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# Studies on the antifungal properties of *N*-thiolated β-lactams

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ABSTRACT

*N*-Thiolated β-lactams had previously been shown to have antibacterial activity against a narrow selection of pathogenic bacteria including *Staphylococcus aureus* and *Bacillus anthracis*, as well as apoptotic-inducing activity in a variety of human cancer cell lines. We now have found that these lactams also possess antifungal activity against *Candida* and other fungi by exerting powerful cytostatic effects that disrupt the structural integrity of cytoplasmic membranes. The mode of action and structure–activity trends of these lactams as antifungals parallel that previously seen in our antibacterial studies.



## 1. Introduction

The rising incidence and prevalence of invasive fungal infections has become an increasing concern. *Candida* species are the most frequently isolated human fungal pathogens.[1] Usually harmless, these microorganisms help constitute the healthy human microflora of the mouth and gastrointestinal tract.[2] However, in a state of altered homeostasis such as during treatment with broad-spectrum antibiotics or immunosuppression, *Candida* species are potent opportunistic pathogens[3] capable of producing infections at almost any site, varying in intensity from acute localized infections to serious invasive infections.[4] The incidence of invasive candidiasis and candidemia has risen rapidly over the past 20 years and has become a significant problem.[5] *Candida* is now the fourth most common cause of nosocomial bloodstream infections in the US, surpassing Gram negative bacilli in frequency.[6] *Candida* infections account for 8% of all septicemias.[7] The patient population at risk for infection by *Candida* has also grown to include those undergoing solid organ and stem cell transplants, those being treated for cancer, immunosuppressive therapy, AIDS, and those from premature birth, with advanced age and recovering from major surgery.[5] The tremendous impact of these infections is apparent in terms of cost, morbidity and mortality. Most strikingly, the attributable mortality to disseminated *Candida* infection is almost 50%.[8]

The etiology of *Candida* infections has also changed over the past 20 years. *Candida albicans* has long been and continues to be the leading etiologic agent of *Candida* infections. However, more recently there has been a growth in the number of cases of non-albicans *Candida* infections.[9] Non-albicans species now account for greater than fifty percent of *Candida* infections.[10] The increased use of azoles such as fluconazole have positively selected for such less sensitive or resistant species as *Candida krusei*, *Candida lusitaniae*, and *Candida glabrata*.[11] *C. glabrata* is now the second most frequent causative agent of candidemia in the US,[12] and has been associated with a digestive or urinary point of entry, especially with catheters.[13] *C. krusei* has been found in patients with solid tumors or leukemia.[13] *C. lusitaniae* is common among urinary and respiratory infections as well as those arising from intravenous catheters and use of broad-spectrum antibiotics.[13] Both *C. parapsilosis* and *C. tropicalis* have both been linked to use of intravenous catheters, contamination of the infusate, and colonization of health care workers.[13] *C. tropicalis* most frequently appears in patients with cancer or leukemia, and *C. parapsilosis* often occurs with long-term parenteral alimentation.[13] Interestingly, in other parts of the world, specifically in Latin American countries, *C. tropicalis* and *C. parapsilosis* usurp *C. glabrata* as the second most common agent of candidemia.[14] The reasons for this are not well known.

Apparently, the genus *Candida* and the infections that result include a rather disparate group of organisms that grow as yeasts, but are not all that closely related,[15] evidenced by the variation of risk factors and even geographical differences in etiology. This presents a challenge in treatment since even the best currently available drugs are plagued by intrinsic and acquired resistance.

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The search for new cellular targets in fungi and the development of novel antifungal drugs is ongoing.

Recent publications from our laboratory have described our investigations of *N*-thiolated β-lactams, a new family of antimicrobial compounds having high selectivity for *Staphylococcus* and *Bacillus* species.[16–22] Their mechanism of action involves the inhibition of fatty acid biosynthesis in microbes expressing high levels of coenzyme A.[23] The compounds also exhibit moderate apoptosis-inducing properties in a variety of human cancer cell lines without discernible toxicity in healthy dermal fibroblasts.[24–27] Consequently, we were interested in investigating whether these compounds could have antifungal properties given the documented observation that *Candida* has high intracellular levels of free thiol which includes coenzyme A.[28] For these investigations, a selection of representative *N*-alkylthio β-lactam compounds was evaluated for in vitro antifungal activity.

## 2. Results and discussion

The structures of the *N*-thiolated β-lactams evaluated for antifungal activities in this study are shown in Figures 1–3.[29] Several types of structural dependencies were evaluated: (1) effect of mono-halogenation and multi-halogenation of the C-4 aryl ring; (2) influence of unsaturation within the C-4 side chain; (3) deviations due to the *S*-alkyl and C3-substituents; and (4) dependency of the absolute stereochemistry of the molecule (Figs. 1–3).

