Review

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Future Medicinal Chemistry

Targeting adhesion in fungal pathogen *Candida albicans*

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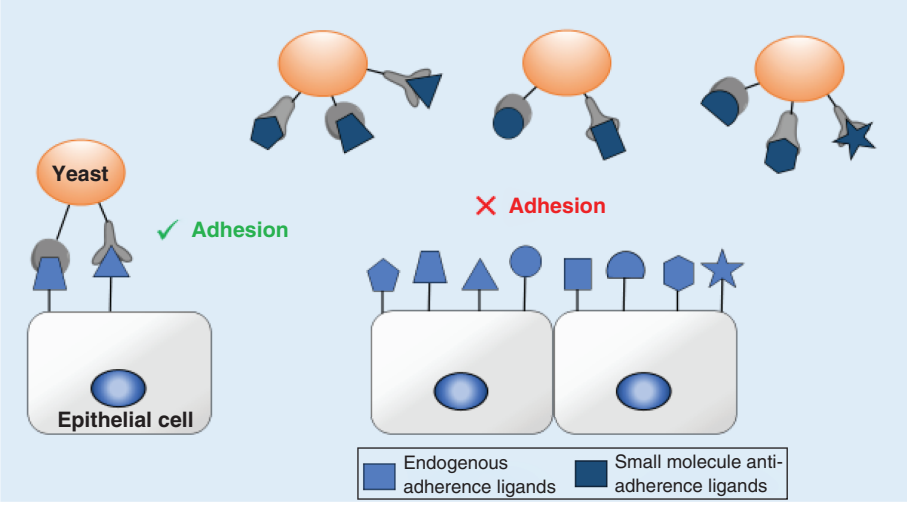
Fungal infections with increasing resistance to conventional therapies are a growing concern. *Candida albicans* is a major opportunistic yeast responsible for mucosal and invasive infections. Targeting the initial step of the infection process (i.e., *C. albicans* adhesion to the host cell) is a promising strategy. A wide variety of molecules can interfere with adhesion processes via an assortment of mechanisms. Herein, we focus on how small molecules disrupt biosynthesis of fungal cell wall components and membrane structure, prevent the localization of GPI-anchor proteins, inhibit production of enzymes involved in adhesion, downregulate genes encoding adhesins and competitively inhibit receptor interactions. As a result, adhesion of *C. albicans* to host cells is reduced, paving the way to new classes of antifungal agents.

Graphical abstract:



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The genus *Candida* includes about 200 different species, with only a few species being opportunistic pathogens of humans, of which *Candida albicans* is the most frequently encountered clinically [1]. It is a commensal colonizer in humans and exists in the gastrointestinal and genitourinary tracts: it is a normal component of the body microbiota where it is found predominantly on mucosa [2]. However, it is known to cause infections when the host becomes immunocompromised. These infections can be superficial and affect the skin (cutaneous candidosis), the mucous membrane of the oral and vulvovaginal cavities, and the fingernails (paronychial and onychial candidosis) [3]. *C. albicans* infections can also be invasive, entering the bloodstream and disseminating to internal organs. Factors that contribute to fungal invasion include: surgery (especially abdominal), burns, long-term stay in intensive care unit,

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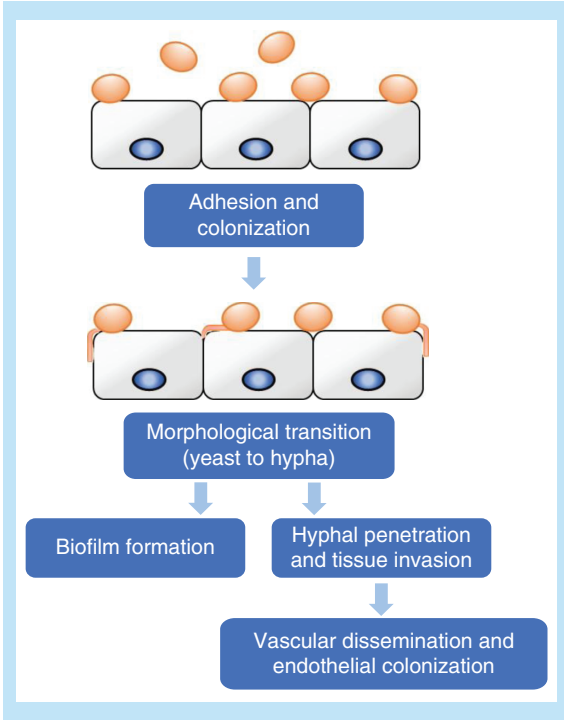

Figure 1. *Candida albicans* adherence and morphogenesis lead to biofilm formation and tissue invasion: adherence of yeast cells to epithelial cell surface is the initial step for *C. albicans* colonization; subsequent yeast-to-hypha transition facilitates biofilm formation, tissue invasion and spread of systemic infection.
Adapted with permission from [12].

disease (e.g., cancer and HIV infection), previous administration of broad-spectrum antibiotics and immunosuppressive agents, antineoplastic chemotherapy, immunosuppression prior to organ transplantation, haemodialysis and central venous catheters [4].

There are three main factors that contribute to the high mortality of invasive fungal infections. First, since conventional microbiological approaches for fungal identification are insensitive, nonspecific and laborious, a trustworthy, timely diagnosis is challenging. Second, the clinical signs and symptoms of an invasive fungal infection may not be present until the infection is at an advanced stage [5]. Third, and most significantly, current antifungal therapies often lack selectivity; the overuse of these drugs have led to the development of antifungal resistance [6].

Antifungal resistance can develop in different ways, depending on the mode of action of the antifungal drug. Resistance mechanisms include reduction in the intracellular accumulation of the drug, decrease in the affinity for the target and counteraction of the drug effect (e.g., overexpression of genes that encode the target proteins) [7]. *C. albicans* has developed resistance to common antifungal drugs used to treat fungal infections, such as the azoles [8], echinocandins [9] and polyenes [10].

C. albicans: infection process

C. albicans relies on a wide-range of virulence factors to infect its host, and these are discussed in great detail in numerous reviews [11–19]. Infections are the invasion and multiplication of pathogenic microbes causing disease. *C. albicans* is a dimorphic fungus: it can exist in the ovoid-shaped budding yeast form, which transitions to the branching filamentous hyphae form [13,14]. To infect its host, *C. albicans* yeast cells adhere to the host cell using adhesins [15], the best studied of which are the agglutinin-like sequence proteins [16] and Hwp1 [20]. After initial adherence, contact with the host cell triggers the yeast-to-hypha transition, directed growth of the hypha occurs through thigmotropism. Invasion into the host cell is then facilitated by invasins, adhesion and physical forces, and occurs via endocytosis or active penetration (Figure 1) [12,17,21]. There is a strong correlation between adherence and ability to colonize and cause disease. For example, *C. albicans* is highly adherent and implicated in a wide range of diseases, while species such as *Candida krusei* and *Candida glabrata* adhere more poorly and are less frequently implicated in disease. Biofilm formation enables the fungi attachment to biotic or abiotic surfaces [18], including medical devices [19].

To prevent *C. albicans* infection, all the stages of this multistep process may be targeted. Recently, research toward new antifungal agents has aimed at the selective targeting of virulence mechanisms as opposed to the killing

of the pathogen, which may increase the selection pressure for development of drug resistance. Thus, strategies seeking the inhibition of *C. albicans* filamentation (i.e., morphological transitions between single-cell budding yeast and filamentous forms) or biofilm formation have received considerable attention and have been reviewed elsewhere [18,22,23]. This review will focus on the anti-adhesion approach, which aims to block the adhesion of the pathogen to the host cell as the first step in the pathogenesis of microbial infections. Interestingly, it is often found that compounds impairing fungal adhesion also have an effect on filamentation or biofilm formation. In this context, a vast amount of research has been carried out into elucidating host–pathogen relationships [24–26].

Anti-adhesion strategy in fungal pathogens

Adhesins are the molecular mediators of adhesion: they are often cell-surface biomolecules, usually (manno)proteins that allow for the attachment of the microbe to host cells or surfaces. Fungal pathogens display a large variety of adhesins on their surface, so they can adhere to several cell types within the host [27–29] and to numerous ligands in various host sites, such as biological fluids, extracellular membrane and basement membranes [30]. The information available on fungal adhesins is more limited than on bacterial ones [31]. This may have contributed to anti-adhesive therapies for bacterial infections having been investigated more extensively than fungal ones [32]. Fungi are complex eukaryotic cells that have structurally unique cell walls, with each species displaying distinctive glycan polymers and proteins [33]. The development of high-affinity ligands is enabled by a detailed knowledge of protein structure. Recently, some fungal adhesins in *Aspergillus fumigatus* (e.g., FleA) have been identified [34,35], fully characterized and crystal structures have been solved [36]. Hence, high-affinity anti-adhesion ligands for this adhesin have been developed [37], proving this approach is also possible for fungal pathogens. The limited number of x-ray crystal structures available for *C. albicans* adhesins has hampered a focused design approach to anti-adhesion agents. Thus, the majority of studies dealing with *C. albicans* inhibition of adhesion discussed herein are carried out without targeting a specific protein.

Disruption of *C. albicans* adhesion by antibodies & biomolecules

The interaction of *C. albicans* with mucosal surface is essential for fungal colonization [38–40]. Early studies pointed to the role of cell surface glycoconjugates as mediators of adhesion for *C. albicans*. Sandin *et al.* showed evidence for mannose-mediated adhesion to buccal epithelial cells (BECs) [41]. Concanavalin A (ConA), a lectin that recognizes mannose and glucose, inhibited the adherence of pretreated yeasts to BECs. This suggests that ConA is binding to and blocking the mannose-containing receptors on the yeast surface or mannose moieties of the indigenous lectin associated with the yeast cell surface. ConA also inhibited the adhesion of pretreated BECs with nontreated yeasts. This result indicates that mannose-containing moieties on the buccal cell surface could be acting as receptors for the *C. albicans* [41]. Also, the presence of α-D-methyl mannoside in the incubation medium during the assay inhibited adhesion of *C. albicans* to the BECs. Critchley and Douglas investigated the effect of several lectins and sugars on the adhesion of *C. albicans* to buccal and vaginal epithelial cells [42]. The adherence of most *C. albicans* strains were inhibited by L-fucose and winged-pea lectin (specific for L-fucose), suggesting that a glycoside containing α-L-fucosyl residues might function as a receptor for these strains. Other lectins, such as wheat-germ agglutinin (specific for *N*-acetyl-β-D-glucosaminyl residues) or peanut lectin (specific for D-galactosyl residues) had little effect on the adherence of these strains. In contrast, the adherence of one *C. albicans* strain was efficiently inhibited by *N*-acetyl-D-glucosamine and wheat-germ agglutinin and was unaffected by winged-pea lectin and peanut lectin. Interestingly, the adhesion of all strains in this study were significantly enhanced by ConA lectin. It was postulated that this effect is due to the fact that ConA has multiple-binding sites and can therefore promote adhesion by cross-linking, acting as bridges between α-D-mannosyl residues on the yeast and epithelial surfaces.

