the likely source of the spike in cases within Rio de Janeiro [3–5,8]. *Sporothrix brasiliensis* has been demonstrated to be a highly successful mammal pathogen, and it is related to zoonotic transmission from infected cats in Brazil [9].

Feline sporotrichosis has a varied range of clinical presentation, but it is frequently a severe condition with the development of disseminated skin lesions and respiratory involvement in infected...
cats [10]. Although human sporotrichosis is not usually severe, disseminated and life-threatening cases have been described in association to S. brasiliensis infection, especially in immunocompromised patients [11].

Infected cats are the most important source of Sporothrix transmission in the Brazilian scenario due to the high burden of yeasts in their lesions. Zoonotic transmission usually occurs through their scratches and bites [3,10]. Although the treatment of feline sporotrichosis is shown to efficiently reduce the fungal burden in lesions of cats contributing to the control of zoonotic transmission, it remains a challenge for veterinary practitioners [12]. Itraconazole frequently in association to potassium iodide is still the treatment of choice, but therapeutic failure, the occurrence of adverse effects, and recrudescence of lesions are described, raising the need for alternative therapeutic options [10].

Several small anti-fungal molecules (1–8) have been reported (Figure 1), targeting different routes to treat fungal infections. The fungal cells, unlike mammalian cells, are encased in a carbohydrate-containing cell wall which has been used as a target to reduce mammalian toxicity and to target specifically the fungus [13]. The fungal cell wall has been targeted with a series of chalcone and quinoline derivatives (1–3) with varying degrees of success [14–16].

As part of those screening efforts, a series of 5-(phenylthio)-3H-1,2-dithiole-3-thione derivatives (4) was also identified, where additional sulfur substitutions improved potency [17]. This was supported by a previous report by DuPont in 1977 [18]. In this program, 7-cyano-7H-(1,2,3,4,5)pentathiepino(6,7-c)pyrazole (5) was developed as a potent anti-fungal in multiple crop species, providing near total control of all fungal growth [18–23]. Two of the three main compounds commercially used to suppress fungal growth are the polysulfide-containing compounds selenium sulfide (6) and pyrithione zinc (7), both used for topical applications (used to treat skin and crops respectively) [24–27].

A major interest of our work has been the reactivity of internal disulfide bridge compounds and their medicinal chemistry applications [28–38]. The high-density polysulfide heterocycles lend themselves to this type of redox-type applications including zinc ejection (in multiple systems) [29–37], anti-viral [29–35], cancer [36,37], and oxidative stress [38].

The pentathiepin functionality (5, 9–17) has been utilized for several different indications including neoplastic diseases, Alzheimer’s, viruses, cancer, bacteria, mycoses, and as an inhibitor of protein kinase C (Figure 2) [18–23,32,39–50]. The DuPont compound 5 showed promising activity against fungus in plants with broad spectrum activity against a wider range of plant disease [18]. While the natural product varacin (9) showed potent inhibition of Candida albicans at a rate 100× that of
5-fluorouracil [39]. More recently, compound 17 has shown good inhibition activity against S. aureus, C. albicans, and C. neoformans with an impressive toxicity profile [50].

![Figure 2. Previously reported biologically active pentathiepin compounds.](image)

2. Results

2.1. Initial Investigation

To investigate pentathiepin as a viable treatment for sporotrichosis, we first screened a symmetric thiophene derivative (13) against six isolates of S. brasiliensis from skin lesions of infected cats. The antifungal susceptibility testing was based on the reference broth microdilution method according to the M38-A2 CLSI guidelines [51] with a few modifications to improve minimal inhibitory concentration (MIC) determination for S. brasiliensis [52]. We screened 13 at two concentrations (4 and 8 µg/mL) to determine if there was any inhibition of fungal growth. We evaluated MIC to suppress both 100% and 50% of the growth of the strains. At the higher concentration, 100% inhibition of growth was observed in all six isolates with the isolate 8607 presenting a slightly higher sensitivity and being the only isolate to respond to this compound at the lower concentration (Table 1).

| Compound | Growth Inhibition Percent | Isolates of S. brasiliensis/MIC (µg/mL) a |
|----------|---------------------------|------------------------------------------|
| 13       | 100% 50%                  | 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 |
| 8        | 100%                      | 0.5 0.5 1 1 2 2                        |

a Mean average (n = 4).