Initial antifungal testing was performed for all 25 compounds by Kirby-Bauer agar disk diffusion[30,31] to assess their relative bioactivities against a variety of *Candida* species, including *C. albicans* (ATCC 2091), *C. glabrata* (ATCC 15126), *C. tropicalis* (clinical isolate), *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 14243), *C. lusitaniae* (ATCC 34449), *Candida kefyr* (ATCC 20409), and *Candida utilis* (ATCC 29950). The results are provided in Table 1.

For the assays, 50 μg of each sample in 10 μL of $CH_2Cl_2$ was used. After 24 h of incubation at 37 °C, the sizes of the growth inhibition zones appearing as clear areas around the cellulose disk were measured and reported as the average of triplicate experiments. Not all of the compounds were active against the seven species of *Candida*, and in fact, *C. krusei* was resistant to all of the compounds. Of those lactams (**1**–**12**) having mono-halogenation within the C4 aryl ring, the fluoro-containing analogues possess the weakest antifungal activity.[18] In fact, as found in our earlier antibacterial studies, it does not appear to be as important which halogen is on the C-4 phenyl ring, but rather where the halogens are positioned.[20] Thus, derivatives having fluoro- or chloro- substituents at the para position have less biological activity than the meta- or ortho-substituted compounds. However, this trend is not observed for the iodophenyl and bromophenyl substituents. On the other hand, the presence of multiple fluoro- or chloro- sub-