Cell surface glycoconjugates on human BEC have also been investigated as possible adhesion receptors for *C. albicans*. Brassart *et al.* [43] studied the ability of soluble glycopeptides and oligosaccharides to inhibit the adhesion of yeast to exfoliated cells. Preliminary studies showed that fucose played a role in inhibiting the adhesion process. Using human milk oligosaccharides as probes, the minimal requirement for activity was found to be the Fucα(1-2)Galβ determinant. This study concluded that this disaccharide, found in cell surface glycoconjugates, may act as a part of a complex adhesion mechanism, which possibly requires multireceptor specificities. In addition, salivary fucosylated glycans and glycoproteins were also able to block *C. albicans* adhesion to BEC [44,45].

Monoclonal antibodies (mABs) have also been investigated as inhibitors of *C. albicans* adhesion. MABs raised against *C. albicans* cell wall components inhibited whole yeast cell binding to BEC [46]. Also, mABs generated against mannosylated *C. albicans* adhesins or secreted aspartyl proteases were used *in vivo* in a rat model of vaginal

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candidiasis and demonstrated significant reductions of fungal burden [47]. mABs directed against the extracellular matrix proteins: collagen, laminin and fibronectin, reduced the adherence of *C. albicans* to HEp-2-cultured cells [48]. The anti-adhesion ability of glucosamine and other simple sugars had been previously reported [49]; a combination of this sugaramine with anticollagen IV antibody reduced yeast-HEp-2 adherence by approximately 70%.

Disruption of *C. albicans* adhesion by small molecules

The majority of research on fungal anti-adhesion ligands based on small molecules has focused on *C. albicans*. Small molecules may display different mechanisms to inhibit the adhesion of fungi to host cells, although in most cases their mode of action is unknown. It is often not clear if the disruption in adhesion caused by these compounds is due to specific interactions with a biomolecular target or is a consequence of nonspecific effects (i.e., hydrophobic and/or electrostatic interactions). It is also important to note that an apparent reduction of adhesion may be observed if the compounds have fungicidal activity; for this reason, it is should be ensured that anti-adhesion assays are carried out well below the concentration range at which the compounds are toxic to the yeast cells. Compounds from both synthetic and natural sources have been tested for their anti-adhesive properties toward *C. albicans*.

A range of adherence assays have been used to test the inhibition of adherence of the small molecules outlined in the following sections. The differences in experimental setup and quantification methods employed in these assays can complicate standardization and the comparison of the anti-adhesion activity across the various studies. The main assays involve measuring the adhesion of *C. albicans* to different substrates: suspended BECs; human cell monolayers; and polystyrene, which provides a useful model for abiotic surfaces. The general procedures for these assays are summarized below.

To determine the ability of compounds to inhibit the adhesion between *C. albicans* and suspended BEC the following steps are followed. *C. albicans* were harvested, washed and re-suspended in phosphat-buffered salin (PBS). The number of yeast per milliliter was determined using a hemocytometer. BEC were collected from volunteers, suspended in a PBS solution and washed three- to four-times by centrifuging. Solutions of BEC and *C. albicans* were mixed using volumes that ensured a higher quantity of yeast than BEC, usually 50–100: one ratio of yeast to BEC. This mixture was incubated at typically 30–37°C for 1–3 h. Cell were then harvested on polycarbonate filters/nylon filter paper, washed with PBS to remove unattached yeast, air dried on slides and stained using Gram stain or crystal violet. The number of yeast attached to 50–200 BEC were counted using light microscopy. This number was compared with a control, which received no treatment. All assays were repeated two- to four-times. The potential anti-adhesion compounds may be added at different times throughout the experiment: in the exclusion or prelabeling assay compounds were added to the yeast before being mixed with BEC; in competitive assay compounds, yeast and BEC are all mixed together; or in displacement assay initial adherence between yeast and BEC was allowed and then compounds were added.

To determine the ability of compounds to inhibit the adhesion between *C. albicans* and a cell monolayer the following steps are followed. A human cell line (generally epithelia) was grown until they formed confluent monolayers in medium at 37°C in cell culture plates. *C. albicans* were treated with potential anti-adhesion compounds in medium at 30°C overnight without shaking. A known amount of yeast cells were added to the established monolayers of human cells. This was incubated at 35–37°C for 45 min to 2 h in 5% (v/v) CO_2, monolayers were washed twice and covered with warm sabouraud dextrose agar. Yeast colonies appearing after 18–48 h at 30°C were counted and compared with the control. The experiments were conducted in triplicate.

To determine the ability of compounds to inhibit the adhesion between *C. albicans* and a polystyrene surface, the following steps are followed. *C. albicans* cells were added to 96-well polystyrene-coated cell culture plates and were allowed to adhere in the presence of potential anti-adhesion compounds at 37°C for 90 min and 24 h in a temperature regulated incubator. Cells were washed with PBS to remove any cells not adhering to the plates. Adhesion was then quantified using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) metabolic assay, AlamarBlue cell viability assay, by adding crystal violet and determining absorbance at 570–590 nm, or through count of colony-forming units and were compared with a control. In some cases, the potential anti-adhesion compounds were added to the plates prior to the *C. albicans.* The compounds were allowed to coat the plate for 12 h at 37°C and then the yeast cells were added to the plates.

Some of the processes involved in yeast adhesion that these molecules target or interfere with include:

- The biosynthesis of cell wall components;
- The localization of GPI-anchored proteins;

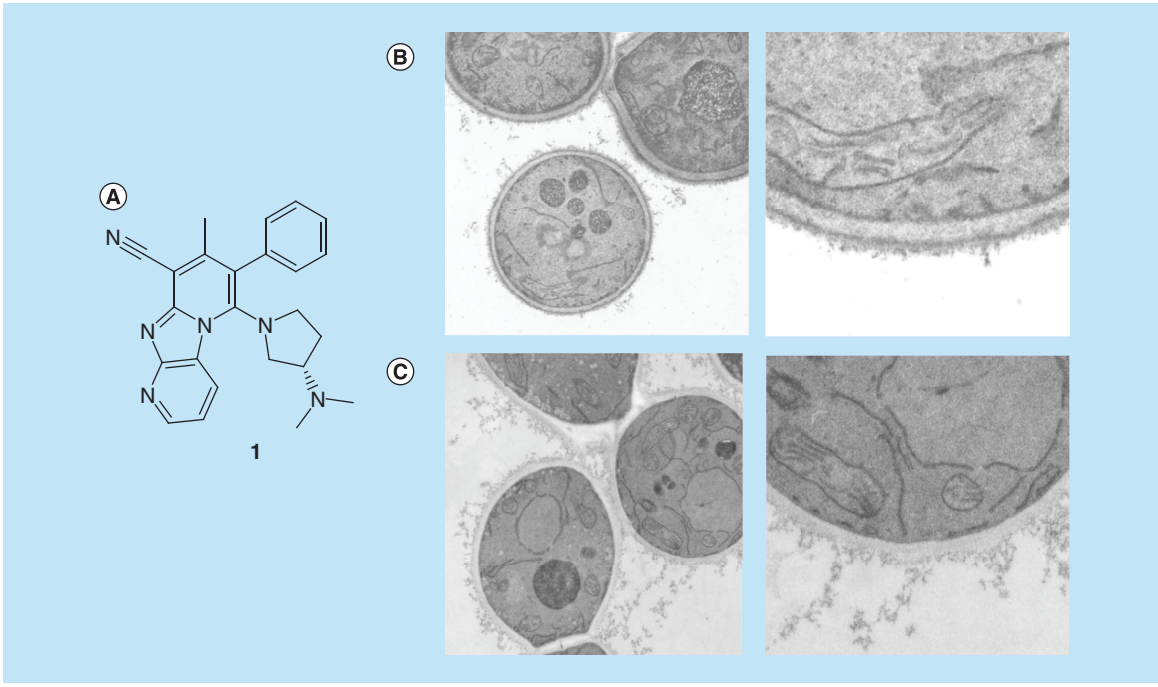

Figure 2. Disruption of the biosynthesis of cell wall components. (A) Structure of the small molecule inhibitor **1** of β-1,6-glucan biosynthesis; **(B)** microscopic images of untreated *Candida albicans* cells; **(C)** microscopic images of *C. albicans* cells treated with compound **1**.
Adapted with permission from [51].

- Inhibition of degradative enzyme production;
- Downregulation of genes that encode for adhesins;
- The disruption of the fungal cell membrane structure;
- The use of small molecules that competitively inhibit the interaction between the fungal adhesin and the host cell receptors.

Some examples relevant for each classification will now be presented.

Disruption of the biosynthesis of cell wall components

β-1,6-glucan is an essential component of fungal cell walls, in particular in *C. albicans*, where it has been reported to retain cell wall proteins. Compound **1** has been identified as an inhibitor of β-1,6-glucan biosynthesis. This resulted in the release of cell wall proteins, impacting in the yeast virulence (Figure 2). It was shown that the adhesion of *C. albicans* to a monolayer of cancerous human lung epithelial cells was significantly inhibited in the presence of compound **1**. At concentrations of 0.1 μg/ml compound **1** reduced adherence by approximately 30%, while at concentrations of 1 μg/ml adherence was reduced by approximately 80% [50].

Anti-adhesion tests of fluconazole (**2**) (Figure 3), a well-known, bis-triazole antifungal drug, were performed by Darwazeh *et al.* [52]. They explored the effect of systemic fluconazole intake on *C. albicans* adhesion to BEC obtained from healthy volunteers on systemic fluconazole therapy. It was found that the fluconazole significantly reduced the number of yeasts adhering to BEC. A 48.7% reduction in adhesion was observed during treatment compared with levels prior to commencement of fluconazole therapy. Several other studies have also shown significant reduction of adherence of *C. albicans* isolates to denture acrylic and BEC after treatment with fluconazole [53–55]. It is known that **2** inhibits the production of lanosterol 14-α-demethylase, an enzyme involved in the biosynthesis of ergosterol, the largest sterol component of the fungal cell membrane. This affects the cell surface composition of *C. albicans* and hence the ability to adhere to BEC.