2.2. Synthesis of Pentathiepin Analogs

We decided to prepare a small array of pentathiepins (13, 16, and 18–24) (Scheme 1). The older methods for preparation of this advanced heterocycle included using an activated sulfur source, such as disulfur dichloride (S2Cl2) or trisulfur dichloride (S3Cl2) or even directly with elemental sulfur (S8), and adding these to an ortho-dithiol, usually under harsh conditions [53–57]. However, a C–H activated route utilizing the 1,4-diaza-bicyclo(2.2.2)octane (DABCO) sulfur monochloride complex enables formation of pentathiepins in one step from commercially available reagents (Scheme 1) [58,59]. The two thiophene analogs (13 and 18) were furnished by treating Hünig’s base and N,N-dibenzylethanamine, respectively, with DABCO and S2Cl2 for 48 h followed by refluxing with triethylamine for a further 2 h to afford 13 and 18 in good and acceptable yields, respectively [52,60]. The pyrrole derivatives were
explored using a series of substituted pyrrole ring systems and treating with DABCO and S₂Cl₂ for 48 hours to access 16, 19, and 20 in good yield [61]. Treatment of N-methylpyrrolidine under analogous conditions allowed access to 21 and 23 in a one-pot reaction. However, under the same conditions, N-isopropylpyrrolidine produced exclusively 22 with the corresponding asymmetric product not observed [62–64]. This was followed by a final analog (24) synthesized by treating N-methylindole with the DABCO sulfur monochloride complex at zero degrees to furnish the final product in excellent yield (70%) [64,65].

![Scheme 1](image-url)  
**Scheme 1.** Synthesis route to pentathiepin derivatives: reagents and conditions. (a) 1,4-Diaza-bicyclo (2.2.2)octane (DABCO), S₂Cl₂, CHCl₃, 0 °C to rt; 48 h, reflux 2 h (13, 30% and 18, 3%); (b) DABCO, S₂Cl₂, CHCl₃, 0 °C; 48 h (16, 36%; 19, 40% and 20, 62%); (c) DABCO, S₂Cl₂, CHCl₃, 20 °C; 48 h (21, 18%; 23, 38% and 22, 16%); (d) S₂Cl₂ (0.8 eq), CHCl₃, 0 °C; 48 h (24, 70%).

### 2.3. Evaluation of Pentathiepin Analogs

We then screened this series of symmetrical derivatives (16, 18–22) against eight skin lesion isolates of *S. brasiliensis* (Table 2). We found that increasing the size of the substituent on the amine of the thiophene (18) removed all activity. The 1,2,5-trimethyl-pyrrole (16) was also inactive, with an N-ethyl derivative (19) and 2,5-diphenyl derivative (20) also showing no activity. Interestingly, the 2,5-dichloro, N-methyl compound (21) showed some activity on two isolates with the 2,5-dichloro, N-isopropyl (22) extending this to three isolates.
Sporothrix brasiliensis is still a significant challenge in the Americas. Its worldwide emergence is clear, whether related or not to zoonotic transmission. Outbreaks have been identified in other regions in Brazil and are currently referred to as the most virulent species among those of clinical importance within the genus. It is estimated that its uncontrolled zoonotic spread from infected cats is responsible for the fact of number of cases in Rio de Janeiro region due to its significant impact on human health. However, not only has the expansion of the disease been identified in other regions in Brazil, but also in other regions of Latin America.

Table 2. Screening results of a symmetrical pentathiepin array against isolates of *Sporothrix brasiliensis*.

| Cmpd | R       | X   | Isolates of *S. brasiliensis*/MIC (µg/mL) a |
|------|---------|-----|---------------------------------------------|
| 13   | N-(Et)₂  | S   | 8 | nt | nt | 4 | nt | 8 | 8 | 8 | nt | 8 | 8 | 8 | 8 |
| 18   | N-(Bn)₂  | S   | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 |
| 16   | CH₃      | N–CH₃ | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 |
| 19   | CH₃      | N–CH₂CH₃ | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 |
| 20   | Ph       | N–CH₃ | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 |
| 21   | Cl       | N–CH₃ | 8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 |
| 22   | Cl       | N–C(CH₃)₂ | 8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 | >8 |

*Mean average (n = 4); nt = not tested.*

Encouraged by our early results, we screened the same set of isolates with two unsymmetrical pentathiepin derivatives (23 and 24) (Table 3). We found antifungal activity across all isolates with both 23 and 24.