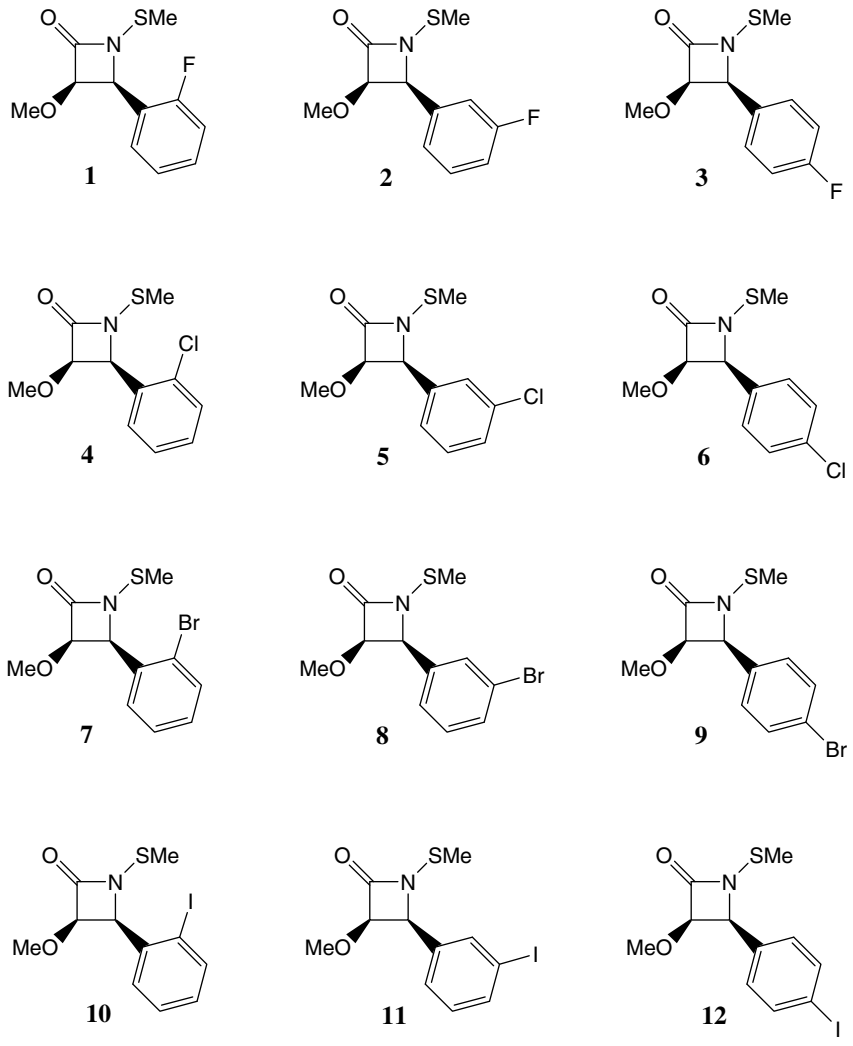



**Figure 1.** *N*-Thiolated β-lactams **1**–**12** bearing a mono-halogenated $C_4$ aryl ring.

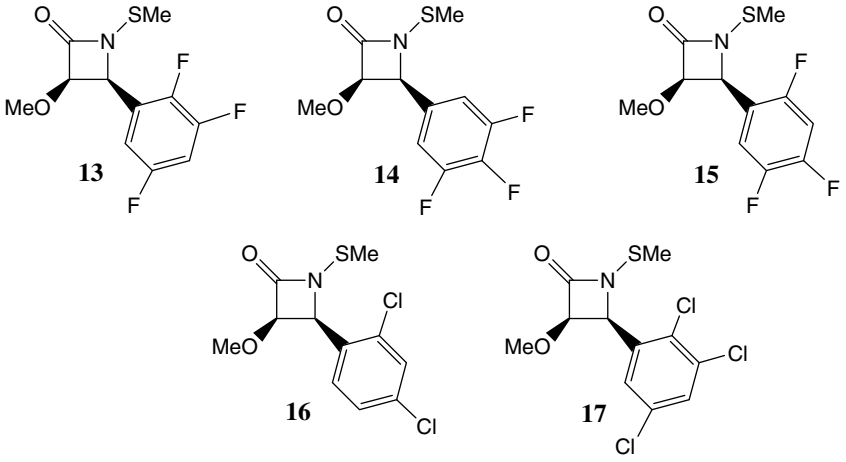



**Figure 2.** *N*-Thiolated β-lactams **13–17** bearing a multi-halogenated $C_4$ aryl ring.

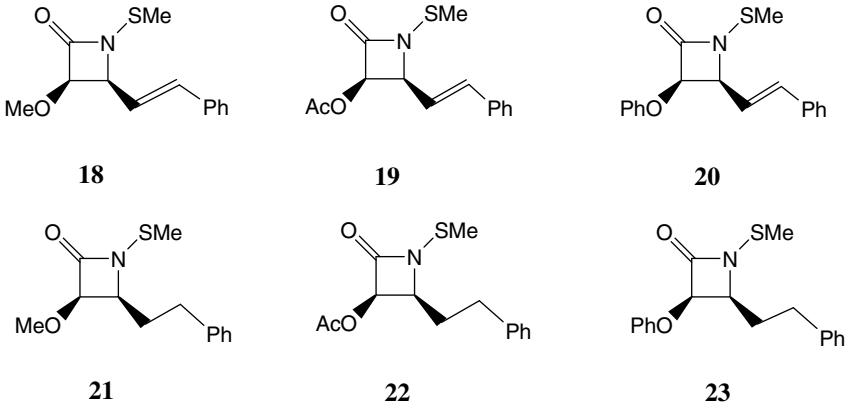
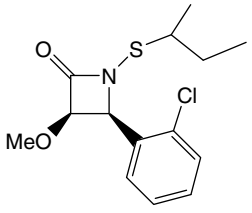



**Figure 3.** *N*-Thiolated β-lactams **18–23** bearing $C_4$ alkenyl and alkyl substituents.

stituents on the aryl ring (compounds **13–17**, Fig. 2) enhances activity. Unsaturation in the C4 side chain (comparing compounds **18–23**, Fig. 3) generally seems to decrease activity.[19] Replacing the C3 methoxy substituent of either compound **18** or **21** for acetoxy or phenoxy also reduced the in vitro activity. Finally, replacement of the *N*-methylthio group of the most active compound, lactam **4**, for *sec*-butylthio (compound **24**) resulted in a marked decrease in antifungal activity against six of the eight *Candida* species examined. This contrasts with the observed activity against *Staphylococcus aureus,* in which the *sec*-butylthio compound **24** showed better in vitro activity than the methylthio analogue **4**.[21] However, for antifungal activity, compound **21** displayed the most potent in vitro activity (Table 1).



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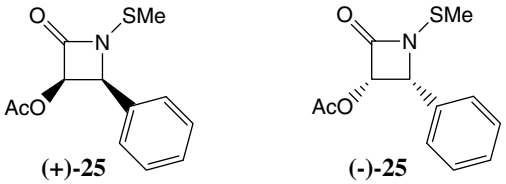
Finally, we also observe that absolute stereochemistry of these lactams has little if any influence on bioactivity, which is evident from the fact that both the (+) and (−) antipodes of lactam **25** have very similar growth inhibition zone sizes against all the *Candida* species examined. This is parallel to what we have observed before for the antibacterial properties.[21]




The structure–antifungal activity profiles of these compounds closely mirror that previously found for *Staphylococcus* and *Bacillus* bacteria, in which differences in the biological activity of different lactams may be more closely related to their ability to diffuse through the cellular membrane than to any specific interactions with a biological target.