Several tetrazole compounds were reported to have anti-adhesion properties against *C. albicans*. Compound **3** (Figure 3) was found to be the most active of a series of 2,5-disubstituted tetrazoles that were tested as inhibitors of *C. albicans* adhesion. This compound efficiently reduced the adherence of *C. albicans* to Caco-2 cells by 86%, at

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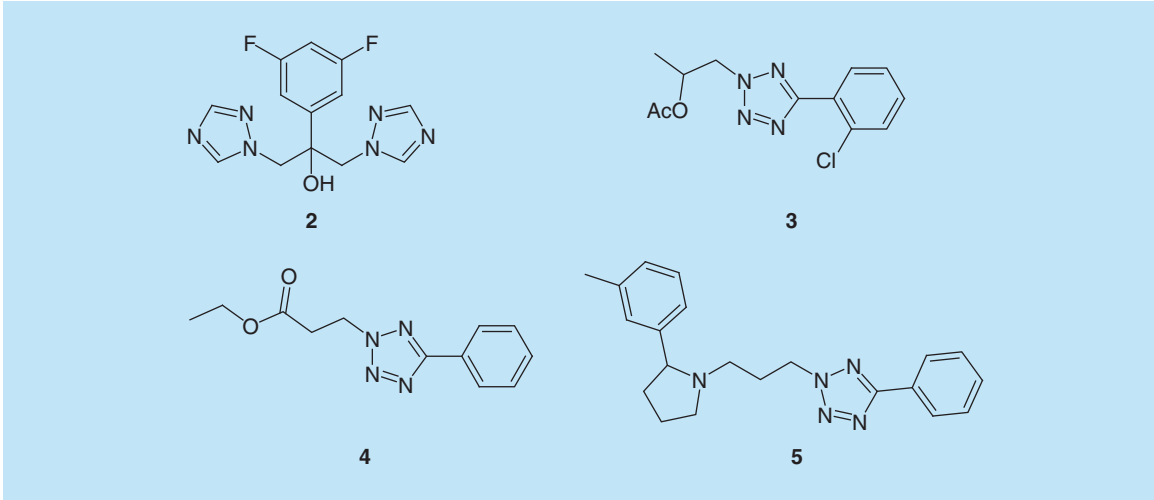

Figure 3. Examples of small molecules that disrupt the biosynthesis of cell wall components. Structures of triazole and tetrazole compounds, including fluconazole (**2**), that show anti-adhesive properties toward *Candida albicans* by disrupting cell wall biogenesis.

16 μg/ml. Compound **3** exhibited minor cytotoxicity to Vero epithelial cells, where the viability of the mammalian cells was only reduced by 13–14% at the highest concentrations (64–256 μg/ml) [56]. More 2,5-disubstituted tetrazoles were tested by Staniszewska *et al.*, compound **4** (Figure 3) reduced the adhesion of *C. albicans* to Caco-2 (human epithelial colon carcinoma cells) monolayer by >60% at 0.0313 μg/ml. Compound **4** has a CC_{50} (50% cytotoxic concentration) of 16 μg/ml against Caco-2, and is nontoxic at 0.0313 μg/ml [57]. Tetrazole derivatives with pyrrolidine scaffolds were also evaluated. Compound **5** (Figure 3) and other derivatives were tested for their ability to prevent *C. albicans* adhesion to TR-146 (buccal mucosa) cell-line monolayer. At high concentration, compound **5** was toxic to mammalian Vero cells (100% reduction of viability at 129 μg/ml), but its cytotoxicity decreased by fourfold at lower concentrations. Compound **5** reduced the adherence of the yeast most efficiently (over 98% reduction at 16 μg/ml). However, at that concentration, the compound caused yeast cell death, so it is likely that this produced the observed reduction in cellular adhesion [58].

Disruption in the localization of GPI-anchored proteins

GPI-anchored cell wall mannoproteins are required for the adhesion of pathogenic fungi, such as *C. albicans*, to human epithelium. Tsukahara *et al.* [59] discovered an isoquinoline derivative, compound **6**, that inhibits the localization of cell wall GPI-anchored mannoproteins in *Saccharomyces cerevisiae* and *C. albicans*. Compound **6** prevented the adhesion of *C. albicans* cells to a monolayer of rat intestinal epithelial cells (Figure 4). The molecular target of this compound was found to be the protein product of a novel gene, *GWT1*. They suggest that GWT1 protein may be involved in the synthesis of GPI, transportation of GPI-anchored protein within the cell and the relocation of the GPI-anchored protein to the fungal cell wall.

Inhibition of degradative enzyme production

Extensive research on the anti-adhesive properties of sulfone derivatives has been conducted in Staniszewska's laboratory [60,61]. Compounds **7** and **8** (Figure 5) were evaluated against the adhesion of a wild-type *C. albicans* strain and three mutant strains (Δ*sap9*, Δ*sap10* and Δ*sap9/10*) to a monolayer of Caco-2 cells. First, the fungi were pretreated with the sulfone derivatives. Compound **7** significantly altered the adherence properties in a concentration-dependent manner. Adhesion of the wild-type *C. albicans* was 10.8-fold lower than the nontreated controls at a concentration of 8 μg/ml, and was 5.2-fold lower at a concentration of 16 μg/ml. The mutant strains displayed a larger inhibition of adhesion. When the yeast cells were post-treated with the sulfone derivatives at 16 μg/ml, there was a very significant reduction of adhesion, ranging from 44.0- to 112.1-fold [60]. Compound **8** also exhibited reduced adhesion, but to a lesser degree than compound **7**.

In another study, structurally similar sulfones were tested against *C. albicans* wild-type and mutant strains Δ*efg1* and Δ*cph1*, which lack morphological transitionality. The various strains showed different susceptibility to the

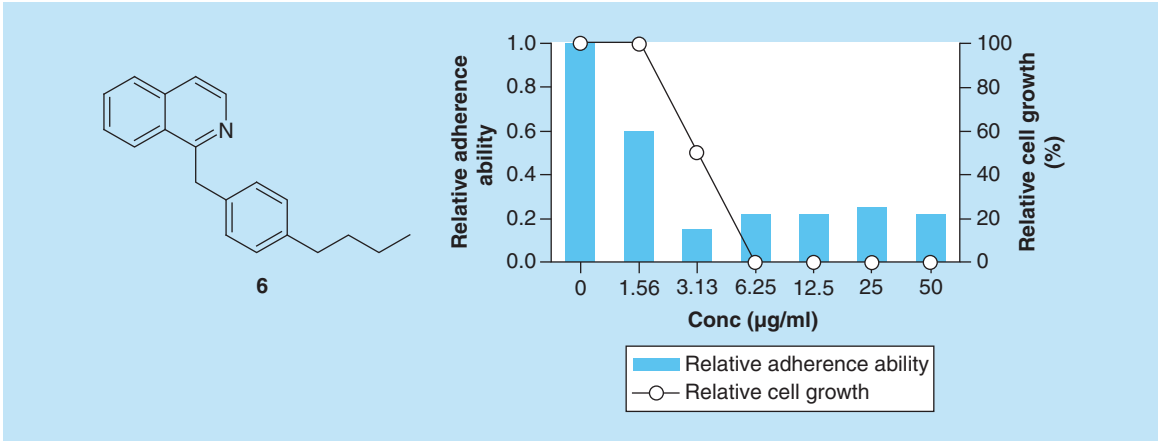

Figure 4. Disruption in the localization of GPI-anchored proteins. Structure of isoquinoline derivative **6** and graph showing the effect of **6** on the adherence ability of *Candida albicans*.
Adapted with permission from [59].

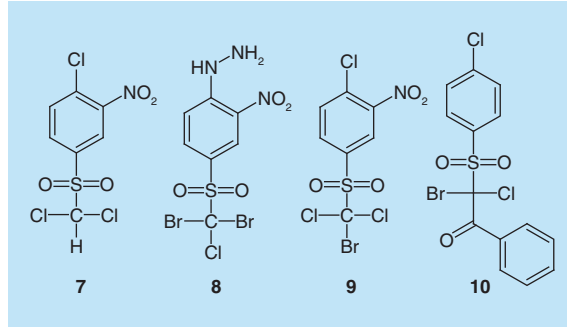

Figure 5. Inhibition of degradative enzyme production. Structure of sulfone-derivatives that have anti-adhesive properties toward *Candida albicans*.

sulfone derivatives. Compound **9** and the previously evaluated sulfone derivatives **7** and **8** (Figure 5) were also tested for their ability to inhibit the adhesion to epithelial cells. At concentrations of 8–16 μg/ml, pretreatment of the cells with sulfones **7** and **9** did not inhibit the adhesion of all the *C. albicans* strains tested. However, at the highest concentration (16 μg/ml) sulfones **7** and **9** inhibited attachment of the wild-type *C. albicans* to the Caco-2 monolayer. Compound **8** affected the adhesion of the tested strains significantly at all the concentrations, with the best results at a concentration of 16 μg/ml in 62% of strains [61].

More studies were carried out on compound **7** and a new β-ketosulfone compound **10** (Figure 5). The yeast cells were pretreatment with the β-ketosulfone (16 μg/ml), which significantly inhibited the adhesion of *C. albicans* to the Caco-2 monolayer, showing a reduction in adhesion of 49.46% (1.97-fold reduction compared with the control) [62].

These sulfone derivatives successfully inhibit degradative enzyme production, for example secreted aspartyl proteases, which have been associated to the enhancement of adhesion processes by the degradation of extracellular matrix and host surface molecules, allowing penetration into host tissues. *SAP2* is downregulated in the presence of these sulfone derivatives. The ability of these compounds to inhibit enzyme production may influence the adhesion of *C. albicans* to host cells.

Downregulation of genes that encode adhesins

Numerous natural products have been tested for their anti-adhesive properties toward *C. albicans* adhesion. These include polyphenols such as curcumin, pyrogallol, magnolol and honokiol, and anthraquinone derivatives such as purpurin, alizarin, chrysazin and emodin. These compounds' mechanism of action involves the downregulation of *C. albicans* adhesin genes.