Table 3. Results of asymmetric pentathiepins against isolates of *Sporothrix brasiliensis*.

| Name     | R      | X         | Isolates of *S. brasiliensis*/MIC (µg/mL) a |
|----------|--------|-----------|---------------------------------------------|
| 23       | Cl     | N–CH₃     | 1 | 1 | 1 | 1 | 0.5 | 0.5 | 1 | 1 | 1 | 1 |
| 24       |        | N–CH₃     | 2 | 4 | 4 | 2 | 2 | 2 | 2 | 2 | 2 |
| Itraconazole (8) |        |           | 0.5 | 0.5 | 0.5 | 1 | 1 | 2 | 2 | 2 |

*Mean average (n = 4).*

Compound 23 presented a lower MIC in comparison to itraconazole (8) in 4/8 cases with the others matching or half as effective. Compound 24 was slightly less active, matching 3/8 and 1 or 2 fold less effective in the other 5/8 isolates. Compound 24 demonstrated a robust dose-dependent inhibition of *S. brasiliensis* growth. This can be observed in Figure 3 with decreasing concentrations (0.5 to 0.015 µg/mL) of 24 having a significant impact on growth of *Sporothrix brasiliensis*.

**Figure 3.** Growth of *Sporothrix brasiliensis* isolate 8584 treated with compound 24 at (A) 0.5 µg/mL (0.172 µM); (B) 0.25 µg/mL (0.086 µM); (C) 0.12 µg/mL (0.041 µM); (D) 0.06 µg/mL (0.020 µM); (E) 0.03 µg/mL (0.010 µM); (F) 0.015 µg/mL (0.005 µM).
3. Discussion

Sporotrichosis is still a neglected disease. It has reached a significant number of cases in Rio de Janeiro region due to the fact of its uncontrolled zoonotic spread from infected cats. However, not only has the expansion of the disease been identified in other regions in Brazil and Argentina, worldwide emergence is clear, whether related or not to zoonotic transmission. Outbreaks have been described on different continents and infected immunosuppressed patients are at serious risk. In addition, *Sporothrix brasiliensis* is the causative agent of zoonotic sporotrichosis in Brazil and is currently referred to as the most virulent species among those of clinical importance within the genus.

The sub-micro molar potencies of the pentathiepins coupled with previous reports of moderate toxicities suggests a low-risk path towards the development of a candidate compound for targeting sporotrichosis in cats and potentially humans [32]. The two asymmetric pentathiepins (23 and 24) both show improvements over the current standard of care (itraconazole). While the symmetrical analogs showed only weak activity, increasing the electron withdrawing nature of the substituents (methyl (16) versus chloro (21)) did increase anti-fungal potency. There is increasing evidence that the pentathiepin functionality itself is not a toxic motif and that rather the electronic contributions pendant arms are what drives non-specific toxicity. The pentathiepin functionality and other high-density sulfur heterocycles provide an exciting opportunity for the development of new clinically relevant compounds highlighted by 23 and 24. The pentathiepin functionality, while still under-explored, has the potential to generate a pre-clinical candidate for treating sporotrichosis in vivo.

4. Materials and Methods

4.1. Isolate Collection

The isolates were previously collected from cats with sporotrichosis seen at the Laboratory of Clinical Research on Dermatozoonosis in Domestic Animals (Lapclin-Dermzoo), Evandro Chagas National Institute of Infectious Diseases (INI), Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil. The strains were initially recovered from the exudate of ulcerated lesions or secretions from nasal cavities that were collected using a sterile swab and cultured on Sabouraud dextrose agar and Mycobiotic Agar (Difco), incubated at 25 °C and observed over 4 weeks for fungal growth. Suspected isolates were sub-cultivated on brain heart infusion agar medium (Difco) at 37 °C, and dimorphism was demonstrated by conversion to the yeast-like form. Then, the isolates were stored in sterile distilled water in the Mycology Laboratory of INI until used.

4.2. General Procedures for Screening

The compounds (13, 16, 18–24) were tested using the broth microdilution technique in accordance with the respective reference protocols of the CSLI (Clinical and Laboratory Standards Institute). The reading of the plates was performed in wells with 100% inhibition of fungal growth (all isolates). All isolates were previously identified by the T3B-fingerprint technique as *S. brasiliensis*, and their antifungal susceptibilities to itraconazole are known [66]. Repetitions of the test were performed to confirm each result.