In follow up to the Kirby-Bauer studies, we then evaluated in vitro activity of the most active lactam, compound **4**, by measuring minimum inhibitory concentration values by broth dilution.[32–34] The results are shown in Table 2.

The MIC was significantly higher at 48 h than at 24 h, suggesting fungistatic behavior in which the fungi overcome the diminishing concentration of active compound. The minimum inhibitory concentration (MIC) was determined against *C. albicans* to be around 8 μg/mL, slightly higher than the clinical agent clotrimazole which displayed an MIC of 2 μg/mL in our assays.

**Table 1**
Kirby-Bauer assays of *N*-thiolated β-lactams **1**–**25** against *Candida*

| Compound | *C. albicans* | *C. tropicalis* | *C. glabrata* | *C. kefyr* | *C. krusei* | *C. lusitaniae* | *C. parapsilosis* | *C. utilis* |
|---|---|---|---|---|---|---|---|---|
| **1** | 15 | 0 | 0 | 15 | 0 | 0 | 19 | 14 |
| **2** | 14 | 0 | 0 | 13 | 0 | 0 | 22 | 16 |
| **3** | 16 | 0 | 0 | – | 0 | 0 | 21 | – |
| **4** | 23 | 21 | 25 | 17 | 0 | 20 | 33 | 22 |
| **5** | 23 | 20 | 21 | 13 | 0 | 13 | 28 | 19 |
| **6** | 0 | 0 | 0 | 10 | 0 | 14 | 0 | 0 |
| **7** | 25 | 21 | 17 | 13 | 0 | 19 | 40 | 25 |
| **8** | 20 | 18 | 17 | 14 | 0 | 15 | 18 | 14 |
| **9** | 25 | 20 | 19 | 17 | 0 | 17 | 23 | 17 |
| **10** | 16 | 15 | 20 | 15 | 0 | 17 | 22 | 15 |
| **11** | 14 | 14 | 19 | 15 | 0 | 19 | 22 | 15 |
| **12** | 16 | 12 | 22 | 19 | 0 | 20 | 25 | 20 |
| **13** | 18 | 24 | 11 | 15 | 0 | 17 | 22 | 16 |
| **14** | 25 | 19 | 18 | 17 | 0 | 25 | 34 | 29 |
| **15** | 25 | 20 | 14 | 16 | 0 | 14 | 22 | 14 |
| **16** | 27 | 23 | 32 | 28 | 0 | 22 | 31 | 21 |
| **17** | 23 | 22 | 31 | 30 | 0 | 23 | 26 | 19 |
| **18** | 27 | 14 | 16 | 14 | 0 | 14 | 13 | 19 |
| **19** | 0 | 0 | 14 | 12 | 0 | 0 | 0 | 13 |
| **20** | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| **21** | 34 | 17 | 20 | 24 | 0 | 15 | 14 | 28 |
| **22** | 0 | 0 | 15 | 13 | 0 | 0 | 0 | 0 |
| **23** | 25 | 19 | 18 | 19 | 0 | 14 | 14 | 21 |
| **24** | 16 | 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| (+)-**25** | 14 | 15 | 0 | 9 | 0 | 15 | 0 | 16 |
| (−)-**25** | 14 | 11 | 0 | 0 | 0 | 10 | 0 | 16 |

The numerical values are the average diameters in mm (for triplicate runs) of the growth inhibition zones appearing around the drug-loaded disk impregnated with 50 μg of substance after 24 h of incubation (37 °C). The following ATCC strains were used: *Candida albicans* (ATCC 2091), *Candida glabrata* (ATCC 15126), *Candida tropicalis* (isolate), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 14243), *Candida lusitaniae* (ATCC 34449), *Candida kefyr* (ATCC 20409), and *Candida utilis* (ATCC 29950).

**Table 2**
Minimum inhibitory concentration (MIC) values of *N*-methylthio β-lactam **4** determined by broth dilution

| Microbe | MIC 24 h | MIC 48 h |
|---|---|---|
| *C. albicans* | <5 μg/ml | <5 μg/ml |
| *C. tropicalis* | 10–15 μg/ml | 30–35 μg/ml |
| *C. glabrata* | 10–15 μg/ml | 10–15 μg/ml |
| *C. kefyr* | 10–15 μg/ml | 35–40 μg/ml |
| *C. lusitaniae* | 10–15 μg/ml | 15–20 μg/ml |
| *C. parapsilosis* | <5 μg/ml | <5 μg/ml |
| *C. utilis* | 10–15 μg/ml | 15–20 μg/ml |

These values are averages (of triplicate trials) of the minimal concentration of test compound needed to completely inhibit fungal growth in broth.