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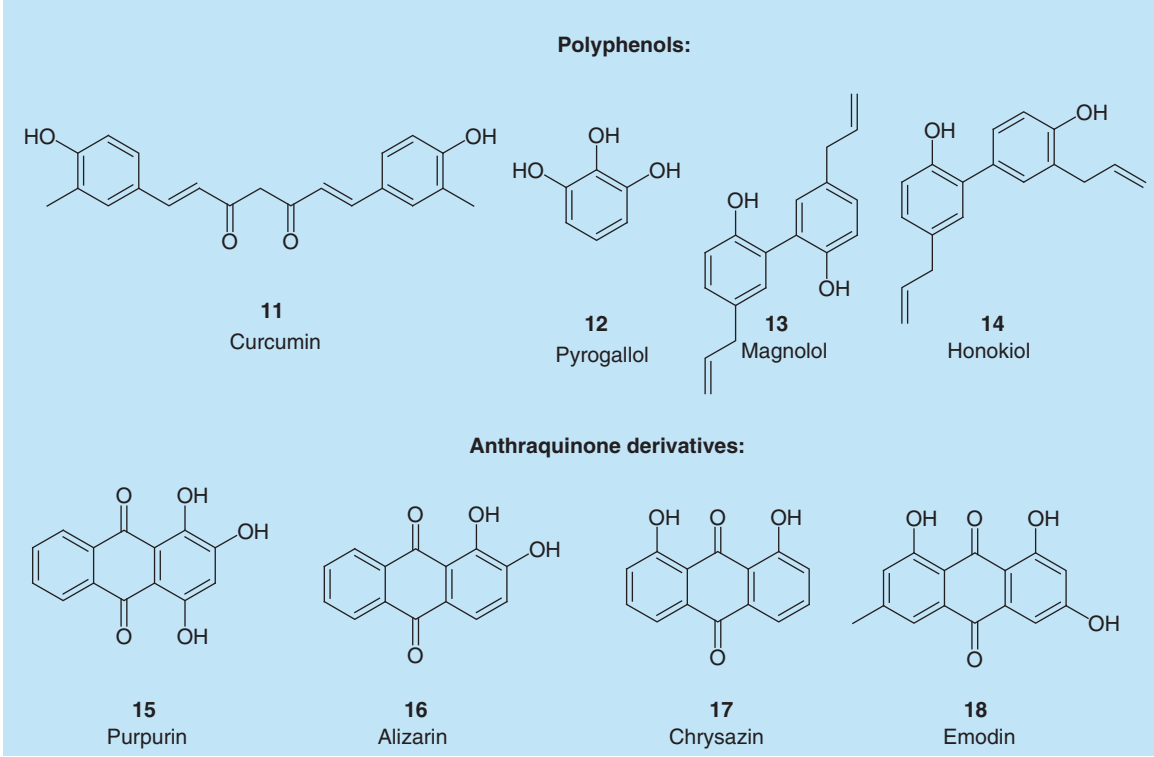

Figure 6. Downregulation of genes that encode adhesins. Structure of polyphenols and anthraquinone derivatives that have been shown to inhibit the adhesion of *Candida albicans* by downregulation of genes that encode adhesins..

Polyphenols

Curcumin (**11**) (Figure 6) is a yellow polyphenol produced by the rhizome of *Curcuma longa*, or turmeric, plants. This compound possesses a wide range of pharmacological properties. In this work, curcumin and curcumin extracts were evaluated for antifungal activity against numerous fungal strains. The ability of curcumin to inhibit the adhesion of *Candida* spp. to human BEC was determined. Curcumin (64 μg/ml) inhibited the adhesion of *C. albicans* to BEC by >50% [63].

In another study, fourteen polyphenols were tested for their antifungal properties. From these compounds, **11** and pyrogallol (**12**) (Figure 6) showed the best antifungal properties. These compounds were also evaluated for their anti-adhesion properties at $SMIC_{50}$ (sessile minimum inhibitory concentrations), 50 μg/ml for **11** and 39.06 μg/ml for **12**. The precoating of coverslips with **11** reduced the ability of *C. albicans* to adhere by 55.3%, while **12** only reduced adhesion by 15.63%. Compound **11** resulted in the downregulation of the expression of Als3 and Hwp1 adhesins, whereas **12** showed no significant downregulation of the adhesins following treatment [64].

In an effort to increase the activity of **11**, curcumin-silk fibroin nanoparticles were developed using silk fibroin (insoluble protein present in silk) as the carrier. These nanoparticles could inhibit the adhesion of *C. albicans* to pulmonary epithelial cells significantly compared with curcumin alone [65].

Magnolol (**13**) and honokiol (**14**) (Figure 6) are lignans found in the bark of species of magnolia. These compounds significantly inhibited the adhesion of *C. albicans* to a monolayer of immortalized rat hepatic stellate cells in a concentration-dependant manner, ranging from 4 to 32 μg/ml; for example, at 4 μg/ml, adhesion was reduced by approximately 15–20% while at 32 μg/ml, adhesion was reduced by >95%. The viability of the mammalian cells were not affected by compound **13** or **14** at the concentration range studied [66]. Compounds **13** and **14** both produced significant downregulation of *HWP1*, *ALS3* and *ECE1* genes which encode adhesins.

Anthraquinone derivatives

Purpurin (**15**) (Figure 6) is a naturally occurring red/yellow dye from the roots of the *Rubia tincorum*, or madder plant. The anti-adhesion properties of purpurin were evaluated at a range of concentrations (10–40 μg/ml).

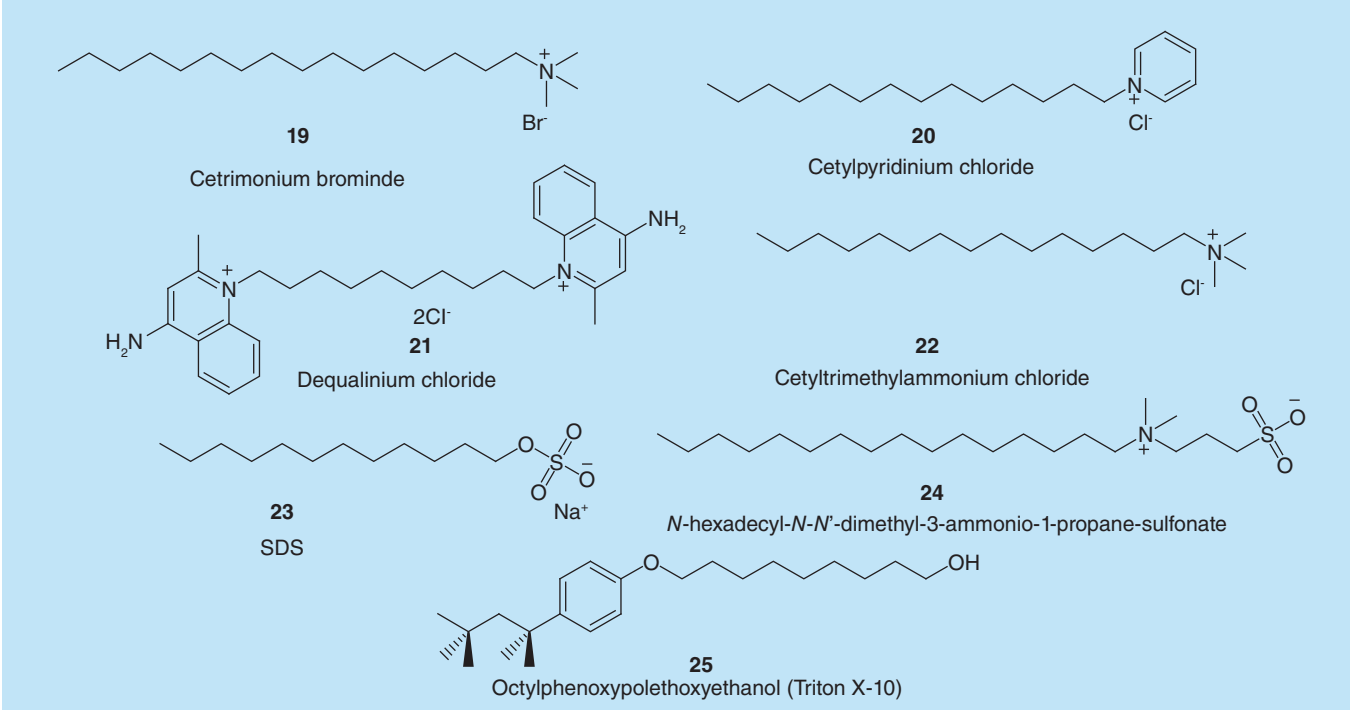

Figure 7. Disruption of cell membrane structure. Structure of compounds that have the ability of reducing the adhesion of *Candida albicans* by disruption of the fungal membrane.

It was found that **15** has an effect on adhesion of *C. albicans* in a dose-dependent manner. Also, expressions of adhesion-related genes, namely *ALS1*, *EFG1* and *HWP1* were decreased in comparison with the control [67].

Alizarin (**16**) (Figure 6) is a red dye derived from the roots of plants of the madder genus. Chrysazin (**17**) (Figure 6) is a synthetic derivative of alizarin. **16** and **17** (2–10 μg/ml) appear to inhibit cell adhesion, biofilm formation and hyphal development in *C. albicans* by regulating hypha-specific genes [68]. **16** also downregulated the expression of several hypha-specific and biofilm-related genes (*ALS3, ECE1, ECE2* and *RBT1*), which affect the adhesive properties of the yeast. The toxicities of compounds **16** and **17** were examined using a nematode model by determining their survival after 4 days. This showed that both **16** and **17** are less toxic then fluconazole.

Emodin (**18**) (Figure 6) is a natural secondary plant product, originally isolated from the rhizomes of *Rheum palmatum*, or Chinese rhubarb, plants. **18** has been tested for its anti-adhesion properties to polystyrene microtiter plates (3–400 μg/ml). In this study, 50 strains of *C. albicans* isolated from vaginas of women in diverse age categories were tested. It was found that this compound could inhibit the adhesion of *C. albicans* to the plates in 30 of the strains, while the adhesion of only 15 of the strains were affected by **18** when the biofilm had been established [69]. Due to the structural similarities to purpurin and alizarin, it is assumed that this compound may also cause downregulation of genes relating to adhesion. Interestingly, emodin added to *Candida* culture also inhibited the phosphorylation of many cellular proteins, leading to the inhibition of protein kinase CK2, which governs the interactions of *C. albicans* with endothelial and oral epithelial cells *in vitro* and virulence during oropharyngeal candidiasis.