4.3. Chemistry

4.3.1. General Procedure to Afford Compounds 13 and 18

Disulfur dichloride (100 mmol) was added dropwise at −15 °C to −20 °C to a stirred solution of DABCO (100 mmol) in chloroform (40 mL). The mixture was stirred at 0 °C for 48 h. The corresponding amine (200 mmol) was added, and the mixture was refluxed for 2 h, filtered, and the solvents were evaporated. The residue was separated by column chromatography (light petroleum and then light petroleum–CH₂Cl₂ mixtures) to afford products 13 and 18.
6,8-Bis(diethylamino)thieno(3,4-f)(1,2,3,4,5)pentathiepin (13)

Orange oil (1.42 g, 30%). Anal. Calcd for C_{12}H_{20}N_{2}S_{6} (%): C, 37.5; H, 5.2; N, 7.3; S, 50.0. Found (%): C, 37.6; H, 5.3; N, 7.3; S, 50.1. 1H NMR (300 MHz, CDCl_3) δ: 3.17 (q, 8H, J = 7.2, 4 × CH_2), 1.11 (t, 12H, J = 7.2, 4 × CH_3). 13C NMR (75.5 MHz, CDCl_3) 152.9 and 125.1 (2 × sp^2 tertiary C), 50.6 (CH_2), 13.0 (CH_3). IR (KBr) v/cm^-1: 2970 (CH), 1500, 1440, 1380, 1240, 1180, 1130, 1090, 840; m/z (EI) m/z 384 (M^+, 46%), 320 (M^+ − S_2, 100%), 287 (65), 259 (95), 244 (65), 231 (69). C_{12}H_{20}N_{2}S_{6} requires (M^+) 383.9951, found (M^+) 383.9943. Consistent with a previous report [60].

6,8-Bis(dibenzylamino)thieno(3,4-f)(1,2,3,4,5)pentathiepin (18)

Orange oil (1.68 g, 21%). Anal. Calcd for C_{32}H_{28}N_{2}S_{6} (%): C, 60.7; H, 4.5; N, 4.4 Found (%): C, 60.4; H, 4.2; N, 4.8. 1H NMR (300 MHz, CDCl_3) δ: 7.30 (s, 5H, Ph), 4.22 (s, 2H, CH_2Ph), 13C NMR (75.5 MHz, CDCl_3) 154.4, 136.9 and 127.1 (3 × sp^2 tertiary C), 128.6, 128.4 and 127.5 (3 × CH Ph), 59.9 (CH_2Ph) IR, (KBr) v/cm^-1: 3080, 3060, 3030, and 2920 (CH), 1600, 1460, 1360, 1180, 1039, 910, 740; m/z (EI) 275 632 (M^+ +6%), 568 (M^+ − S_2, 4%), 477 (28), 356 (52), 287 (25), 91 (100). C_{32}H_{28}N_{2}S_{6} requires (M^+) 632.0577, found (M^+) 632.0557. Consistent with a previous report [60].

4.3.2. General Procedure to Afford Compounds 16 and 19–22

Disulfur dichloride (12.5 mmol) was added dropwise at −25 °C to −35 °C to a stirred solution of DABCO (12.5 mmol) in chloroform (40 mL) under argon. The mixture was stirred at rt for 1 h. The corresponding substituted 2,5-dimethylpyrrole (5.0 mmol) in chloroform (10 mL) was added, and the mixture was stirred at 0 °C for 48 h under argon, filtered, and the solvents evaporated. The residue was separated by column chromatography (light petroleum and then light petroleum—CHCl_3 mixtures) to afford products 16 and 19–22.

6,7,8-Trimethyl-7H-(1,2,3,4,5)pentathiepin(6,7-c)pyrrole (16) yellow solid (4.80 g, 36%) m.p. 157–159 °C. Anal. Calcd for C_{9}H_{11}NS_{5} (%): C, 31.47; H, 3.40; N, 5.25. Found (%): C, 31.75; H, 3.43; N, 5.18. 1H NMR (300 MHz, CDCl_3) δ: 3.40 (3H, s, CH_3), 2.48 (6H, s, 2 × CH_3). 13C NMR (75.5 MHz, CDCl_3) 135.2 (2 × sp^2 tertiary C), 120.5, 31.8 (CH_3), 11.3 (2 × CH_2). IR, (KBr) v/cm^-1: 2930 (C−H), 2860 (C−H), 1520, 1430, 1420, 1370, 1350, 1030, 100, 810, 780. m/z (EI) 267 (M^+, 25), 203 (100), 170 (60), 64 (26), 56 (64). C_{9}H_{11}NS_{5} requires (M^+) 266.9339, found (M^+) 266.9331. Consistent with a previous report [61].