The fungistatic behavior of the lactams was further verified by Trypan blue exclusion assay of the MIC culture in broth after exposure to the antibiotics.[35] The fungal cells treated with 16, 8 and 4 μg/mL of lactam all excluded the dye, indicating that the cells were still viable after exposure. Cells heat-killed for a control culture readily took up the dye, indicating that the cell was non-viable.

Additional in vitro testing of lactam **4** was performed against *Saccharomyces cerevisiae* and *Aspergillus niger* using an agar dilution MIC methodology and indicated similar levels of antifungal activity, with MIC values being 32 μg/mL for *S. cerevisiae* (YM agar) and 16 μg/mL for *A. niger* (potato dextrose agar). Thus, antifungal activity for lactam **4** spans beyond *Candida*.

A broth study was next conducted to determine the fate of the lactam in the presence of *Candida* fungi. For this, lactam **4** was added to a broth culture of *C. albicans*. After 24 h, the broth was extracted with ethyl acetate, and upon evaporation, the organic-soluble residue was examined by proton NMR. We observed that all of the *N*-methylthio lactam **4** was converted cleanly to the *N*-protio lactam indicating the complete removal of the *N*-alkylthio residue from the β-lactam ring. The *N*-protio lactam, meanwhile, was innocuous and induced no further antifungal effect even after prolonged exposure. Thus, it is the *N*-thio moiety and not the β-lactam ring that is essential for antifungal activity.

We also investigated the effects of lactam **4** on *C. albicans* cells by transmission electron microscopy.[36,37] The images shown in Figure 4 compare a normal, healthy cell not exposed to drug (image a) versus a cell treated with lactam **4** at its minimum inhibitory concentration (image b). The severe structural damage to organelles in the treated cells occurs as a consequence of lactam-induced degradation of intracellular membranes.

Damage to the membranes, including mitochondrial and cellular membranes, was observed in the majority of treated fungal cells analyzed, as was disorganization of the cytoplasm, rendering a visible 'scrambling' of its contents. Numerous membrane invaginations were observed for these cells, indicating that extensive damage was caused by the lactam treatment. However, it was not determined whether this damage was irreversible through the TEM analysis. There also appeared to be a shrinking of the protoplast in the treated cells which is most likely due to an obstruction in cell maturation. This was likewise observed for prokaryotic cells previously analyzed, where a lack of cell division was observed for the cells, indicating an obstruction in cell maturation and replication processes. The untreated fungal cells (image a) displayed intact cellular components, including intact mitochondria, vacuoles, protoplasts, nucleus, and cell wall and cell membrane.

## 3. Conclusions

This study has determined that *N*-thiolated β-lactams previously investigated in our laboratory as antibacterial and anticancer compounds also display moderate antifungal activity. This is a most unusual finding, in that until now only a very limited number of β-lactam compounds have ever been observed to act on fungal growth.[32] The time study we conducted over 24 h versus 48 h suggests that the effects of the *N*-thiolated lactams on *Candida* cells are fungistatic. Given the observed structure–activity relationship and the products of the drug's interaction with the cell, it is postulated that the mode of action of these antifungals is similar to that previously observed in *Staphylococcus* bacteria. We postulate that