Disruption of cell membrane structure

Quaternary ammonium compounds

Many quaternary ammonium compounds have been found to have anti-adhesive properties against *C. albicans* and some of their structures are shown in Figure 7. Nonantibiotic, antimicrobial agents (cetrimonium bromide [**19**], cetylpyridinium chloride [**20**] and dequalinium chloride [**21**]) at sublethal concentrations were shown to reduce the adherence of *C. albicans* to human BEC *in vitro* [70].

Research has also been carried out on surfactants **22**–**25** to test their anti-adhesive properties against *C. albicans*: cetyltrimethylammonium chloride (**22**), SDS (**23**), *N*-hexadecyl-*N*–*N'*-dimethyl-3-ammonio-1-propane-sulfonate

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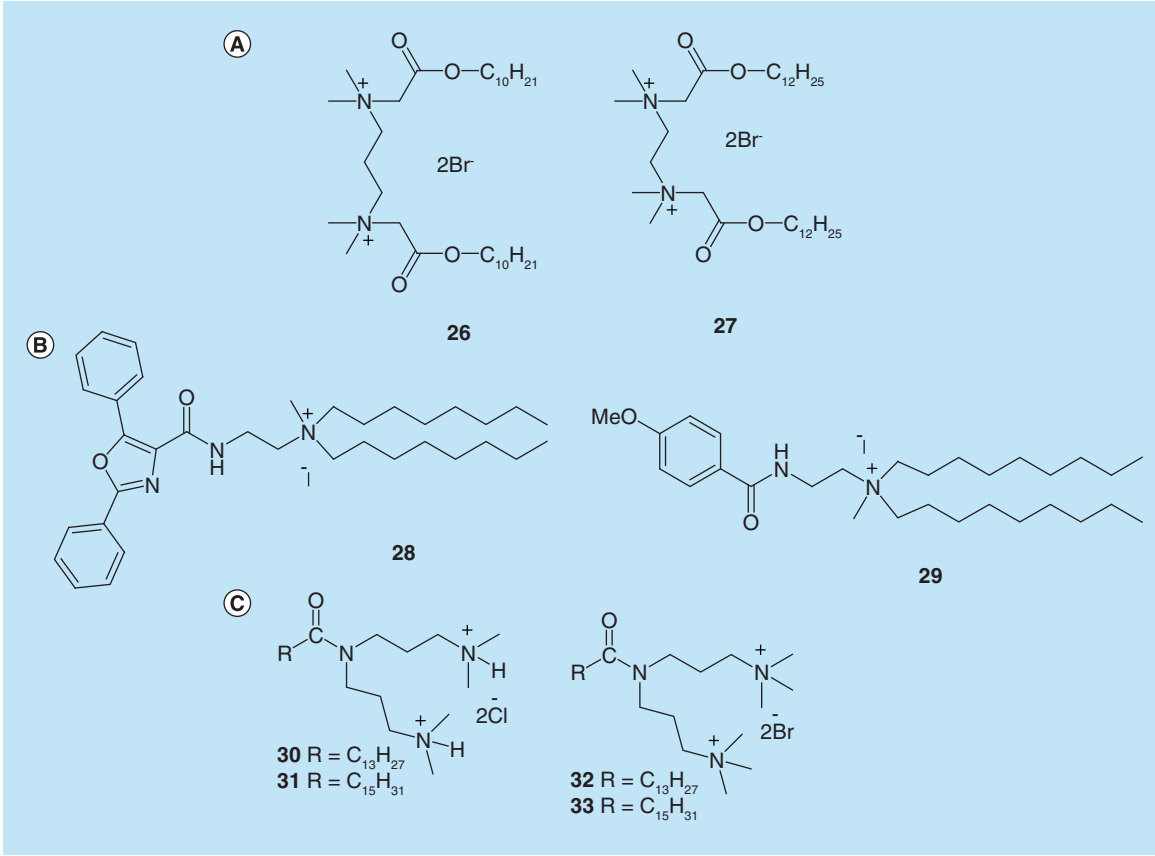

Figure 8. Structures of quaternary ammonium compounds that inhibit the adhesion of *Candida albicans*. (A) Structures of alanine-derived gemini quaternary ammonium salts; **(B)** structures of medium alkyl chain cationic lipo-oxazole and lipo-benzamide compounds; **(C)** structure of double-headed cationic surfactants.

(HPS; **24**) and octylphenoxypolyethoxyethanol (Triton X-100; **25**). All the surfactants tested displayed a decrease in the number of yeasts adhered to BEC; however, the greatest reduction was obtained by **22** (37.1–53.8%) and **24** (32.7–43.5%) in all concentrations tested (0.3–3 μg/ml) and for **23** (28.2–35.8%) and **25** (28.8–30.1%) at the concentrations of 1.5 and 3 μg/ml, respectively [71].

The principal factor which accounts for inhibition of adherence is the denaturation of adhesins on *C. albicans* by the cationic charge of these surfactants. Additional factors include the disruption of the fungal membrane and steric interference on the approach of the microbial cell to the epithelial cell (long hydrophobic chains decrease contact between microbial cell and the substrate). Alteration of cell surface hydrophobicity and cell zeta potential have also been suggested to contribute to the observed activities [71].

A collection of alanine-derived gemini quaternary ammonium salts featuring alkyl chains and spacers of different lengths was tested for inhibition of adherence of *C. albicans* to polystyrene and silicone surfaces (structures of two examples are shown in Figure 8, compounds **26** and **27**). The deposition of gemini quaternary ammonium salts on the polystyrene plate inhibited yeast cell adhesion, with 50% reduction exhibited only at the highest concentrations of 110 μg/ml (**26**) and 117 μg/ml (**27**). Both compounds also exhibited anti-adhesive properties to the silicone catheter, with compound **27** showing slightly better activity [72].

Cationic lipo-oxazoles containing hydrocarbon chains (C_6–C_{13}) and a quaternary ammonium group were evaluated for their antifungal, anti-adhesion and biofilm inhibition properties. Heptyl and octyl chain analogs showed promising antifungal activity. Cationic lipo-oxazole compound **28** (Figure 8B) demonstrated about 60% reduction in adhesion to polystyrene surfaces at a concentration of 12.5 μg/ml compared with the control. Also the cytotoxicity of **28** was determined in mammalian cells, and IC_{50} values of 13.1–45.2 μg/ml were calculated [73]. This cationic agent reportedly reacts with the phospholipid component in the cell membrane, thereby producing membrane distortions often leading to a complete loss of structural organization of the cells.

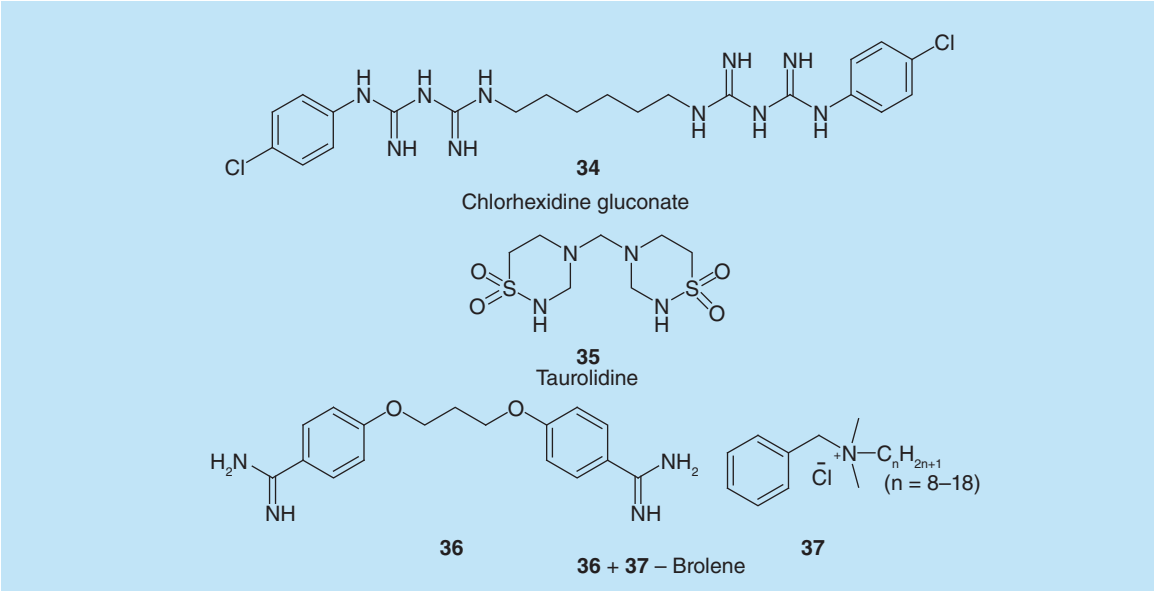

Figure 9. Nonantibiotic antimicrobial agents. Structure of nonantibiotic antimicrobial agents that have been shown to inhibit the adhesion of *Candida albicans*.

The same research group then evaluated lipo-benzamide compounds fused with varying lengths of hydrocarbon chains (C_2–C_{18}) for antifungal activity against *C. albicans*. Quaternary ammonium group containing C_9 hydrocarbon chain derivative **29** (Figure 8B) inhibited the adhesion of *C. albicans* to polystyrene surfaces in a concentration-dependent manner. In particular, at 12.5 μg/ml compound **29** inhibits 90% adhesion compared with the control [74].