7-Ethyl-6,8-dimethyl-7H-(1,2,3,4,5)pentathiepin(6,7-c)pyrrole (19) yellow solid (5.62 g, 40%) m.p. 159–160°C. Anal. Calcd for C_{8}H_{11}NS_{5} (%): C, 34.17; H, 3.95; N, 4.98 Found (%): C, 34.35; H, 3.86; N, 5.09. 1H NMR (300 MHz, CDCl_3) δ: 3.80 (2H, q, J = 7.2 Hz, CH_2); 1.28 (3H, t, J = 7.2 Hz, CH_3). 13C NMR (75.5 MHz, CDCl_3) 134.4 (2 sp^2 tertiary C) 120.8, 40.2 (CH_2), 15.4 (CH_3), 11.0 (CH_3). IR, (KBr) v/cm^-1: 2980 (C−H), 1520, 1470, 1440, 1400, 1380, 1350, 1080, 1010, 760. m/z (EI) 281 (M^+, 13), 249 (2), 219 (14), 217 (100), 185 (13), 184 (53), 152 (12). C_{8}H_{11}NS_{5} requires (M^+) 280.9495, found (M^+) 280.9485. Consistent with a previous report [61].

7-Methyl-6,8-diphenyl-7H-(1,2,3,4,5)pentathiepin(6,7-c)pyrrole (20) yellow solid (12.1 g, 62%) m.p. 252–253 °C. Anal. Calcd for C_{17}H_{13}NS_{5} (%): C, 52.18; H, 3.35; N, 3.58. Found (%): C, 52.35; H, 3.48; N, 3.22. 1H NMR (300 MHz, CDCl_3) δ: 7.46 (10H, m, ArH), 0.34 (3H, s, CH_3). 13C NMR (75.5 MHz, CDCl_3) 139.9 (3 × sp^2 tertiary C), 130.3, 131.1, 128.9 (3 × C−H), 128.5, 125.7, 34.8 (CH_3). IR, (KBr) v/cm^-1: 3050, 1470, 1440, 1430, 1380, 1080, 1010, 920, 810, 780, 750, 700. m/z (EI) 391 (M^+, 20), 327 (100), 295 (52), 280 (15), 118 (53). C_{17}H_{13}NS_{5} requires (M^+) 390.9651, found (M^+) 390.9669. Consistent with a previous report [61].

6,8-Dichloro-7-methyl-7H-(1,2,3,4,5)pentathiepin(6,7-c)pyrrole (21) yellow solid (5.8 g, 38%) m.p. 167–170 °C. Anal. Calcd for C_{5}H_{3}Cl_{2}N_{5} (%): C, 19.5; H, 1.0; N, 4.5. Found (%): C, 19.8; H, 1.2; N, 4.2. 1H NMR (300 MHz, CDCl_3) δ: 3.60 (s, 3H, CH_3). 13C NMR (75.5 MHz, CDCl_3) 123.8 and 121.5 (2 × sp^2 tertiary C), 33.2 (CH_3). IR, (KBr) v/cm^-1: 1460, 1420, 1340, 1100, 780. m/z (EI) 309 (M^+ + 2, 6), 307 (M^+, 9), 245 (39), 243 (48). C_{5}H_{3}Cl_{2}N_{5} requires (M^+) 306.8246, found (M^+) 306.8238. Consistent with a previous report [64].
6,8-Dichloro-7-isopropyl-7H-(1,2,3,4,5)pentathiepino(6,7-c)pyrrole (22) yellow solid (6.4 g, 38%) m.p. 136–139 °C. Anal. Calcld for C_{2}H_{7}Cl_{2}NS_{5}: C, 25.0; H, 2.1; N, 4.2. Found (%): C, 24.7; H, 1.9; N, 4.5. \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ: 5.01 (septet, 1H, J = 6.5, CH), 1.61 (d, 6H, J = 6.5, 2 CH\(_2\)); \(^{13}\)C NMR (75.5 MHz, CDCl\(_3\)) 115.3 and 78.1 (2 × sp\(^2\) tertiary C), 52.1 (CH), 21.7 (CH\(_3\)). IR, (KBr) ν/cm\(^{-1}\): 2980 (CH), 1480, 1400, 1290, 1180, 795 (C–Cl); \(^{13}\)C NMR (300 MHz, CDCl\(_3\)) 33.2 (CH), 148.0, 144.0, 133.0, 124.0, 116.0, 820, 780, 740 (C–Cl).

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