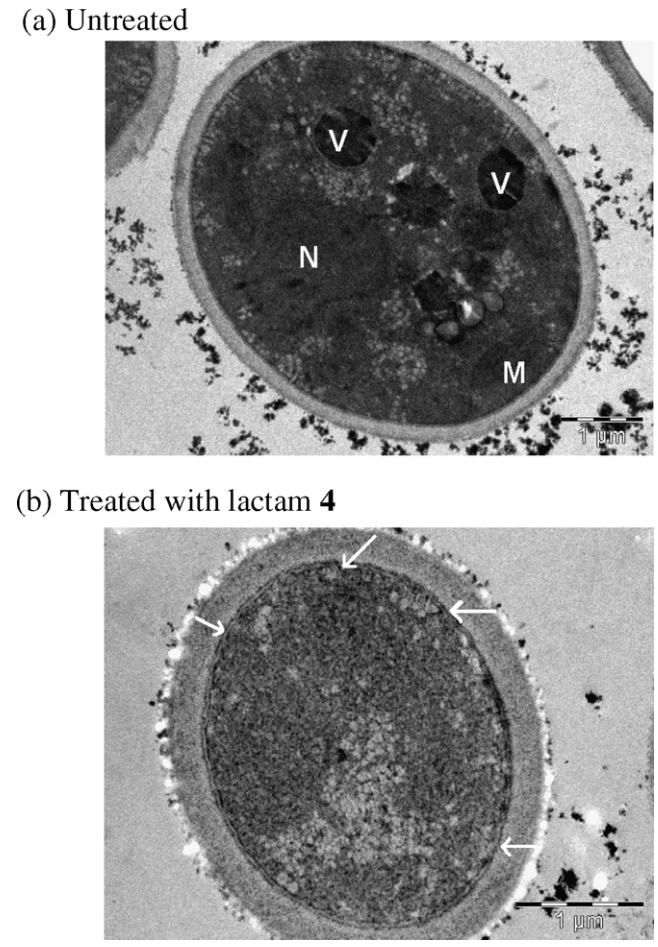



**Figure 4.** Comparison of the effects of *N*-methylthio lactam **4** on *C. albicans* cells. Image (a) a healthy untreated cell showing intact cytoplasm, internal organelles, and cellular membranes (N = nucleus; M = mitochondria; V = vacuole); image (b) a cell treated with lactam **4** in which the compartmentalized organelles are not found, there is obvious disorganization of the cytoplasm, and the cytoplasmic membrane is severely damaged (arrows). No visible changes are seen in the size or shape of the cell wall.

these lactams pass through the fungal cell membrane, and interact covalently with a biological target such as coenzyme A that results in the transfer of the sulfur side chain. This mechanism is primarily supported by the NMR spectra of the product isolated from the cultured media which show that the lactam ring is intact and the sulfur side chain is missing. This is further corroborated by the structure–activity effect in that the sulfur side chain is required for antifungal activity, with *S*-methyl being preferred to *S*-*sec*-butyl, while groups at $C_3$ and $C_4$ can be altered without completely destroying the fungistatic behavior. Further definition of the details or the immediate biochemical consequences of this thio transfer event is not yet available. However, the subtle structure–activity differences seen in fungi versus bacteria suggest that the cellular target of these compounds is likely evolutionarily conserved, such as a common metabolic enzyme having a slightly different structure in bacteria and fungi. These *N*-thiolated β-lactams may thus represent a new therapeutic approach for treating *Candida* and other fungal infections.

## 4. Experimental

### 4.1. General methods

Lactams **1**–**25** were prepared and purified as previously described.[16–22]

### 4.2. Antifungal susceptibility testing

Antifungal activities of the lactams were determined by Kirby-Bauer disk diffusion on agar plates and by minimum inhibitory concentration measurements in liquid broth. Yeast nitrogen base agar was used for Kirby-Bauer disk diffusion assays, and RPMI broth was used for minimum inhibitory concentration (MIC) determinations for the *Candida* strains. *S. cerevisiae* and *A. niger* MIC values were determined using YM agar and potato dextrose agar, respectively, in an agar dilution assay. These agar assays were performed in compliance with the NCCLS guidelines and standards described in NCCLS protocol M7-A2.[34]

#### 4.2.1. Kirby-Bauer agar diffusion assays[30,31]

A suspension of the test microbe equivalent to McFarland standard 0.5 was made in 5 mL of sterile saline solution and swabbed across fresh yeast nitrogen base agar plates, and allowed to dry. Cellulose disks (6 mm in diameter) were impregnated with 10 µL of a 5 mg/mL stock solution of the test lactam dissolved in $CH_2Cl_2$ by pipet, allowed to air dry leaving only the lactam compound on the disk, and the disks were then placed onto the agar surface (three disks per plate). The plates were sealed with parafilm and incubated for 24 or 48 h at 26 °C or 37 °C (depending on the *Candida* strain), and the antimicrobial susceptibilities were determined by measuring the cleared zones of growth inhibition around each disk.

#### 4.2.2. Minimum inhibitory concentration (MIC) assays[32–34]

RPMI broth was prepared following manufacturer's instructions, then aliquoted into test tubes. A 1.6 mg/mL stock solution of lactam in DMSO was diluted to 8 µg/mL, as described in NCCLS document M27-A2, and subsequently serially diluted down to 0.125 µg/mL of lactam in broth. Each tube was then inoculated with 1mL of a standardized solution of $10^6$ cfu/mL of fungi suspended in sterile NaCl solution. After 24 or 48 h of incubation at 26 °C or 37 °C (depending on the *Candida* strain), the MICs were determined by examining the tubes for growth using optical density of the solutions. The lowest concentration of drug inhibiting all fungal growth was determined as the MIC.

#### 4.2.3. Metabolism studies

To a fresh $10^6$ cfu/ml suspension of *C. albicans* in 9 mL of sterile saline was added 1 mL of a 400 µM solution of lactam 4 in DMSO. After 1 h, 10 mL of deionized $H_2O$ was added and the solution was extracted three times with 5 mL of ethyl acetate. The organic layers were combined, dried with magnesium sulfate, and the solvent was removed under reduced pressure. The residue was dissolved in 500 µL of $CDCl_3$, and the chemical structure was elucidated by $^1H$ NMR.