Double-headed cationic surfactants with varying hydrocarbon chain length (n-$C_{13}H_{27}$ and $C_{15}H_{31}$) were synthesized and their antifungal properties were investigated. The ability of these surfactant coatings to inhibit fungal adhesion was tested using different surfaces: polystyrene, silicone, glass and stainless steel. The adhesion studies were focused on C_{14}(DAPACl)$_2$ (**30**), C_{16}(DAPACl)$_2$ (**31**), C_{14}(TAPABr)$_2$ (**32**) and C_{16}(TAPABr)$_2$ (**33**) (Figure 8C). The adhesion of *C. albicans* to polystyrene was not greatly affected by the tested compounds, with the chlorides showing slightly better results. *C. albicans* adhesion to silicone catheters was reduced by C16 dicephalic surfactants **31** and **33**. Significant reduction (~50%) was observed for C_{16}(TAPABr)$_2$ **33** at 100 μM (or 61.5 μg/ml). Unexpectedly, C14 compounds (**30** and **32**) resulted in an increase of *C. albicans* adhesion. Both chlorides (**30** at 47 μg/ml and **31** at 50 μg/ml, 100 μM) drastically reduced the adherence of *C. albicans* to the stainless steel surface. All of these dicephalic surfactants had similar results in the adhesion assays to the glass surface and for most cases, significant reduction in the amount of adherent cells was only achieved at concentrations at high as 400 μM (199–246 μg/ml) [75]. Interestingly, these compounds did not cause DNA leakage from the *C. albicans* cells, with the exception of C_{14}(DAPACl)$_2$ (**31**). Therefore, the mode of action of dicephalic surfactants does not involve cell lysis. Instead, the dicephalic surfactants impacted reactive oxygen species (ROS) production and accumulation of lipid droplets.

Nonantibiotic antimicrobial agents

Nonantibiotic antimicrobial agents, compounds which affect multiple cellular targets in a nonspecific way, have a similar effect as the quaternary ammonia compounds on the adhesion of *C. albicans*. Several studies have shown that chlorhexidine gluconate (**34**) (Figure 9) is able to reduce the adhesion of *C. albicans* cells to BEC at very low concentrations (as low as 0.00005% v/v or 0.5 μg/ml) [70,76–78]. In recent years, in fact, chlorhexidine is often used as a positive control when testing the adhesive properties of other compounds [79–82]. This antifungal impairs the integrity of the cell wall and the plasma membrane entering the cytoplasm resulting in leakage of cell contents and leading to cell death.

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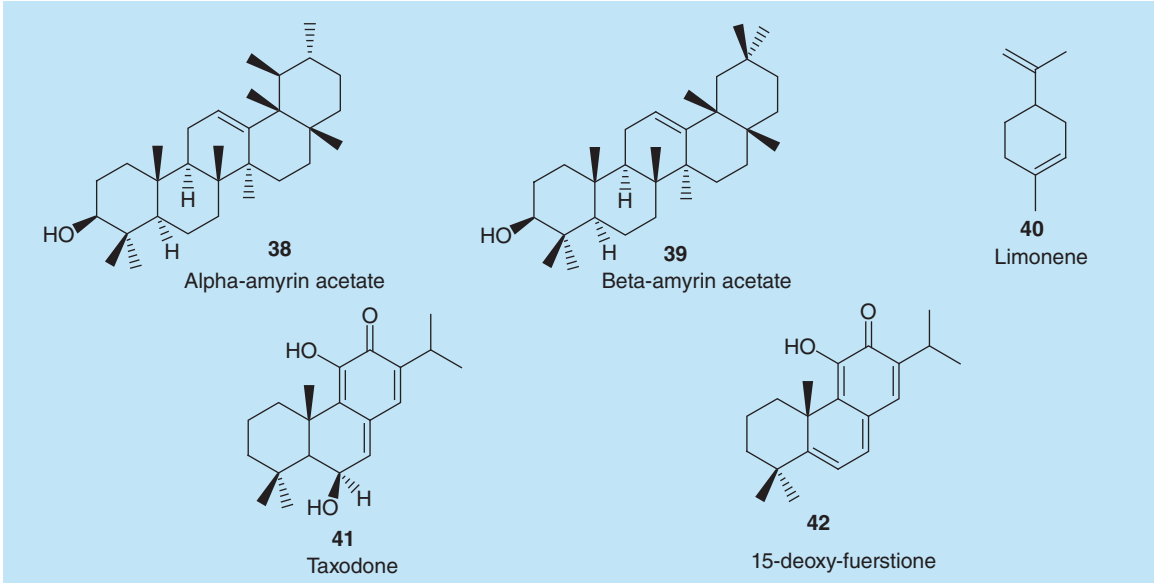

Figure 10. Terpenes and terpenoids. Structure of two terpene derivatives (**38** and **39**), limonene **40** and two terpenoids (**41** and **42**) that inhibited the adhesion of *Candida albicans*.

Another known antimicrobial agent, taurolidine (**35**) (Figure 9), was analyzed for its anti-adhesive properties. **35** at 2% concentration is usually used to prevent infections in catheters. It has been shown to reduce the adherence of *C. albicans* (vaginal isolate) to buccal and uroepithelial cells at concentrations below 0.1% (or 1000 μg/ml) [83].

Brolene is a nonantibiotic, antimicrobial preparation, which contains propamidine isethionate (**36**) as the antimicrobial active agent and benzalkonium chloride (**37**) (Figure 9) as a preservative, both of which are cationic agents. Two strains of *C. albicans*, including one clinical isolate from an oral infection, were employed in this study. At both concentrations examined (10 and 100% w/v, or 0.1 and 1 g/ml), brolene significantly reduced the adherence of *C. albicans* to BEC. These effects were observed for both strains, when either the BEC or yeast were treated. Reductions in adherence ranged from 25.91 to 76.02% [84].

Terpenes & terpenoids

Terpenes are a large and diverse class of natural compounds produced by numerous plants and insects. The amyrins are a family of natural triterpenes, which include α-, β- and δ-amyrin. The antifungal activity of these natural compounds, along with synthetic derivatives, was evaluated against *Candida* species. Of the compounds tested, α- and β-amyrin formiate (**38** and **39**) (Figure 10) and α- and β-amyrin acetate inhibited the growth of all the tested *Candida* species at concentrations of 30–250 μg/ml. In particular, **38** and **39** at 60 μg/ml inhibited the adhesion of *C. albicans* to BEC by 65.3% [85].

Limonene (**40**) is a nontoxic (up to 15 g/day), cyclic monoterpene, which has been shown to inhibit adhesion of *C. albicans* to abiotic surfaces at concentrations 0 of 0.163–2.7 mg/ml [86]. Specifically, limonene inhibited the development and maturation of biofilms by 50% at concentrations of 0.245, 0.163 and 1 mg/ml, respectively, and inhibited adhesion by 90% at 0.681 mg/ml. Proteomic analysis suggests that **40** inhibits *C. albicans* growth by cell wall/membrane damage inducing oxidative stress that causes DNA damage, which leads to apoptosis.

Terpenoids are a large and diverse family of natural compounds derived from terpenes, comprising approximately 60% of known natural products. Two abietane diterpenoids isolated from *Salvia austriaca*, or Austrian sage, taxodone (**41**) and 15-deoxy-fuerstione (**42**) (Figure 10) were tested in *C. albicans*. **41** was found to significantly limit the degree of *C. albicans* adhesion to polystyrene microtiter plates by about 41% at both concentrations of 15.6 and 31.3 μg/ml. Biofilm formation was also inhibited by 75.5 and 76.4% at both concentrations. **42** did not perform as well as taxodone, inhibiting the adhesion of *C. albicans* by 38.3% at 125 μg/ml [87]. It has been proposed that abietane diterpene can cause hydroxyl radical formation, which may cause leakage of the cell contents by disruption of the fungal cell membrane. For terpenoids, the mechanism of action is not fully understood, however, it is postulated that these lipophilic compounds disrupt the cell membrane and that their functional groups affect

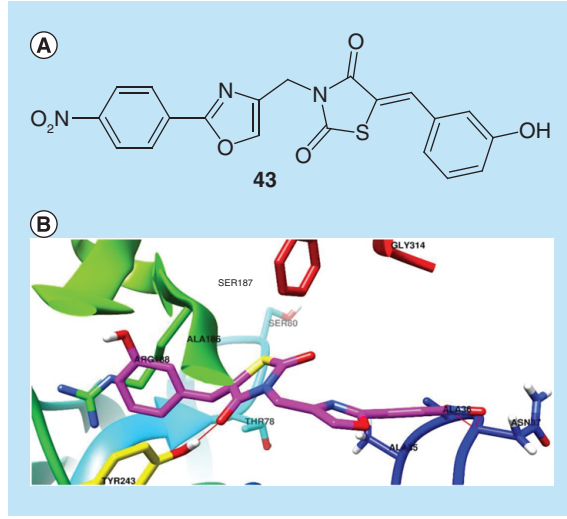

Figure 11. Heterocyclic derivatives. (A) Structure of *N*-(oxazolylmethyl)-thiazolidinedione compound **43**; **(B)** compound **43** docked in the binding site of the *Candida albicans* Als1 surface protein [89].

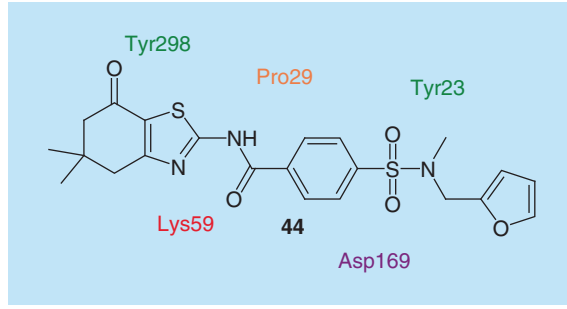

Figure 12. Heterocyclic derivatives. Structure of compound **44**, with amino acids involved in interactions in the binding site of the *Candida albicans* Als3 adhesin. Adapted with permission from [90].

enzyme structure. It has also been suggested that terpenoids inhibit the synthesis of 1,3-β-D-glucan, a component of the fungal cell wall [85,87]. All of these processes impact the adhesion of *C. albicans*.

Blocking the interaction of fungal adhesins & host cell receptors

The inhibition of the interactions between fungal adhesins and host cell receptors can be attempted through several approaches, including: competitive binding with analogs of host cell receptors; binding to specific lectins; use of anti-adhesion antibodies; modulations of adhesins or host cell receptors induced by drugs, enzymes or other substance; use of inhibitors of enzymes involved in biosynthesis of the cell wall components [88]. Some of these processes overlap to some extent with the anti-adhesion mechanisms discussed in previous sections.

Heterocyclic derivatives

The nitro-benzene compound **43** (Figure 11), which possesses various structural moieties present in known antibiofilm agents, was identified by Marc *et al.* [89]. Compounds containing a *N*-(oxazolylmethyl)-thiazolidinedione scaffold were found to be selective inhibitors of *C. albicans* biofilm formation. *In silico* screening suggested that the compounds could act by binding to the *C. albicans* Als surface proteins, especially Als1, Als3, Als5 and Als6, which are well-known adhesion proteins. The binding pocket of these proteins have a large percentage of polar amino acids, which could account for their ability to form polar interactions with various ligands, such as the *N*-(oxazolylmethyl)-thiazolidinediones present in compound **43**.