#### 4.2.4. Trypan blue staining[35]

A 4% Trypan blue solution (w/v) was prepared and filtered through Fisherbrand filter paper, qualitative P8 (Thermo Fisher Scientific, Inc., Pittsburgh, PA). Four tubes of cell suspensions from the MIC assay were used in the procedure: a control tube containing *C. albicans* in Yeast Nitrogen Base (YNB) broth (Difco) and three tubes containing *C. albicans* in YNB broth with 16 µg/ml, 8 µg/ml, and 4 µg/ml of the *N*-thiolated β-lactam PR-02-131. Equal volumes of the Trypan blue solution and the cell suspensions were mixed in microcentrifuge tubes and left at room temperature. For comparison purposes, controls were also performed using live *C. albicans* cells in phosphate-buffered saline and cells that were heat-killed by boiling for 5 min. After an exposure time of 4 min, each sample was loaded into a Cellometer (Nexcelom Bioscience) and cells were observed using bright field optics with an Olympus BX60 epifluorescencce microscope (Olympus America, Inc., Center Valley, PA).

### 4.3. Transmission electron microscopy (TEM) experiments

A concentration of $10^7$ cfu/mL of *C. albicans* in TSB was treated for 4 h with lactam **4** at the MIC concentration dissolved in DMSO and then centrifuged at 10,000*g* for 5 min. The pellet was washed twice with PBS, then resuspended in 5 mL of PBS and fixed with 5 mL of 2.5% glutaraldehyde for 4 h at 4 °C. The fixed cells were centrifuged at 10,000*g* for 5 min and then resuspended in PBS supplemented with 0.1 M sucrose. The cells were then washed twice with PBS, then centrifuged again at 10,000*g* for 5 min and the pellet was embedded in agar for easier handling. The agar blocks were then washed with PBS and post-fixed in 2% osmium tetroxide for 1 h at room temperature. The cells were then washed twice with PBS, then once with 0.9% saline and stained with 1.5% uranyl acetate. The cell-containing agar blocks were then carried through a series of dehydrations using graded ethanol. The cells were infiltrated and embedded in Spurr's Plastic and ultrathin sections were cut using a Sorvall MT-2B ultramicrotome and placed on copper mesh grids. Sections were post-stained using lead citrate for heightened cellular contrast. The grids were then examined on the FEI Morgagni 268D TEM for intracellular makeup.