More recently, *in silico* screening identified 24 potential compounds with the ability to interact with Als3 adhesin of *C. albicans*. Als3 is specific to hypha and hence is involved in the invasion process. Biological assays narrowed the compounds down to five with the ability to inhibit the adhesion and biofilm formation of *C. albicans* to abiotic surfaces at concentrations between 1 and 512 μg/ml. The best performing compound **44** shows hydrogen-bonding interactions with lysine, tyrosine and aspartic acid residues in the binding site of the protein, along with two π–π interactions with tyrosine residues (Figure 12). This compound reduced the adhesion by 96.00% and biofilm formation by 95.33% to the abiotic surface [90].

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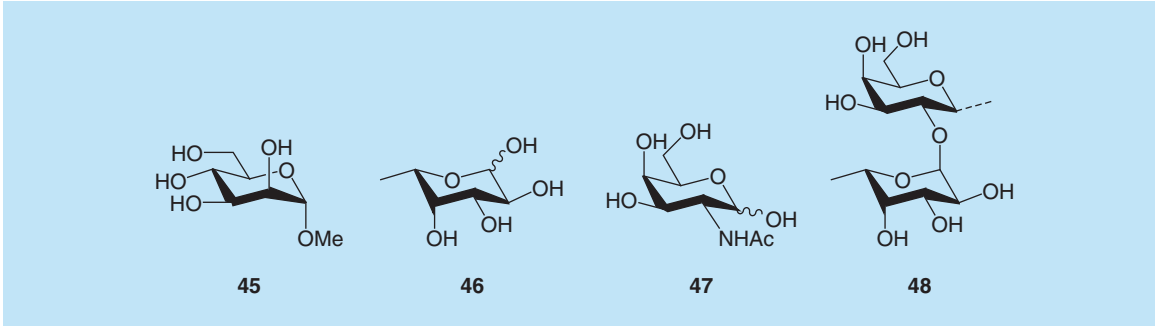

Figure 13. Carbohydrate derivatives. The structure of α-D–methyl mannoside (**45**), L-fucose (**46**), *N*-acetyl-D-glucosamine (**47**), Fucα(1–2)Galβ moiety (**48**), found to inhibit the adhesion of *Candida albicans*.

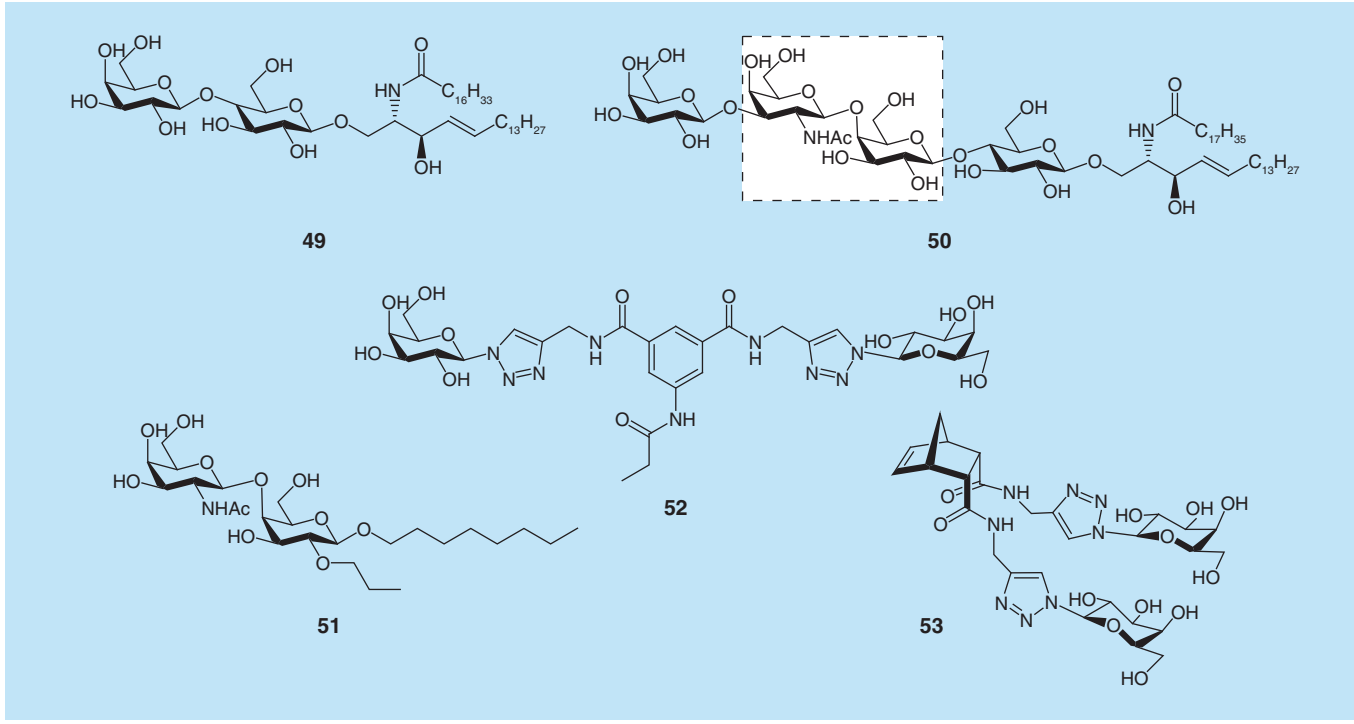

Figure 14. Carbohydrate derivatives. Structure of carbohydrate inhibitors of *Candida albicans* adhesion: lactosylceramide (**49**), asialo-GM_1 (**50**) highlighting the minimal binding sequence, fimbrigal-P (**51**) and divalent galactosides (**52** and **53**).

Carbohydrate derivatives

As discussed previously, early reports indicate that some *C. albicans* adhesins recognize and bind to a broad range of cell surface glycans and carbohydrates [41–45,49]. In these studies, it was found that not only complex oligosaccharides, but also mono and disaccharides (e.g., α-D-methyl mannoside [**45**] [41], L-fucose [**46**] [42], *N*-acetyl-D-glucosamine [**47**] [49], Fucα(1-2)Galβ moiety [**48**] [43], structures shown in Figure 13) can affect the adhesion process of *C. albicans* to human buccal BEC.

More complex carbohydrate-based compounds have been found to participate in the adhesion process of *C. albicans*. Glycosphingolipids in particular can act as adhesion receptors for yeasts. *C. albicans* bound specifically to lactosylceramide (**49**) (Galβ(1-4)Glcβ(1-1)Cer; Figure 14), and required the terminal galactosyl residue for binding [91]. It has also been reported that *C. albicans* yeast form expresses an adhesin that binds to asialo-GM_1 (**50**) (gangliotetraosylceramide: βGal(1-3)βGalNAc(1-4)βGal(1-4)βGlc(1-1)Cer), a glycosphingolipid displayed on the surface of human BEC (Figure 14). It was found that the minimal carbohydrate sequence required for binding was βGalNAc(1-4)βGal [92]. This disaccharide and synthetic derivatives have been shown to inhibit *in vitro* binding of

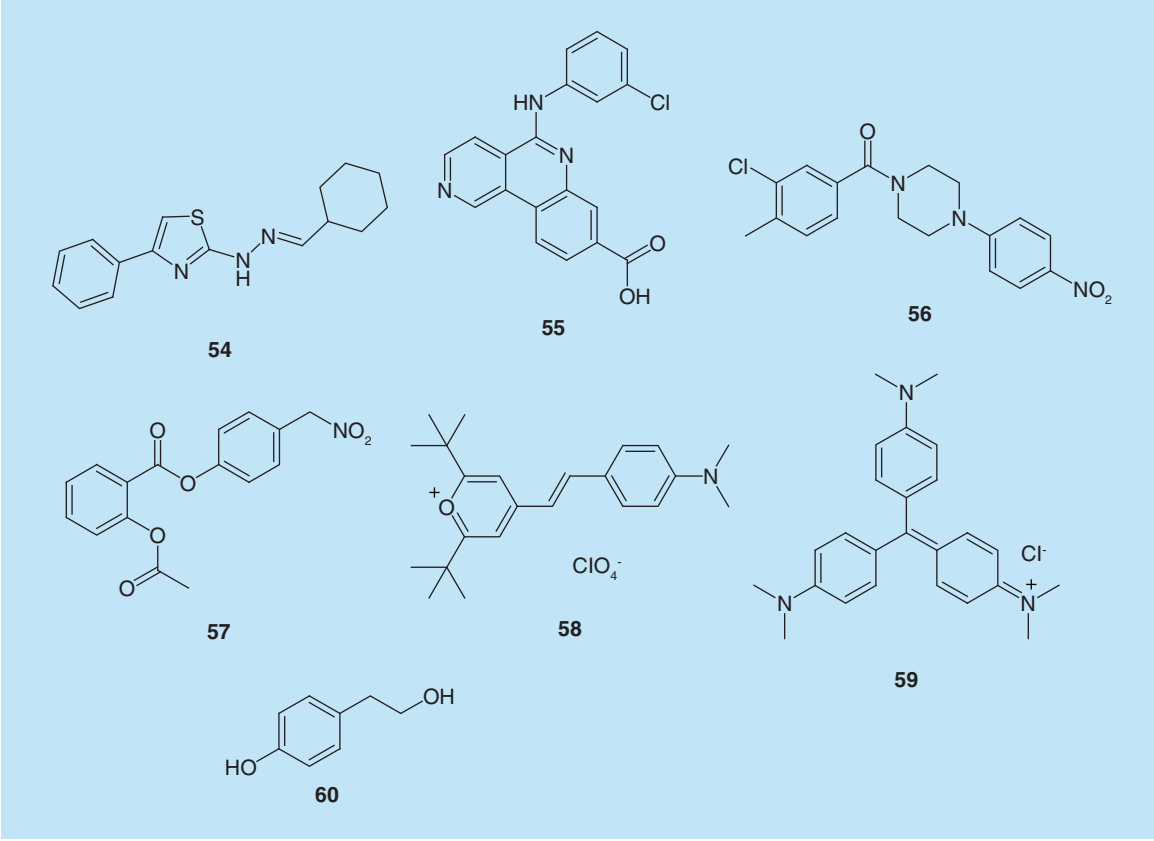

Figure 15. Unknown/other mode of action. Structures of compounds with an unknown mechanism of action that cause reduction of adherence in *Candida albicans*.

BEC to the adhesin and the yeast cells. Fimbrigal-P (**51**) (octyl-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1-4)-2-*O*-propyl-β-D-galactopysanoside) is a propyl-derivative of this disaccharide (Figure 14). The *in vivo* efficacy of **51** was evaluated in a rat model for oral candidiasis. This compound was able to reduce the oral fungal burden, making it a promising candidate for the prevention and treatment of microbial infections in which the pathogen relies on the βGalNAc(1-4)βGal disaccharide to establish adherence [93].

Recently, a small library of aromatic glycoconjugates was evaluated for activity as *C. albicans* adhesion inhibitors [94]. This study showed that divalent galactoside (**52**) (Figure 14) could inhibit adhesion up to 80% when the yeast were pretreated with the glycoconjugate. Also, this compound could displace up to 50% of yeast already adhering to BEC at a concentration of 0.1 mg/ml. In a later study, a second generation of divalent galactosides were assayed for anti-adhesion activity. Compounds with norbornene core such as **53** (Figure 14) presented activity comparable with lead compound **52**, highlighting the importance of the presentation of the two galactosides toward inhibition of *C. albicans* adhesion [95].

Unknown/other mode of action

Thiazole (**54**) and diazanaphthalene (**55**) functionalities are also present in compounds that exhibit anti-adhesion activity and their structures are shown in Figure 15. Compound **54** inhibited adhesion of *C. albicans* to human BEC by 75% at MIC values (1–4 mg/ml for most strains) [96]. This antifungal was also tested on *Cryptococcus* strains. Its mode of action is related to the interference with the antioxidant system and accumulation of superoxide radicals. These destructive events prevent the fungi from employing its virulence factors, therefore inhibiting *C. albicans* adhesion. However, it is not clear if this is a consequence of the fungicidal effect exerted by **54** at MIC. Silmitasertib (CX-4945) (**55**), an antitumor drug which is a selective inhibitor of protein kinase CK2, has been found to have antifungal properties. It effectively inhibited the adhesion phase of the biofilm formation process of *C. albicans* by 42% at the concentration of 62.5 μg/ml (1/4 MIC) [97]. The mode of action of this compound is unknown, but could prevent possible cancer-associated candidiasis.

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Statins are widely used for the control of cholesterol, and function by inhibiting the action of 3-hydroxy-3-methylglutaryl-CoA reductase. In *C. albicans*, statins reduce the synthesis of ergosterol and the abundance of a number of adherence associated proteins (e.g., Cip1p, Pst2p and ABC efflux transporter), leading to a reduction in adherence to BECs. It has been suggested that the altered ergosterol content may influence the composition of the cell wall and the amount or availability of adhesins on the yeast cell surface [98].

Chemical screening of 30,000 compounds by Fazly *et al.* [99] identified filastatin (**56**) (Figure 15) as a small molecule inhibitor of *C. albicans* adhesion, morphogenesis and pathogenesis. Preliminary tests showed that **56** significantly reduced the adhesion of *C. albicans* to polystyrene at a concentration of 9 μg/ml. Interestingly, this compound could also affect adhesion after the *C. albicans* cells had already bound to polystryene. **56** reduced the amount of bound cells when added after adhesion, although not as efficiently as when the compound and cells are co-incubated at the beginning of the experiment. The ability of **56** to inhibit adhesion to monolayers of human lung epithelial cells was also determined, at the same concentration as the previous test (9 μg/ml). **56** significantly inhibited the adherence of green fluorescent protein-labeled *C. albicans* to monolayers of human lung epithelial cells as determined by fluorescence quantitation and microscopy [99]. The mode of action is unknown, but due to the multiple activities of filastatin, it is possible that multiple targets exist.

Previous studies showed that cyclooxygenase inhibitors, such as aspirin, caused a significant reduction in fungal adhesion of fluconazole resistant *C. albicans* at concentrations between 0.18 and 1.8 mg/ml [100]. Nitric oxide (NO) has also been shown to affect adhesion of fungi. NO-releasing xerogel surfaces are effective inhibitors of *C. albicans* adhesion at concentrations as low as 2 pmol cm^{-2} s^{-1} [101]. Madariaga-Venegas *et al.* [102] combined these concepts and evaluated the antifungal/antibiofilm effect of a NO-releasing aspirin (NO-ASA; **57**) (Figure 15) on *C. albicans* isolates from denture stomatitis patients *in vitro*. The ability of *C. albicans* to adhere to polystyrene microtiter plates was evaluated. It was found that **57** (39 μg/ml) caused a decrease in adhesion in all strains tested, ranging from 20 to 77%. **57** inhibited the adhesion of *C. albicans* in a concentration-dependent manner, as the highest tested concentration of 157.6 μg/ml caused the greatest inhibition ranging from 45 to 97%. Interestingly, it was found that the equivalent concentration of aspirin actually increased adhesion of the fungi to the polystyrene plate [102]. **57** has no fungicidal activity, so the observed effects are possibly due to inhibition of PGE_2 synthesis.

In an *in vitro* model of denture stomatitis, compound **58** (Figure 15), a novel antifungal molecule, successfully inhibited the adhesion of *C. albicans* and the development of a biofilm on denture acrylic surfaces. The viability of the biofilm was effected by compound **58**, with 85% reduction in viability when **58** was added before the 1.5 h adhesion phase, 66% reduction when added after, and 97% reduction when added before and after the adhesion phase [103]. This compound is thought to act by causing cell membrane damage.

The effect of sub inhibitory concentrations of gentian violet (**59**) (Figure 15) on the germ tube formation by *C. albicans* and its adherence ability to oral epithelial cells was investigated. At a concentration of 2.4 μg/ml, **59** significantly reduced the adherence ability of *C. albicans* by 57%, with similar results seen for all strains tested [80]. *C. albicans* cells were killed at higher concentrations of **59** (78 μg/ml) while adherence ability and germ tube formation were prevented at subinhibitory concentrations. It is suggested that **59** may inhibit the production of adhesins or disrupt the adhesins already present on the cell surface.

Tyrosol (**60**) is an antioxidant present in natural sources, for example virgin olive oil and wine. **60** has been found to reduce the total biomass and metabolic activity of *C. albicans* at concentrations of 27.6 mg/ml [104]. Colony-forming unit quantification and scanning electron microscope (SEM) observations showed that **60** reduced the number of adhered cells to acrylic surfaces. It was proposed that tyrosol may interfere with the synthesis or expression of filament-associated adhesins on the surface of the cells.

Many essential oils and extracts from plants have also been found to inhibit adhesion of *C. albicans*, but their active compound is generally unknown, as they are composed of complex, unidentified mixtures of both polar and nonpolar natural compounds [105–109].

Future perspective

In light of the antimicrobial drug resistance crisis, fungal infections are becoming recognized as a complex problem for global public health [110,111]. *Candida* spp. are responsible for threatening invasive infections and mucosal candidiasis that affect millions of people [112,113]. The drugs currently used to treat and manage these conditions (namely azoles, echinocandins and polyenes) are still clinically effective, although the toxicity of some of them and the appearance of resistant strains highlight the urgent need for new and improved antifungal agents [114,115]. Since the adhesion of pathogens to surfaces and host cells is critical to initiate infection, molecules that block this process

have therapeutic potential. This strategy has been investigated extensively in bacterial infections [116]; however, the development of inhibitors of *C. albicans* adhesion has encountered some obstacles. In recent years, antifungal drug discovery has focused in a better understanding of the molecular mechanisms of fungal infection; virulence factors, such as fungal cell wall adhesins [29,117], have emerged as promising targets [11,22,71,118]. Antivirulence agents are less likely to be toxic or impaired by the development of resistance than conventional fungicidal drugs; nonetheless, our understanding of the role of adhesion in *C. albicans* virulence mechanisms is still incomplete; this is partly due to lacking detailed structural knowledge of the fungal molecules involved in these complex processes. This has limited structure-based inhibitor design, so the discovery of anti-adhesion compounds against *C. albicans* infections still rely mainly in random compound screening. Therefore, it is also important to develop robust anti-adhesion assays that take into account the physiological environments of cellular adhesion and allow for standardized quantification of activity. An increased awareness of the opportunities that this area of research offers for the discovery of new antifungal agents has recently prompted some breakthroughs, with several compounds undergoing preclinical and clinical trials [113]. The identification of new targets, alongside with the discovery of compounds that target more than one virulence mechanism (i.e., inhibitors of adhesion, morphogenesis and biofilm formation) are promising leads for the development of the next generation of antifungal drugs [119]. Further work addressing these issues should continue to exploit the potential of anti-adhesion therapies not only in *C. albicans* pathogenesis, but also for infections caused by other fungal pathogens.

Executive summary

Candida albicans infections

- *Candida albicans* is a major fungal pathogen responsible for mucosal and invasive infections in immunocompromised hosts.
- Current therapeutic options are limited and often highly toxic, with appearance of resistant strains.
- Targeting adhesion processes of *C. albicans* may lead to new and improved antifungal therapies.

Disruption of *C. albicans* adhesion by antibodies & biomolecules

- Lectins, glycoproteins and antibodies are capable of inhibiting adhesion of *C. albicans* to buccal epithelial cells.

Disruption of *C. albicans* adhesion by small molecules

- Small molecules (synthetic and from natural sources) may have different mechanisms to inhibit the adhesion of *C. albicans* to host cells, which include:
  - targeting the biosynthesis of cell wall components (e.g., inhibitors of β-1,6-glucan biosynthesis);
  - targeting the localization of GPI-anchored proteins;
  - Inhibition of degradative enzyme production (e.g., inhibitors of secreted aspartyl proteases);
  - downregulation of genes that encode for adhesins (e.g., polyphenol and anthraquinone derivatives);
  - disruption of the fungal cell membrane structure (e.g., quaternary ammonium compounds, nonantibiotic antimicrobial agents, terpenes and terpenoids);
  - use of small molecules that competitively inhibit the interaction between the fungal adhesins and the host cell receptors (e.g., heterocyclic and carbohydrate derivatives).

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- **Overview of the molecular mechanisms involved in *Candida albicans* infection process.**

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