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### References

1. Perea, S.; Patterson, T. F. *Clin. Inf. Dis.* **2002**, *1*, 1073.
2. Sobel, J. D.; Ohmit, S. E.; Schuman, P., et al *J. Infect. Dis.* **2001**, *183*, 286.
3. Richardson, M. D. *J. Antimicrob. Chemother.* **2005**, *56*, 5.
4. Wenzel, R. P. *Infect. Dis. Clin. Pract.* **1994**, *3*, 56.
5. Nucci, M.; Marr, K. A. *Emerg. Inf.* **2005**, *41*, 521.
6. Gudlaugsson, O.; Gillespie, S.; Lee, K., et al *Clin. Inf. Dis.* **2003**, *37*, 1172.
7. Pfaller, M. A.; Messer, S. A.; Hollis, R. J.; Jones, R. N.; Doern, G. V.; Brandt, M. E.; Hajjeh, R. A. *Diagn. Microbiol. Infect. Dis.* **1999**, *33*, 121.
8. Wey, S. B.; Mori, M.; Pfaller, M. A.; Woolson, R. F.; Wenzel, R. P. *Arch. Intern. Med.* **1988**, *148*, 2642.
9. Colerman, D. C.; Rinaldi, M. G.; Haynes, K. A.; Rex, J. H.; Summerbell, R. C.; Anaisse, E. J. *Med. Mycol.* **1998**, *36*, 156.
10. Pappas, P. G.; Rex, J. H.; Lee, J., et al *Clin. Inf. Dis.* **2003**, *37*, 634.
11. Trick, W. E.; Fridkin, S. K.; Edwards, J. R.; Hajjeh, R. A.; Gaynes, R. P. *Clin. Inf. Dis.* **2002**, *35*, 627.
12. Pfaller, M. A.; Dickema, D. J. *Clin. Microbiol. Inf.* **2004**, *10*, 11.
13. Dupont, B. *Infect. Dis. Clin. Pract.* **1994**, *3*, S78.
14. Colombo, A. L.; Nucci, M.; Salomao, R., et al *Diagn. Microbiol. Infect. Dis.* **1999**, *34*, 281.
15. *Candida and Candidiasis*; Calderone, R. A., Ed.; SM Press: Washington, 2002.
16. Turos, E.; Konaklieva, M. I.; Ren, R.; Shi, H.; Gonzalez, J.; Dickey, S.; Lim, D. V. *Tetrahedron* **2000**, *56*, 5571.
17. Turos, E.; Long, T. E.; Konaklieva, M. I.; Coates, C.; Shim, J.-Y.; Dickey, S.; Lim, D. V.; Cannons, A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2229.
18. Long, T. E.; Turos, E.; Konaklieva, M. I.; Blum, A. L.; Amry, A.; Baker, E. A.; Suwandi, L. S.; McCain, M. D.; Rahman, M. F.; Dickey, S.; Lim, D. V. *Bioorg. Med. Chem.* **2003**, *11*, 1859.
19. Coates, C.; Long, T. E.; Turos, E.; Dickey, S.; Lim, D. V. *Bioorg. Med. Chem.* **2003**, *11*, 193.
20. Turos, E.; Coates, C.; Shim, J. Y.; Wang, Y.; Leslie, J. M.; Long, T. E.; Reddy, G. S. K.; Ortiz, A.; Culbreath, M.; Dickey, S.; Lim, D. V.; Alonso, E.; Gonzalez, J. *Bioorg. Med. Chem.* **2005**, *13*, 6289.
21. Heldreth, B.; Long, T. E.; Jang, S.; Guntireddygari, S. K. R.; Turos, E.; Dickey, S.; Lim, D. V. *Bioorg. Med. Chem.* **2006**, *14*, 3775.
22. Turos, E.; Long, T. E.; Heldreth, B.; Leslie, J. M.; Guntireddygari, S. K. R.; Wang, Y.; Coates, C.; Konaklieva, M.; Dickey, S.; Lim, D. V.; Alonso, E.; Gonzalez, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2084.
23. Revell, K. D.; Heldreth, B.; Long, T. E.; Jang, S.; Turos, E. *Bioorg. Med. Chem.* **2007**, *15*, 2453.
24. Smith, D. M.; Kazi, A.; Smith, L.; Long, T. E.; Heldreth, B.; Turos, E.; Dou, Q. P. *Mol. Pharmacol.* **2002**, *61*, 1348.
25. Kazi, A.; Hill, R.; Long, T. E.; Kuhn, D. J.; Turos, E.; Dou, Q. P. *Biochem. Pharmacol.* **2004**, *67*, 365.
26. Kuhn, D.; Coates, C.; Daniel, K.; Chen, D.; Bhuiyan, M.; Turos, E.; Dou, Q. P. *Front. Biosci.* **2004**, *9*, 2605.
27. Kuhn, D.; Wang, Y.; Minic, V.; Coates, C.; Reddy, G. S. K.; Daniel, K. G.; Shim, J.-Y.; Chen, D.; Landis-Piwowar, K. R.; Miller, F. R.; Turos, E.; Dou, Q. P. *Front. Biosci.* **2005**, *10*, 1183.
28. Newton, G. L.; Arnold, K.; Price, M. S.; Sherrill, C.; Delcardayre, S. B.; Aharonowitz, A.; Cohen, G.; Davies, J.; Fahey, R. C.; Davis, C. *J. Bacteriol.* **1996**, *178*, 1990.
29. This study was based on the M.S. thesis work of Marci Culbreath, University of South Florida, 2005.
30. Ingroff-Espinel, A. *Clin. Microbiol. News.* **1996**, *18*, 161.
31. National Committee for Clinical Laboratory Standards. Method for antifungal disk diffusion susceptibility testing of yeasts: approved guideline NCCLS document M44-A. National Committee for Clinical Laboratory Standards, Wayne, PA, 2004.
32. Gottstein, W. J.; Eachus, A. H.; Misco, P. F.; Cheney, L. C.; Misiek, M.; Price, K. E. *J. Med. Chem.* **1971**, *14*, 770.
33. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard. NCCLS document M27A. National Committee for Clinical Laboratory Standards, Wayne, PA, 1997.
34. National Committee for Clinical Laboratory Standards. Methods for dilution of antimicrobial susceptibility tests for bacteria that grow aerobically. NCCLS document M7-A2. National Committee for Clinical Laboratory Standards, Wayne, PA, 1997.
35. Kaur, R.; Castano, I.; Cormack, B. P. *Antimicrob. Agents Chemother.* **2004**, *48*, 1600.
36. Bergsson, G.; Arnfinnsson, J.; Steingrimsson, O.; Thormar, H. *Antimicrob. Agents Chemother.* **2001**, *45*, 3209.
37. Wright, R. *Microsc. Res. Tech.* **2000**, *51*, 496.